Background

Active fluorescence methods were developed over 30 years ago to monitor and assess mechanisms of photosynthesis in phytoplankton and higher plants. Active fluorescence methods utilize the relationship between chlorophyll fluorescence and photosynthesis. There are currently instruments on the market that provide active fluorescence values. However, Turner Designs has developed the first solid-state (patent pending) submersible active fluorometer. Users can now access invaluable physiological data real-time at an affordable price.

Applications

Chlorophyll fluorescence is often reduced in algae experiencing adverse conditions such as stressful temperature, nutrient deficiency, and polluting agents. Phytoplankton photosynthetic efficiency is one of the biological signals that rapidly reacts to changes in nutrient availability as well as naturally occurring or anthropogenically introduced toxins (contaminants). The results can be used as an indicator of system wide change or health.
Common applications are:

- Indicator of nutrient status
- Precursor for the onset of Harmful Algae Blooms
- Oceanographic, estuarine, limnological and riverine studies
- Indicator of ballast waters
- Indicator of toxins (contaminants) in the water column

Physiology

Active fluorescence estimates the photochemical efficiency of Photosystem II (PSII) from ratios of fluorescence levels. The PSII system is the membrane protein complex found in oxygenic photosynthetic organisms, which, together with other pigments, harvest light energy to split H_2O into O_2. PSII is comprised of many polypeptides surrounded by a variety of chlorophyll a and b binding proteins, known as light harvesting complexes that funnel light into the PSII. Photosynthesis requires energy transducing protein complexes like PSII that convert sunlight into energy by pumping protons across a gradient, leading to the formation of ATP and NADPH. The following diagrams describe electron flow within reaction centers of the PSII system.

Minimum fluorescence (Fo)

During 'normal' photosynthesis, reaction centers in the PSII are open and there is a free flow of electrons. Each photon of light absorbed by a chlorophyll molecule raises an electron from the ground state to an excited state. During this process the reaction centers of the PSII system are in the open state (figure 1), because electrons are transferred through PSII fast enough to allow more electrons to be donated. The chlorophyll a molecule (P680) acquires excitation energy from electron donors. The P680 then transfers an electron to a primary electron acceptor or plastoquinone (Qa) and the reaction center is now oxidized. There is a constant flow of electrons and minimum fluorescence (**Fo**) is determined. In this state, the probability of fluorescence is very low, while the probability of electrons being used for photochemical conversion (passed to Qa) is very high. During this process fluorometers are able to obtain Fo readings without increasing fluorescence.

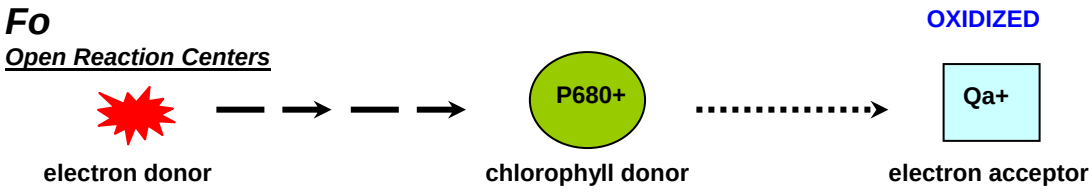

Figure 1. Diagram depicting open reaction centers of the PSII system.

Maximum fluorescence (Fm)

At this step instruments are able to manipulate light, inducing maximum fluorescence. A flash of saturating 'actinic' light is sent to the sample cell. The oxidized reaction center (figure 1) will become completely reduced by increasing the transfer of electrons (more light). The reaction centers are now closed and the electron acceptor (Qa) is completely reduced (figure 2). The flow of electrons is impeded because the Qa pool cannot transfer electrons fast enough to keep up with the influx of photons (from the actinic flash) and the maximum quantum efficiency of PSII (**Fm**) is determined. In this state, the probability of electrons being given off as fluorescence is maximal.

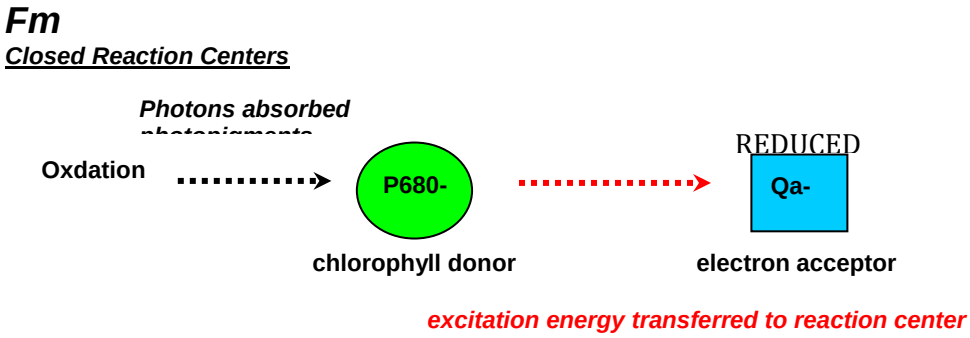

Figure 2. Diagram depicting closed reaction centers of the PSII system.

Variable fluorescence (Fv) and yield

The difference between fluorescence intensities with closed and open reaction centers is known as **variable chlorophyll fluorescence** (**Fv = Fm-Fo**). This corresponds to the part of the absorbed light energy that would be used in photosynthesis if all reaction centers were in the open state. Measuring the fluorescence intensities of Fo and Fm in relative units enables the user to estimate values of the quantum efficiency of the primary photochemical reaction of photosynthesis referred to as the yield (**Fv/Fm**). Changes in the efficiency of electron transfer caused by stress result in a corresponding change in either Fo or Fm (or both), such that Fv/Fm provides a sensitive indicator of cell "health".

PhytoFlash Operation

The PhytoFlash Submersible Active Fluorometer is designed to determine the photosynthetic capacity (yield) of PSII. Nine light emitting diodes (LEDs), at a 465nm wavelength peak, are arranged in a circular array that evenly saturates the sample in the optical cell. Initially three monitoring LED's are activated and minimum fluorescence (**Fo**) or *in vivo* fluorescence is determined (figure 3).

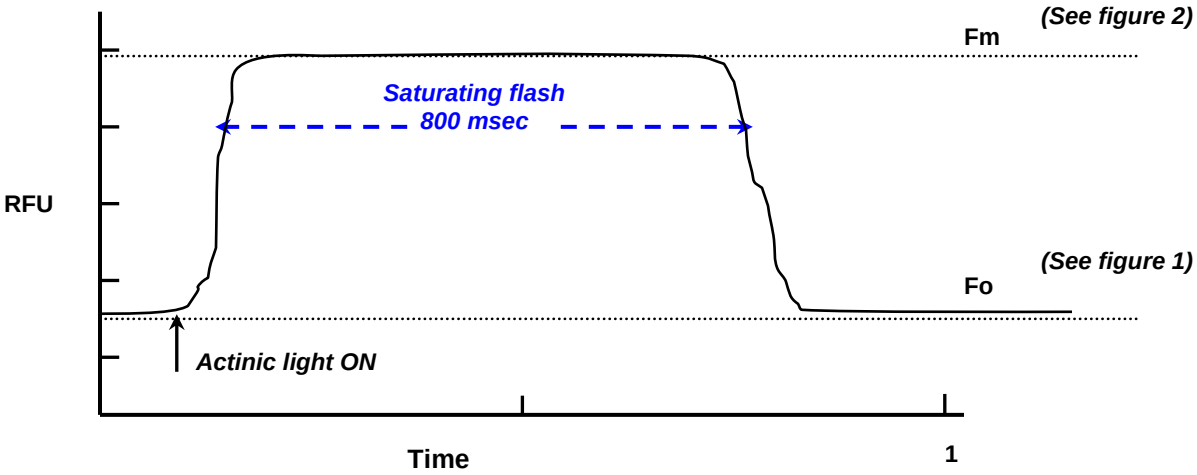

Figure 3. A response curve generated by the PhytoFlash.

At this point six high intensity LED's are activated, actinic light is introduced, and maximum fluorescence (**Fm**) is determined. The duration of the saturating light can be manipulated by the user to last 200-10,000 ms.

Physiological Parameters determined on the PhytoFlash:

Fo	Minimum fluorescence
Fm	Maximum fluorescence
(Fm-Fo)/Fm	Maximum quantum yield of photochemistry in PSII
Blank	Calculated blank value used in calibration

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