Procedure for Measuring Phytoplankton Pigment, Quantum Yield, and Excitation Characteristics using Phyto-PAM
Florida Coastal Everglades LTER

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Equipment:

- Phyto-PAM Phyto-PAM Fluorometer Analyzer (Walz, Germany)
- Phyto-ED
- Phyto-Win Software V 1.45

Supplies:

- quartz cuvettes
- 3 ml sterile syringes
- Nylon sterile filters (25-mm diameter, 0.2-µm pore size)
- Kimwipes

1. Sample collection

1. Water samples are collected in clean, sample-rinsed dark polypropylene bottles filled by hand.
2. Samples are kept at ambient temperature in coolers without ice during the sampling day.
3. Samples are transported to the laboratory and analyzed the same day.

2. Setup

1. Turn on computer and Phyto-PAM machine.
2. Turn off the Emitter-Detector Unit (ED).
3. Launch *PhytoWin* sofware program.
4. Check the *Fluorescence* values (data row F and *Channels* page). Values should be zero when the ED unit is off. A negligible reading of ± 8 is acceptable.
5. Click Report tab to bring up report page. Enter sample run information including date, run name and number, and collection info. Enter the Sample ID before running each sample.
6. Click Light Curve tab and turn on Blue, Green, and Brown in the *Select* box.

3. Sample Analysis

1. Clean cuvette with deionized water and ethanol and dry completely, use Kimwipes to handle and clean the cuvette.
2. Transfer 3 ml of sample into the cuvette and place into ED unit. Keep ED unit cover on whenever possible. When removing the cover, be sure the ED unit is turned off.
3. Turn on the ED unit.
4. From the *Channels* page, press the *Gain* button to run automatic gain adjustment. It often takes 2 or 3 times to settle on a proper gain. Keep pressing *Gain* until the same reading comes up for a few consecutive times.
5. Turn off ED unit.
6. Remove cuvette, discard sample, and clean with deionized water.
7. Filter about 3 ml of sample throught a 0.2 μm filter into clean cuvette.
8. Place cuvette with filtrate into ED unit and turn it on, wait for *Green Light* at the bottom of the screen to come on, stable data measurement.
9. Click the *Zoff* button to set an automatic baseline adjustment for the sample.
10. Turn off ED unit.
11. Remove cuvette and discard filtrate.
12. Transfer 3 ml of sample (unfiltrate) into the cuvette.
13. Place in ED unit and turn it on. Wait for *Green Light*.
14. Click *Start One* button and wait for measurement. Wait for *Green Light*.
15. Click *Chl(Fo)* button and wait for measurement. Wait for *Green Light*.
16. Go to *Light Curve* page by clicking the tab. When light at bottom of page is green, click *Light Curve* button to initiate light curve. When curve is finished, click *Fit* button.
17. Go to *Options* Menu at top of page, and select *Light Curve Fit Parameters*.
18. Copy the data to a Pam Data Sheet.
19. Go to the *File* Menu and Save the report in the appropriate folder.
20. Return to the *Channels* page, click *New Record* button and turn off the *Zoff*.