Support

Application Notes

Learn more about the SCUFA® Submersible Fluorometer

Chlorophyll Reference Papers

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Chlorophyll Discussion Group

Common Questions for the SCUFA® Submersible Fluorometer

· What is the sensitivity of the SCUFA®?

The SCUFA® sensitivity is defined in terms of the minimum detection limits of various analytes. The SCUFA® can detect:

Analyte	Minimum Detection Limit
Chlorophyll *a*	0.02ppb or 0.02 µg/L
Rhodamine WT	0.04ppb or 0.04 µg/L
Turbidity	0.05NTU

· Do I adjust the sensitivity with a gain knob or switch?

There is no manual sensitivity or gain control mechanisms on the SCUFA® . There are three gain settings (x100, x10, x1) on the fluorescence channel and two gain settings on the turbidity channel that are controlled automatically.

With analog output, the user has control over instrument range that will affect the gain settings available. For example, by setting the 0-5V range to 0-20ppb, you will eliminate the need for the x1 gain setting.

To achieve optimal performance, the appropriate calibration standard should be used. An optimal standard will be a standard with a concentration that represents 40-60% of the maximum concentration you will experience in the field.

· Is SCUFAsoft compatible with Macintosh computers?

No, the correct version of SCUFAsoft is not compatible with Macintosh computers.

· How often do I need to calibrate the SCUFA®?

For greatest accuracy, check calibration before every deployment. Verify the need to calibrate by reading a stable, known concentration standard, such as a solid secondary standard, immediately after calibration and again before each deployment to see if the readings have changed significantly. Recalibrate when there is a change in the environment or when the accuracy becomes unacceptable for your study.

· What is the purpose of the solid secondary standard?

The solid secondary standard is designed for recalibration in place of primary standards and to check for instrument performance and drift. It is very stable and does not require special storage conditions.

· What is the concentration of the solid secondary standard?

There is no exact concentration for the solid secondary standard. It is a relative concentration for both Chlorophyll a and Rhodamine WT. You can easily adjust the fluorescence signal of the solid standard and use it as a reference value for future calibrations as well as check for electronic drift.

· What is the power consumption if using internal data logging?

When using internal data logging, the total power consumption is dependant on the sampling rate set by the user. If the sampling rate is set to an interval longer than 1 reading every 5 seconds, the unit will power down into sleep mode (60mA). The instrument will consume 60mA when logging data.

· How long can the SCUFA® stay submerged with anti-fouling screens?

The Copper Anti-fouling System is intended to be used for fluorometer deployments of extended periods (>1 day). The copper components should be installed prior to instrument calibration.

The copper components will slowly dissolve in water and need to be monitored for wear. It is recommended that all components be changed after two months of use.

· How do I attach the SCUFA® to a CTD?

To integrate a SCUFA® with a CTD, two pieces of hardware are required; an integration cable and a mounting bracket. If possible, the integration should be conducted by the CTD manufacturer. If this is not possible, you must contact the appropriate cable/connector vendor to have an integration cable made that will allow communication between the fluorometer and CTD.
An integration cable consists of two in-line connectors, locking sleeves and a cable of specified length, usually 3-4 feet.

The in-line connector required for the SCUFA® is an Impulse 8-pin, female connector (Impulse P/N: MIL-8-FS), the locking sleeve is P/N MCDLS/F. The maximum cable length is 50m.

The bulkhead connector on the SCUFA® is Impulse P/N: MCBH-8-MS.
The wiring for the SCUFA bulkhead connector is as follows:

1 V Batt +
2 V Batt -
3 RS-232 ground
4 RS-232 T1 Out
5 RS-232 R1 In
6 V Out 1
7 V Out 2

8 ground

· **How do I set the 0-5V Outputs?**

Setting the 5V to a value greater than the 0V activates analog calibration for the channel of interest; fluorescence and/or turbidity. When analog output is activated, the Internal Data Logging (IDL), if purchased, is automatically disabled and the IDL screen will be faded out.

Activating the analog signal output should follow instrument calibration. By calibrating first, you can then set the 0V and 5V to calibrated values. For example, if you calibrated with a 10ppb solution and know that you will not exceed 100ppb in the field, you can set 5V to equal 100. By doing this you can optimize the resolution and accuracy of your analog data and interpret your analog data with a calibration coefficient. In this example, the calibration coefficient would be 20 (5V=100ppb, 0.5V = 10ppb).

Once set, the analog output will be activated upon the next power up as long as the unit is not connected to the portable computer.

· **What if I need a longer integration cable?**

We recommend using a deployment cable no longer than 50m to avoid electrical signal decay. If a deployment cable with an AC power supply and RS-232 connector is required, Turner Designs offers 20m and 50m versions (P/N 2000-970 and P/N 2000-980).

Deployment cables to be used with a DC power source and analog signal output should be purchased directly from Impulse Enterprise or the appropriate distributor.

Please contact Impulse Enterprise for information on their products and distributors.

Impulse Enterprise
8254 Ronson Road
San Diego, CA 92111
tel: 800-327-0971
fax: 858-565-1649
e-mail: impulse@impulse-ent.com

· **What is the flow-through cap for?**

The flow-through cap is an optional accessory that can allow the SCUFA® to be used in a flow-through mode. The cap is installed over the optics and has inlet and outlet ports to connect with a plumbing system. A common use of the cap is in conjunction with Sea-Bird CTD systems that can use a submersible pump to pump water through all of the probes. Another use could be to use the cap on a ship or in the lab with an external pump.

The cap is not necessary for use. The SCUFA® has been designed as an open-optics unit, meaning it can operate successfully with high levels of

ambient light without the need for a pump.

· How can I use the SCUFA® in the laboratory?

There are two ways that the SCUFA® could be used to analyze samples in the laboratory. The optical head of the SCUFA could be immersed into sample solution. The SCUFA® should be held at least 2" off the bottom of the container. The second option is to use the flow-through cap (P/N 2000-900) with an external pump or syringe.

CHLOROPHYLL ANALYSIS

· How does the SCUFA® detect and quantitate chlorophyll in water?

Chlorophyll a naturally absorbs blue light and emits red light. The SCUFA® will detect chlorophyll by transmitting an excitation beam of light in the 440nm (blue) range and by detecting the light emitted by the sample in the 680nm (red) range.

· What is in vivo chlorophyll analysis?

In vivo chlorophyll analysis is the fluorescent detection of chlorophyll in living algal and cyanobacterial cells in water. In this technique, the excitation light from the fluorometer passes through the untreated sample water and
excites chlorophyll within the living cells of the algae present. Due to the nature of light, cells and other dissolved and particulate materials in the water will affect the excitation light before it reaches the chlorophyll molecules. Examples of interfering materials include other plant pigments and degradation products, dissolved organic matter, turbidity, and cell morphologies. Therefore, in vivo analysis is a semi-quantitative tool. In vivo numbers
should correlate well with each other but rarely can they be used as actual chlorophyll a concentration measurements until correlated with extracted chlorophyll a data.

· Could I calibrate with extracted chlorophyll a?

No. Methanol, 90% acetone and other organic solvents will react with the SCUFA's delrin housing. The SCUFA® should not be used to measure extracted chlorophyll a. Please use a separate fluorometer such as the Turner Designs 10-AU or TD-700 Fluorometers to determine actual chlorophyll concentrations.

· What environmental factors cause error in in vivo chlorophyll analysis?

Temperature has an inverse relationship with fluorescence. In a vertical profile, as temperature decreases, the fluorescence will increase independent of chlorophyll concentration. The SCUFA® is equipped with temperature
compensation to automatically correct data for temperature effects.

Light history will have significant affects on the fluorescence in algal cells. Cells will fluoresce more chlorophyll per cell when in darker environments than in well lit zones. One way of reducing the effects of

light is to use the
flow-through cap when sampling natural waters. By using a flow-through cap and an external pump, the algal cells will be dark-adapted before entering the fluorometer, significantly reducing fluorescence error caused by variations in the light history of the cells.

Dissolved organic matter (DOM), chlorophyll degradation products, and turbidity can also affect fluorescence response. If these factors are suspected to be significant it is worth conducting a quick study to look at the effects by comparing the fluorescence from filtered and non-filtered water samples from below the photic zone where chlorophyll concentrations would be at a minimum.

FLUORESCENCE

· **What variables will effect the linearity of a sample?**

Fluorescence intensity is typically directly proportional (linear) to concentration. When a concentration is too high, light cannot pass through the sample to cause excitation; thus very high concentrations can have very low fluorescence (concentration quenching). The fluorometer reading rises at a decreasing rate and eventually
begins to decrease, even though the concentration is still increasing. Diluting a sample 1:1 or some other convenient ratio may check linearity. If it is linear, the reading will decrease in direct proportion to the dilution.

· **How does photochemical decay affect fluorescence?**

Many fluorescent molecules can be bleached or destroyed by light (fading of dyes in the sun). Ultraviolet light, especially, can cause certain molecules to break down. Fluorescence readings decrease as the molecules are
destroyed. Rate of destruction varies depending upon environmental factors, temperature. Fluorescein, for example, is destroyed rapidly in sunlight. Rhodamine WT, however, is adequately stable for field studies. For chlorophyll measurements, samples and standards need to be kept in the dark until read. All flow measurements should employ opaque delivery hoses to minimize photochemical interference.

· **What are the advantages of fluorescence?**

Sensitivity: Limits of detection depend to a large extent on the properties of the sample being measured. Detectability to parts per billion or even parts per trillion is common for most analytes. Fluorometers achieve 1,000 to 500,000 times better limits of detection as compared to spectrophotometers.

Specificity: Spectrophotometers merely absorb light. Spectrophotometric techniques are prone to interference problems because many materials absorb light, making it difficult to isolate the targeted analyte in a complex matrix. Fluorometers are highly specific and less susceptible to interferences because fewer materials absorb and also emit light (fluoresce). And, if non-target compounds do absorb and emit light, it is rare that they will emit the same wavelength of light as target compounds.

Simplicity and Speed: Fluorometry is a relatively simple analytical technique. Fluorometry's sensivity and specificity reduce or eliminate the sample preparation procedures often required to concentrate analytes or remove interferences from samples prior to analysis. This reduction in or elimination of sample preparation time not only simplifies, but also expedites the analysis.

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