Enhancing DL-based Cell Segmentation of Microalgae with Classical Image Processing Priors

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***Abstract*—We present a hybrid approach for the automatic detection and segmentation of *Nannochloropsis Oceanica, Wild Type* (NocWT) microalgae cells, combining classical image processing techniques with deep learning. Initially, we apply traditional computer vision methods to detect and count cells efficiently, but these struggle with challenges such as morphological variability and overlapping structures. To overcome these limitations, we incorporate the *Segment Anything Model* (SAM), a state-of-the-art segmentation framework leveraging a transformer architecture pre-trained on large-scale datasets. Instead of relying solely on SAM's general capabilities, we guide its segmentation using pre-segmented regions derived from classical methods, improving accuracy in delineating complex cell boundaries. The proposed method is evaluated on a manually annotated dataset of bright-field microscopic images, ensuring reliable performance assessment despite the dataset's limited size. By integrating the interpretability of traditional approaches with the adaptability of deep learning, our method achieves robust and precise microalgae segmentation, demonstrating the advantages of a complementary strategy over standalone state-of-the-art techniques.**

***Index Terms*—Microalgae, automatic detection, cell counting, segmentation bright field microscopy, image processing**

I. Introduction

Microalgae are a valuable resource for sustainable biomass production, with applications in food, cosmetics, and biofuels [1]–[3]. Due to their high photosynthetic efficiency, microalgae convert solar energy into biomass at a rate much higher than other crops, requiring fewer water resources and avoiding extensive land use. Their use aligns well with circular economy principles, as algal biomass can be repurposed for applications like fertilizer production [4] or biogas energy recovery [5]. However, large-scale industrial adoption is hindered by challenges that limit their competitiveness compared to other biomass sources.

A major barrier to industrial microalgae production is the difficulty in scaling laboratory methods to manufacturing processes [6]. Expanding cultivation systems introduces complexities in managing environmental factors like temperature, light, and nutrients, all critical for growth. Additionally, industrial environments increase the risk of contamination by pathogens, which can reduce productivity and biomass quality [7]. The lack of reliable predictive models that describe the interaction between environmental factors and production performance is another challenge, complicating process optimization and increasing operational costs [8]. Addressing these issues requires advanced monitoring and automation technologies to enhance efficiency and sustainability in large-scale production.

To ensure stable production, integrating advanced image processing tools is crucial for real-time monitoring of microalgal cultures. These systems must capture and analyze microscopic images of algal cells, detecting early signs of stress before irreversible damage occurs. Automated analysis, powered by AI models, enables continuous monitoring and process optimization, improving reliability and reducing labor and costs.

In this paper, we present a hybrid approach for the automatic detection, counting, and segmentation of *Nannochloropsis Oceanica, Wild Type* (NocWT) microalgae cells, leveraging the strengths of both classical computer vision techniques and state-of-the-art deep learning models. Our method is tested on a manually annotated dataset of bright-field microscopic images. While the dataset is limited in size due to the need for high-quality ground-truth validation, it remains representative of typical cell morphology and imaging conditions, ensuring the reliability of performance evaluation. The first phase of our study employs traditional image processing, optimizing a combination of filters and operations to accurately detect and count cells. While these classical methods offer efficiency and interpretability, they often struggle with challenges such as variations in cell morphology, overlapping structures, and non-uniform lighting conditions, which can compromise segmentation accuracy. To address these limitations, we integrate an advanced segmentation strategy based on the *Segment Anything Model* (SAM) [9], a cutting-edge deep learning framework that

leverages transformer architectures pre-trained on large-scale image datasets. Unlike conventional deep learning models that require extensive task-specific training, SAM autonomously isolates elements in an image with minimal supervision. By providing pre-segmented regions as guidance, we refine and enhance SAM's segmentation output, enhancing its ability to delineate complex cell boundaries. By combining the precision of traditional methods with the adaptability of AI-driven segmentation, our approach enhances accuracy and reliability in microalgae cell analysis, demonstrating the advantages of a complementary methodology over standalone techniques.

II. Related works

Microalgae image analysis has been the focus of extensive research, particularly in detection and classification [10], [11]. However, a critical aspect often overlooked is the preservation of morphological structures, which can be achieved through precise segmentation. While numerous methods for general cell segmentation exist [12], the specific application to microalgae presents unique challenges due to their diverse morphologies, potential for overlapping colonies, and low contrast against complex backgrounds. To address this, various studies have explored both semantic and instance segmentation approaches, each presenting advantages and limitations.

The study in [13] compares semantic segmentation of diatoms[1], which assigns class labels at the pixel level, and instance segmentation, which delineates individual diatom instances. Findings highlight that while Mask-RCNN exhibits robust separation of individual objects, overall accuracy is dependent on the efficiency of the Region Proposal Network (RPN). Conversely, SegNet [14] displays superior sensitivity but lower specificity, often misclassifying cellular structures as background debris. Moving beyond traditional segmentation methodologies, the work in [15] proposes AlgaeSeg-YOLO, a modified YOLOv8n-seg [16] architecture integrating a convolutional (CBF) module. This enhances mean Average Precision (mAP) while maintaining computational efficiency. However, this method still faces challenges in scenarios with severe algal overlap, limiting its effectiveness in densely packed microalgae samples.

Complementary research has focused on real-time processing and visibility constraints in microalgae segmentation. FastSAM [17] successfully segments colonies and filaments in complex aquatic environments. Nonetheless, its performance is hindered by low image contrast when detecting smaller elements. Similarly, [18] employs Pairwise Deep Learning Features (PDLF) within a SegNet framework, leveraging a combination of Shi-Tomasi descriptors [19] and VGG-16 features [20] to enhance the segmentation of barely visible microorganisms. While this improves accuracy, it introduces increased computational complexity and requires fine-tuning.

Beyond domain-specific models, generalist approaches have been investigated to tackle bioimaging and object extraction challenges. DeepCell [21] is a freely available deep learning framework developed for biological image analysis. It provides a collection of pre-trained models specialized in tasks like single-cell segmentation, tracking, and classification in microscopy images. CellSAM [12] represents an innovative solution that integrates the Segment Anything Model (SAM) with a CellFinder, a transformer-based object detector designed to identify cellular structures, to improve segmentation accuracy. This approach enables accurate zero-shot segmentation, demonstrating the potential of combining broad AI-driven models with domain-specific object extraction techniques. Most existing approaches rely solely on deep learning for feature extraction. Incorporating conventional image processing techniques for cell detection can provide valuable priors, aiding in the initialization of segmentation methods and ultimately enhancing overall segmentation quality.

[1]Single-celled algae.

TABLE I: Description of the cell dataset.

Dataset name	**# of total images**	**Image size [px]**
BF_NocCCAP849/10	155	1920×1080
Factor scale [µm/px]	**# of annotated images (Ground-truth)**	
10 µm/78 px	155 (Detection/Counting)	30 (Segmentation)

III. Cell data

A. Image dataset

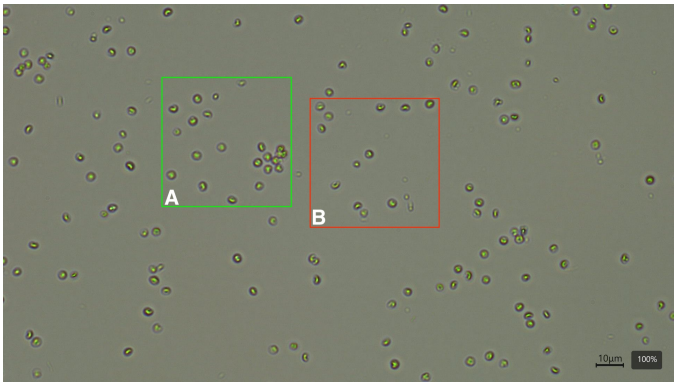
The dataset used in this study consists of high-resolution images obtained through optical microscopy, or bright-field (BF) microscopy, a widely used technique for observing biological samples. In this method, a beam of light from a source below the microscope slide passes through the sample. Regions of higher density absorb more light, creating contrast and allowing the visualization of cellular structures. This technique enables precise assessment of cell morphology and dimensions, with clear boundaries.

The images in this dataset contain numerous uniformly sized cells of *NocWT* distributed across the focal plane. A representative image from the validation dataset is shown in Fig. 1a. Their high density and clarity make them suitable for tasks like cell identification, enumeration, and segmentation.

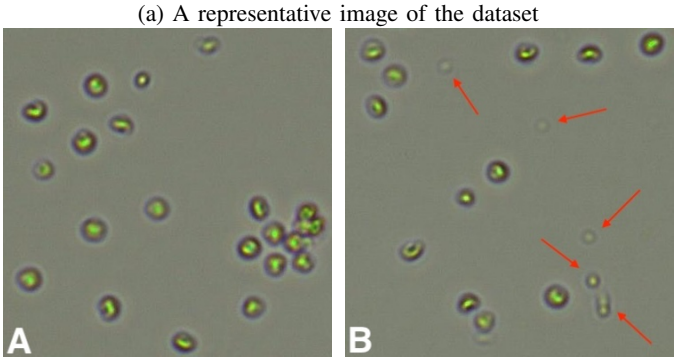
Bright-field images provide information on cell morphology, size, and spatial distribution (see Fig. 1b). However, artifacts from debris, dirt, air bubbles, or residual organic substances may introduce noise, appearing as dark regions in the images (see Fig. 1c). These artifacts can cause false positives in cell identification and segmentation, affecting analysis accuracy. A detailed summary of the dataset is presented in Table I.

B. Ground-truth annotation strategy

To ensure a reliable evaluation of computational methods, we generate ground-truth data for both detection and segmentation. The segmentation ground-truth is manually created using ProCreate [22], which provides standard image editing tools. The annotation process begins with an automated selection of cell boundaries (using the proposed algorithm), followed by manual refinement to ensure accurate delineation



(a) A representative image of the dataset



(b) Details of cell morphology (c) Artifacts

Fig. 1: Bright-field image of cells of *Nannochloropsis oceanica* strain CCAP 849/10.

before exporting the final masks as binary images. For cell detection and counting, the ground-truth is established by analyzing and correcting the obtained results. ImageJ [23] is used to systematically identify and assess false negatives and false positives, which are relatively low.

Despite rigorous annotation procedures, human error remains a challenge in GT generation. Manual segmentation is influenced by the annotator's expertise and the morphological complexity of the structures. In cell detection and counting, ambiguous elements resembling cells, as well as partial cells along image borders, introduce uncertainty. Due to the labor-intensive nature of annotation, ground-truth segmentation data is generated for only a subset of the images. Table I provides a summary of the total number of annotated images.

IV. Methods

One of the key features of SAM is the ability to utilize visual prompts—such as points, bounding boxes, or rough masks—to guide the segmentation process. These prompts provide SAM with contextual cues, allowing it to focus on specific objects or regions within an image. In our approach, we leverage SAM by using as prompts the cells previously segmented through a classical image processing pipeline, as shown in Fig. 2. By inputting these pre-segmented regions as guidance, we refine and enhance SAM's output, improving its ability to delineate complex cell boundaries.

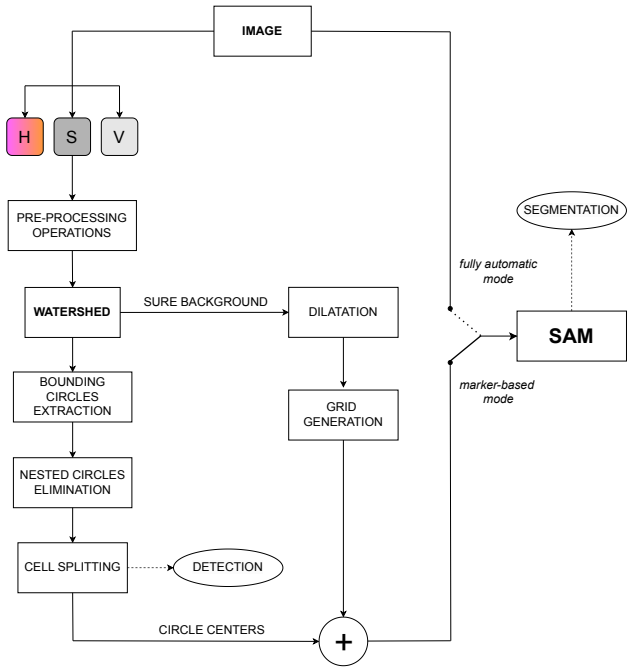


Fig. 2: The proposed cell segmentation framework.

A. Pre-processing and traditional segmentation

To ensure robust cell analysis, the image pre-processing pipeline addresses microscopy artifacts while preserving cellular features. It begins with Gaussian blur to reduce noise, followed by HSV conversion, utilizing the Saturation channel to enhance boundaries. CLAHE [24] improves contrast, and a Bilateral Filter prevents noise amplification. Otsu thresholding [25] generates a binary mask, defining sure background and foreground. Background is extracted via dilation, while a Distance Transform refines the foreground by weighting pixels based on proximity to the background. The unknown region, obtained as their difference, serves as input for the Watershed segmentation algorithm [26], [27] to delineate cell boundaries.

B. SAM prompting strategy

Cell coordinates for SAM prompts are extracted through Watershed-based instance segmentation, identifying distinct markers for each object. Minimal enclosing circles are computed to define center coordinates and radii, with nested circles filtered to retain the largest in overlapping sets. A dynamic threshold excludes smaller circles, mitigating noise. Segmentation inaccuracies in closely apposed cells are addressed by flagging bottleneck-shaped objects for validation based on foreground pixel proportion. Flagged candidates undergo reprocessing with Watershed [27], refining separation through adjusted sensitivity. This workflow optimizes segmentation by eliminating redundancy, adapting thresholds, and addressing errors, ensuring robust coordinate extraction for SAM.

Additionally, background points are identified to guide SAM segmentation: the sure background mask from Watershed is dilated using an elliptical kernel, expanding cell borders. A grid of equidistant points is generated across the image,

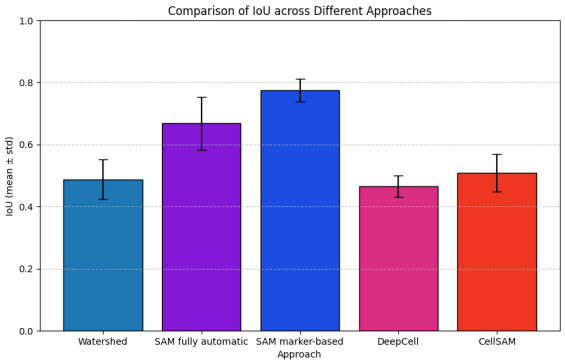


Fig. 3: Performance comparison of the different methods.

excluding those within the dilated mask. These background points complete the input array, enhancing SAM's segmentation precision. All segmentation results are obtained with SAM (ViT-H), ensuring high-capacity feature extraction and robust performance in delineating cell boundaries.

V. Results

In this section, we present the segmentation performance obtained using the classical Watershed algorithm and SAM guided by pre-segmented regions from classical methods as prompts. By comparing these methods with other state-of-the-art techniques, such as fully automatic SAM, DeepCell, and CellSAM, we evaluate the strengths and limitations of each approach in detecting and delineating NocWT cells. This analysis highlights the impact of incorporating prior knowledge from traditional segmentation techniques to enhance the accuracy and reliability of deep learning-based segmentation.

Segmentation quality is assessed using the *Intersection over Union* (IoU), which measures the overlap between the predicted mask and the ground truth mask. In this study, IoU is calculated globally, considering all segmented cells as a single binary mask to ensure consistency across different methods.

A. Watershed algorithm

As shown in Fig. 3, the Watershed algorithm provides inadequate segmentation results, with IoU values below 0.5. This underperformance is due to the non-uniform cell surface, with varying intensities that cause the algorithm to incorrectly place the watershed barrier at intensity variations, leading to incomplete segmentation. Post-processing techniques like dilation or appropriate filtering could improve IoU, but they risk distorting cell boundaries.

B. Marker-based SAM

While the Watershed algorithm does not achieve perfect cell segmentation, it proves highly effective for individual cell detection. This makes it a valuable prior, as these detections serve as reliable prompts for guiding SAM, refining its segmentation while preserving crucial cell structures. The performance of these cell detections, evaluated against the ground truth, is quantitatively assessed and reported in Table II, demonstrating consistently high metrics (above 0.95) with low standard deviation, indicating both accuracy and stability.

TABLE II: Cell detection performance.

Precision		**Recall**		**F1-score**	
μ	σ	μ	σ	μ	σ
0.998	0.004	0.963	0.027	0.980	0.009

Initialized with these detected cell prompts, the marker-based SAM approach offers substantially improved performance compared to the Watershed method, as visualized in Fig. 3. Leveraging the identified coordinates as prompts effectively guides the segmentation process, enabling the system to distinguish cells from the background with remarkable precision. This targeted approach not only enhances accurate cell boundary delineation but also minimizes the impact of impurities and background noise.

C. Comparison with SoA

To further evaluate our approach, we compare its performance with those of fully automatic SAM segmentation [9], DeepCell [21], and CellSAM [12], as shown in Fig. 3.

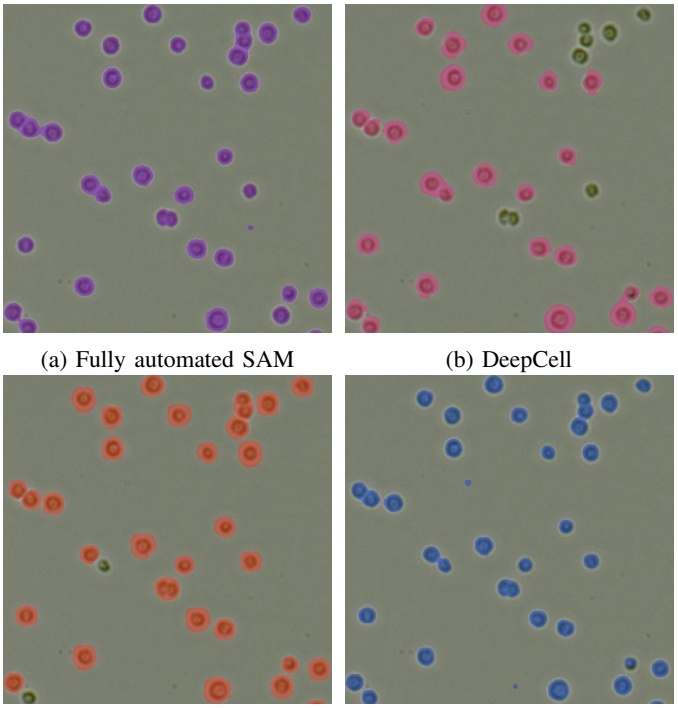
The automatic SAM approach offers versatility, requiring no user-provided prompts. However, without prior guidance, it is more sensitive to noise, segmenting any distinguishable element in the image, including the halos around cells. As a result, the masks tend to be larger, incorporating artifacts and reducing overall accuracy, as shown in Fig. 4a.

Results show that the SAM marker-based approach consistently outperforms also other relevant benchmarks, such as DeepCell [21], a deep-learning framework with pre-trained models for single-cell segmentation, and CellSAM [12], a SAM-based approach optimized for biomedical imaging. In fact, both exhibit limitations, as they tend to miss some cells while oversegmenting others due to sensitivity to halos, ultimately resulting in less precise cell delineation compared to our approach, as shown in Fig. 4b and Fig. 4c, respectively.

Among SAM variants, the marker-based method achieves superior segmentation with significantly lower computational costs. While automatic SAM requires about one minute per image on a consumer-grade GPU, the marker-based method delivers high-quality results in roughly 4 seconds, making it more practical for large-scale analysis.

VI. Conclusion

This work describes a hybrid approach for automated detection, counting, and segmentation of *Nannochloropsis Oceanica, Wild Type* (NocWT) microalgae cells. Combining classical image processing with deep learning, we leverage the strengths of both. Traditional methods excel at detection and counting but struggle with segmentation due to morphological variations and overlapping cells. To address this, we guide the *Segment Anything Model* (SAM) using pre-segmented regions derived from classical methods, refining cell boundary delineation. Comparisons with fully automatic SAM, DeepCell, and CellSAM highlight the advantages of integrating prior



(a) Fully automated SAM (b) DeepCell

(c) CellSAM (d) Our method

Fig. 4: Qualitative comparisons of results.

knowledge into AI-driven segmentation. Our marker-based strategy optimizes both accuracy and efficiency, processing images in just four seconds on a consumer-grade GPU, about 15 times faster than unassisted SAM. This hybrid approach balances precision, interpretability, and speed, offering a scalable solution for high-throughput microalgae image analysis.

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