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QUANTIFICATION OF CHLORELLA - ALGAE BY IMAGE ANALYSIS

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ABSTRACT

In order to raise the production it is necessary to monitor the growth of the algae periodically. The main aim of this work is to develop an automated tool that provides reliable and accurate results for counting the chlorella algae cells from the images taken from chlorella ponds. Recent developments in computer image analysis techniques allow more accurate quantification of algae growth in ponds through images which are taken by microscopic enabled mobile phones. In this paper, we propose a technique for quantifying the chlorella algae to increase the production using Image J software, which is freely available over the internet. This software includes the methods for calibrating measurements, scaling the images, assessment of the size of the object and its thickness, cell counting and analysis of area. The counting has been done on several microscopic images and finally discussion has been made by comparing the results achieved by the proposed method and the conventional manual counting method. The proposed method is very simple and easy one.

KEYWORDS: **Chlorella, image processing, Image J, Image analysis and automatic counting**

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INTRODUCTION

The need to fulfill the ever increasing global energy demand caused the intensive use of fossil fuels like coal, petroleum and natural gas during the last century and nowadays they represent more than the 80% of the energetic resources. *Chlorella vulgaris* has a great potential as a resource for biodiesel production due to faster growth and easier cultivation.[1] Moreover unlike traditional oilseed crops, microalgae cultures do not need of herbicides or pesticides and can be performed in ponds or photo bioreactors on unsuitable for agricultural purposes, minimizing damages caused to eco and food-chain systems and without compromising the production of food, fodder and other products derived from crops.[2] Actually, one of the most promising feedstock for biodiesel production is unicellular algae.[3,4] A widely used technique to measure the growth of chlorella is manually counting algae cells using microscope. Accurate quantification of this chlorella is critical in growth measurement of algae. Due to the complex nature of cell, it remains a difficult task to segment it from the background and count automatically.[5,6,7] A semi-automated algorithm was developed which enabled automated identification of apoptosing Retinal Ganglion Cells (RGCs) labeled with fluorescent Annexin-5 on DARC (Digital Angiography Reading Center) images.[8] Counting cells is a crucial procedure not only in evaluating the success of a treatment and also in microscopic images.[9,10] Watcharin et al. proposed an algorithm to count blood cells in urine sediment using ANN (Artificial Neural Network) and hough transform.[11] J.G.A. Barbedo presented a method for counting of microorganisms that use a series of morphological operations to create a representation in which objects of interest are easily isolated and counted.[12] Circular Hough Transform based approaches for counting and splitting are discussed.[13,14] Despite various techniques are used for analyzing the microscopic images, many researchers are still counting the cells manually since the existing system requires specific knowledge.[15] The old conventional counting method under microscope gives an unreliable and inaccurate result depends on clinical laboratory technician skill. Consequently, there is a need for sophisticated, reliable and easy to use technique to improve the productivity of algae and getting export's opinion easily. In this paper, we describe a simple and useful technique for assessing the production of chlorella with the help of image analysis software – ImageJ freely available on internet.

(i) What is Chlorella?

Chlorella is a single cell fresh water green algae probably one of the first organisms seen on the earth. It is an edible form of algae and it consists of green plant pigments, chlorophyll, vitamins, minerals, and protein, fiber and omega fatty acids. Due to its high nutritional profile, it is also called as super food. Generally the size of the chlorella cells are 10-30 microns. Now a day, Chlorella turns the attention of industrialists due to its photosynthetic process. ie. Converting the polluted water into irrigation water and converting Carbon di oxide into fresh oxygen which is the most important one to safeguard our future generation. Research is also going on for preparing bio fuel and bio feed from its fat content.

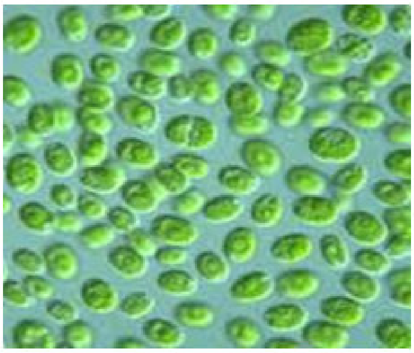
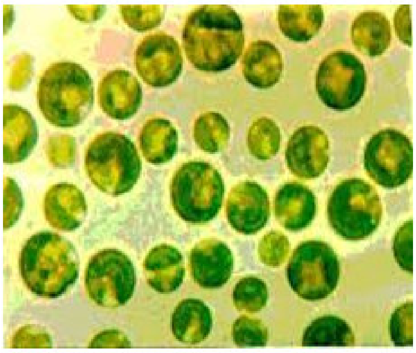



Figure 1
Sample microscopic view of Chlorella images

(ii) Production System of chlorella

Due to the significance of chlorella for producing food, bio fuel, medicine and cleaning the environment, its production techniques are very important. There are two major types of commercial production systems are used for chlorella mass cultivation. They are
1. Open pond system and
2. Photo bio reactor.
Open pond system is highly successful one and an open pond can be constructed with different sizes like 250 m^2, 500 m^2 and 1000 m^2. But the optimum pond size is 10 X 50 m (500 m^2) and height 45cm. As per worldwide standard pond size is 1:5 ratio length and width. Pond can be made with cement pond or PVC pond liners. PVC pond liners are cheaper and faster than cement tanks. However, due to the high equipment costs bioreactor tanks are not widely used. Once the algal cultures have reached sufficient density, the cells can be harvested.

Figure 2
Sample Chlorella pond images

(iii) ***Benefits of Chlorella***

Some of the benefits of clorella are listed below:

- It contains Lutein which is essential for normal vision.
- It protects skin against damage due to sun light.
- It reduces diabetes, skin cancer, and sunburn and artery diseases.
- It has anti-inflammatory and anti-cancer properties.

(iv) ***Monitoring***

It is necessary to produce Chlorella at mass level for identifying and eradicating the contamination at initial stage.

MATERIALS AND METHODS

The following materials can be used to design an automatic tool for counting the chlorella.

(i) Mobile microscopy and image capturing

Microscopic enabled mobile phones are available in the market. The cost of the microscopic enabled mobile phones has fallen in recent years. Making use of those mobile phones chlorella pond images are captured easily. Here the digital images are available in the mobile phone. We can send those mobile images to the system for further processing through Bluetooth, whatapp, email and so on.

(ii) Image requirements (Requirements for Image analysis)

A standand desktop or laptop and a monitor with a graphics card provide sufficient tools to perform image analysis.

(iii) Image J software

There are various image analysis tools and packages available in the market. These programs have many interfaces and uses in a particular situation, cost also can be extremely high and there may be a chance of license restrictions to use the package. Image J is written in java it can be freely downloaded from the internet. This was developed by Wayne Rasband. Image J is a freeware .We can download it from the internet. Once it is installed a rectangular box is displayed on the system containing the menu and tools like the Fig 3. The menu bar contains File, Edit, Image, Process, Analyze, Plugins, Window and Help.

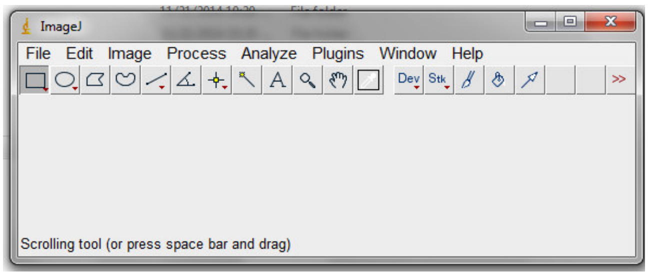


Figure 3
Image J front screen

ImageJ deals with many types of image data. Some formats are opened without the need of third party plug-ins like TIFF (Tagged Image File Format), GIF, JPEG, PNG), DICOM (Digital Imaging and Communications in Medicine), BMP and PGM.[16,17]

SYSTEM ARCHITECTURE

The overall system design is given in the Fig.4. Using the microscopic enabled mobile phones images are taken from chlorella ponds. The real world images have some unwanted information by default. We have to apply some preprocessing steps to remove the unwanted information from the images and then the images are converted into binary form.

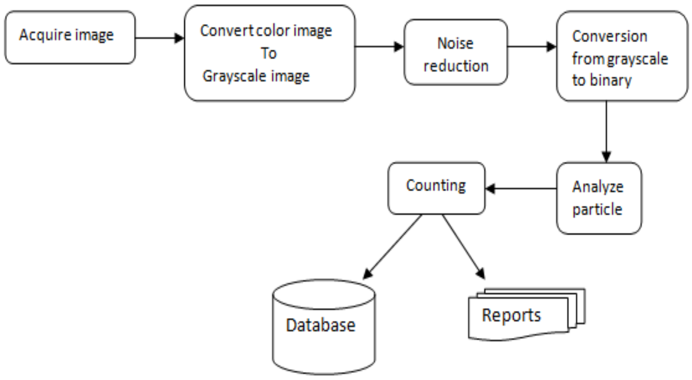


Figure 4
Overall System Architecture

PROCEDURE OF THE PROPOSED SYSTEM

The front page of ImageJ will look like in Fig 2 when the exe file is executed. The following steps were developed and optimized based on the microscopic images obtained from several types of chlorella ponds. The entire counting process is done as follows:

Step 1: Some sample images are chosen from the acquired image set.

Step 2: The selected images are converted into gray scale image which gives more clarity for counting the algae cells.

Step 3: Gray scale images are again converted into binary image by using the Make Binary option in the process menu.

Step 4: To count the algae cells choose analyze menu and then select Analyze Particles which is shown in Fig.9.

Step 5: The algae cell particles are analyzed by using the various options like outline option, overlay option and no option. All objects are considered when no option is chosen. When the outline option is selected, the boundaries of the algae cells will be shown. The number will be displayed for each and every algae cell when overlay option is selected.

Step 6: A summary of the algae count is shown in the window like Total area, Average size and mean value.

RESULTS

The sample images are selected for processing by choosing the file option in the main menu which will be displayed in the Fig 4. The selected sample images are shown in Fig.6 (a) and Fig.7 (a). The converted gray scale images are shown in Fig 6(b) and Fig.7(b). . Fig 6(c) and Fig 7(c) depicts the binary images. The Fig.10 depicts the results of analyzed particles with various options.In analyze particle menu the show option has drop down menu. From the drop down list we have to choose any one of the option for analyzing further ie., counting the algae cells. If no option is selected from the dropdown menu, it will consider all objects in the image and will not differentiate the objects from the background. If outline is chosen, for all selected objects it will show only the boundary of the objects. If overlay outline is chosen, it will show the number for each and every object in the image which will be used to count the number of objects in the image. Moreover, we have some check boxes like Display results, summarize the results and clear results for selection in the analyze particles menu. If we want to select display results option, it will show the total number of objects presents in the image and the size of each and every object and their mean values are tabulated as in the Fig.11.The summarize option will display the entire detail about the selected image for processing which is shown in Fig.12. Here the same image is tested two times that is why it shows the same results for two times.Actual size of the algae cells are also calculated in X and Y directions. It is observed that the results obtained by the above steps offer a good conformity with the manual counting method.

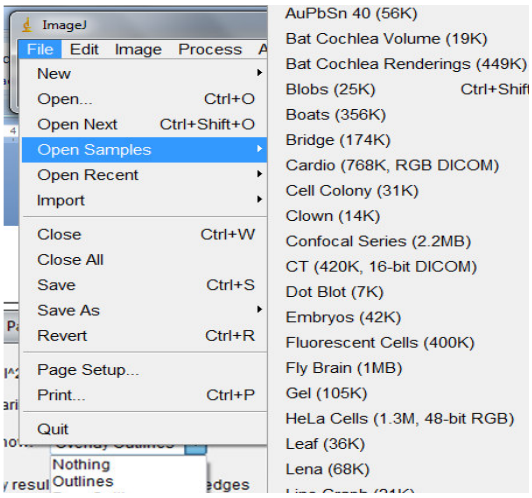


Figure 5
Selection of Sample images

Figure 6
(a) Color image (Sample -1) (b) Gray scale image (c) Binary image

Figure 7
(a) Color image (Sample -1) (b) Gray scale image (c) Binary image

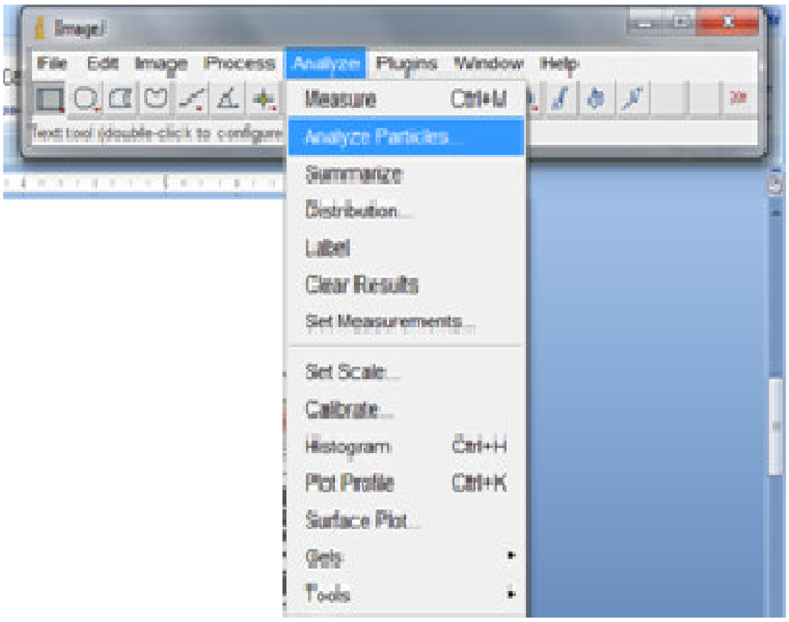


Figure 8
Analyze menu option in imageJ

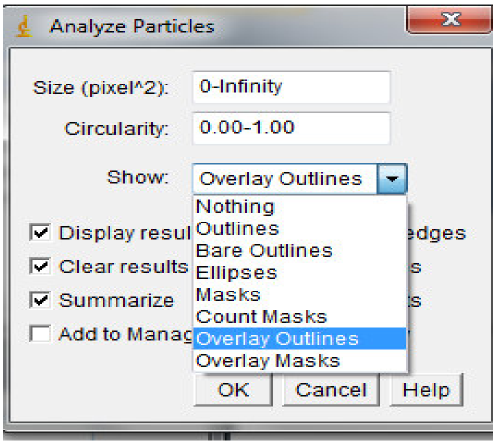


Figure 9
Analyze Particle menu in ImageJ

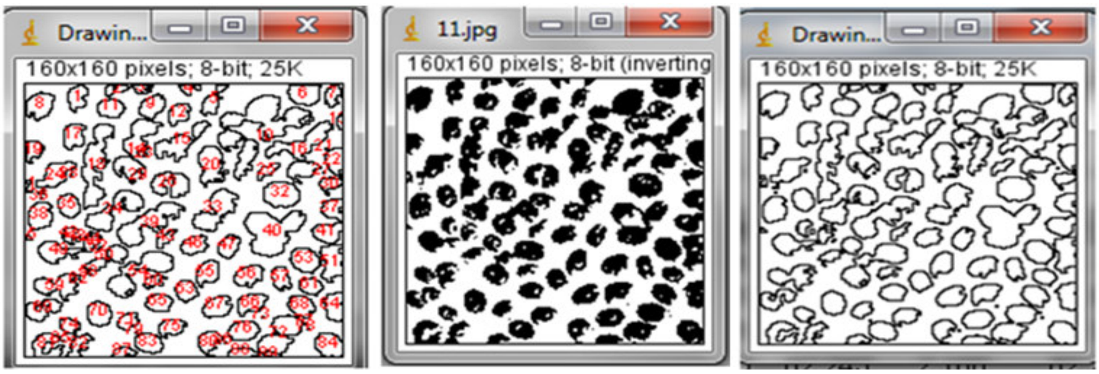
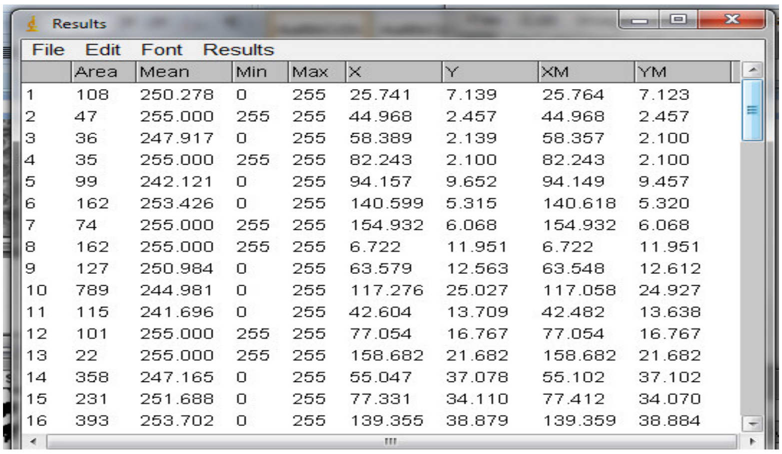


Figure 10
(a)Bared outline (b) nothing (c) Outline

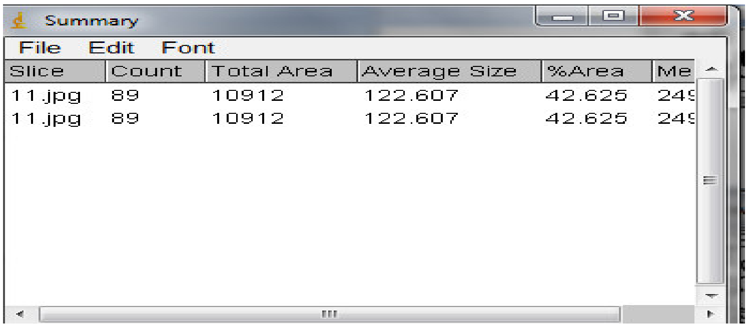


Results

File Edit Font Results

	Area	Mean	Min	Max	X	Y	XM	YM
1	108	250.278	0	255	25.741	7.139	25.764	7.123
2	47	255.000	255	255	44.968	2.457	44.968	2.457
3	36	247.917	0	255	58.389	2.139	58.357	2.100
4	35	255.000	255	255	82.243	2.100	82.243	2.100
5	99	242.121	0	255	94.157	9.652	94.149	9.457
6	162	253.426	0	255	140.599	5.315	140.618	5.320
7	74	255.000	255	255	154.932	6.068	154.932	6.068
8	162	255.000	255	255	6.722	11.951	6.722	11.951
9	127	250.984	0	255	63.579	12.563	63.548	12.612
10	789	244.981	0	255	117.276	25.027	117.058	24.927
11	115	241.696	0	255	42.604	13.709	42.482	13.638
12	101	255.000	255	255	77.054	16.767	77.054	16.767
13	22	255.000	255	255	158.682	21.682	158.682	21.682
14	358	247.165	0	255	55.047	37.078	55.102	37.102
15	231	251.688	0	255	77.331	34.110	77.412	34.070
16	393	253.702	0	255	139.355	38.879	139.359	38.884

Figure 11
Results of each and every object present in the image



Summary

File Edit Font

Slice	Count	Total Area	Average Size	%Area	Me
11.jpg	89	10912	122.607	42.625	24ç
11.jpg	89	10912	122.607	42.625	24ç

Figure 12
Summarize in ImageJ for analyzing the particles

All sample images were tested and algae cells were counted by using this automatic counting and manual counting methods. The comparison results are shown as graph in the Fig.13.

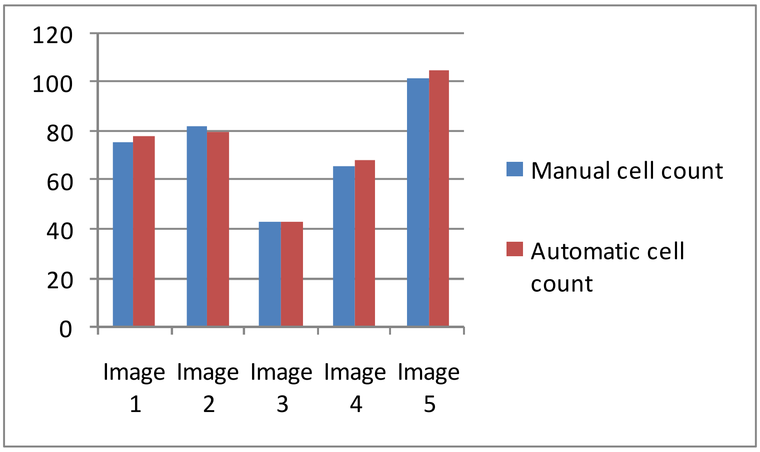


Figure 13
Comparison chart

DISCUSSION

Cell counting has numerous applications in the area of medical image processing.[9,10,11,12] Using the ImageJ software in the proposed system the chlorella algae cells are counted easily. When the cells are counted then the suggestions are given for harvesting to raise the production by the proposed system itself. ie., If the algae cell count is 75 or more than 75 then that is good for harvesting. If the count is less than 75 we can suggest that to wait for two more days so that we can improve the production.The algae growth can be estimated by using the the numerous direct methods like algae biomass, packed cell volume, pigment contents and cell counts.Generally, the cell counting is considered as tedious and laborious work for many samples. The counting of algae cells manually on an image taken from microscope enabled mobile phone by a lab technician is on an average of 3 minutes. By using our automated system for knowing the total count is on an average of 10 to 12 seconds. Hence this technique has the advantages of being fast, accurate and non-labour intensive.This work does not require any specific knowledge of image processing and drastically improve the performance of the system. The automatic cell counting process is an efficient one and provides as correct results. It acts as an efficient tool for counting the algae cells and also a time consuming one. Nonetheless the foreign bodies in algae ponds will adverse the nature of normal algae growth under the scope of this subject that has to be monitored and earlier identification of foreign bodies are the future direction of this work which will definitely improve the production. Sometimes the shape of the algae cells may vary due to damage or the sectioning process.[18] The local contrast variability will reduce the accuracy of automated analysis.[19] In case of poor quality images with various shaped structures yet to be tested. There are no studies we can find which have developed automated techniques for counting of algae cells. This limits the comparability of our automated cell detection method to other methods.

CONCLUSION

This paper presents a methodology for counting of algae cells through the microscopic images using ImageJ. Results indicate that the counting of algae cells offer remarkable accuracy. The installation of this system is very cost-effective and time consuming one. It is also very time effective as manual counting is a very tedious job and time consuming.

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