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1. Specifications

Details regarding some of these specifications can be found in other parts of the manual. WETStar carries a manufacturer's two year limited warranty.

Mechanical

Size:	Pressure housing—6.7 x 2.7 in (17.1 x 6.9 cm) Overall height (including bulkhead connector and tubing fittings)—10.2 in (25.7 cm)
Weight	in air: 1.7 lb (0.8 kg) in water: 0.25 lb (0.1 kg)
Rated Depth:	600 meters
Housing:	Copolymer plastic

Electrical

Response Time:	0.17 sec
Input:	7–15 VDC
Output:	0–5 VDC (analog) 0–4095 counts (digital)
Power requirement:	< 450 mW (analog) < 900 mW (digital)

Optical

Dynamic Range:	0.03 –75 µg/L standard (other ranges available on request)
Sensitivity:	≥ 0.03 µg/L
Wavelength Excitation:	460 nm
Wavelength Emission:	695 nm

Specifications are subject to change without notice.

2. Theory of Operation

The WETStar miniature fluorometer allows the user to measure relative chlorophyll concentrations by directly measuring the amount of fluorescence emission from a given sample of water. The sample media is pumped through a quartz tube mounted through the long axis of the instrument. Chlorophyll, when excited by the presence of an external light source, absorbs energy in certain regions of the visible spectrum and emits a portion of this energy as fluorescence at longer wavelengths. WETStar uses two bright blue LEDs (centered at approximately 470 nm and modulated at 1 kHz) to provide the excitation. Blue interference filters are used to reject the small amount of red light emitted by the LEDs. A detector, positioned at 90 degrees to the axis of the LED mounts, measures the emitted light from the sample volume. The approximately 0.25 cm^3 sample volume is defined by the intersection of the excitation light with the field of view of the detector, within the quartz flow tube. A red interference filter is used to discriminate against the scattered blue excitation light. The red fluorescence emitted at 90 degrees is synchronously detected at 1 kHz by a silicon photodiode. The amplified and demodulated voltage output of the photodiode is provided to the user for connection to a digital voltmeter or an A/D converter. Figure 1 shows a simple view of how WETStar works. In the actual instrument there are two LEDs, doubling the excitation light, as well as mirrors and lenses to optimize the instrument's performance.

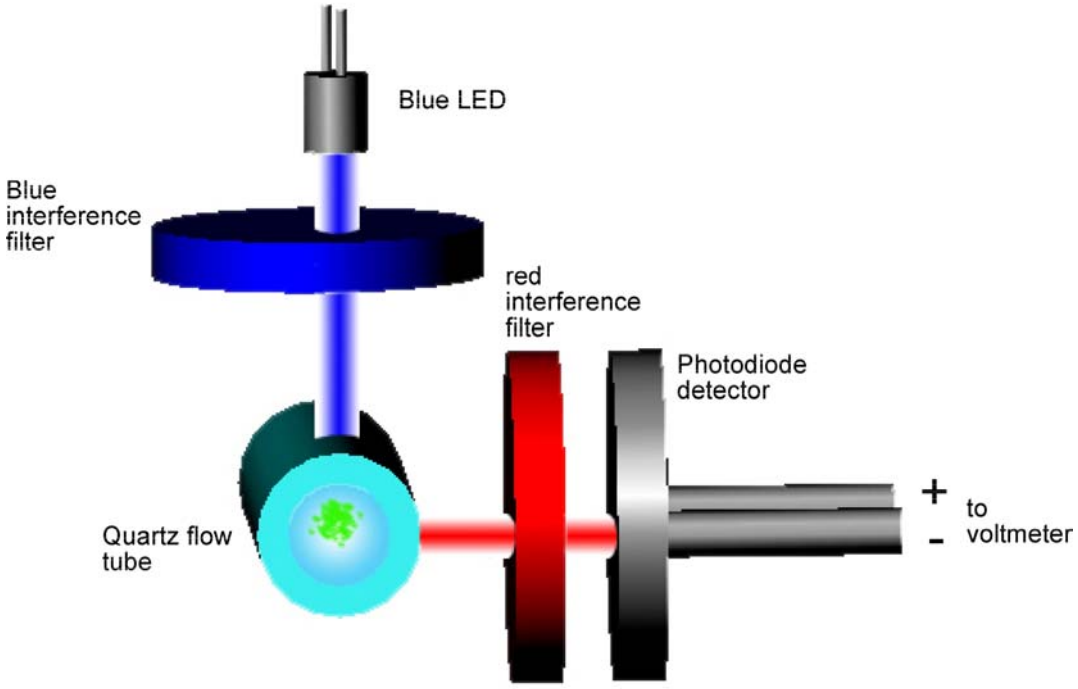

Figure 1. Absorption path of blue excitation light

3. Instrument Operation

3.1 Setup

WETStar is a simple analog output instrument. Its output of 0–5 VDC is proportional to the amount of fluoresced light emitted at approximately 685 nm. This value is, in turn, proportional to the chlorophyll concentration in the sample volume. A four-pin bulkhead connector and matching pigtail provide the power, ground, analog out and analog return signals. The pinouts are clearly described in the Technical Reference section. The analog out and return signals are connected to a data acquisition system of your choice. WETStar is designed to connect directly to many CTD systems and is compatible with other platforms that can provide power and accept a 0–5 VDC analog signal.

WETStar is optionally available with digital output. This is accomplished using a 12-bit analog-to-digital converter: full scale analog output is 5 volts whereas full scale digital is 4095 counts. This manual generally refers to analog instrument's ouput. Details of the digital instrument output are in Appendix A.

The standard WETStar delivery consists of the instrument itself (a small acetal copolymer cylinder with a miniature four-pin connector), tubing nipples which allow a small pump to be connected to the instrument, a short pigtail lead with mating connector, a small stick of fluorescing plastic material for functionality checks, the manual and a calibration sheet.

3.2 Initial Checkout

As delivered, WETStar is typically configured for one of two measurement ranges; 0.03–75.0 µg/L or 0.06–150 µg/L. This is done at WET Labs using a specific concentration of Coproporphyrin III Tetramethyl Ester and adjusting the electronic gain of the WETStar for a corresponding specific output value. A dilution series using Coproporphyrin III Tetramethyl Ester is also performed to ensure the dynamic range of the instrument is adequate and to establish the instrument's linearity. As is the case with other fluorometers, detailed calibration must be done by the user to determine the actual zero point and scale factor for his/her particular use. The techniques used to characterize the instrument are discussed in Section 6.

The four-pin bulkhead connector was chosen for its size and reliability. The mating pigtail makes a very tight fit. This is alleviated by applying a thin coat of silicon grease to the rubber skirt of the bulkhead connector. Like all such connectors, one lines up the bump on the pigtail with the hole for the large pin on the bulkhead connector. Push straight in without wiggling the pigtail from side to side. After the connector slides on, give an additional push to remove any trapped air and "seat" the connector and screw the lock collar securely to the bulkhead connector. To remove the pigtail, unscrew the lock collar then grasp the body of the connector (not the wire)

and pull straight out. Many connectors are damaged by rocking the pigtail connector from side to side as they are pulled out.

Electrical checkout of WETStar is straightforward. Apply 7–15 volts DC to the instrument to provide power to the LEDs and electronics. Ensure that positive voltage is applied to Pin 3, and common or ground is applied to Pin 1 (the large hole in the bulkhead connector). See Section 4.2 for a sketch of the pinouts of WETStar and the supplied pigtail. A 9-volt transistor radio battery makes an ideal power supply for bench testing. With the proper voltage applied to Pin 1 and Pin 3, the blue LEDs should illuminate the quartz flow tube. This light can be seen when looking straight into the flow tube. Connect Pin 2 (analog out) and Pin 4 (analog return) to a digital multi-meter. With the flow tube clean and dry, the analog output voltage should read approximately 0.2–0.5 VDC. Insertion of the fluorescent plastic material should produce a signal level at or near saturation (~5 VDC).

3.3 Deployment

WETStar can be deployed in either a non-pumped flow through mode or a pumped configuration. We highly recommend using a pump because, as one would expect from considerations of phytoplankton physiology, there is a flow rate dependence of the signal. A pump, used during calibration and during field work, will provide a consistent flow and ensure the highest quality data. We supply threaded tubing nipples for the inlet and outlet flow tube ports to aid in plumbing a pump and/or water traps. If you deploy WETStar in a flow through mode, best results will be obtained by lowering the instrument steadily at 0.2 to 1.0 meter per second. This is compatible with the descent rate requirements of many small CTDs.

If the instrument is used in a free flow mode, it is important to ensure that the flow tube inlet/outlets are “seeing” a clear water path during descent. Since WETStar’s size makes it easy to tuck away inside a cage, this can present a problem. One solution would be to add Tygon tubing to the fittings on the flow tube that are in turn connected to water traps (funnel type devices which are mounted with their wide end facing in the direction of deployment).

If you use a small pump to flush the flow cell, the recommended flow rate is in the range of 10 to 30 ml/sec. Laboratory tests have shown that, for phytoplankton cultures, increasing the flow rate up to 30 ml/sec decreases the signal but slightly improves the signal to noise ratio. The flow rate which provides the best signal to noise ratio is 25 ml/sec. A good pump for this purpose is Sea-Bird Electronics’ SBE-05T, which is a small, low powered pump which has an adjustable motor speed so that flow rate can be precisely controlled. Flow rate dependence is discussed further in the Technical Reference section.

3.4 Data Collection

The simplest form of data collection consists of connecting the analog output voltage directly to a digital multimeter. This is recommended for bench top checkout and troubleshooting. This method relies on hand logging the voltage values of interest. For actual data collection, WETStar must be connected to a host system that will receive the analog voltage output and digitize it. Many oceanographic instruments such as CTDs, radiometers, and data loggers are equipped with analog input channels and carry on-board A/D converters.

Adding the instrument to a CTD or other host solves several other problems. Since the data is merged with the CTD data, correlating the WETStar output with depth or time is done automatically. If one is building a logger or interface, it will be necessary to provide some pressure or time reference to stamp the fluorescence data, tying it to the rest of the physical data.

WETStar's output is limited to a current of 10 mA or less. Its output impedance is approximately 500 ohms that effectively limits the drive current. Therefore, the electrical signal will degrade over a long electrical wire due to the electrical resistance of the cable. For best results, the analog signal should be fed directly into an A/D converter and the digital signal should be sent up the cable. One such option is to use WET Lab's DH-4 data logger, a sub-surface data logging system that can handle up to three analog signals simultaneously, as well as two digital signals if necessary.

3.5 Data Analysis

WETStar's response is linear over the measurement range provided. The offshore version has a measurement range of approximately 0.03 to 75.0 µg/L while the coastal version has a measurement range of 0.06 to 150 µg/L. Because of the varied environments in which each user will work, it is important to do calibrations using similar seawater as you expect to encounter *in situ.* Please refer to calibration section for further details. This will provide an accurate blank and equivalent phytoplankton types for calculating the scale factor, thereby providing an accurate and meaningful calibration. Once a zero point has been determined and a scale factor established, the conversion of DC volts to chlorophyll concentration is straightforward using the equation:

$$[Chl]_{sample} = (V_{sample} - V_{blank}) * \text{Scale Factor}$$

where $[Chl]_{sample}$ = Concentration of a chlorophyll sample of interest (µg/L)
V_{sample} = voltage output when measuring a sample of interest (VDC)
V_{blank} = measured signal for a sea water blank (VDC)
Scale factor = multiplier in µg/L-volts

$$\text{Scale factor} = [Chl]_{standard} / (V_{standard} - V_{blank})$$

where:

$V_{standard}$ = measured signal for a known chlorophyll concentration (VDC)

V_{blank} = measured signal for a sea water blank (VDC)

$[Chl]_{standard}$ = known chlorophyll concentration (µg/L)

For example, if the blank reading was 0.05 VDC and a known concentration of 65 µg/L provided a signal of 4.650 volts, your scale factor would be:

65.0 µg/L / (4.650 - 0.050) VDC = 14.13 µg/L-volt

This simple relationship is then applied to your analog output signal to provide the direct conversion of the analog output voltage to chlorophyll concentration.

3.6 Upkeep and Maintenance

WETStar is a very compact instrument and its maintenance can be easily overlooked. However, the miniature fluorometer is a precision instrument and does require a minimum of routine upkeep. After each cast or exposure of the instrument to natural water, flush the instrument with clean fresh water, paying careful attention to the flow tube. Soapy water will cut any grease or oil accumulation. The tube is high quality quartz that can easily be broken or scratched so use caution. Do not use a dowel or stiff brush in the tube. A long cotton swab works nicely for cleaning the tube. At the end of an experiment, the instrument should be rinsed thoroughly, air-dried and stored in a cool, dry place. Solvents such as methanol may also be used to clean the tube.

4. Technical Reference

4.1 Optics

WETStar's optical path begins with the two blue LED light sources located near the top of the flow tube (Figure 2). The LEDs are directly across from each other, providing a uniform excitation light field. The LEDs emit wideband output light centered at approximately 470 nm. This light is channeled through blue interference filters, which reject any red light emitted by the LEDs. The blue light enters the 7 mm (ID) quartz flow tube, providing an excitation source for chlorophyll fluorescence. The fluoresced light is detected by a silicon photodiode detector. The resulting product can be measured as a 0–5 VDC signal on the output pins of the WETStar. This voltage is proportional to the chlorophyll concentration of the sample measured. WETStar is primarily designed to measure the concentration of chlorophyll containing phytoplankton, which absorb light of wavelengths between 400 and 520 nm and emit light between 670 and 730 nm.

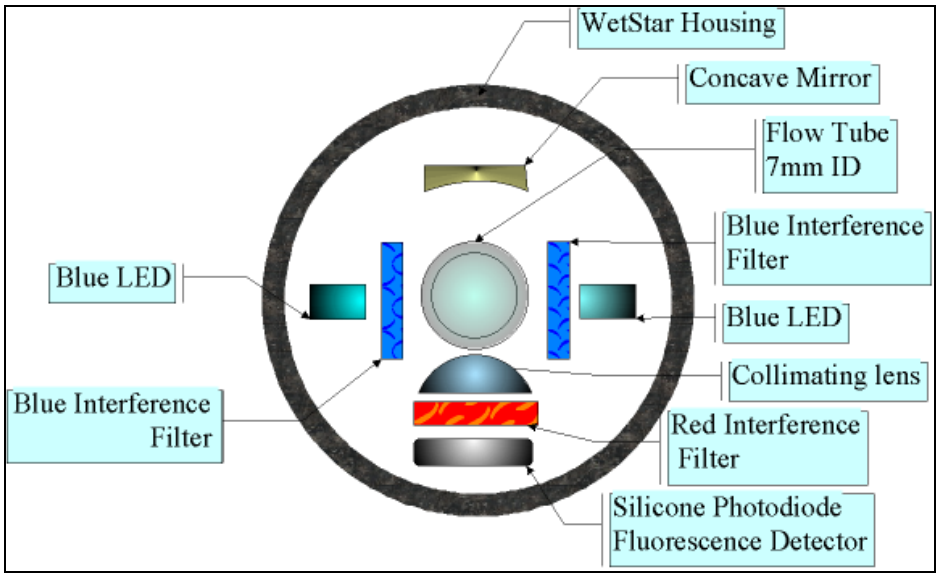

Figure 2. WETStar interior components

A red interference filter centered at approximately 685 nm. This filter is used to discriminate against scattered blue light, allowing only the red fluoresced light to pass through to the detector. A mirror and lens help optimize the detector performance. The sample volume is approximately 0.25 cm^3.

4.2 Electronics

WET Star is designed to draw a minimum of power and be very robust electronically. It uses a modulated LED light source for excitation rather than the ordinary flash lamp technology that many other fluorometers use. This allows a consistent power draw, lowering the noise floor and power consumption. The basic electronic feed is

through a four-pin Impulse or SeaCon VSG-4-PBCLM miniature bulkhead connector (Figure 3.)

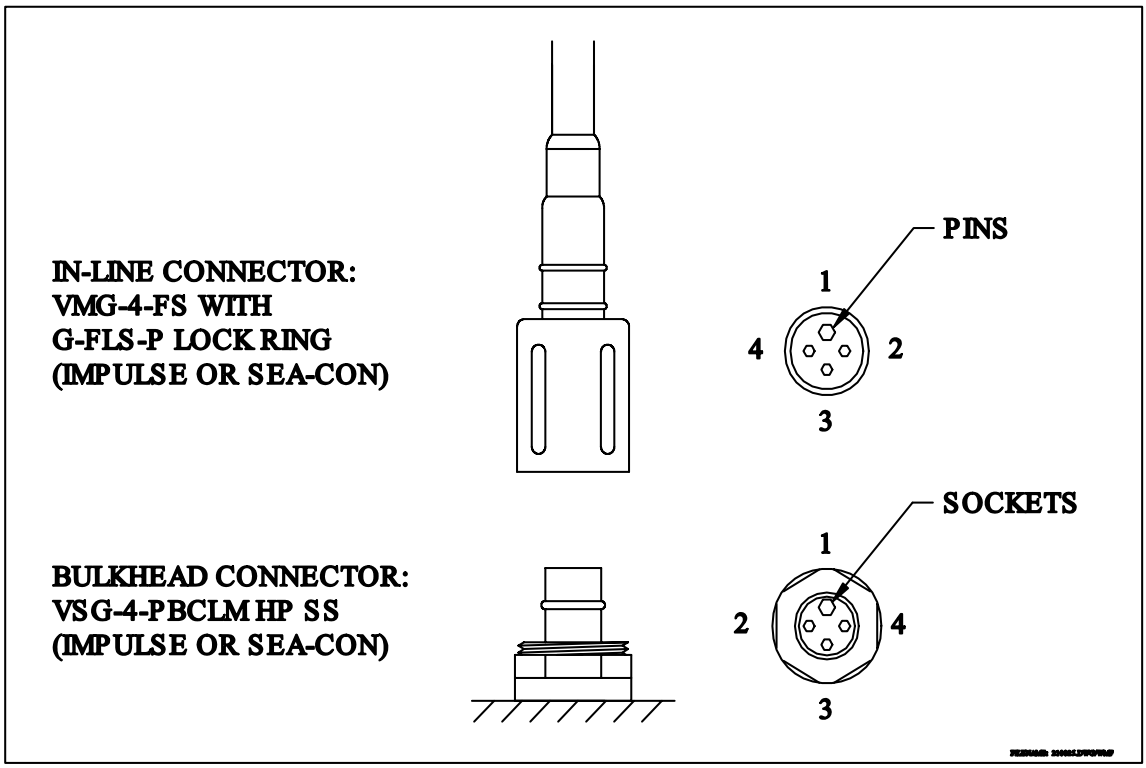

Figure 3. WETStar Bulkhead Connector

Socket/Pin	Function
1	Common (ground)
2	Analog out
3	7–15 VDC power in
4	Analog return

4.2.1 Circuitry

A 32 kHz crystal oscillator is at the heart of the WETStar electronics. A divider circuit takes the output of this 32 kHz oscillator, and creates a 1 kHz signal that drives the LED and synchronous detector circuits.

The 1 kHz red fluorescence signal is detected by a standard photodiode detector. This signal is then amplified by a high-gain two-stage op-amp circuit. The amplified signal is fed into a synchronous detector operating at 1 kHz, in phase with the LED flash rate. By synchronously detecting the fluorescence signal, ambient light and noise is rejected. After this detection, the signal is passed

though an offset circuit that adjusts the output level to close to zero volts when no fluorescence is present.

The final output circuit contains two op-amps, one with a gain of 1.0, and the other configured as a voltage follower, connected to a low pass filter. This low-pass output filter gives the instrument a response time of 1/6th of a second. The output circuit is capable of delivering a maximum current of 10 mA.

A high-efficiency switching power supply circuit is employed to provide the circuit with a tightly regulated plus and minus 5 volts DC supply. This power supply operates over an input voltage range of 7–15 volts DC. The instrument case is not connected to ground.

Caution

Do not deliver more than 15 VDC to the WETStar. The WETStar is protected against high transient voltage events with a Transient Suppression Device. When voltage transients higher than 15 VDC occur the transient voltage suppression device turns on and shunts this voltage to ground. If an input power higher than 15 VDC is applied to the WETStar, this device will turn on. If left in this condition the transient voltage suppression device will fail and damage to the WETStar may occur.

4.3 Flow Rate Dependence

Fluorescent signals from phytoplankton samples exhibit some dependence on the flow rate of the sample water through the measurement chamber. Because of WETStar's unique size and flow tube technology, providing for a uniform flow rate is highly desirable. For this reason, we recommend using a small submersible pump with a known flow rate. Free-flow (un-pumped) measurements are possible, but care must be taken to use a steady profiling rate on the order of one meter per second to provide the proper flushing of the flow tube. It is important to note that when profiling in free-flow mode, it is possible for the descent of the profiling package to come to a stop or even reverse direction briefly due to sea-state and ship motion. Figure 4 shows how the voltage output of the WETStar can vary with flow rates ranging from about 3 to 30 ml/sec. A small, constant volume pump was placed in line with a closed loop flow system fitted with a throttle valve to control the flow rate. It should be noted that the relative response only varies by 15–18 percent over the entire range so that useful data can be obtained even in situations where the flow rate is unknown.

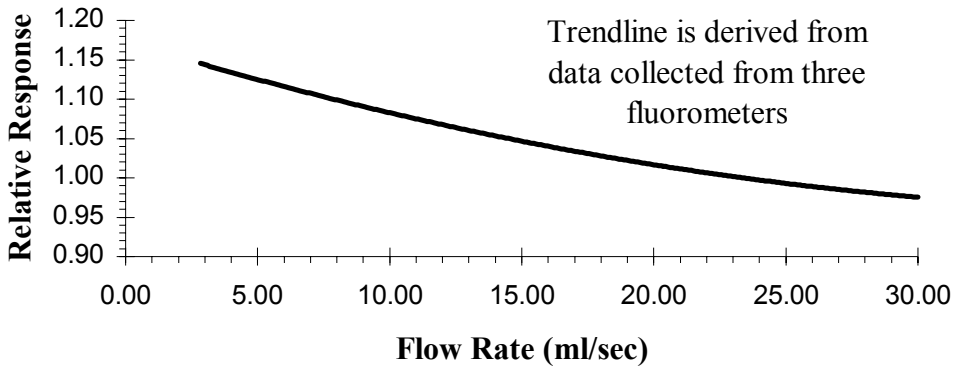

Figure 4. Flow rate dependence for Thalassiosire Weissflogii.

Output voltages are normalized to the voltage value recorded at 24 ml/sec. Using the same flow rate in the field that was used during one's calibration will ensure consistent results.

5. Characterization

Prior to shipment, each WETStar is characterized to ensure that it meets the instrument's specifications. WET Labs characterization procedure is described in the following paragraphs.

5.1 Measurement Apparatus

To measure the WETStar output voltages for tuning and characterization, the fluorometer is connected to a 16-bit analog-to-digital (A/D) converter. The A/D outputs the voltages in a standard RS-232 serial text format that is collected with a terminal program. A spreadsheet is then used to perform calculations on the collected values.

5.2 Procedure

There are two main steps in the tuning and testing of the WETStar. The first step, "Characterization," is performed before the unit is put in its enclosure. The second step, "Final Testing" is done with the unit completely assembled.

5.2.1 Coproporphyrin Standard Preparation

The current characterization standard is 0.5 mg/l (500 µg/l) of Coproporphyrin III Tetramethyl Ester solution. Coproporphyrin III Tetramethyl Ester is available in powdered form from Sigma-Aldrich (www.sigma-aldrich.com) in 1 or 5 mg vials.

WARNING
If you are not experienced or trained in the safe and proper techniques of working with acids, seek proper assistance.

To make a 0.5 mg/l concentration of stock solution, dissolve 1 mg of Coproporphyrin III Tetramethyl Ester in 40 ml of 6 normal hydrochloric acid (HCl) and 960 ml of pure water. This will yield one liter of 1 mg/l solution. Dilute this solution by a factor of two to make a 0.5 mg/l stock solution.

Due to variability in the purity of the Coproporphyrin and the ability to precisely measure and handle a powdered substance such as Coproporphyrin III Tetramethyl Ester, WET Labs uses an ac-9 spectral absorption and attenuation meter to fine-tune the stock concentration. We have determined that a 0.5 mg/l solution of Coproporphyrin III Tetramethyl Ester has an absorption value of 4.86 m^{-1} at 412 nm. We fine-tune the stock solution by adding pure water or more concentrate Coproporphyrin solution, depending on which way the concentration needs to move. This standard is tied to a series of Isochrysis chlorophyll extractions performed by Richard Davis, Oregon State University, and WET Labs, Inc., at University of California, Santa Barbara.

It is important to note that fluorescence observed in the natural environment can deviate widely over varying conditions. Given this, the Coproporphyrin standard should not directly be compared to chlorophyll. The standard is used in the tuning of WETStar as a reference, not as a calibration.

Refer to the next section, “Calibration” for details on performing a laboratory or field calibration on the WETStar.

The WETStar sensitivity is adjusted to be within certain limits when a controlled Coproporphyrin standard is introduced into the sample volume. The standard is nominally equivalent to 50 µg/l of chlorophyll. On the WS3S models, the output voltage is adjusted for 3.0 volts (+/- 0.150 volts), and on the WS1S, the output is 1.5 volts (+/- 0.150 volts).

The output is adjusted by setting the gain in several operational amplifier stages in the WETStar. Gain, or sensitivity, is set with fixed-value resistors.

5.2.1.1 Pure Water Blank

Pure, de-ionized water is used to set the “zero” voltage of the WETStar. This zero voltage is set for approximately 0.090 volts (+/- 0.020 volts) on both WETStar models.

The WETStar employs an offset voltage circuit. Water blank is adjusted with two fixed voltage divider resistors.

5.2.1.2 Response Time (Time Constant)

The specified time constant for the WETStar is 0.167 seconds. This time constant is the RC value, computed by 1/RC.

To verify the time constant, the step response is observed on an oscilloscope. A sample is introduced that produces a full-scale reading. The sample is then quickly removed, and the decay is observed on the oscilloscope. The output voltage must reach a value of 66 percent of the original within 0.167 seconds. A nominally full-scale output is obtained after six time constants.

5.2.2 Final Testing

5.2.2.1 Pressure

To ensure the integrity of the housing and seals, the WETStar is subjected to a wet hyperbaric test before final testing. The testing chamber applies a water pressure of at least 50 PSI. The rated depth of the WETStar is 600 meters. WET Stars are spot checked once every 10 units to the full rated depth.

5.2.2.2 Mechanical Stability

Before final testing, the WETStar is subjected to a mechanical stability test. This involves subjecting the unit to mild vibration and shock. The air, water, and Coproporphyrin voltages must remain the same before and after the mechanical stability test.

5.2.2.3 Temperature Stability

To verify temperature stability, the WETStar is immersed in an ice bath. The starting temperature is typically 25–30 degrees Celsius, and the ending temperature is 1–5 degrees Celsius. A voltage sample is collected every 30 seconds, with a 0.5 second smoothing. Specifications assert that the maximum variation per degree Celsius is 1.25 mV.

5.2.2.4 Electronic Stability

This value is computed by collecting a sample once per minute for twelve hours, or more. The smoothing time for this one sample is 0.5 seconds. After the data is collected, the minimum and maximum values are determined, and the difference between these two is divided by the number of hours the test has run. The result is the stability value listed on the characterization sheet. The stability value must be less than 2.0 mV/Hour.

5.2.2.5 Full Scale Verification

The specified maximum output of the WETStar is nominally five volts. The verify this, a Coproporphyrin solution equivalent to 90 µg/l is introduced into the sample volume on the WS3S. A full-scale reading of 5 Volts (+/- 0.1) should result. On the WS1S, a solution equivalent to 170 µg/l is used to verify full scale.

5.2.2.6 Noise

The noise value is computed from a standard deviation over 60 samples. These samples are collected at one-second intervals for one minute. The smoothing (averaging) time for these samples is 0.5 seconds. A standard deviation is then performed on the 60 samples, and the result is the published noise on the characterization sheet. The calculated noise must be below 1.5 mV.

5.2.2.7 Final Water Blank Test

De-ionized, pure water is introduced into the sample volume. The output voltage must be 0.090 volts (+/- 0.020 volts) on both WETStar models. This value is recorded on the characterization sheet.

5.2.2.8 Final Coproporphyrin Standard Test

As with the initial Coproporphyrin standard adjustment, a stock solution (see above) is placed in the sample volume. This solution is equivalent to 50 µg/l of chlorophyll. On the WS3S, an output voltage of 3.0 volts (+/- 0.80 volts) is required. For the WS1S, the output voltage must be 1.5 volts (+/- 0.80 volts). This value is recorded on the characterization sheet.

5.2.2.9 Voltage and Current Range Verification

To verify that the WETStar operates over the entire specified voltage range (7–15 volts), a voltage-sweep test is performed. The WETStar is operated over the entire voltage range, and the current and operation is observed. The total power consumption (voltage times current) must remain below 450 mW over the entire voltage range.

5.2.2.10 Linearity

Linearity tests are performed on every WETStar. This linearity test consists of a complete Coproporphyrin dilution series. The linear regression "R-squared" value must be better than 0.9900.

6. Calibration

WETStar is shipped pre-configured to provide accurate, linear response over one of two dynamic ranges (0.03–75.0 or 0.06–150 µg/l). However, because of the myriad calibration techniques and different properties of the natural waters from which "blanks" will be prepared, it is important that an experiment specific calibration is done before (and after) each major cruise or event. The key to obtaining high quality data is to determine the instrument's response to the conditions that will be found in the field. Because of the many different applications involving fluorometric chlorophyll determinations, detailed calibration of the instrument must be done by the user. Note: this section is provided by Richard Davis, Oregon State University.

6.1 Introduction and Caveats

The purpose of calibrating an *in situ* fluorometer is to be able to convert its in-water signal to an absolute value of chlorophyll-*a*. In theory this should be a simple process of measuring the voltages from the instrument obtained from a dilution (or addition) series of a phytoplankton culture of known concentration, linear regress the recorded voltages against chlorophyll concentration and then obtain a calibration coefficient. Problems arise from the fact that the optical properties of phytoplankton are functions of size, shape, pigmentation, taxonomic composition, photo-adaptation and physiological status. For example, exposure to supersaturating light will cause an immediate (time scale of seconds) depression in fluorescence with any change in chlorophyll concentration happening very much slower (time scale of hours) (Kiefer 1973, Cullen et al.1988). However, since it is unreasonable to calibrate a fluorometer with every species of phytoplankton at all different physiological states, one has to simply be aware of the problems and go forth. Thus any conversions of *in situ* fluorometry into chlorophyll-*a* concentration are estimates at best and guesses at worst. Over the time scale of a mooring deployment a fluorometer will probably estimate chlorophyll-*a* within a factor of 2 (Lorenzen 1966).

6.2 Calibration Protocol

The following procedures involve using some improvisational techniques and equipment. For example, the Sea-Bird SBE-5 pump is designed for submersible work and may prove difficult or impossible for some people to use in the way described. This calibration procedure also assumes the person performing calibration is already familiar with the spectrophotometric extraction method for determining chlorophyll concentration.

Calibration should be a two-phase process. The first phase, a serial addition procedure, occurs in the lab, the second, a simple correlation procedure, in the field.

6.2.1 Lab Calibration

Materials Needed:

- WETStar fluorometer
- SBE pump
- cables for fluorometer and pump
- tubing for fluorometer
  - inlet
  - outlet
- thermometer
- scint vials w/ 10 ml 90% acetone
- set of volumetric pipettes
  - 5 ml
  - 10 ml
  - 25 ml
- pipette bulb
- large tub for immersing instruments
- lab notebook
- DC power supply
- digital voltmeter
- ring stand w/ 2 large clamps
- 50, 100 and 500 ml graduated cylinders
- phytoplankton culture
- stopwatch
- some 500 ml beakers
- GF/F filters
- forceps
- filter rig w/ pump, tubing, traps

It is critical that the entire calibration process be performed under non-varying conditions. Changes in temperature or light will affect the *in vivo* fluorescence of the phytoplankton culture and possibly the instrument. It is recommended that the room be dimly lit and that the culture be allowed to sit in the calibration area for 30 minutes prior to use. The instrument should be equilibrated to calibration temperature for at least 4 hours.

Obtain a culture of late logarithmic phase phytoplankton and some of the culture media. Use a species of phytoplankton (or at least genera) that you are likely to encounter in the field. If time, energy and materials allow use two species of different groups (e.g., a diatom and a chrysophyte) for comparison purposes. Absolute concentration of chlorophyll-*a* in the culture should be no more than 50 µg/l (you can start to see color by eye at about 20 µg/l, at which point the culture is starting to slow down and enter the stationary phase). Filter approximately 50–100 ml onto a GF/F filter for spectrophotometric chlorophyll determination. You should be able to easily see color on the filter. If you can't, filter more. Place the filter into 10 ml of 90 percent acetone and store in a freezer for 24 hrs. Record the optical density of the solution at 750, 664, 647, and 630 nm. Calculate pigment concentrations by:

$$C_a = 11.85*OD_{664} - 1.54*OD_{647} - 0.08*OD_{630}$$
$$C_b = 21.03*OD_{647} - 5.43*OD_{664} - 2.66*OD_{630}$$
$$C_c = 24.52*OD_{630} - 1.67*OD_{664} - 7.60*OD_{647}$$

where a, b, and c denote chlorophyll-*a*, -*b* and -*c*, respectively, and all optical densities have had the 750 nm signal subtracted.

Set up the WETStar fluorometer and pump on a ring stand according to Figure 5. Provide power to the fluorometer and pump via a DC power supply. Record all voltages from the fluorometer with a voltmeter. Power up the fluorometer and let it warm up for 10 minutes. At the end of this period record the voltage as the air blank. Begin pumping a known volume of culture medium through the fluorometer. Check for and clear bubbles. Allow the fluorometer to stabilize and record the voltage as the seawater blank. Add an aliquot of culture to the blank culture medium with a volumetric pipette. The aliquot should be enough that you would expect to see an increase in the fluorometer voltage between 0.5 and 1 volt. In the scenario of the WETStar set up for the lower range (0–75 µg/l), a culture containing approximately 50 µg/l chlorophyll, and a blank culture medium volume of 400 ml the aliquot would be approximately 25 ml. After the reading has stabilized record the voltage and volume added. Continue with the serial additions until you saturate the fluorometer. Clean the fluorometer after calibrating.

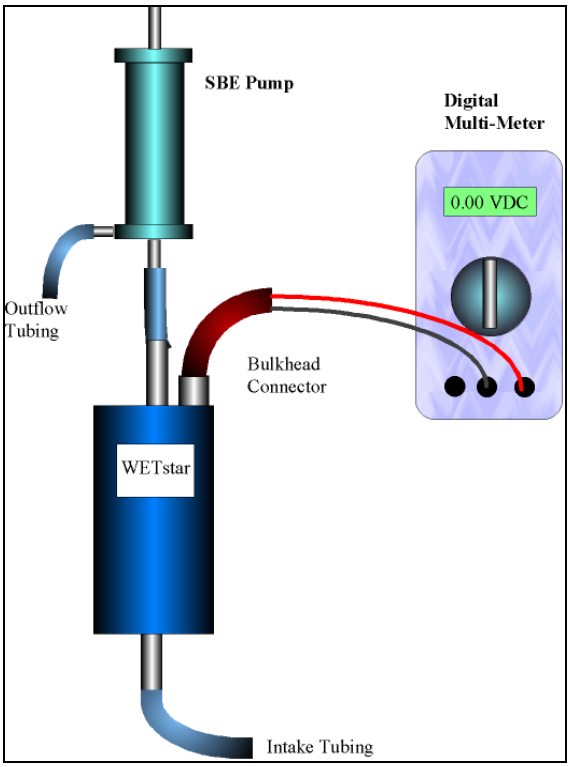

Figure 5. Instrument Setup

6.2.2 Field Calibration

There are two goals to the field calibration. The first is to verify that the lab calibration is valid in the field. The second is to detect any changes in the fluorometer over time. To achieve these goals chlorophyll-*a* samples should be taken as often and as close as possible to the mooring. Record the time that the Niskin bottle containing the sample was tripped. Preferably samples would be taken throughout a 24 hr period to investigate the effects of irradiance on *in situ* fluorescence.

6.3 References

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Appendix A: Digital WETStar

The WETStar is available with optional digital output that varies from the analog WETStar with respect to output and bulkhead connector configurations as detailed below. Note that most digital WETStars use a four-pin connector; the six-pin is a custom configuration.

Connectors

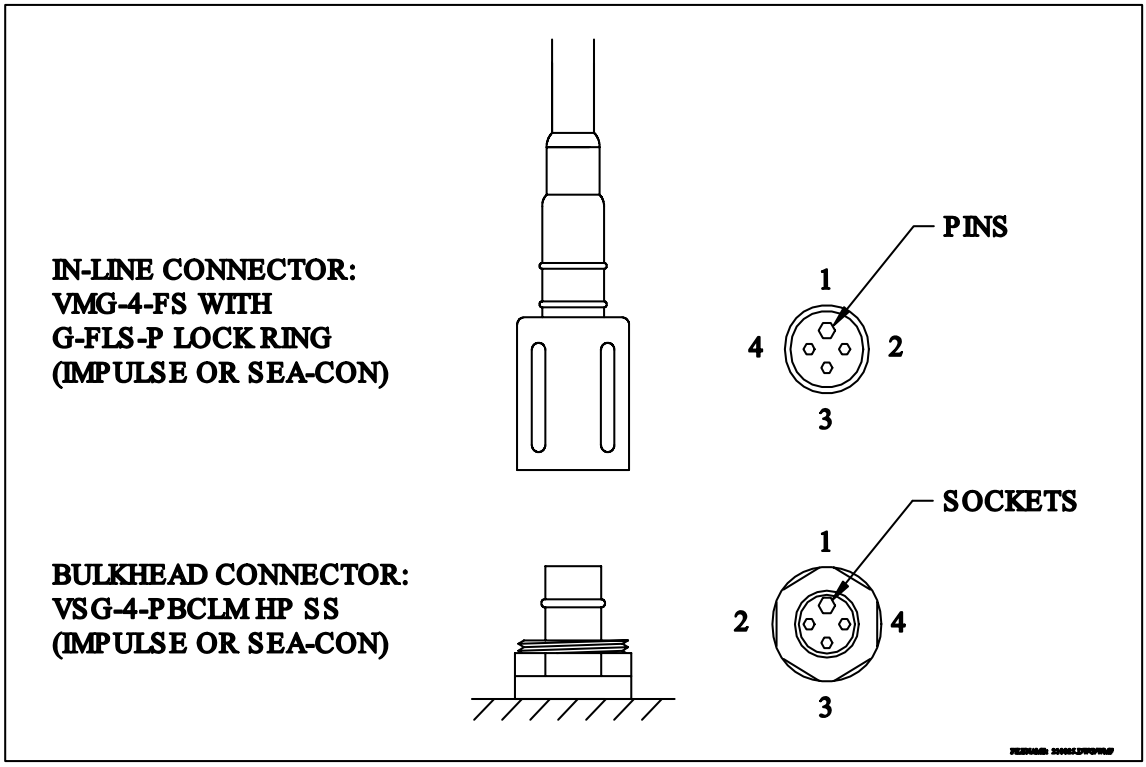

Figure 6. 4-pin digital WETStar connector schematic

Socket/Pin	Function
1	Ground
2	Analog +
3	V+
4	RS-232

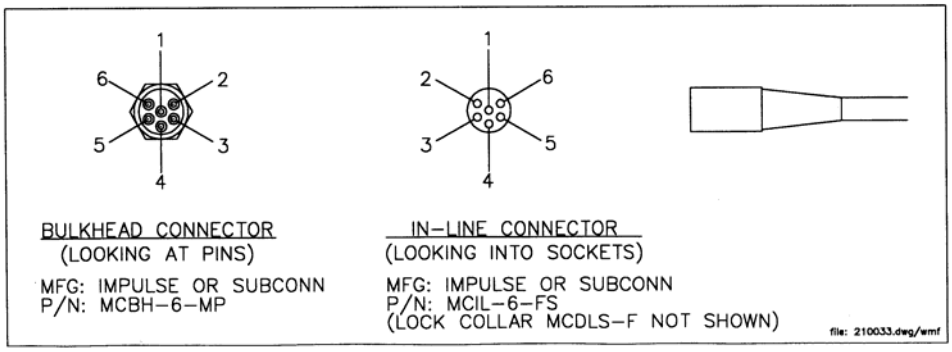

Figure 7. 6-pin digital WETStar connector schematic

Pinout summary for digital WETStar connectors

Pin (or Socket)	Function
1	Power ground
2	RS-232 (RX)
3	Analog ground
4	V in
5	RS-232 (TX)
6	Analog output

Input power of 7–15 volts DC is applied to pin 4. The power supply current returns through the common ground pin. Data is sent out the Serial Output pin (pin 5).

Output Format

The RS-232 output from the digital WETStar is a single column of numbers whose values range between 0 and 4095 counts. Note that RS-232 protocol is limited to a cable length of 15 feet. WET Labs' experience is that these cables can be significantly longer, but they should be tested before deployment.

Serial Port Configuration

Data Rate	Data type
9600 baud	8 data bits, 1 stop bit, no parity, no flow control

WETStar

User's Guide

The user's guide is an evolving document. Please check our website periodically for updates. If you find sections that are unclear, or missing information, please let us know.

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Revision History

Revision	Date	Revision Description	Originator
A	10/07/99	Begin revision tracking	H. Van Zee
B	01/03/00	Change Specifications, Delete section 3.3 (DCR 6)	C. Moore
C	01/06/00	Update document (DCR 8)	D. Hankins
D	03/29/00	Reorder chapters for consistency (DCR 19)	H. Van Zee
E	12/12/00	Update document and illustrations (DCR 75)	D. Hankins, H. Van Zee
F	03/12/01	Correct Copro standard preparation (DCR 90)	D. Hankins
G	11/26/01	Revise references to excitation wavelengths (DCR 164)	J. Kitchen
H	01/23/02	Correct terminology in section 2 and update Figure 1 (DCR 188)	H. Van Zee
I	04/09/02	Add digital WETStar capabilities (DCR 213)	S. Campos. H. Van Zee
J	10/25/02	Delete reference to Schott glass (DCR 246)	S. Campos
K	05/12/03	Add four-pin digital connector diagram and functions (DCR 300)	M. Everett