Recent Advances of Structural Biology via Single Particle Cryogenic Electron Microscopy and the Remaining Challenges

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ABSTRACT

Single-particle cryogenic electron microscopy (cryo-EM) has revolutionized molecular studies in life science by enabling high-resolution structure determination of biological macromolecules without requiring crystallization. This revolution was propelled by the advent of direct electron detectors, advanced algorithms, and modern cryo-EM with increased high-throughput data collection schemes, where routine acquisition of a large dataset containing sub-millions to millions of particle images can be achieved in a single day. Compared to X-ray crystallography, this technique has unique strength in simultaneous capturing of a mixture of solution conformations reflecting functional states to aid the mechanistic understanding of dynamic biological process. However, as single cryo-EM imaging is performed with low-dose electron exposure (10-40 $e^-/Å^2$) to minimize radiation damage, the produced images are highly noisy with signal-to-noise ratios (SNRs) typically approaching ~0.1 or lower. As a result, reconstructing accurate 3D structures from such data presents a challenging inverse problem, for which the solution entails a multi-step workflow of image analysis that progressively harnesses the weak signals from the noises. In this lecture, I will first briefly explain the workflow including the major bottle met by 2D classification for particle image curation and other challenges. In addition, I will gives a number of successful examples of structure determination using this approach from our laboratories to illustrate how this method has been utilized to solve issues ranging from greenhouse gas to fish farming.

CRISP: A Modular Platform for Cryo-EM Image Segmentation and Processing with Conditional Random Field

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ABSTRACT

Distinguishing signal from background in cryogenic electron microscopy (cryo-EM) micrographs is a critical yet challenging step due to the inherently low signal-to-noise ratio (SNR), the presence of contaminants, variable ice thickness, and densely packed particles of heterogeneous sizes. Recent image segmentation methods, which operate at the pixel level, offer several advantages over traditional object-detection approaches: they enable more accurate suppression of false positives via segmented blob mass, improve particle centering by leveraging full brightness profiles, and more reliably detect irregularly shaped particles. However, the low SNR in cryo-EM data makes it difficult to obtain accurate pixel-level annotations for training, and in the absence of standardized evaluation platforms, most existing segmentation pipelines rely on ad hoc design choices.

Here, we present a modular platform for generating high-quality reference segmentation maps automatically. The platform supports flexible combinations of segmentation architectures, feature extractors, and loss functions, and integrates a novel Conditional Random Fields (CRF) module that uses class-discriminative features and a regularized optimization scheme to refine coarse predictions into fine-grained masks. On synthetic datasets, models trained with our reference labels achieve over 90% accuracy, precision, recall, Intersection-over-Union (IoU), and F1 score at the pixel level. Furthermore, we demonstrate that the predicted segmentation maps can be directly used for particle picking, yielding higher-resolution 3D density maps from real cryo-EM datasets — matching expert-curated reconstructions and surpassing the performance of state-of-the-art particle-picking tools.

Keywords: cryogenic electron microscopy; image segmentation; image processing, conditional random fields

A Robust Hierarchical Linear Model for Cryo-EM Analysis

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ABSTRACT

Cryo-electron microscopy (cryo-EM) is essential for determining high-resolution three-dimensional maps of biological macromolecules, which subsequently facilitate atomic model generation. The atomic models and their corresponding cryo-EM maps are archived in the Protein Data Bank (PDB) and the Electron Microscopy Data Bank (EMDB), respectively. In this study, we present a robust hierarchical modeling approach that quantitatively links cryo-EM maps with atomic models. Our model accounts for parameter variations based on atom types and amino acid categories, effectively capturing the nuanced influences of the local electron distribution environment. By applying the minimum power divergence method, we derive robust estimates that minimize the impact of outliers. The effectiveness and robustness of our approach have been validated through applications to both simulated and real datasets.