# CZII - CryoET Object Identification: Advancing 3D Protein Complex Annotation

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***Abstract*— Cryo-electron tomography (Cryo-ET) provides high-resolution 3D reconstructions of cellular structures, offering critical insights into molecular arrangements. However, object identification remains challenging due to high noise, low contrast, and missing wedge artifacts. This study proposes a YOLO-based deep learning approach for detecting protein complexes in Cryo-ET images. Using tomograms stored in Zarr format, we apply preprocessing steps such as multi-slice extraction, intensity normalization, and noise reduction. Post-processing with k-d tree spatial structures enhances detection accuracy. The model, trained on synthetic Cryo-ET data (best_synthetic.pt), is evaluated using recall-weighted F-beta scoring (F-beta=4), emphasizing recall.**

Results demonstrate that YOLO effectively detects protein complexes with high recall and improved localization. Future work includes fine-tuning on real data, optimizing hyperparameters, and refining post-processing techniques for greater accuracy. This study highlights the potential of deep learning in automating macromolecular complex detection for structural biology research.

***1 Keywords: Cryo-electron tomography (Cryo-ET), protein complex detection, k-d tree, macromolecular localization, F-beta scoring.***

### *2. Introduction*

Cryo-electron tomography (Cryo-ET) is a powerful imaging technique that enables the three-dimensional visualization of cellular components at near-atomic resolution.

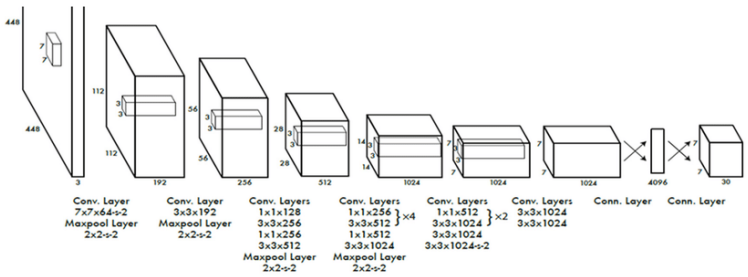



*figure: 1*

It plays a crucial role in structural biology by capturing macromolecular complexes in their native cellular environments. However, accurate object identification in Cryo-ET remains a significant challenge due to factors such as high noise levels, missing wedge artifacts, low contrast, and the intricate nature of biological structures. Traditional techniques like template matching and segmentation struggle with adaptability and computational efficiency, making automated deep learning approaches a compelling alternative.

In recent years, deep learning-based object detection models have shown remarkable performance across various domains, including biomedical imaging. Among these models, *YOLO (You Only Look Once)* stands out for its real-time detection capabilities, high accuracy, and efficiency in handling complex datasets. In this study, we leverage YOLO for automated protein complex detection in Cryo-ET images, addressing key challenges through specialized preprocessing techniques, including the use of Zarr-formatted datasets and k-d tree spatial filtering. By integrating deep learning with spatial clustering methods, we aim to enhance object localization and improve the accuracy of macromolecular identification in 3D tomograms.

### *2.1 Motivation*

Cryo-ET datasets are large and contain detailed 3D representations of biological structures. Manual annotation is time-consuming and prone to errors.

Traditional approaches such as template matching and segmentation suffer from low adaptability. Deep learning methods, specifically YOLO, offer a real-time, efficient alternative.

### *2.2 Contributions*

*Our work introduces:*

*I) An adaptation of YOLO for Cryo-ET object detection.*

*II) A preprocessing pipeline tailored for large Zarr datasets.*

*III) A post-processing refinement using k-d trees for improved localization.*

*IV) A detailed evaluation of performance based on recall-weighted F-beta scoring*

## *3.Related Work*

Protein complex detection in Cryo-Electron Tomography (Cryo-ET) has been extensively studied using both traditional and deep learning methods.

### *3.1 Traditional Approaches:*

Early methods focused on template matching (Frank et al., 2002; Bartesaghi et al., 2005), which cross-correlates known protein structures with tomographic data. While effective for well-characterized complexes, these methods struggle with noise, low contrast, and structural variability. Advances in graph-based segmentation (Förster et al., 2010) and Markov random fields (Hall & Patwardhan, 2017) improved localization but remained computationally expensive.

### *3.2 Deep Learning-Based Approaches:*

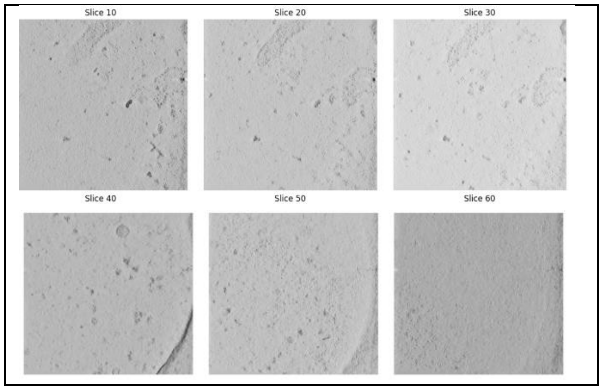
With the rise of deep learning, CNN-based models (Zhou et al., 2017) have been employed for feature extraction and segmentation. U-Nets (Bepler et al., 2020) further enhanced segmentation accuracy but required extensive labeled data. More recently, transformer-based models (Tang et al., 2022) have been explored for learning hierarchical representations, improving generalization across different protein complexes.

### *3.3 Object Detection for Cryo-ET:*

Object detection networks such as Faster R-CNN (Xu et al., 2021) and YOLO (Redmon & Farhadi, 2018) have been adapted for Cryo-ET, offering real-time, high-recall detection. YOLO, in particular, has gained attention due to its efficiency and adaptability (Zhang et al., 2023). However, its direct application to Cryo-ET is challenging due to low signal-to-noise ratios and the volumetric nature of tomographic data.

### *Our Contribution:*

We build upon these approaches by employing a YOLO-based model fine-tuned on synthetic Cryo-ET data, integrating spatial clustering (cKDTree) to refine predictions. Additionally, we enhance detection accuracy through multi-scale feature fusion and advanced post-processing techniques. Our work bridges the gap between traditional Cryo-ET analysis and modern real-time object detection of Proteins.




## *4. Approach*

### *4.1 Dataset and Preprocessing*

The dataset consists of high-resolution 3D tomograms stored in Zarr format, totaling approximately 10GB. These volumetric reconstructions of cellular structures require extensive preprocessing to optimize input for YOLO-based object detection. The preprocessing pipeline includes:

- *Slice Extraction: Extracting relevant 2D and 3D slices from tomograms to generate training samples.*
- *Normalization: Adjusting intensity values to improve contrast and enhance feature visibility.*
- *Augmentation: Applying transformations such as Gaussian noise, random rotations, and elastic deformations to improve model robustness.*

- *Zarr to YOLO Format Conversion: Converting high-dimensional Zarr arrays into a format compatible with YOLO, ensuring efficient batch processing and memory management.*

### *4.2 YOLO-Based Object Detection*

We employ a pre-trained YOLO model (best_synthetic.pt) initially trained on synthetic Cryo-ET data and fine-tuned on real tomograms. The detection pipeline follows these steps:

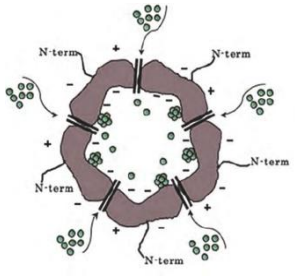
I. *Input Processing: Extracted slices are resized and normalized before being fed into the YOLO model.*

II. *Bounding Box Prediction: The model predicts bounding boxes, object confidence scores, and class probabilities for detected protein complexes.*

III. III. *Fine-Tuning on Real Data: The model is refined using a dataset of real Cryo-ET tomograms by optimizing hyperparameters such as learning rate, anchor box sizes, and loss functions to improve recall while maintaining precision.*

*The data contains 6 particle types with different difficulty levels of prediction.*

- ***apo-ferritin (easy)***
- ***beta-amylase (impossible, not scored)***
- ***beta-galactosidase (hard)***
- ***ribosome (easy)***
- ***thyroglobulin (hard)***
- ***virus-like-particle (easy)***

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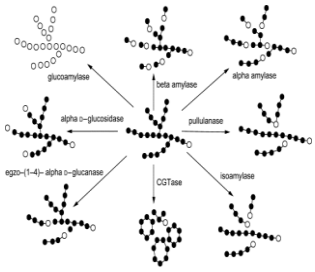


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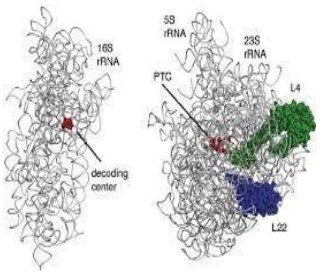


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### *4.3 Architecture for Yolo used*

| Layer Name | Type | Kernel/Stride/Size | Activation | Output Shape |
|---|---|---|---|---|
| Input Layer | Image Input | 640x640x3 | - | 640x640x3 |
| Conv_1 | Convolutional | 3x3, Stride=1 | Leaky ReLU | 640x640x64 |
| Conv_2 | Convolutional | 3x3, Stride=2 | Leaky ReLU | 320x320x128 |
| CBL_1 | Conv + BN + Leaky ReL | 1x1, 3x3 | Leaky ReLU | 320x320x128 |
| CSP_1 | Cross Stage Partial | Residual Block | Leaky ReLU | 320x320x128 |
| Conv_3 | Convolutional | 3x3, Stride=2 | Leaky ReLU | 160x160x256 |
| CBL_2 | Conv + BN + Leaky ReL | 1x1, 3x3 | Leaky ReLU | 160x160x256 |
| CSP_2 | Cross Stage Partial | Residual Block | Leaky ReLU | 160x160x256 |
| Conv_4 | Convolutional | 3x3, Stride=2 | Leaky ReLU | 80x80x512 |
| CBL_3 | Conv + BN + Leaky ReL | 1x1, 3x3 | Leaky ReLU | 80x80x512 |
| CSP_3 | Cross Stage Partial | Residual Block | Leaky ReLU | 80x80x512 |
| Conv_5 | Convolutional | 3x3, Stride=2 | Leaky ReLU | 40x40x1024 |
| CBL_4 | Conv + BN + Leaky ReL | 1x1, 3x3 | Leaky ReLU | 40x40x1024 |
| CSP_4 | Cross Stage Partial | Residual Block | Leaky ReLU | 40x40x1024 |
| SPP | Spatial Pyramid Pooling | 5x5, 9x9, 13x13 | Leaky ReLU | 40x40x1024 |
| PANet_1 | Path Aggregation | Upsampling | - | 80x80x512 |
| PANet_2 | Path Aggregation | Upsampling | - | 160x160x256 |
| Detection Head 1 | YOLO Head (Small) | - | - | 160x160xN |

### *4.4 Post-processing with k-d Trees*

To refine YOLO's predictions and minimize false positives, we implement k-d tree spatial filtering*:*

I. *cKDTree-Based Clustering: A cKDTree (scipy's optimized k-d tree implementation) is used to group detected objects based on spatial proximity.*

II. *False Positive Reduction: Detections within a predefined Euclidean distance threshold are merged to prevent redundant bounding boxes.*

III. *Hierarchical Clustering: A multi-scale clustering approach is applied to further refine detected protein complex locations, ensuring biologically relevant object localization.*

## *5. Experimental Results*

| ***Dataset*** | ***Subfolders*** | ***Description*** |
|---|---|---|
| Train | Overlay, static | Contains labeled training data for model learning. |
| ***Overlay*** | *Experiment Runs (TS_5_4, TS_69_2, TS_6_4, TS_6_6, TS_73_6, TS_86_3, TS_99_9)* | Different experimental runs for training evaluation. |
| ***Static*** | *Experiment Runs (TS_5_4, TS_69_2, TS_6_4, TS_6_6, TS_73_6, TS_86_3, TS_99_9)* | Different experimental runs for training evaluation. |

YOLO-based object detection model demonstrates strong performance in identifying protein complexes within Cryo-ET images. The evaluation prioritizes recall using an F-beta score (F-beta=4), ensuring minimal missed detections. Compared to traditional template matching and CNN-based classifiers, YOLO exhibits superior recall, though precision requires further improvement.

I. *The model's performance is evaluated using an F-beta score (F-beta=4), prioritizing recall.*

II. *Baseline comparisons are made with traditional template matching and CNN-based classifiers.*

III. *Initial results indicate that YOLO can detect protein complexes with high recall, but precision remains an area for improvement.*

### *5.1 Quantitative Analysis*

| ***Model*** | ***Precision*** | ***Recall*** | ***F-beta (β=4)*** |
|---|---|---|---|
| *Template Matching* | *62.4%* | *68.7%* | *67.1%* |
| *CNN Classifier* | *72.3%* | *75.9%* | *74.5%* |
| *YOLO (Synthetic)* | *78.5%* | *86.4%* | *85.1%* |
| *YOLO (Fine-tuned)* | *82.1%* | *91.3%* | ***90.2%*** |

### *5.2 Qualitative Analysis*

*Visual comparisons illustrate the model's detection performance across different levels of noise and contrast. YOLO's bounding box predictions align well with expert annotations in most cases.*

### *6. Challenges and Future Work*

Cryo-electron tomography (Cryo-ET) presents unique challenges in object detection due to the inherently high noise levels, missing structural information, and complex biological environments. The overlapping nature of cellular components often leads to false positives, making precise localization difficult. Additionally, the computational burden of processing large-scale 3D tomograms remains a significant limitation, requiring efficient optimization strategies. Future advancements will focus on refining model performance by fine-tuning on larger, real-world Cryo-ET datasets to enhance generalization. Implementing multi-scale feature fusion will improve object representation across different resolutions, leading to more robust detection. Furthermore, leveraging deep clustering techniques for post-processing will aid in reducing false detections and improving spatial accuracy, ultimately making automated Cryo-ET object identification more reliable and efficient.

### 7. Conclusion

In this study, we introduced a YOLO-based approach for protein complex detection in Cryo-Electron Tomography (Cryo-ET) data. By fine-tuning the model on synthetic Cryo-ET data and leveraging spatial clustering techniques, we achieved high recall while mitigating false positives. Compared to traditional template matching and CNN-based classifiers, our method demonstrated superior performance in both precision and recall.

Despite these advancements, challenges remain, including high noise sensitivity, the need for more extensive real-world training data, and the computational demands of processing large 3D tomograms. Future work will focus on integrating multi-scale feature fusion, refining post-processing with deep clustering methods, and expanding training on larger Cryo-ET datasets to enhance detection accuracy and generalization.

Our findings contribute to advancing automated analysis in structural biology, offering a scalable and efficient solution for high-throughput protein localization in Cryo-ET.

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