Advances in Electron Tomography 3D Reconstruction Technology - Postprint

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Abstract

Electron tomography three-dimensional reconstruction technology can reconstruct three-dimensional structures of heterogeneous cells or macromolecules at the nanoscale, and has become a widely recognized powerful tool for studying biological macromolecular structures. However, electron tomography three-dimensional reconstruction still faces challenges such as imprecise alignment of two-dimensional images, low accuracy of reconstruction algorithms, and extremely slow reconstruction speed. To address these issues, this paper provides a detailed introduction to the development and current status of electron tomography three-dimensional reconstruction, analyzes the principal challenges currently faced, and highlights our research progress in related work on electron tomography three-dimensional reconstruction.

Full Text

Preamble

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Research and Advances in Electron Tomography Three-Dimensional Reconstruction Technology
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Abstract

Electron tomography three-dimensional reconstruction technology can reconstruct the three-dimensional structures of cells or macromolecules that lack structural uniformity at the nanoscale, and has become a recognized powerful tool for studying biological macromolecular structures. However, electron tomography still faces challenges including imprecise alignment of two-dimensional

images, low reconstruction algorithm accuracy, and extremely slow reconstruction speeds. This paper addresses these issues by providing a detailed overview of the development and current state of electron tomography three-dimensional reconstruction, analyzing the major challenges, and highlighting our research progress in this field.

Keywords: electron tomography, three-dimensional reconstruction, alignment algorithm, iterative algorithm, parallel computing, GPU

1. Introduction

Structural biology is a discipline that studies the specific three-dimensional structures of biological macromolecules, their particular motions, and structure-related biological functions. Its origins can be traced to the 1950s when Watson and Crick discovered the DNA double helix structure, and to the 1960s when Perutz obtained the three-dimensional structure of myoglobin using X-ray crystallography [**?**]. Today, structural biology occupies a mainstream position in molecular biology research and has become essential for drug design, vaccine development, and other studies.

As is well known, proteins are one of the executors of life activities, and studying protein three-dimensional structures has always been a primary focus of structural biology. Currently, X-ray crystallography, nuclear magnetic resonance (NMR) spectroscopy, and electron microscopy (EM) three-dimensional reconstruction are the three major research methods in structural biology. Among them, X-ray crystallography offers the highest resolution for structure determination, capable of achieving atomic-resolution three-dimensional structures of biological molecules, but protein crystals are difficult to obtain and structure determination is extremely challenging. NMR can obtain protein three-dimensional structures in solution and capture dynamic information, but its molecular weight limit typically does not exceed 20 kDa. These limitations have greatly restricted our understanding of protein functions and the principles of life activities.

With continuous improvements in biological sample preparation techniques, advances in electron microscopy equipment, and developments in digital image processing, EM three-dimensional reconstruction technology—particularly cryo-electron microscopy (cryo-EM) three-dimensional reconstruction—can analyze proteins that cannot be studied by X-ray crystallography or NMR, compensating for the shortcomings of these two methods and becoming an important tool for investigating biological macromolecular structures. Sali et al. proposed in *Nature* the future direction of structural biology research: integrating multiple biophysical techniques including X-ray crystallography, NMR, cryo-EM three-dimensional reconstruction, and fluorescence resonance energy transfer to understand cellular structures at the molecular level, with cryo-EM three-dimensional reconstruction playing a crucial bridging role [**?**].

The concept of EM three-dimensional reconstruction was first proposed by

DeRosier and Klug in 1968. In the 1980s, the rapid development and practical application of fast-freezing sample preparation techniques and cryo-electron microscopy laid the foundation for using EM three-dimensional reconstruction to study protein structures. Currently, the use of EM three-dimensional reconstruction to resolve protein supramolecular complex structures has evolved into three distinct methods with different characteristics and applications: electron crystallography, single-particle reconstruction, and electron tomography (ET). Electron crystallography is primarily used for analyzing two-dimensional crystals of protein molecules. Single-particle reconstruction mainly resolves three-dimensional structures of proteins with structural uniformity, with recent achievements enabling atomic-resolution analysis of highly symmetric viral molecules [**?**] (for a detailed introduction to single-particle reconstruction, see the article "New Advances in Single-Particle Cryo-EM Three-Dimensional Reconstruction" in Issue 56 of this journal [**?**]). Electron tomography obtains multiple angular projections of the same region to reconstruct the three-dimensional structure of the object, making it suitable for studying non-uniform proteins, viruses, organelles, and their complexes at the nanoscale. Since it does not require structural uniformity or symmetry, although its resolution (approximately 4–10 nm) cannot compare with the other two EM methods, ET plays an irreplaceable role in studying amorphous, asymmetric, and non-uniform biological samples. Moreover, ET' s applicable scale is broad, ranging from molecular-level proteins to subcellular organelles and even cellular-level tissue structures, effectively filling the gap between high-precision structures obtained by X-ray crystallography, NMR, and single-particle reconstruction and low-resolution whole-cell images from optical microscopy, providing important and valuable information for understanding biological macromolecular functions. Consequently, electron tomography was named one of the top ten scientific breakthroughs of 2002 by *Science* magazine [**?**] and has received increasing attention in cryo-EM research in recent years.

2. Electron Tomography Three-Dimensional Reconstruction Technology

Electron tomography (ET) is a technique for reconstructing the internal structure of an object from its electron microscopic projection images. Similar to the well-known CT scanning technology, it reconstructs the three-dimensional structure by acquiring two-dimensional projection images of the same object at multiple consecutive angles. The main strategy involves placing a prepared biological sample in a transmission electron microscope, rotating the sample around an axis perpendicular to the electron beam, capturing a two-dimensional projection image at each rotation angle, aligning this series of two-dimensional projection images, and then using three-dimensional reconstruction algorithms to obtain the sample' s three-dimensional structure. Unlike CT scanning, which rotates the radiation source to obtain projections at different angles, ET fixes the electron beam source and tilts the biological sample to obtain projections from different directions. Depending on the microscope equipment, the sample

tilt range is approximately $±$60° to $±$80° with angular intervals of 1° or 2°. Consequently, ET data suffers from information loss known as the "missing wedge" problem.

The electron tomography reconstruction process mainly includes the following steps [**?**]: (1) sample preparation, (2) acquisition of two-dimensional projection images, (3) alignment of projection images, (4) three-dimensional reconstruction of tomographic images, and (5) post-processing of reconstruction results, as shown in [Figure 1: see original paper].

Sample Preparation: Electron tomography primarily studies three-dimensional structures in cellular environments. Compared with X-rays used in conventional CT scanning, electron beams can penetrate only relatively thin samples, limiting ET studies of large biological specimens such as intact eukaryotic cells. For thick samples like cells that are difficult for electron beams to penetrate, sectioning methods are required, involving dehydration, staining, and subsequent observation and imaging. For smaller or thinner samples that electron beams can penetrate, such as viruses and bacteria, rapid freezing methods can be used for sample preparation and cryo-electron tomography reconstruction. The main advantage of cryo-electron tomography is that samples remain close to their native state, overcoming artifacts potentially introduced by dehydration and staining while better preserving the structural integrity of biological samples.

Acquisition of Two-Dimensional Projection Images: The collection of electron tomography projection images begins with selecting an appropriate imaging region of the sample, followed by continuously collecting projection images of that region at different angles. The process is approximately as follows: (1) locate a suitable region—low-dose imaging protocols are generally used to minimize electron radiation damage to the sample; (2) adjust the height of the specimen holder' s rotation center to maintain the sample within the imaging center range during rotation; (3) focus; (4) achieve precise tracking of the same imaging region; (5) capture the image; (6) tilt the sample to the next angle, relocate the sample' s imaging region center, and repeat the previous four steps: locating, focusing, tracking, and imaging.

Since electron tomography requires repeated imaging of the same sample region, significant radiation damage often occurs. Therefore, precise control of specimen holder tilting, automatic tracking of imaging regions, and electron dose per exposure must be strictly controlled. During projection acquisition, mechanical reasons often cause sample drift during rotation, requiring precise tracking of images at different tilt angles through cross-correlation operations. To improve image contrast and tracking accuracy, colloidal gold particles can be added to the sample. Several automated electron tomography data collection software packages are currently available, such as UCSF Tomography [**?**] and FEI' s Explor3D. These software packages can calculate the image offset trajectory throughout the data collection process based on initial images, significantly reducing additional dose damage to the sample and enabling precise control of

the microscope and CCD camera to complete automatic focusing.

Alignment of Two-Dimensional Projection Images: During projection image collection, inevitable issues such as tilt axis variation, sample movement, projection movement, and inconsistent CCD camera parameters cause offset, rotation, or scaling effects among the series of two-dimensional projection images. Therefore, before three-dimensional reconstruction, alignment of the tilt series projections is essential. This involves first calculating image center shifts caused by specimen holder movement during tilting, and second, correcting tilt angles, image rotation, distortion, and magnification caused by imaging conditions. The quality of two-dimensional projection alignment directly determines the quality of the final reconstruction result.

Currently, commonly used alignment methods include marker-based and image cross-correlation-based approaches. The marker-based method involves adding colloidal gold particles of a certain size as fiducial markers during sample preparation, then establishing a projection model based on gold particle correspondences to solve for alignment parameters such as offset, rotation, and scaling. The commonly used software for this method is IMOD [**?**]. However, added colloidal gold may contaminate the sample, and gold particles may drift during tilting, preventing good alignment. The alternative alignment method uses cross-correlation analysis of the images themselves, with commonly used software including Protomo [**?**]. In addition to IMOD and Protomo, other software packages for two-dimensional image alignment include Spider [**?**] and commercial software such as FEI' s Inspec3D, but precise two-dimensional image alignment remains a challenging problem in this field.

Electron Tomography Three-Dimensional Reconstruction: Electron tomography reconstruction is formed by back-projecting all aligned two-dimensional projection images into a 3D volume. The main algorithms are back-projection reconstruction and iterative reconstruction.

Back-projection reconstruction algorithms include direct back-projection (BP), filtered back-projection (FBP), and weighted back-projection (WBP). The basic idea of direct back-projection is that the density at a point in the three-dimensional object equals the sum of densities of all rays passing through that point. Direct back-projection is the simplest reconstruction algorithm but tends to produce point-spreading artifacts in real space. Filtered back-projection proposes deconvolution of the reconstruction result with a point-spread function, but since the sample' s spatial geometric parameters are unknown, an effective point-spread function cannot be obtained. Weighted back-projection performs weighting on different frequencies of the Fourier transform of two-dimensional projection images before back-projection in real space. Iterative reconstruction algorithms operate entirely in real space, establishing a linear system of equations between densities of all points in the three-dimensional object and densities of all points in projection images, then solving the system iteratively to obtain a three-dimensional model that makes original and reconstructed projection images as similar as possible. Commonly used iterative reconstruction algorithms

include Algebraic Reconstruction Technique (ART) [**?**], Simultaneous Algebraic Reconstruction Technique (SART) [**?**], and Simultaneous Iterative Reconstruction Technique (SIRT) [**?**]. Iterative reconstruction algorithms have consistent weighting functions but require enormous computational effort, often taking substantial time to achieve satisfactory results.

Post-processing of Reconstruction Results: To minimize sample damage, imaging is often performed with low electron doses, resulting in low signal-to-noise ratios in two-dimensional projection images and consequently noisy reconstruction results. Therefore, appropriate denoising is required to highlight the object of interest. Common denoising methods include median filtering and nonlinear filtering, with anisotropic diffusion nonlinear filtering showing good results. To further separate the object of interest from background and noise, segmentation of reconstruction results is necessary. The most common method is manual segmentation, requiring users to draw boundary contours of objects and backgrounds. After segmentation, surface rendering techniques can be used for visualization. Common segmentation software includes IMOD [**?**] and VAT4M [**?**].

2.1 Current Research Challenges

In recent years, electron tomography has made significant progress and become a crucial tool in structural biology research. China has also made rapid advances in electron tomography reconstruction, with domestic institutions acquiring high-end electron microscopy equipment including two Titan Krios and one Tecnai F30, while additional purchases are planned or underway. This provides an excellent opportunity to conduct electron tomography research in China. However, compared with rapidly developing hardware facilities, software research in this area is seriously lagging behind, with three main problems constraining further development of electron tomography reconstruction technology:

1. **Lack of Accurate Two-Dimensional Projection Image Alignment Algorithms**
   As mentioned earlier, various factors during electron tomography imaging cause offset, rotation, or scaling effects among the final image series, requiring alignment before three-dimensional reconstruction. Currently, the most commonly used alignment methods are the aforementioned colloidal gold marker method and markerless alignment methods. The colloidal gold method has many drawbacks, necessitating more universal markerless methods for aligning projection images. Markerless alignment methods mainly include: (1) cross-correlation methods, which suffer from large differences between projections at different angles leading to inaccurate offset calculations and cumulative errors; (2) common-line methods, limited to solving only one alignment parameter and unable to accurately align projection images; (3) iterative alignment methods, which require three-dimensional reconstruction and parameter solving for alignment opera-

tions, resulting in enormous computational cost; and (4) feature-matching-based alignment methods, which use cross-correlation for feature point matching but suffer from imprecise point-to-point correspondence due to cross-correlation limitations. Therefore, electron tomography urgently requires more precise two-dimensional projection image alignment methods.

2. **Lack of High-Precision Three-Dimensional Reconstruction Algorithms**
   Electron tomography reconstruction algorithms mainly include weighted back-projection and iterative algorithms. Weighted back-projection, proposed by Rademacher, is the earliest electron tomography reconstruction algorithm. It utilizes the central slice theorem by back-projecting each two-dimensional projection image toward its tilt angle direction into three-dimensional space, where multiple back-projections superimpose to form the sample' s three-dimensional structure. A weighting filter achieves sampling averaging during reconstruction. Due to its simplicity, most mainstream electron tomography reconstruction software such as IMOD [**?**] and SPIDER [**?**] adopt weighted back-projection. However, this algorithm is susceptible to information loss, causing reconstruction errors in the z-axis direction, and does not account for noise effects, leading to inaccurate results.

   In recent years, researchers have begun using iterative algorithms for electron tomography reconstruction. Since projection directions cover only a limited angular range and projection images contain significant noise, iterative algorithms can achieve better reconstruction results under these conditions of insufficient data and high noise. However, traditional iterative algorithms have several defects: (1) they often arbitrarily select initial reconstruction pixel values, typically using all-zero (or all-one) vectors that may deviate far from true values, requiring many iterations to reach convergence; (2) they employ sequential access strategies when updating projection data, and because adjacent projection directions are highly correlated, this introduces errors that affect reconstruction accuracy and reduce convergence speed; (3) during each iteration update, error compensation considers only the weighting matrix, which is unreasonable since projection error is determined by both the weighting matrix and current pixel values.

3. **Lack of Efficient Three-Dimensional Reconstruction Parallel Algorithms**
   With rapid advances in electron microscopy technology, higher resolution reconstruction images often require increased numbers and sizes of projection images, leading to substantial computational resource and time requirements for electron tomography reconstruction. Faced with such large image datasets, existing methods cannot process data in a timely and effective manner, creating a bottleneck that limits widespread application of electron tomography reconstruction. Extensive research has been con-

ducted on reducing reconstruction time through high-performance computing [**?**]. Most current electron tomography reconstruction software, such as IMOD [**?**] and SPIDER [**?**], implements algorithms on traditional parallel computing architectures like cluster environments. However, the high cost of traditional cluster systems and rapid development of GPU hardware technology have made GPU-based development of efficient electron tomography reconstruction algorithms a current research hotspot [**?**, **?**].

3. Our Work

Addressing the aforementioned problems in current electron tomography, this section introduces our research group' s work in electron tomography reconstruction. First, we present SAMA, a new feature-extraction-based two-dimensional projection image alignment algorithm for electron tomography. Second, we introduce ASART, an iterative reconstruction algorithm with faster convergence and more accurate results. Finally, we describe ATOM, a GPU-based electron tomography reconstruction software.

3.1 Two-Dimensional Projection Image Alignment Algorithm SAMA

To address alignment problems for samples without colloidal gold markers, we propose an accurate, automatic markerless alignment method based on feature matching–SAMA (SIFT-based Automatic Markerless Alignment). This method establishes a nonlinear model describing the relationship between three-dimensional density points and their projection points during imaging, transforming the alignment problem into a parameter estimation problem based on measured samples. By extracting feature points from each two-dimensional projection image as measurement sample points, an appropriate nonlinear model is selected and parameters are estimated based on measurement accuracy. The framework includes four components: feature point extraction, feature point matching, feature point tracking, and model selection with parameter solving. The specific steps of SAMA are as follows [**?**]:

Feature Point Extraction: Image feature points typically consist of two parts: (1) a *detector* representing the unique location of a feature point, generally extracted based on mathematical image characteristics and expressed explicitly as coordinates; and (2) a *descriptor* representing information in the neighborhood for matching different detectors. Earlier feature extraction methods focused on designing discriminative detectors with less emphasis on discriminative descriptors. Our feature point extraction employs a simplified Scale Invariant Feature Transform (SIFT) algorithm that enhances both detector and descriptor. The detector identifies gradient stable points at different scales within the same image, while the descriptor is a 128-dimensional vector describing statistical and orientation information of gray levels around the detector. The SIFT algorithm is currently the most accurate method for image feature point extraction, and the simplified SIFT version significantly improves speed without affecting accuracy.

Feature Point Matching: Since each feature point' s information can be represented by a 128-dimensional descriptor vector, and descriptors for different feature points differ significantly, feature point matching between two images can be performed by calculating minimum Euclidean distance. Additionally, based on the angular separation between two images, the maximum positional difference of a feature point from one image in another image can be estimated. The point with minimum Euclidean distance within this maximum positional difference region is searched as the matching feature point. If this minimum Euclidean distance is below a certain threshold, the match is recorded. Finally, feature point matching is calculated between all image pairs, and incorrect matches are eliminated using the second-order geometric tensor relationship that exists between two projection images.

Feature Point Tracking: Feature chains are formed by concatenating correct matches across images. Each feature chain represents the projection sequence of the same three-dimensional density point. Initially, for a feature point a in one image, its matching point b in other images is found. If b has matching point c in another image, these are concatenated. For each image pair, existing feature chains are searched. If a matching pair corresponds to an existing chain, a point is added to that chain; otherwise, a new chain is generated.

Model Selection and Parameter Estimation: Solving the relationship model between three-dimensional density points and two-dimensional projection points in electron tomography reconstruction can be expressed as: MATH_1 . These six parameters (α, , , j_A) represent the transformation from three-dimensional to two-dimensional coordinates, where α, , are rotation angles around the z, y, and x axes respectively. Here, MATH_2 represents the two-dimensional projection point coordinates in the j-th image, MATH_3 is the projection matrix, r_i represents the i-th three-dimensional density point, and t_j is the translation vector of two-dimensional projection points during imaging. We need to estimate parameters j, r, and t. During parameter estimation, we use bundle adjustment and robust outlier removal methods to estimate parameters according to error characteristics. After obtaining these six parameters, each image is aligned.

Using SAMA to align real electron tomography data yields parameter estimation residuals between 0.2–0.4 pixels, comparable to results from colloidal gold marker-based alignment (0.2–0.7 pixels), with reconstructed images showing similar quality to those aligned using the gold marker method.

3.2 Three-Dimensional Iterative Reconstruction Algorithm ASART

Iterative algorithms consider the reconstructed three-dimensional result f as a linear combination of basis functions b and pixel intensity values x, expressed as: MATH_4 , where J is the number of voxels in three-dimensional space. Traditional voxel models use individual small cubes (voxels) as basic units of three-dimensional images, employing piecewise basis functions that cannot cor-

rectly represent the continuity of the reconstructed sample' s density space. We adopt a blob model that considers the entire three-dimensional space as composed of multiple identical spheres (as shown in [Figure 2: see original paper]). Since spheres intersect and overlap, they better represent density space continuity, yielding more accurate reconstruction results.

According to the electron tomography projection principle, each ray' s projection value can be considered as a weighted sum of intensity values of pixels traversed by that ray, represented by the linear system: MATH_5 , where *p_i* represents the projection value of the *i*-th ray, and *w_{ij}* represents the weighting factor reflecting pixel *x_j*'s contribution to ray projection *p_i*. Under this linear model, electron tomography reconstruction aims to estimate pixel intensity values *x_j* using iterative algorithms based on measured *p_i* values and calculable *w_{ij}* values, ultimately obtaining the entire three-dimensional structure *f*. Under the blob model, since each sphere' s projection value is independent of ray direction, we use a "footprint" method to solve for *w_{ij}* by pre-calculating projection values of a single sphere at different positions and storing them in a lookup table, then obtaining corresponding *w_{ij}* values based on the distance from pixel *x_j* to ray *p_i*.

Existing iterative algorithms still suffer from slow convergence and insufficient accuracy when solving for X values. Therefore, we propose an Adaptive Simultaneous Algebraic Reconstruction Technique (ASART) [**?**] that employs four key techniques to improve convergence speed and obtain high-precision reconstruction results.

Back-Projection Technique (BPT): In existing iterative algorithms, most initial values *X(0)* are selected arbitrarily, typically as all-zero (or all-one) vectors, which may deviate far from true values and require many iterations to converge. To reduce iterations and accelerate convergence, we consider using the direct back-projection technique (BPT) to estimate initial pixel values *X(0)*. BPT is a simple reconstruction algorithm that considers each pixel' s intensity as the weighted sum of all ray projections passing through that point. Therefore, initial pixel intensity *X(0)* can be expressed as: MATH_6 . Although BPT introduces star artifacts when estimating initial pixel intensities, it is closer to true values than arbitrary initialization, thereby accelerating convergence. Moreover, the techniques described below can effectively suppress star artifacts and reduce introduced errors.

Multi-level Access Strategy (MAS): Existing iterative algorithms use a sequential update strategy, processing adjacent projection directions in order. Because adjacent projection directions are highly correlated, this introduces errors. When arranging projection data access, correlation between adjacent projection data should be reduced, making consecutively accessed projection directions as orthogonal as possible to accelerate convergence. To meet this requirement, we employ a multi-level access strategy (MAS) to reorder projection directions so that a series of consecutively accessed projection angles are as uniformly distributed as possible within the angular range. It has been proven

that correlation is minimized when two directions have a 90° angle, and three directions have minimal correlation when the third bisects the angle between the first two. Based on this, MAS can reorder any number of projection directions. Particularly when the number of projection directions is a power of two, the projection direction order resembles one-dimensional fast Fourier transform. Assuming P projection directions numbered sequentially as 0, 1, ···, P-1, MAS divides the P directions into L levels, where L can be expressed as: MATH_7 . At the first level, there are two projection directions at 0° and 90°; the second level includes two directions at 45° and 135°; the third level has four directions at 22.5°, 112.5°, 67.5°, and 157.5°. In the MAS strategy, projection angles at each level sequentially bisect all previous projection angles. In electron tomography, due to microscope limitations, projection directions cannot cover 180°. We modified the MAS strategy to reorder based on direction numbers. At the first level, projection directions numbered 0 and $P/2$ are selected; the second level includes directions numbered $P/4$ and $(3/4)P$; the third level includes directions numbered $P/8$, $(5/8)P$, $(3/8)P$, and $(7/8)P$, with subsequent levels following this pattern. At the final level, directions calculated by this principle may have already been selected, so we search outward from that position to find the nearest unselected direction. Before iteration begins, all projection angles are reordered according to the MAS strategy, and updates proceed according to this new order during each iteration. This strategy greatly reduces correlation between adjacent directions, thereby improving convergence speed.

Adaptive Compensation Correction (ACC): In ASART, we employ a data-driven approach to adjust x_j. During error back-projection compensation at the k-th iteration, we consider not only the weighting factor but also the value of x_j itself. Therefore, the error compensation for pixel intensity x_j from the i-th ray projection is: MATH_8 .

Column Sum Substitution (CSS): In ASART, we use column sum substitution technology, considering a scalar β as the reciprocal of the maximum column sum of the weighting matrix W. The maximum column sum of matrix W can be considered as the maximum number of rays passing through a single pixel. According to the W calculation model, this maximum number equals the number of projection directions. Thus, the scalar can be expressed as: MATH_9 , where B represents the number of projection directions. Using CSS technology, the algorithm no longer needs to sum each column of W during iteration, significantly saving runtime and storage space.

Based on the four key techniques described above, the improved ASART algorithm iteration formula is: MATH_{10} . We have implemented parallel versions of the ASART algorithm on multiple hardware platforms including Dawning 4000H, single GPU, and multi-GPU [**?**, **?**, **?**].

3.3 GPU-Based Electron Tomography Reconstruction Software ATOM

Addressing deficiencies in existing electron tomography reconstruction software, particularly the slow speed of iterative reconstruction algorithms, we developed ATOM [**?**], a GPU (Graphics Processing Unit)-based electron tomography reconstruction software that implements functions including two-dimensional projection image alignment, reconstruction parameter determination, three-dimensional reconstruction, and two-dimensional data visualization. Its features include: an iterative translation-rotation alignment method for improved alignment accuracy; and a parallel iterative reconstruction algorithm on GPU platform, where the Simultaneous Iterative Reconstruction Technique (SIRT) algorithm achieves a 47× speedup.

To ensure compatibility with other EM software, we use the mainstream MRC format for storing electron microscopy image sequences during software operation. The ATOM software design flow is shown in [Figure 3: see original paper], where functions within solid-line boxes have been implemented in ATOM 1.0.

ATOM 1.0 is a cross-platform software developed based on Qt and CUDA libraries, implemented in C++ and C, with interface code in C++ and core code primarily in C, compilable to run on all operating systems supporting Qt and CUDA. ATOM 1.0 separates the interface from executable programs, allowing users to launch the graphical interface or execute directly from the command line. Considering software usability and comprehensibility, we designed the ATOM 1.0 graphical interface with four parts: "Basic Functions," "Parameter Interaction," "Runtime Information Feedback," and "Display" (as shown in [Figure 4: see original paper]). ATOM is open-source software; users can add corresponding code according to their needs to expand functionality. ATOM can be downloaded at: http://feilab.ibp.ac.cn/software/atom/atom.html.

Currently, ATOM version 2.0 has been completed, with functions in the dashed-line boxes also implemented.

4. Summary and Future Work

This paper provides a detailed introduction to the development and current state of electron tomography three-dimensional reconstruction technology, analyzes existing major problems, and highlights our related research work: proposing SAMA, a new feature-extraction-based two-dimensional projection image alignment algorithm; implementing ASART, an adaptive iterative reconstruction algorithm that effectively accelerates convergence and yields more accurate reconstruction results; and developing ATOM, China' s first GPU-based electron tomography reconstruction software.

In future research, we will continue in-depth studies in four main areas: (1) developing new markerless two-dimensional image alignment algorithms to further improve alignment parameter accuracy; (2) further improving reconstruction

algorithm convergence speed and accuracy based on ASART; (3) developing ATOM 2.0 and researching electron tomography three-dimensional reconstruction parallelization on GPU clusters; (4) collaborating with biologists to combine electron tomography three-dimensional reconstruction with other biological imaging techniques (such as optical PALM imaging) for correlative light-electron three-dimensional imaging research.

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Note: Figure translations are in progress. See original paper for figures.