ROBERT GORDON UNIVERSITY ABERDEEN
SCHOOL OF COMPUTING

BSc. (Hons) Computer Science

Automated Microscopy Cell Counting using Neural Networks

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Introduction

Aphanizomenon is a filamentous cyanobacteria that inhabits fresh water. Cyanobacteria can produce toxins which degrade water quality, are dangerous to the health of both humans and aquatic animals, and incur a significant economic impact. [1]

Counting the number of cyanobacteria cells in a water sample provides a significant measure of its quality. Presently, this kind of cell counting task is usually performed by eye by expert microbiologists. Overlapping cells, variations in shape and size, and the subtle differences between species make this challenging. In addition, different human observers count inconsistently [2], and the task is tedious and time-consuming.

The project aim is to develop a novel artifact as part of a reliable automatic counting solution for filamentous cyanobacteria cells. This artifact will take the form of a machine-learning model, trained on a novel image dataset.

Data Annotation

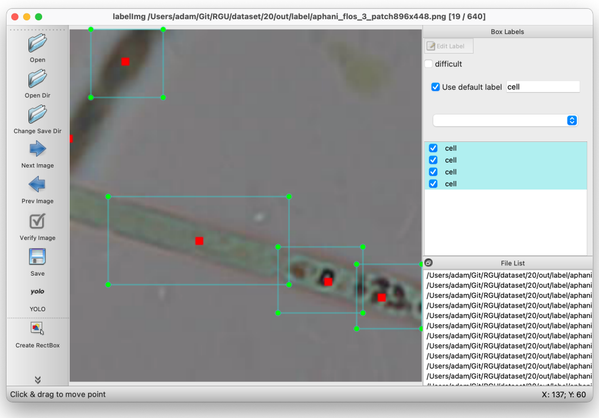

Figure 1: LabelImg

A sample of 20 images was taken from a novel dataset of 311 microscope images of *Aphanizomenon.* These were split into 640 patches, which were then reannotated using LabelImg; based on the existing annotation (a red dot drawn at the centre of each cell), bounding boxes were drawn to encompass the entire cell in each instance. These annotations were then interpretable by YOLOv5, a state-of-the-art model for object detection & localisation. [3]

Modelling and Results

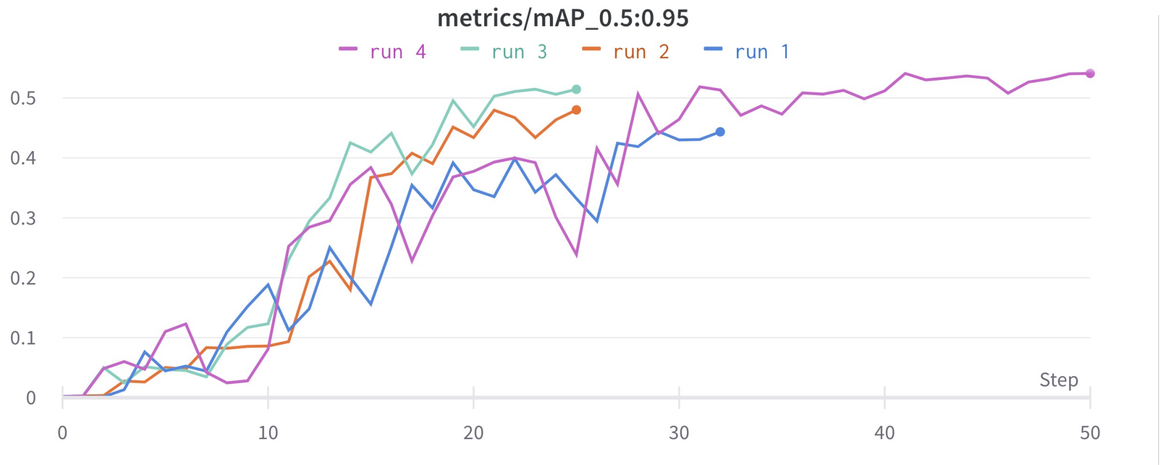

Figure 2: `mAP_0.5:0.95` for all 4 runs of training

Image	Ground Truth	Model 1	absError	percentageError	Model 2	absError	percentageError	Model 3	absError	percentageError	Model 4	absError	percentageError
34	81	15	66	81.48	258	177	218.52	256	175	216.05	198	117	144.44
39	58	8	50	86.21	125	67	115.52	144	86	148.28	123	65	112.07
41	86	12	74	86.05	192	106	123.26	192	106	123.26	176	90	104.65
44	102	14	88	86.27	305	203	199.02	274	172	168.63	253	151	148.04

Figure 3: Ground truth count alongside count, absolute error, and percentage error for each model

A preexisting YOLOv5 model is unsuitable for this use case, so the chosen model (YOLOv5s) was retrained 4 times with varying configurations of number of epochs and batch size. The dataset annotations were also edited to include more partially-visible and out-of-focus cells, and the train/test+val split was changed from 50/50 to 70/30.

Of these, the fourth run produced the 'best' model by metrics. It achieved an `mAP_0.5:0.95` of 0.54 (average precision over all IoU thresholds between 0.5 and 0.95, with a step of 0.05), and its `mAP_0.5` of 0.90 suggested that 90% of cells were successfully localised with an IoU of at least 0.5. However, a comparison of model counts versus ground-truth counts (Fig. 3) revealed that this model consistently counts more than double the number of cells actually present in the image.

Qualitative evaluation of the model's predicted bounding boxes (Fig. 4) shows that its localisation of cells is good, especially in single trichomes where each cell is clearly separable from those around it; but it can be confused by overlapping and out-of-focus cells, where it is vulnerable to false positive errors from detecting cells multiple times. It also occasionally classes pieces of foreign material on the slide as cells. The model's counting capability requires further work.

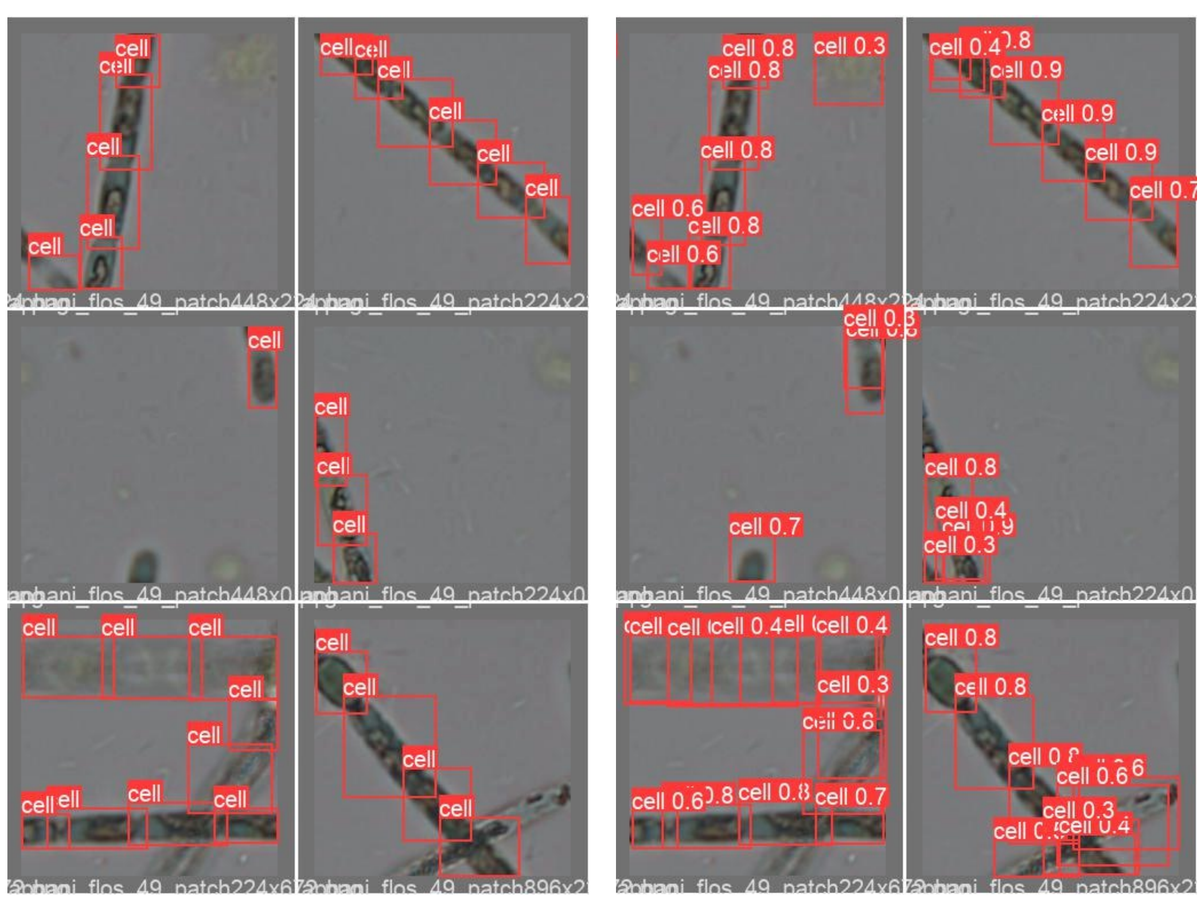

Figure 4: Ground-truth annotations (left) & best model's predicted bounding boxes (right)

Conclusion

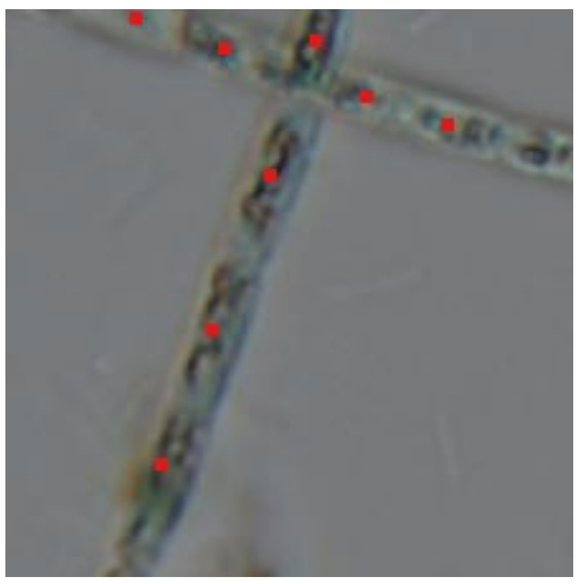

Figure 5: An image of Aphanizomenon from the dataset

The detection-based approach to counting filamentous cyanobacteria holds promise. Future work must investigate the particularly difficult problem of cells and trichomes which overlap or are out-of-focus, as well as false positive errors produced by foreign material present in the image. It must also take into account that metrics for an object-detection model are not necessarily meaningful in a counting use case. It is possible that an increase in mAP does not correlate with an increase in counting accuracy, but may correlate with increased false positives. The dataset used was small since annotation is highly time-consuming, but adding more data to the training set after the 2nd run generally improved mAP and count accuracy, as did increasing the number of training epochs. Both factors should be further investigated.

Acknowledgments

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References

[1] Baek et al., 2020. Identification and enumeration of cyanobacte- ria species using a deep neural network. Ecological Indicators, 115, 106395.
[2] Culverhouse et al., 2003. Do experts make mistakes? a comparison of human and machine identification of dinoflagellates. Marine Ecology Progress Series, 247, 17–25.
[3] GitHub.com. YOLOv5 [Internet]. 2022. Available from: https://github.com/ultralytics/yolov5