An Artificial Neural Network Model for the Cell Density Measurement of *Spirulina platensis* for Controlled Closed Algal Culturing System

Aaron U. Aquino[#1,] Ma. Veronica L. Bautista[#2], Camille H. Diaz[#3], Matthew Edward M. Fernandez[#4], Aileen P. Guzman[#5], Albert A. Matias[#6]
Electronics Engineering Department
Technological University of the Philippines
Manila, Philippines
[1]aaron_aquino@tup.edu.ph
[3]camilledz07@gmail.com

Abstract **–This study presents a dynamic model for the cell density measurement of *Spirulina platensis* by utilizing Artificial Neural Network (ANN). The RGB and lux value are the input nodes and the cell density is the output node of the ANN structure. The vision system developed is composed of camera and photodetector. The lux value from the photodetector was used as training data for the neural network, as well as the RGB values extracted from the images captured by the camera. MATLAB is used to structure the hidden values of the network. Based on the result of the trained network, using 24 nodes, the neural network model develop shows a low mean-squared error of 0.0047813 and a fast learning time of 2 seconds. Upon validation, the network shows that the two sets of data has a strong correlation.**

Keywords – Artificial Neural Network, Spirulina platensis, cell density, image processing

I. Introduction

An accurate and reliable cell count is very vital in the study of eukaryotic cells for different purposes such as cell culture management in biological research, diagnostic titration of cell population, and in-process controls in industrial bioprocesses [1-3]. Due to its affordability, manual cell counting using hemocytometer has been one of the most commonly used methods in different fields [4]. However, the process is very time consuming and susceptible to human error. It is subject to different results depending on expertise of the analyst [5-6].

In the Philippines, manual cell counting is commonly used in most of the laboratory in various fields. Just like what medical technologist do in counting the white blood cells, red blood cells and other chemical test which involves cell counting. Local researchers in the fields of biology also do the same process in counting cells of an animals, plants or even micro algae. This results in prolonging data analysis and outcome of research investigation. The development of an accurate and dependable automated system for cell counting will lead to advancement not only in the fields of medicine but also in the agricultural sectors as well.

In the previous years, some studies are conducted in order to improve the state of this manual cell counting process and to ease the task associated with it. To mention some of it, Yamanishi H. et. al developed a disposable plastic hemocytometer chamber (C-Chip) which determine the leukocyte count in cerebrospinal fluid by comparing its perfo`rmance to the traditional cell counting process. Results shows that C-Chip counting chamber is comparable in FR chamber. However, significantly low cell counts were yielded if the C-Chip were exposed to cold environment [7]. Another one is the study of Herrera D. which focuses on the validation of three trypan blue exclusion-based methods, manual, semi-automated and automated for validation. Results shows that the automated method efficiently analysed huge number of samples [8]. Another one is a computer aided system for red blood cell classification in blood smear image developed by Tomari R. et. al. Results shows that the system is reliable and effective in classifying normal and abnormal RBC [9].

Due to the difficulties experience by conducting manual cell counting, the researchers will model an artificial neural network that measures cell density of Spirulina platensis that will be used particularly for closed algal culturing system. The neural network model will be a significant attempt for the development of some system specifically for the automation of manual cell counting. This will benefits researchers and many professions in various fields whose task depends on the manual cell counting procedure.

II. Design of the Vision System

The system uses web camera which capture image and extract RGB value from the sampling cube and a photodetector which measures the sample’s turbidity. The Artificial Neural Network (ANN) correlates the RGB and lux in order to measure the cell density.

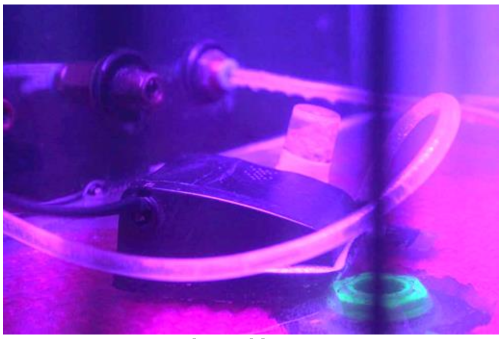

Fig. 1 Submersible Water Pump

Fig. 1 shows the submersible water pump installed inside the culturing tank. The water pump is used in order to get a sample and fill the sampling cube.

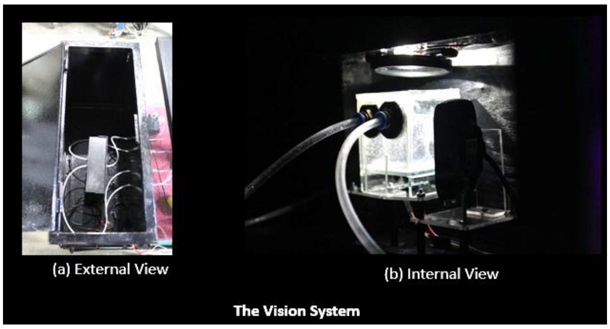

Fig. 2 Vision System (a) Internal and (b) External View

The vision system of the device gather two important data, RGB value and lux, which the Artificial Neural Network needs as an input to correlate in order to detect the cell density of the culture.

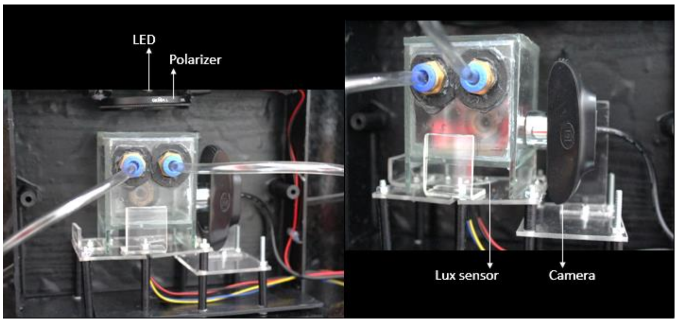

Fig. 3 Parts of the Vision System

Figure 3 shows the parts of the vision system. A 12V LED, the light source, is placed above the sampling cube with a lux sensor located beneath it. A polarizer is used in order to correct the light intensity coming from the LED. The web camera will capture an image periodically. The Matlab program will extract the RGB value of the image that will be used as a data by the neural network by correlating the lux value at the same instance.

Fig. 4 Sample image for feature extraction

III. Artificial Neural Network Model

A. *Artificial Neural Network (ANN)*

Artificial Neural Network (ANN) is a very powerful tool and a new method of computing which is originally modelled based on how the human brain behave in processing information. Furthermore, ANN can be used in developing new technology in various fields by creating different structural model. It is also used to effectively measure and predict data which is beneficial for diverse fields and application such as taxonomic identification, predictive growth model, cardiac complication predictions after surgery and many more [10-13].

The learning process of ANN is based on the relationships between sets of input and output data. The first process is to present an input and output vector to the input and output neurons to the network respectively. The training data sets and errors are computed based on the comparison of the ANN output and training data sets. To update the weights of the training neurons, error derivatives are used. The training continue until the errors are less than the recommended values [14].

B. *Dynamic Modelling*

Figure 5 summarized the processes involve in designing an ANN dynamic model.

The network configurations are listed as follow:

- Network Inputs: RGB and lux value
- Network Output: Cell Count
- Network Type: Feed-forward back propagation
- Number of Layers: 1 Layers with 24 nodes
- The data are divided into two parts: training data and validation data.

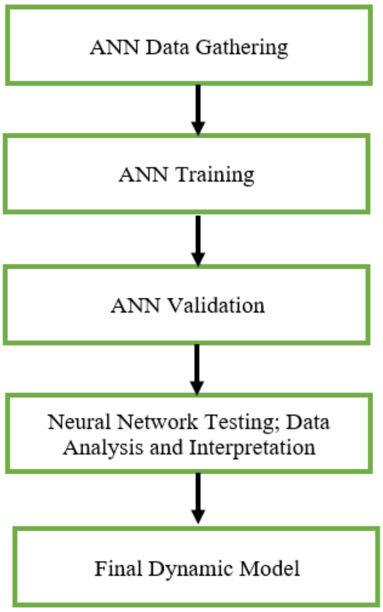

Fig. 5 ANN Dynamic Model Design Process

- **Artificial Neural Network Data Gathering**

The researchers tested the vision system functionality and at the same time gather data for the artificial neural network (ANN) at South East Asian Fisheries Development Center (SEAFDEC). Guided by Ms. Mary Jane M. Sayco, an algal expert, the researchers cultured 8L NPK solution with 10% *Spirulina platensis* inoculum. The RGB and lux value are extracted via vision system and get 5 mL of samples from the culture tank every 30 minutes for manual cell counting in order to know cell density with its equivalent RGB and lux values. Table 1 shows the sample training data for the network gathered using the vision system.

Table 1 ANN Training Data (Samples)

R	G	B	Lux	Cell Count
119.807	149.542	155.244	178	0
126.172	166.721	168.492	112	19.00941
133.607	174.898	176.359	116	77.81978
128.054	170.915	174.424	85	19.60346
118.164	165.974	170.683	86	15.44515
124.371	171.787	174.765	86	137.2242
114.995	161.67	164.752	91	166.9264
126.432	166.748	166.609	116	228.1129
126.764	166.561	167.738	141	202.569
131.053	171.711	172.462	155	176.4311
130.785	171.877	172.396	155	185.9358

- **Artificial Neural Network Training**

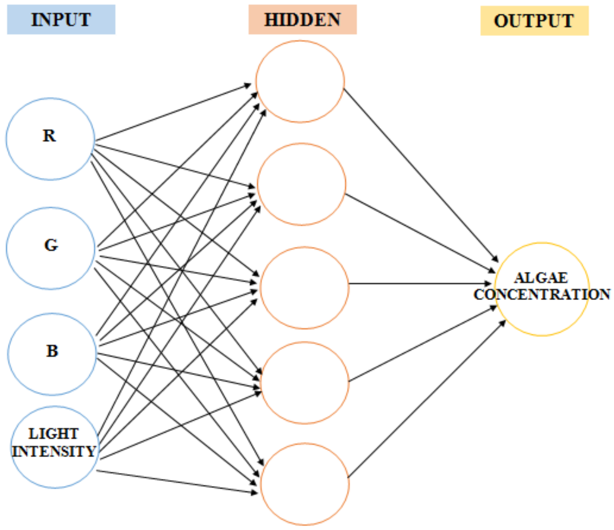

Fig. 6 ANN Structure

Figure 6 shows the diagram of the artificial neural network. RGB values extracted from the images of the system together with the lux values from the photodetector will be used as training data for the neural network. The neural network will be trained to correlate the training data to the algal concentration of the system. MATLAB will be used to structure the hidden values of the network in order to back propagate the algal concentrations given any reading of RGB and lux value to be used in optimizing the yield of the system.

The researcher uses MATLAB's artificial neural network in training and correlating the RGB and lux value of the culture in order to know the cell density of the current culture. From the 300 sampling data gathered by the researchers, 200 were used as training data for the ANN and 100 data were used as validation data.

IV. Results and Discussion

A. *Neural Network Training Regression*

As it is shown in Table 1, the researchers tested varying hidden node sizes to determine the optimal size fit to use for the neural network. Optimal parameters are defined as being of lower node size with low mean squared error (MSE) and a value of R close to 1. Based on the results, node 24 shows the based performance based on its MSE, regression and the number of nodes.

Table 2 Node Size vs. Network Training Parameters

Hidden Node Size	Mean Squared Error	R	Learning Time (Iterations)	Seconds
4	0.0068107	0.91676	25	1
6	0.0050823	0.90865	26	1
8	0.0037668	0.92529	31	1
10	0.011434	0.89141	24	0
12	0.0077775	0.93968	40	1
14	0.0069816	0.92602	29	1
16	0.0061991	0.90889	25	0
18	0.010369	0.92307	27	1
20	0.0050472	0.90943	24	1
22	0.011854	0.9574	52	4
24	0.0047813	0.96345	30	2
26	0.0031728	0.90595	20	1
28	0.0075295	0.93718	29	2
30	0.0082668	0.95431	33	2
35	0.010404	0.92436	24	3
40	0.010145	0.93222	23	2
45	0.014603	0.90134	27	3
50	0.0097202	0.91859	26	3
55	0.0062095	0.90228	24	3
60	0.0076395	0.89959	25	10
65	0.002504	0.85517	20	3
70	0.006437	0.94723	25	4
80	0.011687	0.89415	25	5
90	0.010562	0.94003	25	48
100	0.006051	0.93378	25	7
120	0.011805	0.93599	22	8
140	0.0090892	0.85764	30	15
160	0.0041934	0.93266	20	80
180	0.0048652	0.94616	24	17

B. Neural Network Training Regression

Figure 7 shows the regression plot of the network with a hidden node size of 24. It can be observed that most of the points corresponding to the data are aligned to the curve which suggests that the neural network successfully modeled most of the training data.

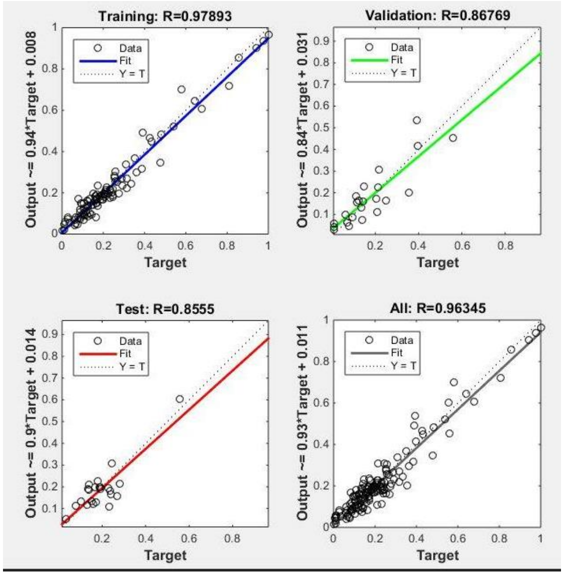

Fig. 7 Regression plots for Training, Validation and Test data.

C. Neural Network Training Regression

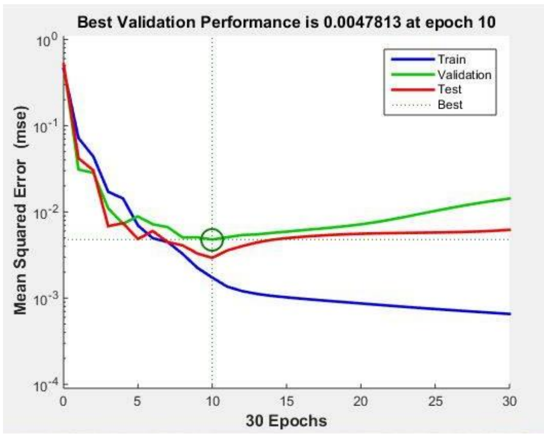

Fig. 8 Neural Network Training Performance plot.

Figure 8 shows the performance of the network during training with a hidden node size of 24. The plot shows how the network performed until it reached its minimum performance gradient as the network tries to update its node values. It can be observed that best validation performance occurred at its 10th adjustment.

D. ANN Validation through F-Test

Table 3 F-Test for Validation Data

Sample	R	G	B	Lux	From CC	From ANN
1	129.65	167.18	149.69	47	191.282	362.002
2	116.47	172.89	150.33	47	186.53	606.439
3	113.1	157.41	138.13	43	390.881	502.828
4	114.01	165.98	141.95	40	307.715	609.399
5	115.59	155.7	137.55	51	408.702	487.181
6	124.04	172.95	150.57	65	293.458	560.343
7	124.68	173.78	151.06	72	205.539	604.747
8	123.05	172.3	150.46	78	203.757	640.271
9	125.06	175.41	150.6	85	686.121	706.243
10	128.26	179.9	154.34	78	330.289	690.173
11	122.35	173.22	147.17	73	365.931	819.58
12	125.66	177.34	150.94	69	270.884	723.582
13	122.78	173.95	146.87	69	509.69	820.003
14	139.52	194.97	163.88	61	558.401	526.933
15	140.87	196.49	164.95	55	1036.61	422.477
16	123.62	175.61	146.03	46	787.702	636.465
17	130.32	186.15	154.37	41	839.978	565.84
18	134.65	192.3	157.06	40	1049.08	536.237
19	114.1	163.4	133.07	38	854.235	755.299
20	117	163.93	136.28	37	1214.82	576.413
21	114.62	167.2	135	45	850.671	1028.07
22	132.57	187.8	154.15	40	1471.45	546.81
23	121.39	172.85	139.41	42	1394.22	752.762
24	135.07	193.4	155.04	46	1861.73	725.274
25	124.22	187.96	143.65	37	2126.68	1681.87

The output of the created neural network and the manual cell count is tabulated side by side as shown in Table 3 and have undergone F-test to prove whether the two sets of data are related and originated from the same data set. From the result of this statistical test tabulated at Table 4, it shows that the F is less than the F critical and thus the null hypothesis that the two variances between the two populations are equal is accepted. It can therefore be concluded that the two sets of data are related and originated from the same data set and that the output of the network based on the RGB and lux values of the culture are valid.

Table 4 F-Test for Data Validation

F-Test Two-Sample for Variances

	From CC	*From ANN*
Mean	0.46422297	0.440098
Variance	0.07863828	0.0645553
Observations	100	100
df	99	99
F	1.21815301	
P(F<=f) one-tail	0.16392183	
F Critical one-tail	1.39406126	

V. Conclusion

Based on the experimentation and results conducted by the researchers, the data shows that the neural network develop were able to achieved a fewer number of nodes with a faster learning time of 2 seconds and a low Mean Squared Error of 0.0047813 using 24 nodes. As compared with node 65 that has an MSE of 0.002504 and regression of 0.85517, node 24 is still better since its regression is much closer to 1 and the network is much simple since the number of nodes are lesser. This results denotes that it is possible to model fewer variable even without bearing too much error. In addition, the researchers were able to successfully model the neural network for the cell density measurement of Spirulina for closed algal culturing system upon validation.

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