AUTOMATED CELL COUNTER FOR *DUNALIELLA* UNDER LABORATORY CONDITION

MARY JANE MAGNO-TAN[1,2], JONAS GERARD C. RIGOR[2], JOSE R. SAN JUAN JR.[2], AND ARMIN S. CORONADO[1,3]

[1]*Institute for Science and Technology Research, Office of the Vice President for Research, Extension and Development, Polytechnic University of the Philippines, Sta. Mesa, Manila*
[2]*Department of Computer Science, College of Computer and Information Sciences, Polytechnic University of the Philippines, Sta. Mesa, Manila, Philippines*
[3]*Department of Biology, College of Science, Polytechnic University of the Philippines, Sta. Mesa, Manila, Philippines*

Abstract: In order to maximize the potential of *Dunaliella* sp. as feedstock for biodiesel production, the laboratory culture conditions must be fully understood to obtain high yield and good quality lipids. However, optimizing culture conditions need rigorous daily monitoring of algal growth that entails time-consuming protocol like manual counting of cells under the microscope. This research developed a cost-effective system that utilizes Haar Cascade Algorithm as classifier, to automatically count *Dunaliella* sp. cells in order to calculate the culture cell density and generate data through graphs. The Automated Cell Counter has a percentage accuracy of 87.75% and percentage performance of 87.75% using F-measure (F1-score). Moreover, the precision (exactness) of the system and recall (sensitivity of the classifier) has values of 72.76% and 71.3%, respectively. Analysis of Variance (ANOVA) revealed that the calculated cell density from automated cell counting and from manual counting done by domain experts of *Dunaliella* sp. is not significantly different ($\alpha_{0.05}$<0.609). Therefore, the Haar Cascade Algorithm can be used as classifier to count *Dunaliella* sp. cells.

Keywords: *Haar Cascade Algorithm, Dunaliella, classifier, automated cell counter, image processing*

1. INTRODUCTION

In microalgal cultivation, measuring the cell number to monitor the growth rate of the algal culture is a long-standing problem (Cordova-Matson *et al.*, 2009). Cell concentrations in microalgal cultures are typically expressed as cell count per unit volume (cells mL^{-1}), which refers to the cell density. Hemocytometer is widely used to manually count the microalgal density. However, this technique in calculating the cell density in the microalgal cultures is tedious and time consuming (Imamoglu *et al.*, 1991).

Precision and reproducibility are the major constraints in the manual counting of microalgal cells. Although hemocytometer has a known volume in each chamber, slight increase in the liquid volume can be encountered upon filling the chamber. Thus, significant variations in estimating the cell count are common errors when using this technique (Nielson *et al.*, 1991).

One way in resolving the problem of counting algal cells is to develop an automatic cell counter. This would provide speedy analysis and consistent results, and free up

personnel for other tasks (Sjostrom *et al.*, 1998). Several attempts were made to produce hardware for counting viable cell lines, which showed accuracy and precision comparable to manual cell counting without the tedious and long hours of laborious manual count (Invitrogen, 2009). However, the hardware is specific only to mammalian cell lines. Furthermore, these automatic cell counters require the use of dye in determining the viable cells that are considered for counting. At present, there is no available software for microalgal cell counting specifically for *Dunaliella* sp. that does not require the use of staining reagents to automatically count the cells. Previous automated cell counting systems used expensive image processing tools and techniques like template matching, active contours and level set techniques (Young *et al.*, 1998; Bamford, 1998; Li *et al.*, 2010). Haar Cascade Classifier has been used to classify cyst images with a total correct classification rate of 86%; while it has been used to detect human face with a 95% accuracy rate.

Thus, this research attempted to develop a cost-effective and efficient system to estimate cell density of *Dunaliella* sp. cells using a Haar Cascade Classifier-based automated cell counter. The system also aimed to aid microalgae cultivators to make timely decisions based on the plotted growth curve derived from the daily record of cell density (Viola & Jones, 2001; Han, 2014).

1. METHODOLOGY

2.1 Software development

The system was developed using Oracle VM Virtualbox that created an Ubuntu Server-Virtual Machine. Python programming language was used as the programming tool and virtualenv was used to create isolated Python environments. OpenCV, an open source image processing library was used in processing the digital images. Haar Cascade Algorithm was used to classify and count *Dunaliella* sp. cells. Bootstrap and Django Templates were used for front-end development, while Python and a Django web framework were used for backend development. The sample images that were used for the training and testing of the classifier during the implementation were collected from the Polytechnic University of the Philippines' Center for Life Sciences Research, Institute for Science and Technology Research (PUP CLSR-ISTR).

2.2 System architecture

Figure 1 shows the general system architecture. The system is composed of three major parts: the Image Processing, the Classification using Haar Cascade, and the Interpretation of Data. The digital image of *Dunaliella* sp. under hemocytometer was the input to the system. The image undergoes preprocessing (grayscaling) technique to increase the classifier's accuracy in detecting blurry and unclear images. Grayscaling the image would make it monochromatic and easier for the system to classify the cells.

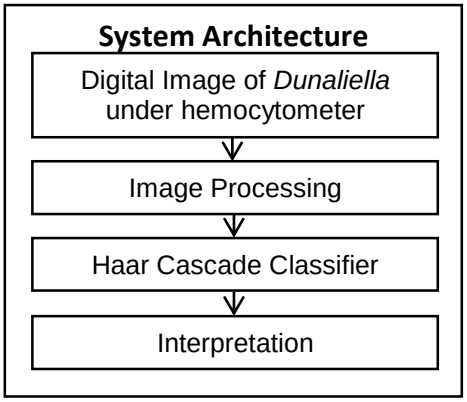


Figure 1. System architecture.

The preprocessed image is used as input to the Haar Cascade Classifier, an object detection method where a cascade function is trained from a lot of positive and negative images. The image is scanned per area (block) and all the recognized cells by the classifier are counted. The system then computes the total cell count from the five (5) blocks of the two (2) chambers of the hemocytometer. The total count is used to compute the cell density that is represented as a graph.

2.3 Data analysis

The digital image of *Dunaliella* sp. cells under the hemocytometer was counted from the blocks of each chamber (Figure 2) and used for the computation of the cell density. Only the cells inside the boundary lines of each block is counted. The cell count and cell density from the automated system was compared to the manual counting of the cells, which was done by domain experts from the PUP Center for Life Science Research.

Each chamber of the hemocytometer holds five (5) blocks (Figure 3): Block A (Upper left numbered 1), Block B (Upper right numbered 2), Block C (Lower left numbered 3), Block D (Lower right numbered 4), and Block E (Middle).

The digitized image is scanned by the algorithm per block and all the recognized cells are counted, after which the total cell count from the five (5) blocks in each of the hemocytometer's two (2) chambers is computed. The total count is used to compute the cell density.

Different cultures of *Dunaliella* cells were subjected to ten (10) trials with each trial replicated thrice. Replication is the number of distinct experimental units under the same treatment where the treatments are assigned to the cells at random, allowing each of the samples to have an equal probability of receiving a treatment, providing a basis for estimating the error variance. Each trial is obtained from water samples with different concentrations. Trials are obtained regardless of the concentrations of the treatment.

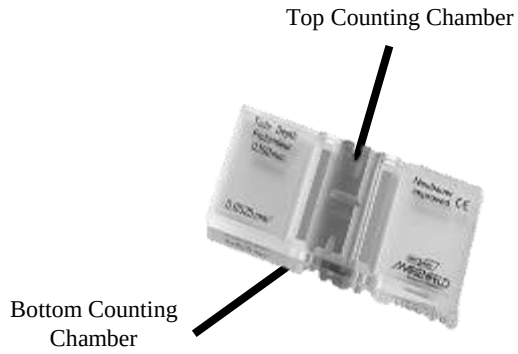


Figure 2. Image of the hemocytometer showing the counting chambers.

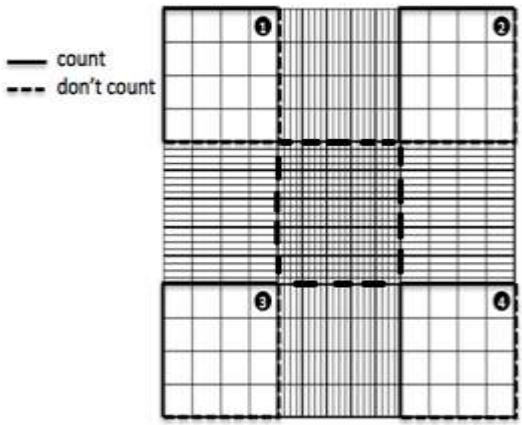
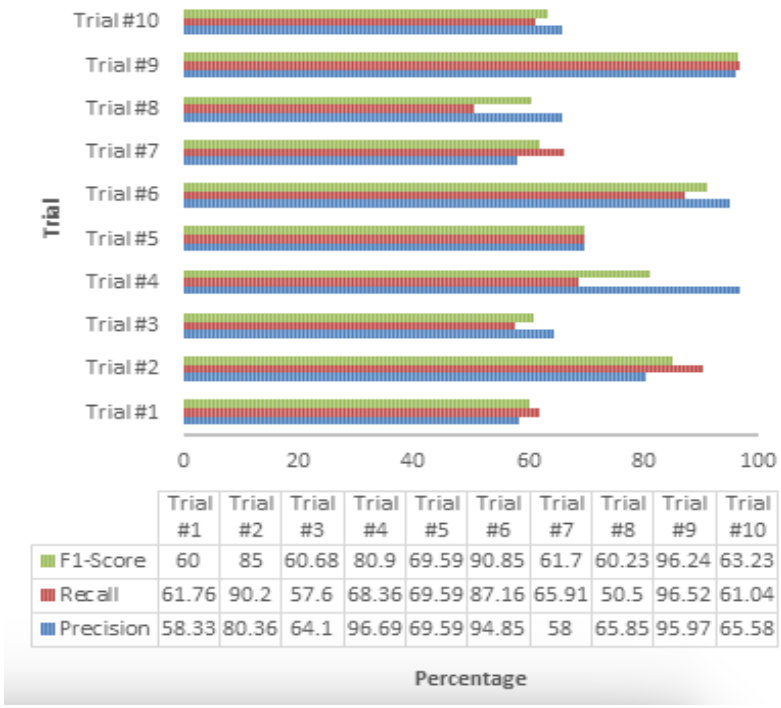


Figure 3. Hemocytometer under microscope showing areas to be counted.

Results of the experiment were recorded in the experiment paper which shows the computed mean of the cell counts from the three replicates for each trial, for both manual and automated counting.

To test the accuracy of the system in classifying *Dunaliella* cells, ten (10) trials were done. The degree of accuracy was determined using the computed values for the classifier's Precision, Recall and F1-score, which are based on confusion matrices. To determine if the automated count is comparable to the manual count done by the domain experts, Analysis of Variance (ANOVA) was used to determine if there is a significant difference between the automated and manual cell counting.



	Trial #1	Trial #2	Trial #3	Trial #4	Trial #5	Trial #6	Trial #7	Trial #8	Trial #9	Trial #10
F1-Score	60	85	60.68	80.9	69.59	90.85	61.7	60.23	96.24	63.23
Recall	61.76	90.2	57.6	68.36	69.59	87.16	65.91	50.5	96.52	61.04
Precision	58.33	80.36	64.1	96.69	69.59	94.85	58	65.85	95.97	65.58

Figure 4. Performance of the automated cell counter per trial.

2. RESULTS AND DISCUSSION

3.1 Measurement of accuracy

Using the data gathered from each block of the hemocytometer, the performance of the system's classifier per trial was computed. Figure 4 presents the computed Precision, Recall and F1-Score of the classifier for each trial conducted. Results showed that the F1-Score, Recall, and Precision from the ten (10) trials are 74.93%, 71.37% and 72.76%, respectively. The resulting values do not differ significantly ($p>0.05$) for each trial. Moreover, the cell count derived between manual counting done by domain experts and the automatic cell counting is not significantly different ($\alpha_{0.05}<0.609$). Thus, the software can recognize and count microalgal cells to estimate the cell density of the algal culture.

3.2 Image detection

Figure 5A shows the good quality images of cells which are accurately detected by the Haar Cascade algorithm.

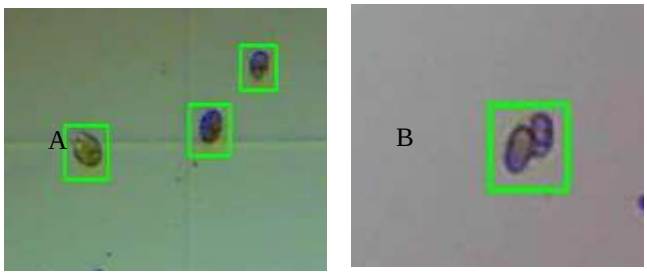


Figure 5. Digitized images of *Dunaliella* sp. cells (A) as recognized by the system; (B) overlapping cells counted as one cell by the system.

Images that are not blurred are accurately detected and correctly counted by the algorithm. The images that are fed to the Haar Cascade classifier should be of good quality for the algorithm to have higher accuracy and ease in detecting the cells.

On the other hand, overlapping cells are counted as one because the algorithm has not been trained to distinguish two or more overlapping figures from each other (Figure 5B).

3. CONCLUSIONS

The accuracy rate of the system is within the acceptable limits and can therefore be utilized as an alternative method to the traditional manual counting of *Dunaliella* sp. cells using hemocytometer.

The accuracy of the system in counting algal cells is affected by the quality of the digitized images. Clear images and those without overlapping cells enjoy higher accuracy in cell counting. It is recommended to use image preprocessing techniques and tools to increase the classifier's accuracy in detecting from initially blurry and unclear images. Grayscaling the image would turn it monochromatic and make it easier for the system to classify the cells.

The classifier's accuracy may be improved by training it with a better data set. A good positive set of images should contain *Dunalliela* cells with different orientation, size and shape; while a good negative set of images can contain any images under the hemocytometer that does not include *Dunalliela* cells.

The current system can only detect and count *Dunalliela* sp. cells because the classifier was trained with *Dunalliela* cells only. Future researchers can add more species that can be classified by the system by training it with images of cells from different species.

The algorithm was not trained to count overlapping cells as two or more. It is then recommended that culture samples be properly diluted to avoid overlapping of cells prior to capturing digital image and uploading to the system.

4. RECOMMENDATIONS

Modifications may be applied to the program so that overlapping cells can be detected and not counted as one.

The classifier may be enhanced to include a forecasting system which alerts the user when the declination phase starts, and by integrating a decision support system that suggests or advises experts when to harvest and sub-culture the microalgae.

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