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WORKS FOR ME 1

Application of PHYTO-PAM-II (Compact Version) For Running Rapid light curves on Cyanobacterial samples

COMMENTS 0

Forked from Application of PHYTO-PAM-II (Compact Version) on Aureococcus anophagefferens cultures for photosynthetic efficiency and quantum yield of PSII

DOI
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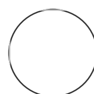
Gwen Stark: Forked protocol from Emily E.Chase Aureococcus PHYTO-PAM II protocol;



Gwen Stark

ABSTRACT

A protocol used to acquire photosynthetic efficiency (F_v/F_m) and quantum yield of photosystem II (Y(II)) of *Microcystis aeruginosa* cultures using far-red acclimation.

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PROTOCOL CITATION

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FORK NOTE

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KEYWORDS

photosynthetic efficiency, quantum yield, Fv/Fm, HAB, chlorophyll fluorescence, Cyanobacteria

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GUIDELINES

The collection of quantum yield and photosynthetic efficiency is highly sensitive to modifications in sampling protocol.

MATERIALS TEXT

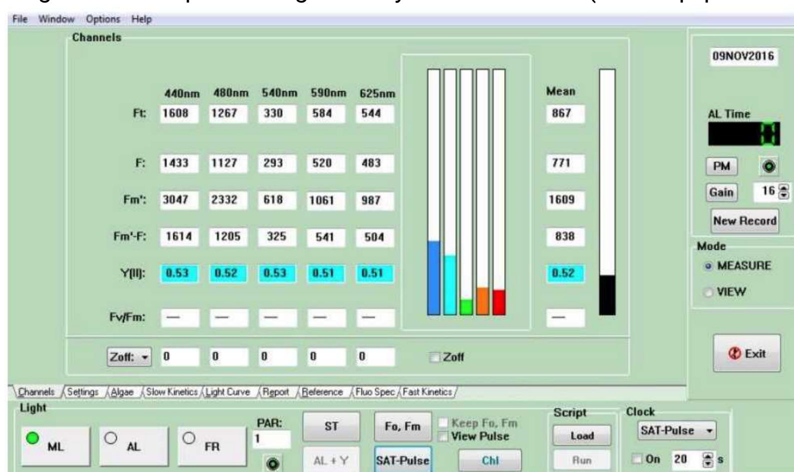
PHYTO-PAM-II Compact Version and components
Laptop with a USB port, and PhytoWin_3 installed
Measurement cuvettes
At least 3 mL of culture material for each sample
70% Ethanol
KimWipes
Necessary materials and setup for both dark adapting cultures, and a no light or low light environment for sampling

BEFORE STARTING

Familiarise yourself with the PHYTO-PAM-II equipment, manufacturer's provided manual, and the basics of chlorophyll fluorescence parameters for full use of data collected and accuracy of results.

EQUIPMENT PREPARATION

1 Start the PhytoPAM-II Compact Version up by toggling the power, and plugging in the charger. Connect the equipment to a laptop or computer by USB. Select the PhytoWin_3 program from the computer, select the appropriate measuring head you are using (Compact Version = "Phyto Compact Unit"). In order to take measurements ensure that the program is in "MEASURE" mode. "VIEW" mode is exclusively for reviewing record files (i.e. data collected). Ensure the "ML" (measuring light) light is selected (signalled by a green light), and that "AL" (actinic light) and "FR" (far red) are not selected. Wait for the light directly under the PAR input to be green before proceeding with any measurements (if the equipment is not ready the light will be red).



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2 Adjust the Phyto-Pam settings (settings tab). Depending on your culture, settings that work for one species may not work for another, and there may be some troubleshooting involved to give you accurate Fv/Fm readings and RLC measurements. The best way to test if your saturation pulse (which you adjust in the settings) is sufficient for your sample is to check the saturation pulse kinetics. You can do this by clicking the "View Pulse" check box. A distinct plateau should be observed from your pulse kinetics if maximum flourescence yield was reached from your applied saturation pulse. Keep all settings consistent when running an experiment. Once you decide what works best for your cultures do not adjust it at any point in the experiment, this alone will change your output data.

It's important to remember for your experimental design that you can not compare Fv/Fm from one species to another, all measurement comparisons should be kept within each species/strain.

SAMPLE PREPARATION

3 For each biological replicate of your cyanobacteria culture, 3mL of culture will need to be transferred into a quartz cuvette that comes with the Phyto-Pam. It is best to leave your cultures in their treatment conditions until you are ready to run your rapid light curves. Minimize exposure time as much as possible while extracting your sample for processing. After transferring culture into quartz cuvette, place the cuvette into the optical port and cover the port with the darkening hood.

4 Before acquiring your data, auto-adjust the gain on each sample run. This is important for your signal-to-noise ratios. On environmental samples, it may be best to run the zero-offset function (Zoff- determination) to blank the Phyto-pam II before your runs. To do this you will want to filter the cells out from your sample.

5 For acquiring F_o and F_m, run a low-light far-red acclimation on your 3mL of prepared culture. Far-red light induces photosystem I, oxidizing the PQ pool in cyanobacteria. An empty PQ pool will "open" up Photosystem II, which should result in maximal (F_m) readings. Dark acclimation is not recommended for cyanobacteria, since it will cause electron flow from respiratory processes to reduce the PQ pool.

There is also evidence to suggest that no acclimation or a low-light acclimation is best for cyanobacteria, but it may be best to test weak far-red acclimation v. no acclimation/low-light to see what gives you the most consistent results with your specific cultures.

MEASUREMENT ACQUISITION

6 Experimental measurements can now be collected.

7 Add 3 mL of the culture to be measured into the cuvette, wipe down the sides with a KimWipe, place into the measuring head and cover with the cap. Auto-adjust gain and run your far-red light acclimation. Turn on the measuring light (ML button). Wait for the indicator lamp to turn green (from red).

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8 You can now proceed with taking Fv/Fm measurements, or running a rapid light curve. If only Fv/Fm is desired, you can click the "Fo, Fm" button. For running a rapid light curve, go to the "light curve tab". You can adjust your PAR and exposure time for each rapid light curve step by clicking "edit" and filling in the steps appropriately. To run the light curve, hit "start". Ideally, you will want your ETR curve to hit a maximum peak and level off, or start to drop (indicative of photoinhibition) once higher PAR values are reached. If you are not generating typical curves in your ETR window, you will have to trouble shoot and adjust your settings or your light curve steps, make sure your saturation pulse is appropriate for your culture as well.

9 Measurements have now been automatically recorded in the "Channels" and "Algae" tab. Check relevant wavelengths and/or "Algae" settings for comparing samples/treatments. Ethanol (70%) should be used to clean the cuvette between different treatments.

For Microcystis we have recorded the "blue" algae results on the "Algae" tab.

10 Acquire data for all samples, record, and shut down the program, toggle the machine off, and unplug the charger and USB connection. Data can be exported to USB/ hard-drive as a .csv file by going into "view mode".

It is recommended to store the equipment for prolonged periods (long term) with a full charge. If the equipment is sitting unused for months it is advisable to charge it to full capacity on occasion to prolong the life of the battery.

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