Feasibility of Instance Segmentation of Phytoplankton using Brightfield Microscopy & Deep Learning

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Abstract

Harmful algal blooms (HABs) can have significant negative economic, environmental and health impacts. Therefore the real-time monitoring of phytoplankton is becoming increasingly critical for proper management of water bodies. This work demonstrates that instance segmentation on phytoplankton is possible on a small dataset with a large number of classes. This is accomplished in three main steps. First, 596 images of 21 different unialgal cultures were captured using brightfield microscopy. Second, these raw images were processed using traditional computer vision methods to rapidly create a binary mask. Finally, these raw images and binary masks were used to train a deep learning instance segmentation model. Experimental results show that high instance segmentation performance can be achieved for certain algae types and mixed performance for others by finetuning a Mask R-CNN deep convolutional neural network with a small but highly diverse dataset of different phytoplankton. These results show promising progress towards building a real-time on-site monitoring phytoplankton system.

1 Introduction

Freshwater and marine harmful algal blooms (HABs) can have a negative impact on different aspects of the the economy and human health. For example, HABs can negatively affect our drinking water, recreational use of water, the tourism industry, and aquaculture [1, 2]. The standard method to identify algae in water requires a highly trained human taxonomist to manually identify and count these algae through a microscope [3]. This process is both tedious and time consuming, as well as can be prone to human error when the taxonomist is fatigued. In addition, professional taxonomists are in decline [4] and systematic biases between different taxonomists result in variation in labels between professionals [5]. Many organizations that conduct water monitoring of algae, such as drinking water utilities or aquaculture farms, do not have a trained taxonomist on site. This further delays any analysis by a number of days as a water sample must now be shipped off site. Therefore an automated method to conduct on-site real-time analysis of phytoplankton is of high priority to ensure proper operations.

Given the prevalent use and high performance of deep learning methods across many industries and data types, it's natural to explore the efficacy of deep learning to segment phytoplankton in microscope images. Some recent examples of this can be by Ruiz *et al.* [6] and by Bergum *et al.* [7]. Ruiz *et al.* looked at 126 images of 10 different diatoms and created an instance segmentation classifier and found that the Mask R-CNN model had the highest performance. Bergum *et al.* created their own dataset of 126 images and segmented copepods using the Mask R-CCN architecture. In this work we collected 596 images of 21 different types of algae and trained a Mask R-CNN model to explore the efficacy of segmenting a high number of different phytoplankton classes. In order to train the deep learning model we create ground truth labels using traditional computer vision approaches. We discuss the benefits and challenges of both the traditional computer vision approach to create binary images as well as the deep learning approach for instance segmentation of phytoplankton.

2 Methodology

2.1 Overview

Given a raw image of a pure algal strain there are two steps to explore the efficacy of instance segmentation with deep learning. The first step is to segment each object the image using traditional computer vision methods by creating a binary mask. Ideally a human expert would annotate these raw images, however, given the simplicity of the images and knowledge that every organism present in a single raw image belongs to the same class, it is faster to use traditional computer vision methods to create a binary mask. The second step is to use this mapping from raw inputs to binary masks to train a deep learning model to accomplish the task of instance segmentation.

2.2 Image Binarization

To generate a mapping from an input image to a binarized mask involves three steps. First, flatfield correction was applied to correct for any illumination variation across the image. Next, Otsu thresholding was used to create a binary image of different regions. Then, given these regions, different morphological operations were used to create a binarized image. Given the variation across the different phytoplankton, the exact morphological operations change for a given phytoplankton type. This was done by manually tweaking the different parameters for a given phytoplankton type. This reinforces the need to create an end-to-end classifier that does not require manual changing the morphological operations for a given organism type. Hence deep learning methods for instance segmentation are a natural choice to explore.

2.3 Instance Segmentation

There are a number of segmentation deep learning approaches, each with different advantages and disadvantages. In their recent survey, Minaee *et al.* [8] discuss convolutional networks, recurrent networks, attention based models, generative and adversarial approaches, and other methods. Given the high performance when compared to other instance segmentation methods [8], and to match recent work [6, 7], the commonly used benchmark Mask R-CNN model [9] was used in this paper.

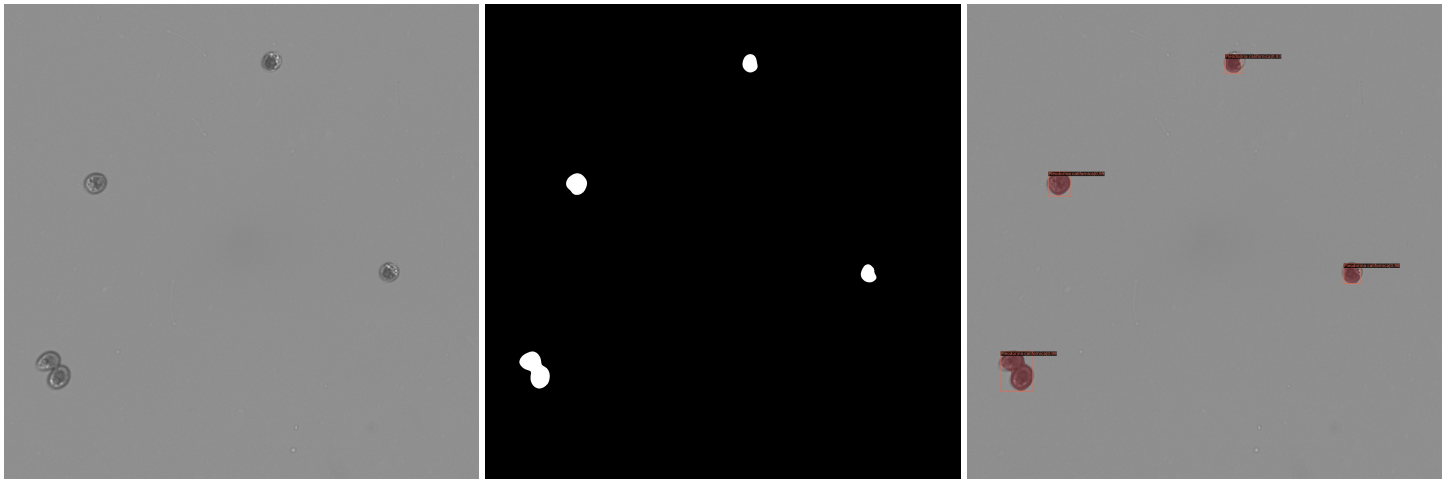
3 Experimental Setup

3.1 Phytoplankton Selection

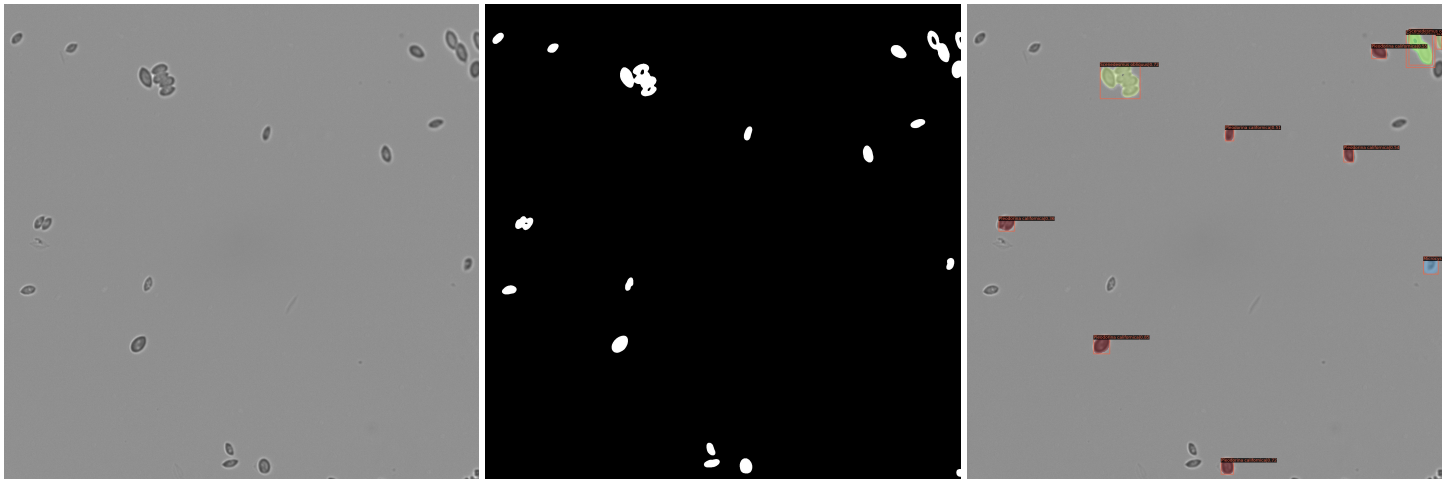
A number of different algae were selected from the Canadian Phycological Culture Centre (CPCC) at the University of Waterloo and the Charles Trick Lab at Western University. In alphabetical order the 21 types of algae that were imaged were: *Ankistrodesmus falcatus*, *Chlorella kessleri*, *Closterium sp.*, *Cylindrospermum sp.*, *Dolichospermum sp.*, *Euglena gracilis*, *Fistulifera pelliculosa*, *Merismopedia sp.*, *Microcystis aeruginosa*, *Monoraphdium contortum*, *Navicula pelliculosa*, *Nostoc sp.*, *Pediastrum duplex*, *Pleodorina californica*, *Pseudanabaena sp.*, *Scenedesmus obliquus*, *Scenedesmus quadricauda*, *Staurastrum johnsonii*, *Stenomitos tremulus*, *Tetradesmus obliquus*, and *Trichormus variabilis*.

3.2 Data Collection

Data was collected using SAMSON: A **S**pectral **A**bsorption-fluorescence **M**icroscopy **S**ystem for **ON**-site-imaging of algae [10]. Given the modular design, SAMSON can be configured to use different wavelength LEDs, optics, and filters when collecting data. For this instantiation, SAMSON was configured as a standard brightfield microscope with a monochromatic sensor. A total of 596 images were collected by using a Luxeon white LED as a light source, with a 40x objective lens, and the FLIR Grasshopper 3 NIR monochromatic camera.



(a) Pleodorina californica raw image *(b) Pleodorina californica binary image* *(c) Pleodorina californica prediction*



(d) Scenedesmus obliquus raw image *(e) Scenedesmus obliquus binary image* *(f) Scenedesmus obliquus prediction*

Fig. 1: The raw image (left), binarized image (middle) and prediction (right) for both *Pleodorina californica* (top) and *Scenedesmus obliquus* (bottom). Notice the prediction colours for *Pleodorina californica* (red) and *Scenedesmus obliquus* (yellow) and how many *Scenedesmus obliquus* (yellow) have been classified as *Pleodorina californica* (red) in the bottom right image.

3.3 Machine Learning Framework

Given the small dataset, only a train and test set were created with an 80/20 split. This resulted in 477 images in the training dataset and 119 images in the test dataset. Given the readily available GitHub repositories, OpenMMLab's framework [11] was used to access a pretrained Mask R-CNN model. Using the training dataset, the model was then fine-tuned for 12 epochs using the Stochastic Gradient Descent (SGD) optimizer with a learning rate of 0.02, momentum of 0.9, and weight decay of 0.0001. Standard image augmentation methods were applied when training.

4 Results & Discussion

4.1 Image Binarization

Given the raw images from the microscope, as seen in Figure 1(a) and Figure 1(d), the binary images produced by using traditional computer vision can be seen in Figure 1(b) and Figure 1(e). Given the simplicity of the images, and the knowledge that each image is of a known unialgal strain, traditional computer vision methods are faster to build a quick dataset for prototyping. However, using this approach results in undesired artifacts such as (1) holes / gaps in the binary images, as seen in Figure 1(e), as well as (2) missing fine-grained morphological features like antenna on certain phytoplankton. These artifacts come from attempting to build a single computer vision pipeline for all 21 phytoplankton types. In order for the pipeline to segment one algae type results in these artifacts appearing in other phytoplankton types. Therefore, to properly build on this feasibility study, it is highly recommended that a human expert annotate the raw images.

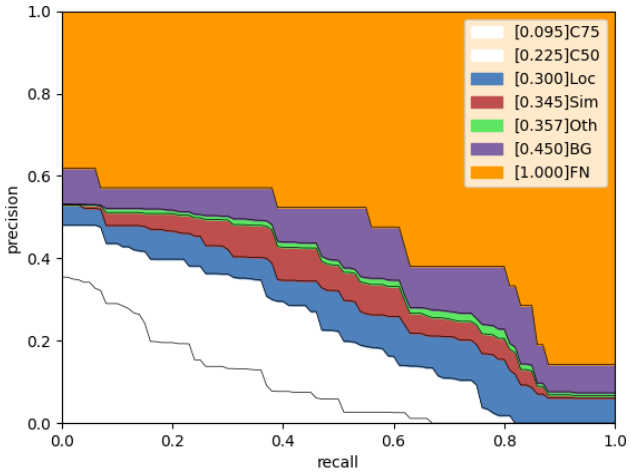
4.2 Instance Segmentation

Two examples of the resulting instance segmentation from the trained model can be seen in Figure 1(c) and Figure 1(f). As seen in Figure 1(c), our trained model correctly segmented and classified all the instances of *Pleodorina californica* (red). In general the *Pleodorina californica* class performed well, as seen by the precision recall curve in Figure 2(b). This demonstrates that the model is able to both correctly segment and classify phytoplankton.

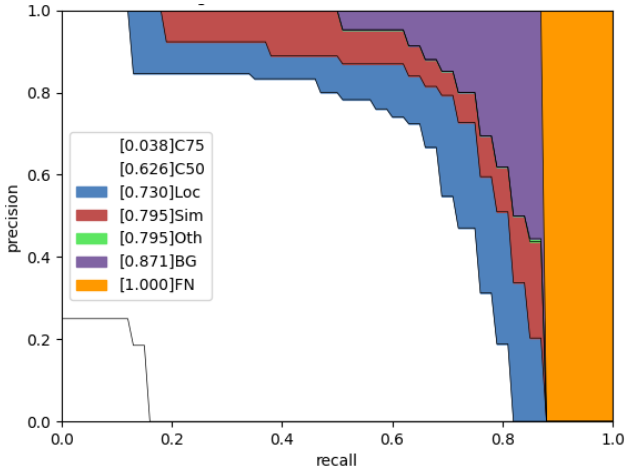
However, this performance can not be observed across the entire dataset. Figure 2(a) illustrates that the overall performance of the classifier was moderate. This was due to many classes not correctly segmenting the desired area, or incorrectly classifying a segmented area. For example, in Figure 1(f) all the organisms are *Scenedesmus obliquus*. However, many of these organisms are not segmented by the classifier, and in this specific image only one classification of *Scenedesmus obliquus* (yellow) is correct. The majority of the instances are classified incorrectly as *Pleodorina californica* (red). This demonstrates that the machine learning model unperformed when segmenting and classifying, likely as a result of the very small dataset available.

5 Conclusions & Future Work

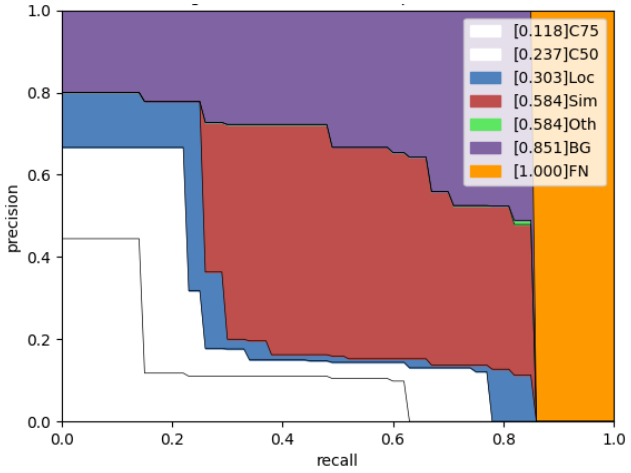
This work illustrates that instance segmentation is feasible for 21 different phytoplankton cultures when imaging with a brightfield microscope housed with a monochromatic camera and when fine-tuning a pretrained Mask R-CNN deep learning model. While the overall performance was moderate, many classes achieved high performance and were properly segmented and classified. In addition, the poor performance can be attributed to a very small dataset with a high number of classes, which is not ideal when training machine learning models.



(a) Precision Recall Curve for all classes



(b) Precision Recall Curve for *Pleodorina californica*



(c) Precision Recall Curve for *Scenedesmus obliquus*

Fig. 2: The Precision Recall (PR) curves for (a) the average overall classes, (b) for the *Pleodorina californica* class as seen in Figure 1 (top), and (c) for the *Scenedesmus obliquus* class as seen in Figure 1 (bottom).

Given this initial validation there are a number of experiments and tests that should be conducted to further validate this approach. First, to improve this work one must increase the amount and quality of data. Second, in this study ground truth regions were not reviewed by a professional algal taxonomist. To further improve this approach a larger dataset must be created and human experts trained in phytoplankton taxonomy must validate the ground truth labels. Third, other deep learning pretrained instance segmentation models should be explored to determine the optimal architecture for this task. Fourth, opposed to monochromatic sensor, a colour senor should be used. Alternatively, additional wavelength bands, such as fluorescence images, could be used to improve classification results. Finally, future work must explore mixed lab samples as well as environmental samples before this approach can be used in a real-world environment.

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Contributions

JLD and AW conceived and planned out the work. JLD collected images from samples provided by the CPCC. JLD, ECF, KJE and MMA collected images from samples provided by the Charles Trick lab. JLD and JAP developed the code base and ran the machine learning experiments. JLD wrote the manuscript and all authors reviewed the manuscript.

References

[1] W. W. Carmichael and G. L. Boyer, "Health impacts from cyanobacteria harmful algae blooms: Implications for the north american great lakes," *Harmful algae*, vol. 54, pp. 194–212, 2016.

[2] A. M. M. Ibrahim, "Review of the impact of harmful algae blooms and toxins on the world economy and human health," 2007.

[3] E. G. Bellinger and D. C. Sigee, *Freshwater algae: identification, enumeration and use as bioindicators*. John Wiley & Sons, 2015.

[4] A. McQuatters-Gollop, D. G. Johns, E. Bresnan, J. Skinner, I. Rombouts, R. Stern, A. Aubert, M. Johansen, J. Bedford, and A. Knights, "From microscope to management: the critical value of plankton taxonomy to marine policy and biodiversity conservation," *Marine Policy*, vol. 83, pp. 1–10, 2017.

[5] P. F. Culverhouse, R. Williams, B. Reguera, V. Herry, and S. González-Gil, "Do experts make mistakes? a comparison of human and machine indentification of dinoflagellates," *Marine ecology progress series*, vol. 247, pp. 17–25, 2003.

[6] J. Ruiz-Santaquiteria, G. Bueno, O. Deniz, N. Vallez, and G. Cristobal, "Semantic versus instance segmentation in microscopic algae detection," *Engineering Applications of Artificial Intelligence*, vol. 87, p. 103271, 2020.

[7] S. A. Bergum, "Object detection and instance segmentation of planktonic organisms using mask r-cnn for real-time in-situ image processing." Master's thesis, NTNU, 2020.

[8] S. Minaee, Y. Y. Boykov, F. Porikli, A. J. Plaza, N. Kehtarnavaz, and D. Terzopoulos, "Image segmentation using deep learning: A survey," *IEEE Transactions on Pattern Analysis and Machine Intelligence*, 2021.

[9] K. He, G. Gkioxari, P. Dollar, and R. Girshick, "Mask r-cnn," *2017 IEEE International Conference on Computer Vision (ICCV)*, Oct 2017.

[10] J. Deglint, L. Tang, Y. Wang, C. Jin, and A. Wong, "SAMSON: Spectral Absorption-fluorescence Microscopy System for ON-site-imaging of algae," *Journal of Computational Vision and Imaging Systems*, vol. 4, no. 1, p. 3, Dec. 2018. [Online]. Available: http://openjournals.uwaterloo.ca/index.php/vsl/article/view/324

[11] K. Chen, J. Wang, J. Pang, Y. Cao, Y. Xiong, X. Li, S. Sun, W. Feng, Z. Liu, J. Xu, Z. Zhang, D. Cheng, C. Zhu, T. Cheng, Q. Zhao, B. Li, X. Lu, R. Zhu, Y. Wu, J. Dai, J. Wang, J. Shi, W. Ouyang, C. C. Loy, and D. Lin, "MMDetection: Open mmlab detection toolbox and benchmark," *arXiv preprint arXiv:1906.07155*, 2019.