An Introduction to Measuring Chlorophyll *a* Using the Hydrolab DataSonde 4a with the Turner Designs Fluorescence Sensor

INTRODUCTION

Turner Designs has developed a high performance, compact submersible fluorescence sensor designed specifically for integration into Hydrolab Series 4a multi-parameter instruments. Although small, the sensor does not compromise measurement performance including sensitivity and dynamic range. Also, the excellent turbidity rejection ensures superior detection limits in a wide range of environmental conditions.

Three automatically controlled gain ranges provide a wide measurement dynamic range of 0.03 to 500 µg/L for chlorophyll *a* and 0.04 to 1000 ppb for rhodamine WT. When integrated into Hydrolab systems, the gains are automatically controlled based on signal strength of the sample. Hydrolab will offer the Turner Designs fluorescence sensors for Chlorophyll *a*, Rhodamine WT, and Phycocyanin (available soon) as standard options on Series 4a instruments. Other fluorophores, including Phycoerythrin and the tracer dye Fluorescein will also be available soon as non-standard options with extended lead times.

PERFORMANCE

The challenge for any *in situ* fluorescence sensor is to effectively separate the extremely large ambient light signal from the extremely small fluorescent signal. Fluorometers do this by comparing the signal from when the LED light source is ON and OFF. The sensitivity, accuracy and response time of a particular sensor is largely dictated by the speed and manner by which this comparison is conducted.

Measuring chlorophyll *a* concentrations using the Hydrolab DataSonde with the Turner Designs fluorescence sensor can be distinguished from the competition on several fronts. The most significant distinction is in the fluorescence signal circuitry design and performance. The Turner Designs sensor has a proprietary high performance analog signal processing unit that identifies and removes the ambient light signal before the fluorescence is amplified. Once the fluorescence signal is isolated it is then amplified and outputted to the Hydrolab system. This system of signal processing is beneficial on several fronts. Firstly, it generates a signal with lower inherent signal 'noise' level because the ambient light signal is identified and removed early in the signal processing prior to amplification. Secondly, the response time is fast due to the high speed of the LED flashing and analog signal processing. Lastly, the sensitivity is further optimized since electrical noise from the detection circuitry is removed concurrently with the ambient light signal.

Through side-by-side comparisons with the other chlorophyll sensors integrated into competitors' multi-parameter systems available on the market it is possible to draw some distinctions between the Turner Designs sensor and the competition. The competitive sensors tested appear to use firmware instead of hardware to compare the LED ON and LED OFF signals. Unlike the Turner Designs sensor that identifies and removes the ambient light signal early in the process, the competition amplifies both signals. By amplifying these signals, the inherent 'noise' in the detection electronics is also amplified resulting in a significantly noisier signal (see Figure 1 vs. Figure 2). This inherent noise can be reduced to a degree through longer averaging which slows response time. In addition, the competitive design results in a magnification of errors that occur in the detection electronics (error is proportional to the size of the signal which depends on ambient light and fluorophore concentration) making it a challenge to obtain stable readings when looking at the small differences from two very large signals. The consequence of this is that there is a much greater deviation in readings over time, perhaps why they do not list a detection limit.

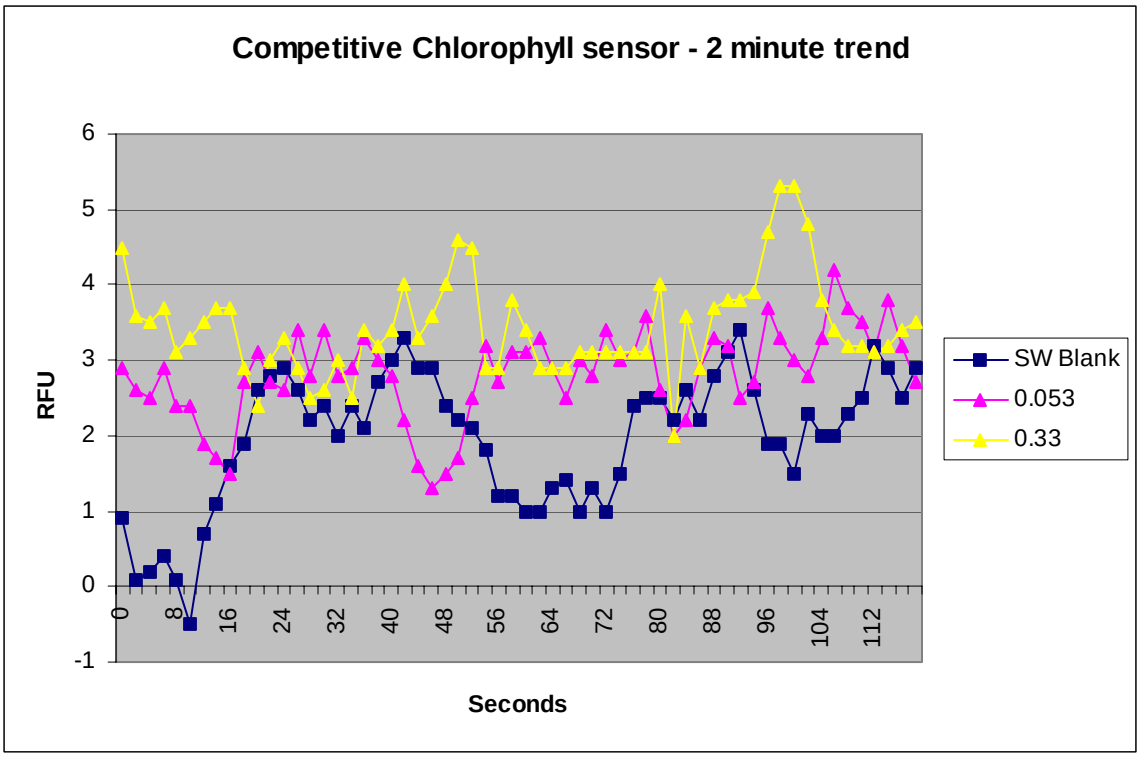

Figure 1: Data collected from a competitive sensor from a blank solution and two algal culture samples containing 0.05µg/l chlorophyll *a* and 0.33µg/l chlorophyll *a* over 2 minutes of continuous sampling.

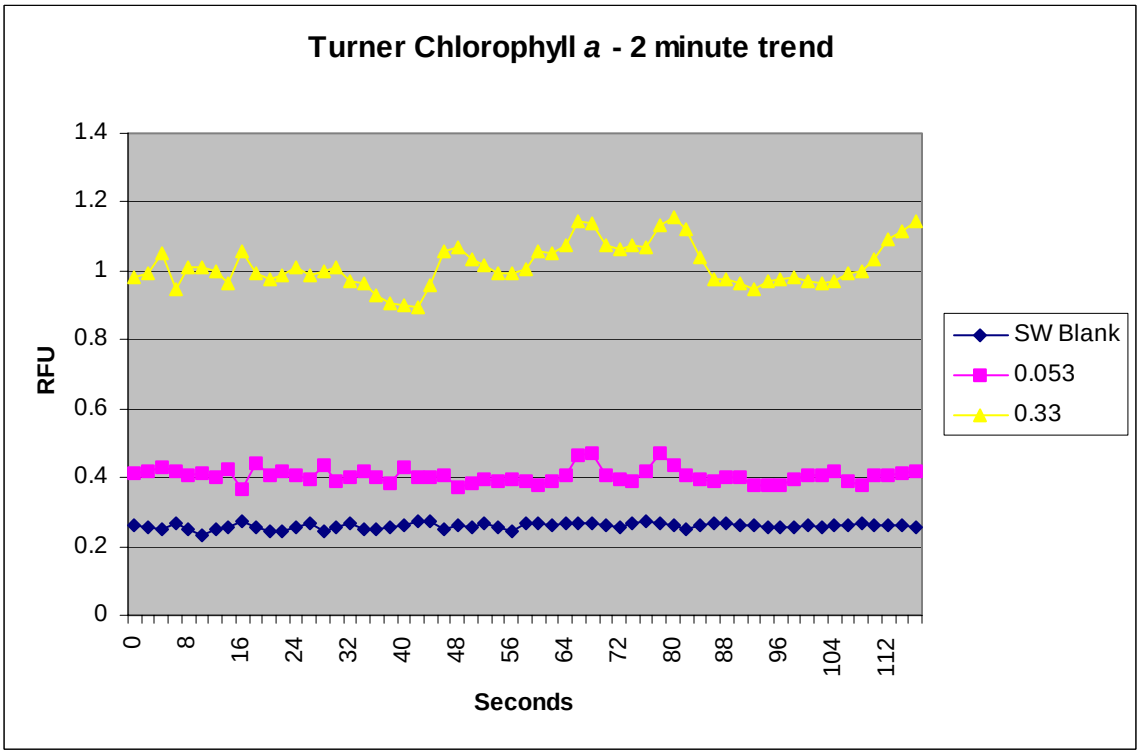

Figure 2: Data collected from a Turner Designs Chlorophyll *a* sensor from a blank solution and two algal culture samples containing 0.05µg/l chlorophyll *a* and 0.33µg/l chlorophyll *a* over 2 minutes of continuous sampling.

Some competitive sensors use a long moving average in order to deal with noise and to improve detection limits with relatively simple electronics and optics. However, the use of a relatively long averaging period results in a slower response time. In laboratory tests a competitive sensor took up to 60 seconds to reach the actual signal level of a sample (see Figure 3). Exact response time of competition will depend on the signal level. The moving average is weighted to allow for faster response with sudden changes (ex: 3a+2b+1c/3=x).

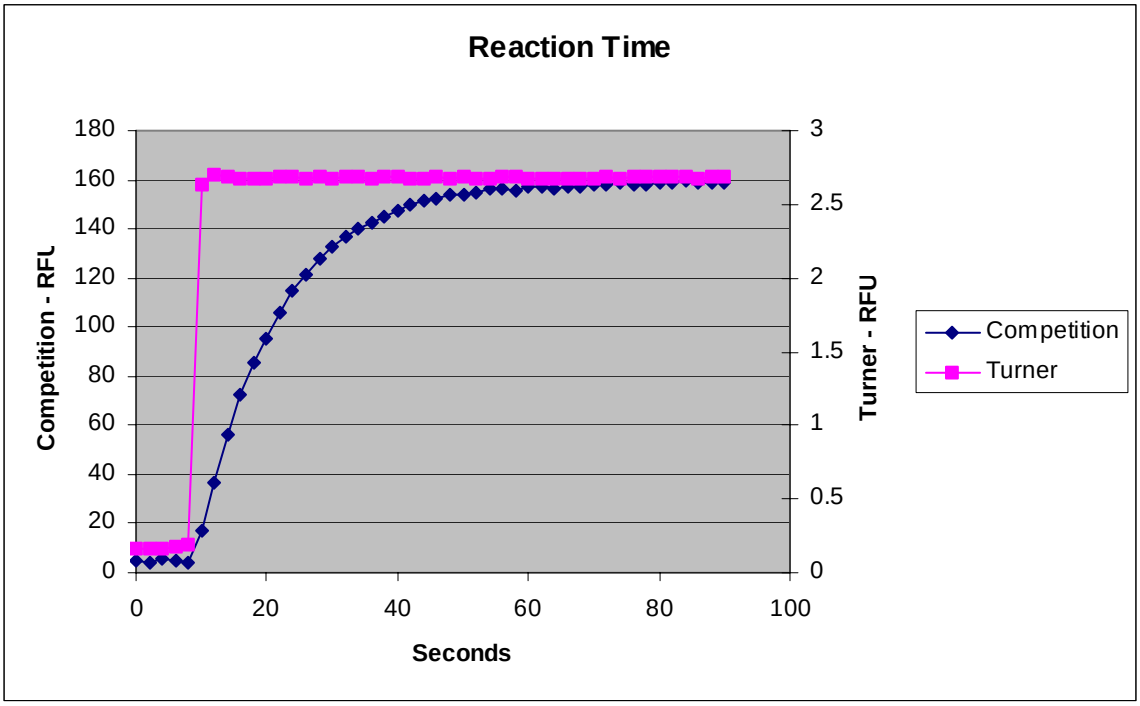

Figure 3: Data collected from a Turner Designs Chlorophyll *a* sensor and a competitive sensor over the course of 90 seconds. Both sensors were submerged in a blank solution of DI water with a magnetic stirrer in each beaker. 1ml of concentrated rhodamine WT dye was injected and the response time of the sensor was measured.

In summary, the advanced signal processing circuitry of the Turner Designs sensor results in:

- a more sensitive lower noise due to electronic filtration of ambient light, efficient optical coupling and quality optical components
- fast response through electronic filtration of ambient light
- wide dynamic range through the use of three, automatically controlled gain(sensitivity) settings (X1, X10, X100)
- excellent turbidity rejection due to small sample volume design and high quality optical filters

SOLID, SECONDARY STANDARDS

Turner Designs unique Secondary Standards provide a simple and quick way to check for correct operation of the sensor. A secondary standard is basically a substance that fluoresces at wavelengths similar to the substance being measured. Turner Designs offers secondary standards for many applications that are stable for years with no special storage requirements. The Secondary Standard can be adjusted so that when attached to the sensor, the sensor generates a desired output voltage, normally the voltage corresponding to a known chlorophyll or dye concentration.

The stability of the Chlorophyll *a* sensor can then be quickly and easily verified at a desired later time by measuring the sensor output voltage with the same secondary standard. The stability and accuracy of standards is a critical factor in fluorescence measurements. In cases such as chlorophyll measurements, where standards are not stable over the long term, a stable secondary standard can considerably simplify procedures, is relatively inexpensive, and can provide confidence in the accuracy of readings.

IN VIVO CHLOROPHYLL ANALYSIS

In vivo analysis is the direct measurement of chlorophyll *a* in living algal cells, without extraction or chemical treatment. The obvious advantage of *in vivo* analysis is rapid, on-the-spot measurement eliminating the delays for extraction and laboratory measurement. For qualitative analysis, *in vivo* measurement alone may answer the analyst's questions. For quantitative determinations, the *in vivo* data are compared with other measurements, including fluorometric extractive data. For a more detailed discussion of *in vivo* chlorophyll methodology, refer to the Turner Designs' *In Vivo* Chlorophyll E-Support section of their web page or contact them for a copy on CD.

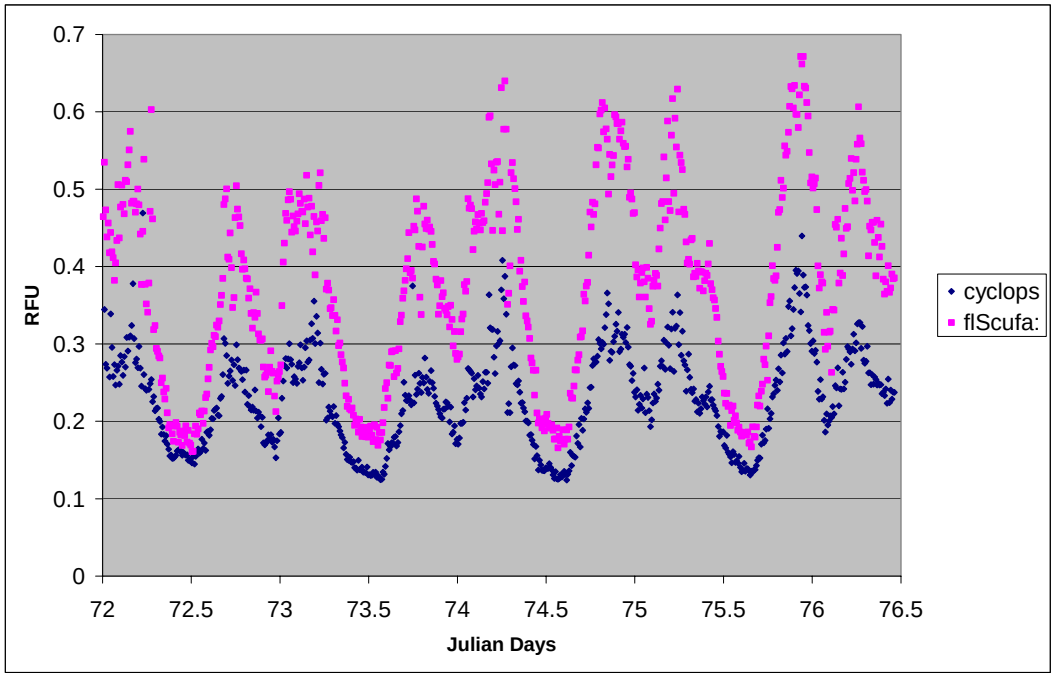

Figure 4: *In vivo* chlorophyll data collected from side-by-side SCUFA and the new integrated Chlorophyll *a* submersible fluorometers (Cyclops) deployed in San Francisco Bay, CA. Diurnal fluctuations of algal biomass are clear from both data sets. The data collected from the integrated Chlorophyll *a* and SCUFA submersible fluorometers correlate extremely well.

TURNER DESIGNS SENSORS AVAILABLE WITH THE DATASONDE 4a

Hydrolab Series 4a instruments are available with the following optical configurations:

- Chlorophyll *a* – Standard Option
- Rhodamine WT tracer dye – Standard Option
- Phycocyanin [cyanobacterial pigment – freshwater] – Standard Option (Available Soon)
- Fluorescein tracer dye – *Special* Option (Available Soon)
- Phycoerythrin [cyanobacterial pigment – marine] – *Special* Option (Available Soon)

The optics on an individual sensor cannot be changed once purchased. Each application will have unique performance specifications.