Application Note: In Vivo Chlorophyll Concepts and Concerns

In vivo chlorophyll analysis is the measurement of chlorophyll fluorescence within a living cell. The advantage of this type of analysis is that it is quick and simple and does not require special sample preparation or extraction. It allows the user to measure 100's to 1000's of samples a day. However, without comparisons to extractive analysis, *in vivo* readings are qualitative in nature.

Research purposes include:

- Vertical and horizontal profiling of algal biomass
- Algal management for:
    - Drinking water facilities
    - Fisheries and Aquaculture
- Environmental impact and long term monitoring programs
- Mapping and tracking algal blooms
- Mixing and upwelling in natural waters

For questions such as:

- How are the algal populations distributed in the water column?
- Is there an algal bloom developing? Where is the bloom?
- Is the algal population increasing or decreasing over time?
- What are the effects of pollution on a natural event on the ecosystem?

Variation:

The following biological and environmental issues affect *in vivo* results:

1. **Health of the organism**
   Healthy phytoplankton will produce a LOWER fluorescence response per unit chlorophyll than senescent "dying" phytoplankton cells.

2. **Light history of the organism**
   Phytoplankton cells adapted to bright light conditions will produce a LOWER fluorescence response per unit chlorophyll than dark-adapted phytoplankton.

3. **Morphology of the cell**
   Composition and shape of the cell and surrounding cellular material can interfere with the fluorescent signal.

4. **Light adaptation of the cell**
   The amount of chlorophyll per cell can vary due to its light availability.

5. **Turbidity**
   Can cause scattering or shading effects depending upon the chemical composition of the turbidity.

Basic Calibration Concepts:

1. Collect a representative water sample from the body of water to be studied.

2. Blank the instrument. Ideally, a blank sample should represent the constituents of the body of water being studied excluding the phytoplankton. This can be accomplished by filtering a sample of water through a 0.45 um filter to remove the phytoplankton. Deionized water or artificial seawater can be used as substitutes if filtered sample water is not available.

3. Calibrate your fluorometer with the sample collected from step 1, assigning it a relative value. Example: 50 RFU (relative fluorescence units).

4. Read all other samples for relative increases or decreases in fluorescence (RFU's). To minimize erroneous readings caused by changes in algal pigments due to light availability, taking sample readings at approximately the same time each day is recommended, preferably at dawn.

Quantitative Analysis:

Using periodic extractive chlorophyll a analysis in conjunction with *in vivo* analysis can provide more quantitative results when relative information is not enough.

1. Calibrate the instrument as discussed above.

2. Proceed with the study, collecting periodic *in vivo* samples for extractive analysis. We suggest collecting samples anytime there are suspected or expected changes in the environment or water quality. You may find you will have different, *in vivo* chlorophyll to extracted chlorophyll, correlations for different field stations or for different times of the year.

For best results, keep the collected sample cold and in the dark until filtered. Filter the samples as soon as possible (within a couple of hours after collection). If after filtering your sample the filter is not immediately extracted in solvent, make sure to freeze the filter for later extraction to reduce degradation of chlorophyll.

3. At the exact time of sample collection note the *in vivo* chlorophyll fluorescence value of the particular sample and label.

4. In the lab, extract the collected *in vivo* samples as per the EPA 445.0 protocol and analyze your extracts with a calibrated fluorometer or spectrophotometer. Determine the correlation between the extracted concentration and *in vivo* recorded value.

Instruments:

Turner Designs offers a complete line of versatile research instruments and field based monitoring instruments. Our research instruments can be used for both *in vivo* and extracted chlorophyll analysis as well as many other applications. Our monitoring instruments are designed to detect *in vivo* chlorophyll, and in some cases other parameters such as turbidity, depending on the instrument chosen.

Research Instruments include:
10-AU Field Fluorometer
Trilogy Laboratory Fluorometer
*Aqua*fluor Handheld Fluorometer

Monitoring Instruments include:
*Aqua*fluor Handheld Fluorometer
AlgaeWatch/Cyanowatch on-line Fluorometer
C6 Multi-Sensor Platform
Cyclops-7
PhytoFlash

CHLOROPHYLL ANALYSIS:

Instrument	In Vivo	Extracted
Turner 10-AU	**YES**	**YES**
Trilogy	**YES**	**YES**
Aquafluor HandHeld	**YES**	**YES**
Cyclops-7	**YES**	**NO**

AlgaeWatch/Cyanowatch	**YES**	**NO**
PhytoFlash	**YES**	**NO**
C6 Multi-Sensor Platform	**YES**	**NO**

Standards:

Turner Designs also offers primary and secondary calibration standards for chlorophyll analysis. Our primary standards are pure chlorophyll *a* in 90% acetone. The concentration has already 950).
Solid, secondary standards are also available for each instrument. The solid standard is made of a very stable fluorescent material that fluoresces at the same wavelengths as chlorophyll *a.* The standards can be used to quickly and easily check for instrument drift and to re-calibrate after an initial calibration with a primary been determined and each standard comes with quality control data. We have primary standards that are available for fluorometers (P/N: 10-850) and spectrophotometers (P/N: 10-950).

Further information can be found on our website:

EPA link for 445.0 protocol:
http://www.epa.gov/nerlcwww/m445_0.pdf

Effects of turbidity on *in vivo* chlorophyll fluorescence
http://www.turnerdesigns.com/t2/esci/turbidity_effects.html

Primary and Secondary Calibration Standards
http://www.turnerdesigns.com/t2/instruments/standards_main.html

Frequently Asked Questions and Fluorescence Tutorial
http://www.turnerdesigns.com/t2/doc/tutorials/main.html

10-AU In Vivo Calibration Method
http://www.turnerdesigns.com/t2/doc/appnotes/998_0037.html