When comparing phytoplankton physiological parameters researchers must be cognizant of the fact that active fluorometers utilize varying flash protocols to determine parameters such as minimum fluorescence (Fo), maximum fluorescence (Fm) and quantum efficiency (yield). Absolute values of these parameters can not be directly compared between instruments that utilize different flash protocols (multiple versus single turnover systems), but rather trends of yields, which is represented as a ratio (Fv/Fm), can be compared. This paper describes the multiple turnover protocol the Turner Designs' PhytoFlash utilizes and comparisons to the gold standard DCMU herbicidal method and instruments utilizing single turnover systems.

Currently several instruments are available that will determine photosynthetic mechanisms utilizing either multiple turnover (MT) or single turnover (ST) techniques. Both techniques provide an accurate assessment of the quantum efficiency of phytoplanktonic cells. Please see Turner Designs' application notes for a full description of active fluorescence (http://www.turnerdesigns.com/t2/doc/appnotes/S-0071.pdf).

DCMU (herbicidal electron inhibitor) Technique and PhytoFlash Comparison

Over 30 years ago researchers showed that the electron-inhibitor, DCMU (3-(3,4-dichlorophenly)-1,1dimethylurea), could be used to achieve maximum fluorescence (Fm) determined through standard fluorometry. The DCMU technique has been widely accepted and used as a proxy for photosynthetic health of phytoplankton. Since then instrument development has increased the use of variable fluorescence as a research tool, however this technique is still used as the 'gold standard' for variable fluorescence.

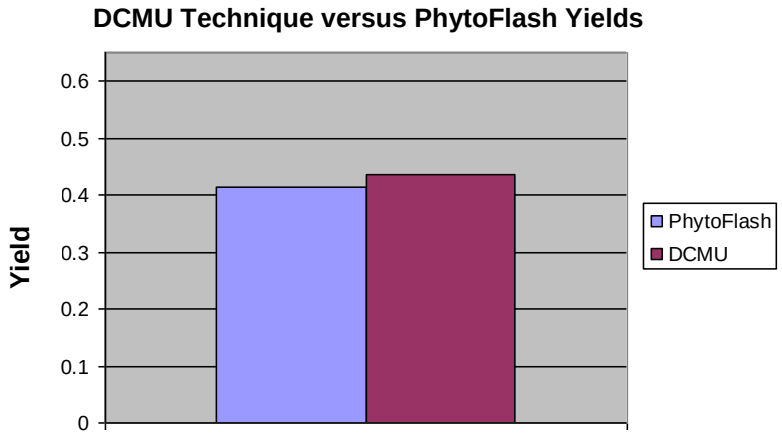

Figure 1. Yield comparisons of the diatom *Thalassiosira weissflogii* utilizing the fluorometric DCMU herbicide technique and the Turner Designs' PhytoFlash Active Fluorometer (n=4).

Multiple Turnover System (MT)

Minimum fluorescence (Fo) is determined through standard fluorometric techniques. The active fluorometer will excite algae at or near 460nm, cells in turn will emit light at 685nm. Photosynthesis is not enhanced during this process. After Fo is determined MT systems utilize a long single saturating flash (200-10,000ms) to achieve Fm (maximum fluorescence). During the saturation the MT system allows repeated charge separation processes until the electron acceptors of PSII are reduced or have stopped completely. The MT system determines variable fluorescence (Fv or Fm-Fo) and the yield of phytoplankton can be calculated (Fv/Fm).

The Turner Designs' PhytoFlash and the Heinz Walz's Pulse Amplitude Modulated Active fluorometer utilize similar flash protocols under a multiple turnover system. Data below shows a comparison of two multiple turnover systems utilizing varying concentrations of algae.

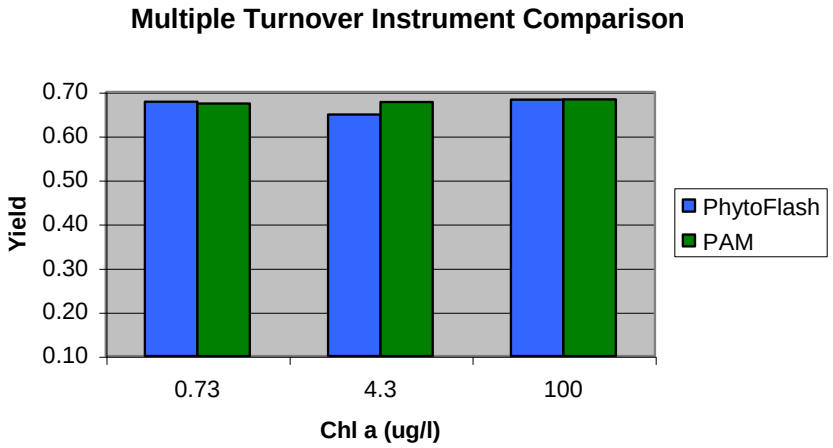

Figure 2. Comparison of Turner Designs' PhytoFlash submersible active fluorometer (MT) and the benchtop PAM Walz active fluorometer (MT) using green algae (*Dunaliella*) cultures at different concentrations.

Single Turnover System (ST)

Minimum fluorescence (Fo) is achieved in the same manner as MT systems. ST systems vary from MT systems in that they utilize multiple short flashlets (10-100μs) leading up to Fm (maximum fluorescence). The ST system only allows one charge separation during the flash and reduces only the primary acceptor of PSII. The Fm, determined by a ST system, may be smaller leading to a lower yield. The ST system determines variable fluorescence (Fv or Fm-Fo) and the yield (quantum efficiency) of phytoplankton can be calculated (Fv/Fm).

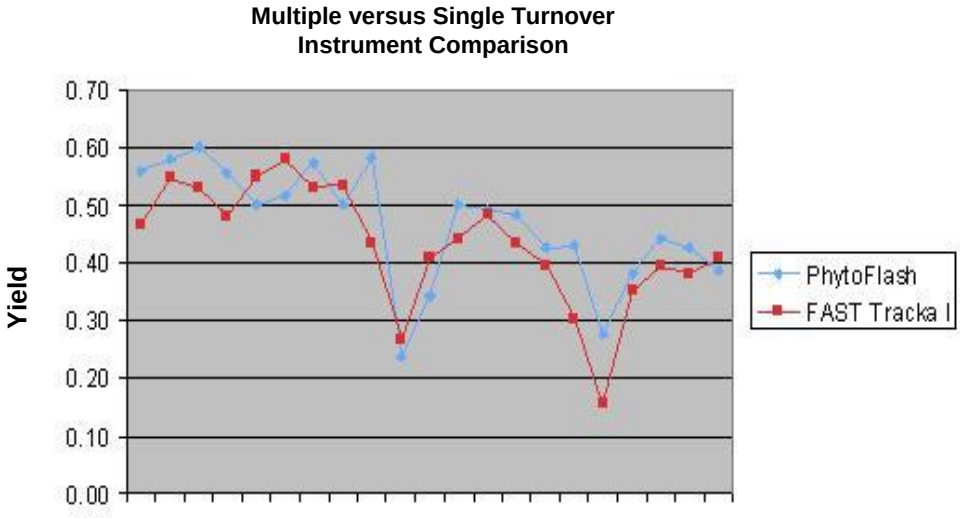

Figure 3. Comparison of Turner Designs' PhytoFlash and Chelsea's Fast Tracka I during combined profiles in Monterey Bay. The PhytoFlash was deployed with a shade cap.

Operation of Active Fluorometers

It is important that the user operate active fluorometers under suggested methods in order to obtain accurate photosynthetic parameters. Data can be misinterpreted if samples are evaluated under varying light regimes or instrument-blanking protocols differ. The following provides general guidelines for dark or light adaption and blanking procedures. Other procedures are also accepted by the scientific community and are included in the reference section.

♦ Blanking

It is important for the researcher to determine the appropriate blank when calibrating an active fluorometer depending on study site. Blanks are generally filtered seawater, artificial seawater, or deoinized water. If colored water is highly variable at the study site users can zero the PhytoFlash and post-calculate yields after blanks are determined back in the laboratory. Blanking becomes increasingly important when working in waters with low chlorophyll *a*. Yield measurements may be near or at the minimum detection limit of the instrument therefore an error in blanking will have a great effect on the final calculation.

♦ Dark Adaption

To obtain pure unbiased irradiance photosynthetic parameters it is suggested to collect fluorescence data, including active fluorescence, in the evening or during dark conditions. At this point cells are dark adapted so that reaction centers of the PSII are open. When reaction centers are open, and at maximal state, Fo is low. The optimal duration of dark-adapted samples varies depending on location and/or researcher. Some researchers dark adapt samples between 1 and 2 minutes while others dark adapt no less than 15-30 minutes. It is the researcher's responsibility to determine the appropriate time necessary for dark adaption. The PhytoFlash is equipped with a dark flow cap (http://www.turnerdesigns.com/t2/instruments/phytoflash.html#accessories) designed to allow users to manipulate various sample speeds necessary for dark adaption.

♦ Light Acclimation

Light history of the cell can have an affect on active fluorescence parameters. Light acclimated yields integrate all processes downstream of PSII as well as the degree of PSII closure. In the light adapted state photosynthesis light curves (with the addition of a light meter) and *in situ* maximum PSII efficiency can be determined to yield useful information about recent light stress. The PhytoFlash is equipped with a shade cap (http://www.turnerdesigns.com/t2/instruments/phytoflash.html#accessories) that allows for continuous flow of light adapted samples over the sensor while shielding the optics from ambient light.

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