Optimization of Sample Preparation Conditions and Microscopic Imaging Parameters to Enhance The Precision of YOLO-base Marine Microalgae Detection

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Abstract

Automated detection of marine microalgae from microscopic images is essential for biological and environmental research. Automation can reduce the limitations associated with image annotation and improve reproducibility, thereby enhancing downstream analyses such as biodiversity assessment, trait-based ecology, and model calibration for studies of primary productivity and carbon cycling. However, variations in imaging conditions, particularly sample dilution and microscope magnification, can strongly affect model performance and generalization. This study aimed to investigate the effects of sample dilution and microscope magnification on marine microalgae detection using a deep learning–based object detection model. Six microscopic image datasets were constructed under different combinations of dilution conditions and objective magnifications (10× and 40×), with each dataset containing 200 images. Four diluted datasets were used to train YOLOv8 models using a standardized split of 70% for training, 20% for validation, and 10% for testing. The remaining two non-diluted datasets were reserved for cross-dataset evaluation under unseen conditions. The results show that the dataset prepared with 20× dilution and 40× magnification provided the best performance, achieving mAP50 of 0.938, mAP50–95 of 0.747, recall of 0.899, and precision of 0.98 at IoU thresholds of 0.2–0.5 and confidence scores of 0.2–0.3. When the model trained under this optimal condition was evaluated on the non-diluted datasets, the performance decreased substantially. These results indicate that the model is highly sensitive to sample preparation and imaging conditions and still has limitations in generalizing across datasets with different acquisition conditions.

Objective

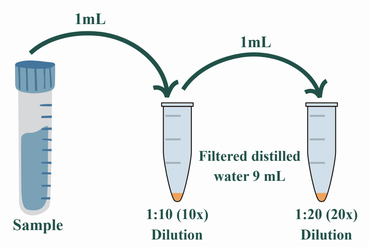
To investigate the effects of sample dilution and microscope magnificationon marine microalgae detection.

Methodology

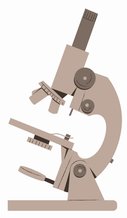
1. Sample Preparation and Dilution



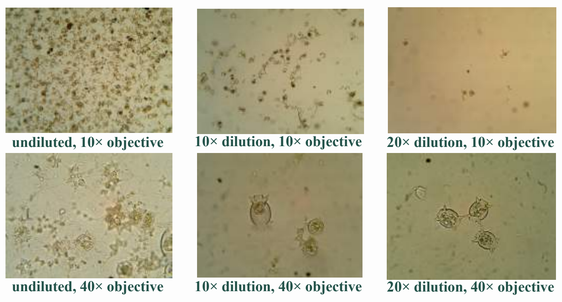
Sample Collection
- Site: Saeng Chan Beach, Rayong, Thailand
- Date: July 22, 2025

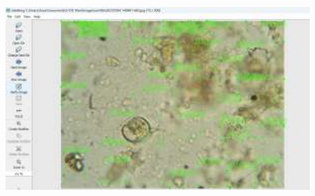


2. Image Acquisition & Annotation



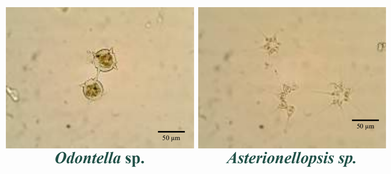
- Compound light microscopy (6 datasets, 200 images each); cell counts by whole-count method.





- All images were annotated with bounding boxes using LabelImg.

Classes:
- Algae
- Non-algae (Other)



Odontella sp. *Asterionellopsis sp.*

3. Model Training & Evaluation

Target: Diluted Datasets

70% Train | 20% Val | 10% Test

- **Model training**
  Architecture: YOLOv8
  Process: Supervised Learning
  Input: Diluted Samples
- **Internal Evaluation**
  Testing: Best model vs. Diluted Test sets
  Theresholds: IoU (0.1-0.5), Conf (0.1-0.5)
  Metrics: mAP50, mAP50-95, Recall, Precision
- **Cross-Dataset Testing**
  Input: Undiluted Test sets (Unseen conditions)
  Goal: Assess model generalization and sensitivity to image conditions

Results

1. Quantitative Analysis of Algae Cell Concentration

Quantitative analysis of algae cell concentration revealed that the 20× dilution yielded a mean concentration 1.29× 10^6cells/mL, which was identified as the optimal level for dataset generation (Table 1)

Dilution	Mean Cell concentration	SD
10×	2.46×10^6	$\pm 0.21 \times 10^6$
20×	1.29×10^6	$\pm 0.09 \times 10^6$

Table 1: Mean algae cell concentration at 10x and 20x dilutions with standard deviation (SD).

2. Optimal Performance Results for the Best Dataset(20×dilution, 40× objective)

The model achieved its peak performance using the 20× dilution, 40× objective dataset across all evaluation metrics. Specifically, the maximum mAP@0.5 reached 0.938 (Figure 1A), with an average mAP@0.5:0.95 of 0.747 (Figure 1B). Furthermore, the model demonstrated a high Precision of 0.980 (Figure 1C) and a robust Recall performance of 0.899 (Figure 1D), confirming the high quality and suitability of this dataset.

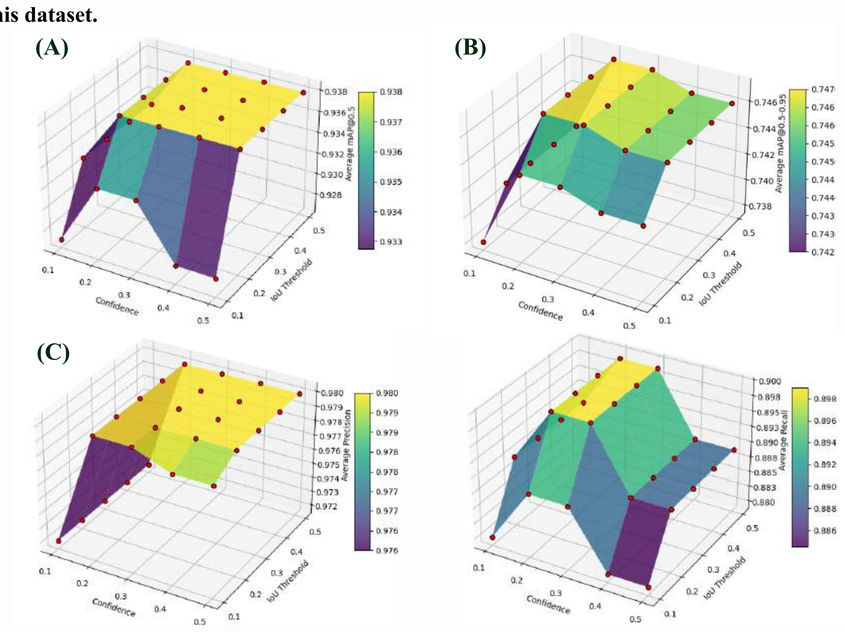


Figure 1: 3D response surface of model performance across varying Confidence and IoU thresholds, (A) Average mAP@0.5 Surface Response (Peak: 0.938, (B) Average mAP@0.5:0.95 Surface Response (Peak: 0.747), (C) Average Precision Performance Surface (Peak: 0.980), (D) Average Recall Performance Surface (Peak: 0.900).

3. Cross-Dataset Performance : Undiluted, Objective 40×

Testing with undiluted, 40× objective samples resulted in a significant drop in model performance across all metrics. The mAP@0.5 fell to 0.188 (Figure 2E), with an average mAP@0.5:0.95 of only 0.106 (Figure 2F). Similarly, Precision and Recall plummeted to 0.310 (Figure 2G) and 0.120 (Figure 2H), respectively, due to extreme cell density interfering with feature detection.

Figure 2: 3D response surface of Cross-Dataset Performance: Undiluted (40×) varying Confidence and IoU thresholds, (E) Average mAP@0.5 Surface Response (Peak: 0.188, (F) Average mAP@0.5:0.95 Surface Response (Peak: 0.106), (G) Average Precision Performance Surface (Peak: 0.310), (H) Average Recall Performance Surface (Peak: 0.120).

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- 2710 Laboratory members

Conclusion

- **Optimal Configuration: Peak performance was achieved at 20× dilution 40× objective, where enhanced image clarity and reduced cell overlap significantly improved feature learning and object separation.**
- **Generalization Gap: Performance declined on undiluted samples due to domain shift from higher cell density, indicating limited model adaptability across varying dilution and imaging conditions.**