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Recent Advances in Single-Particle Cryo-Electron Microscopy Three-Dimensional Reconstruction: A Postprint

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Abstract

Single-particle cryo-electron microscopy (cryo-EM) three-dimensional reconstruction has become a recognized powerful approach for studying biological macromolecular structures, owing to its unique advantages over other research methods. However, cryo-EM micrographs exhibit extremely low signal-to-noise ratios, and acquiring high-resolution results necessitates collecting vast amounts of image data, rendering the three-dimensional reconstruction process extremely time-consuming. To address these challenges, this article presents a comprehensive overview of the development and current state of single-particle cryo-EM three-dimensional reconstruction, analyzes the major challenges currently faced, and highlights our research progress in this field.

Full Text

Preamble

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Recent Advances in Single-Particle Cryo-Electron Microscopy 3D Reconstruction

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Abstract

Single-particle cryo-electron microscopy (cryo-EM) 3D reconstruction has become a widely recognized powerful technique for studying the structures of biological macromolecules, offering unique advantages over other methods. How-

ever, cryo-EM micrographs exhibit extremely low signal-to-noise ratios, necessitating the collection of massive image datasets to achieve high-resolution results, which makes 3D reconstruction exceptionally time-consuming. This paper provides a comprehensive overview of the development and current state of single-particle cryo-EM 3D reconstruction, analyzes the major challenges, and highlights our research progress in this field.

Keywords: cryo-electron microscopy; 3D reconstruction; particle image recognition; adaptive dynamic scheduling; ParaEMAN

1. Introduction

With the completion of the Human Genome Project and whole-genome sequencing initiatives for various model organisms, biologists have recognized that understanding life processes requires structural and functional studies of proteins—the products of gene expression. According to the fundamental principle that "structure determines function," determining the three-dimensional structures of protein-based biological macromolecules is essential for elucidating their biological functions. Consequently, structural biology, which employs X-ray crystallography, nuclear magnetic resonance (NMR) spectroscopy, electron microscopy, and computational biology as its primary research tools, will play an increasingly important role [1].

Electron microscopy has evolved through the integration of electron microscope technology with image processing techniques. Compared to the mature structural biology methods of X-ray crystallography and NMR, electron microscopy offers distinct advantages: it can directly obtain molecular morphology and phase information, and it can analyze proteins unsuitable for X-ray crystallography or NMR. With improvements in biological sample preparation, advances in electron microscope instrumentation, and developments in digital image processing technology, electron microscopy has become a recognized powerful tool for studying biological macromolecules, supramolecular complexes, and subcellular structures [2].

Electron microscopy encompasses three independent techniques: electron crystallography, single-particle 3D reconstruction, and electron tomography. Among these, single-particle 3D reconstruction has become a crucial and irreplaceable method in structural biology research because it only requires processing electron micrographs of randomly dispersed macromolecules without the need for crystallization, and its performance improves with larger molecular sizes. The best resolution achieved by single-particle reconstruction currently reaches approximately 4 Å [3]. However, cryo-EM images have extremely low signal-to-noise ratios, requiring the collection of vast amounts of image data (nearly 100,000 particle images) to obtain high-resolution results. Furthermore, each particle image requires computational determination of its projection orientation and various processing steps including fast Fourier transforms, making the total reconstruction time extraordinarily demanding—typically

requiring about 10^6 CPU hours—and current methods can only process 20,000–40,000 particle images [4]. Therefore, developing fast and accurate 3D reconstruction methods leveraging high-performance computing systems is particularly urgent and critical.

To enhance the application and research of cryo-electron microscopy, the Biological Imaging Center of the Chinese Academy of Sciences' Protein Science Research Platform will soon install the world' s most advanced cryo-field transmission electron microscope, the Titan Krios. Additionally, the major Knowledge Innovation Project of the Chinese Academy of Sciences launched in 2008, "High-Performance Computing Research for Protein Science," has designated single-particle cryo-EM 3D reconstruction as one of its two primary applications.

2. Single-Particle Cryo-EM 3D Reconstruction

The concept of electron microscopy 3D reconstruction was first proposed by D. De Rosier and A. Klug in 1968 [5], while cryo-EM technology was pioneered by K. Taylor and R. M. Glaeser in 1974 [6]. After more than three decades of development, cryo-EM has become a powerful tool for studying the structure and function of biological macromolecules. Cryo-EM 3D reconstruction primarily involves preserving identical biological macromolecule samples at liquid nitrogen or liquid helium temperatures, performing two-dimensional imaging using transmission electron microscopy, and subsequently analyzing these 2D projection images to reconstruct the 3D structure [7].

2.1 Principles of Electron Microscopy 3D Reconstruction

De Rosier and Klug's 3D reconstruction theory utilizes a series of electron micrographs projected from different directions to reconstruct the three-dimensional configuration of the specimen. They introduced the concept and methodology of using digital image processing techniques for 3D reconstruction of biological macromolecules from electron micrographs. The mathematical foundation of electron microscopy 3D reconstruction is the central section theorem and Fourier transform. The central section theorem states that the Fourier transform of a function' s projection along a particular direction equals the section function of that function' s Fourier transform passing through the origin and perpendicular to the projection direction [2]. Therefore, the theoretical basis of electron microscopy 3D reconstruction is that the Fourier transform of a 3D projection image equals the central section (a section passing through the origin) in the 3D Fourier transform of the object that is perpendicular to the projection direction, as illustrated in Figure 1 [Figure 1: see original paper] [8]. Each electron micrograph represents a 2D projection of the object; Fourier transformation of a series of micrographs taken from different projection directions yields sections with various orientations. When a sufficient number of sections are obtained, 3D information in Fourier space can be assembled, and inverse Fourier transformation yields the 3D structure of the object, as shown in Figure

2 [Figure 2: see original paper] [2]. This method has found broad applications, from cell organelles without fixed structural characteristics and macromolecular complexes to macromolecular crystals, evolving into a practical method for protein structure determination.

Figure 1. Central Section Theorem
(a) 3D model of a biological macromolecule; (b) Projection of the 3D model in (a) along one direction; (c) Fourier transform of the projection; (d) The Fourier transform of a 2D projection of the 3D model equals a central section of the Fourier transform of the original 3D model. The four sections shown correspond to four different projection directions (0°, ~45°, 90°, ~135°).

2.2 Principles of Single-Particle Cryo-EM 3D Reconstruction

Single-particle cryo-EM is an important method for obtaining 3D reconstructions of biological macromolecules. The so-called single-particle method involves structural analysis of purified, discrete molecular particles. Its fundamental principle is to improve the signal-to-noise ratio by aligning and averaging projection micrographs of identical biological macromolecules in real space, thereby enhancing the structural information common to all particles, and finally reconstructing the 3D structure from single-particle micrographs with various projection directions in 3D space. The main steps of single-particle cryo-EM are shown in Figure 3 [Figure 3: see original paper] [9]: (1) Prepare vitrified-hydrated samples of chemically and structurally homogeneous biological macromolecules; (2) Select sample areas with optimal particle density and vitreous ice thickness most likely to produce the best images; (3) Set optimal parameters (such as defocus value, magnification, and electron dose) and record numerous images of these sample areas; (4) Select projections of discrete molecules using manual or semi-automatic procedures; (5) Calculate the relative orientations between different images through various image processing methods to reconstruct a 3D structural model of the biological macromolecule; (6) Perform final structural analysis and evaluation, docking atomic coordinates of protein structures obtained from crystallography or NMR into the 3D density map.

Figure 4 [Figure 4: see original paper] summarizes the complex image processing pipeline involved in single-particle cryo-EM 3D reconstruction, from 2D projection images to 3D models [8]:

Image Acquisition: Due to the extremely low signal-to-noise ratio of electron micrographs, acquiring as many particle images as possible is necessary to obtain high-resolution molecular 3D models. The current primary method is manual selection, which is a time-consuming and tedious task; manually selecting hundreds of thousands of particle images is practically impossible. Additionally, this stage requires image denoising and downsampling processing; details can be found in [10].

Contrast Transfer Function (CTF) Correction: The objective lens of a transmission electron microscope is not an ideal convex lens. Due to spherical

aberration, defocus, and other factors, the final micrograph results from the action of the contrast transfer function and various noise functions, rather than representing the true sample projection potential. Therefore, CTF correction is essential during reconstruction. The common approach involves first estimating the power spectral density (PSD) of particle images, then fitting the PSD using a theoretical CTF model to correct the CTF phase [11,12].

2D Image Analysis: Based on the acquired particle image dataset, each image must undergo rotation, translation, and alignment processing to obtain average images and symmetry information for each viewing direction.

Image Classification: Before 3D reconstruction, particle projection images must be classified to ensure all images in each class belong to the same projection direction; otherwise, the reconstruction results will be severely compromised [13]. Classical pattern recognition and clustering techniques are typically employed, such as feature extraction, auto-/cross-correlation analysis, hard clustering, and fuzzy clustering [2,14,15].

Angle Assignment: After classification, the projection direction for each class must be determined. This is generally accomplished by comparing particle images with computationally simulated projections. To reduce noise effects, the average image of each class is typically used to represent that class.

3D Reconstruction: According to the central section theorem, the Fourier transform of each particle image equals a central section in the 3D Fourier space of the original model. Therefore, based on the projection directions of each class, the 3D Fourier space can be reconstructed. Direct inverse Fourier transform or weighted back-projection, followed by multiple iterations of refinement, yields the final 3D structural model of the molecule.

2.3 Single-Particle Cryo-EM 3D Reconstruction Software EMAN

EMAN, developed by Steven J. Ludtke and colleagues at the National Center for Macromolecular Imaging, was first released in 1999 [16] and has become one of the world's most widely used and highest-resolution single-particle reconstruction software packages. Using EMAN for single-particle 3D reconstruction from electron micrographs involves three basic steps: (1) Particle picking—selecting images of biological sample particles from electron micrographs and saving all selections; (2) Initial model generation—creating a coarse initial 3D model from the selected particle images, which generally cannot meet expected resolution requirements; (3) Model refinement—iteratively optimizing the initial model until the 3D model achieves the required resolution or converges. Among these steps, model refinement is the most time-consuming, accounting for the vast majority of the total reconstruction time, and it directly determines the resolution of the final molecular structure.

Each iteration of model refinement involves four main operations, as shown in Figure 5 [Figure 5: see original paper] [17]:

Step 1: Projection Operation: The input is a 3D model. For the first iteration, this is the initial model generated in the previous step; for subsequent iterations, it is the new model from the previous iteration. The projection operation calculates 2D projections of the input model along a set of Euler angles (, ,). These projections serve as templates for comparison with molecular particle images in subsequent operations. In the following discussion, we denote the 2D projections generated by this operation as T , T , ···, where T corresponds to Euler angles (, ,).

Step 2: Classification Operation: This evaluates the similarity between each particle image P and the projections generated in the previous step, and classifies the particle images accordingly. For particle images P , P , ···, the algorithm assesses their similarity with each projection as follows: Given any projection T , first align P with T through rotation and translation to find the optimal rotation angle and translation vector that maximizes similarity. Then, treat the grayscale values of corresponding pixels in P and T as x- and y-coordinates of points in a 2D plane, and perform least-squares linear fitting on these points. The similarity s(i,j) between T and P is determined by the fitting error: larger fitting errors yield lower similarity, and vice versa. Finally, identify the projection T_c(j) with highest similarity to P , satisfying s(c(j),j) = max s(i,j). Assign P to class C_c(j) and set (_c(j), _c(j), _c(j)) as P ' s projection orientation. Repeating this process for every particle image yields a set of particle image classes C , C , ···containing images that satisfy the classification condition.

Step 3: Averaging Operation: This generates an average image for each class of particle images produced above. The algorithm consists of two stages: (1) Particle screening—calculate a threshold cut(i) and remove from class C any particle images with similarity below this value. The screened class C ' thus contains only particles satisfying the similarity condition. (2) Compute the average image using the remaining particles. Each pixel' s grayscale value in the average image is the mean of grayscale values from all screened particles at that location. Assuming the average image generated from particle class C is a , and since C ' s corresponding projection T and all particles in C share projection angles (, ,), we consider a ' s projection angles to be (, ,) as well.

Step 4: Reconstruction Operation: This generates a new 3D model from all average images obtained in the previous step. First, compute the Fourier transforms of all class average images to obtain a set of frequency spectra. Then insert each spectrum into the 3D Fourier space. According to the central section theorem, each spectrum corresponds to a section passing through the origin in 3D Fourier space, perpendicular to the average image' s projection direction. After all spectra are correctly positioned in 3D Fourier space, inverse Fourier transformation of the 3D volume data yields the new 3D model.

2.4 Current Research Challenges

Although single-particle cryo-EM 3D reconstruction has made significant progress in recent years and become a crucial and irreplaceable tool in structural biology, numerous computational challenges remain that constrain its further development. Two areas require particular attention:

1. **Fast and Accurate Particle Image Recognition Algorithms:** Due to the low electron dose used in electron microscopy, protein particle images have extremely low signal-to-noise ratios. To improve the signal-to-noise ratio and achieve high-resolution 3D models, a large number of particle images must be collected—typically hundreds of thousands of raw particle images are needed for atomic-resolution structures [18]. Currently, the most reliable particle selection method remains manual picking, which is unimaginable for hundreds of thousands of images. Particle recognition algorithms have long been a research hotspot in cryo-EM 3D reconstruction. A special issue of the *Journal of Structural Biology* (Vol. 145, 2004) systematically summarized automated particle selection methods, categorizing them into five types [10]: (1) template-based methods, (2) edge detection-based methods, (3) intensity comparison methods, (4) texture-based methods, and (5) neural network methods. Despite important advances in recent years [19], automated particle selection remains a bottleneck in single-particle reconstruction, necessitating the development of fast and accurate algorithms.

2. **High-Performance Computing:** In cryo-EM 3D reconstruction, each protein particle image requires computational determination of its projection orientation, followed by 3D structure determination using the central section theorem and Fourier transforms. Moreover, the 3D reconstruction model requires multiple iterations of optimization, making the entire process extremely time-consuming—typically requiring 10^6 CPU hours. For example, the structure of the 15 bacteriophage published in *Nature* in February 2008 was reconstructed from 36,259 particle images using Purdue University' s Condor computing resource, taking several months (10^6 CPU hours) to achieve 4.5 Å resolution [20]. Existing computational capabilities and methods cannot process cryo-EM experimental data in a timely manner, severely limiting practical applications. Therefore, developing fast and accurate 3D reconstruction algorithms using high-performance supercomputing environments is critical and urgent.

3. Our Research

This section highlights our group' s research contributions to cryo-EM 3D reconstruction, focusing first on high-performance computing aspects and then on protein particle recognition.

3.1 Parallel Single-Particle Reconstruction Software ParaEMAN

Single-particle cryo-EM 3D reconstruction involves multiple computational modules with diverse characteristics, making a single, global parallelization strategy unsuitable. We developed different parallel strategies for EMAN' s main processing modules (projection, classification, averaging, reconstruction) and implemented the parallel software ParaEMAN, achieving a speedup of 508.5× on 1,024 cores of the Dawning 5000A high-performance computing system.

The core challenge in ParaEMAN' s design is dynamic scheduling of computational tasks: N particles are divided into n classes by the *classesbymra* program, where class i contains k particles, and the *classalign2* program then computes the average for each class. Conventional parallelization of *classalign2* would further divide the k particles in class i among processes or threads, but this strategy performs poorly due to imbalanced particle numbers across classes. For classes with few particles, each process or thread receives minimal data, and the performance gain from parallelization cannot compensate for multi-threading/multi-processing overhead, resulting in overall performance degradation. To address this, our group proposed a Self-Adaptive Dynamic Scheduling (SADS) strategy that effectively parallelizes the classification operation [21,22].

SADS treats the averaging operation for each class as an independent task assigned to different processes. Unlike conventional methods that parallelize one classification operation at a time, this strategy considers all class-averaging operations simultaneously, distributing classes to processes based on their processing times to equalize total processing time across processes. The primary challenge is estimating each task' s processing time. Since the averaging operation has time complexity O(k), where k represents the number of particle images in class i, we model task processing time using the linear function:

$$t = a \cdot k + b$$

where a and b are coefficients dependent on system configuration. In practice, we update these coefficients using actual execution times from the previous iteration. Assuming the coefficient values in iteration j are a(j) and b(j), and the number of particle images in class i is k (j), the SADS framework is:

1. Initialize: $a(0) = 0$, $b(0) = 0$

2. For j = 0 to ITER-1 do:
   2.1. Estimate task processing time: $t (j) = a(j) \cdot k (j) + b(j)$
   2.2. Assign tasks to different processes based on estimated times
   2.3. Execute tasks and record actual running time m (j) for each task
   2.4. Update coefficients for next iteration using actual running times:
   $a(j+1) = (\Sigma m (j) \cdot k (j)) / (\Sigma k (j)^2)$
   $b(j+1) = (\Sigma m (j)) / n$

In this framework, step 2.2 distributes m tasks among p processes based on estimated execution times to achieve balanced workload. This problem reduces

to integer programming:

Minimize max Σ class(p) t
Subject to: class(p) = {1,2,···,m}
class(p) class(p) = for p p

This is an NP-hard optimization problem. By appropriately simplifying the problem and employing dynamic programming, we developed an effective scheduling algorithm.

3.2 Cryo-EM Single-Particle Image Recognition Methods

The extremely low signal-to-noise ratio of cryo-EM particle images and the random orientation of molecular particles pose significant challenges for particle selection. To address this, we have experimented with multiple particle image recognition algorithms, including histogram information entropy, improved AdaBoost algorithms, Bayesian classification, minimum distance classification, and correlation matching, which effectively reduce the false positive rate (FPR) and false negative rate (FNR) of particle selection. Our main contributions include:

- **Histogram Information Entropy Method:** Based on the observation that grayscale histogram distributions differ between particle and non-particle regions while being similar among regions containing the same particle type, we proposed an image recognition method using histogram information entropy. The information entropy is calculated as:

  V = Σ|p - q |
  V = Σp · log(p /q)

  where p and q represent grayscale distributions of the template and candidate region, respectively, and n represents the number of gray levels. V denotes the difference in grayscale histogram information entropy between template and candidate region, while V represents relative entropy (or Kullback-Leibler distance), indicating image differences.

- **Improved AdaBoost Algorithm:** Drawing inspiration from the AdaBoost algorithm commonly used in face recognition and adapting it to cryo-EM image characteristics, we proposed an optimized AdaBoost method using a divide-and-conquer principle to improve recognition accuracy. The approach divides the entire sample set during the training phase, learns on sub-samples, and combines the resulting strong classifiers weighted by sub-sample proportions [23]. For each sub-sample, a strong classifier is trained:

  H (x) = Σ h (x)

  where h (x) represents weak classifier values, denotes thresholds identified by the weak learning algorithm, f(x) represents feature values, x denotes a Haar feature, represents weak classifier error probability, and n

indexes the strong classifier for the nth sub-sample. The full-sample-space strong classifier is formed by linear combination of sub-sample classifiers:

$H(x) = \Sigma w\ H\ (x)$

where H(x) is the full-sample classifier and w , w , ···, w are weights for each sub-sample.

- **Bayesian and Minimum Distance Classifiers:** Leveraging the advantages of Bayesian and minimum distance classifiers for feature classification, we proposed classification methods based on these approaches. The Bayesian classifier principle uses prior probabilities of objects to calculate posterior probabilities (the probability of belonging to a particular class) via Bayes' formula, selecting the class with maximum posterior probability. We employed a Gaussian pattern class Bayesian classifier, where classification patterns follow Gaussian density. The 2D Bayesian discriminant function is:

  $d\ (x) = \ln P(w\ ) - ½\ln|C\ | - ½[(x-m\ )\ C\ ^{1}(x-m\ )]$

  where w represents the j-th pattern class and P denotes probability. The distance classifier exploits the property that molecular particles exhibit clustered distributions in multi-dimensional feature space, determining distances from unknown pattern vectors to cluster centers in this space:

  $D(x) = \min\ ||x - (1/N\ )\Sigma x\ W\ \ x||$

  where x is an unknown pattern vector, N is the number of pattern vectors in class W , and W represents the number of pattern classes.

We tested these methods on public cryo-EM particle image benchmark datasets [24], with selection performance in terms of false positive rate, false negative rate, and recognition accuracy shown in Table 1 .

4. Summary and Future Work

This paper detailed the development and current status of single-particle cryo-EM 3D reconstruction, analyzed major challenges, and highlighted our research progress: (1) a Self-Adaptive Dynamic Scheduling (SADS) strategy that effectively addresses computational task scheduling issues in cryo-EM 3D reconstruction; (2) development of the parallel reconstruction software ParaEMAN based on SADS; and (3) implementation of multiple particle image recognition algorithms that significantly reduce false positive and false negative rates in particle selection.

Future research will focus on three directions: (1) investigating high-performance cryo-EM 3D reconstruction algorithms to further improve ParaEMAN; (2) developing fast and accurate particle image recognition algorithms to enhance selection accuracy; and (3) exploring novel reconstruction algorithms, such as those based on spherical harmonics.

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