

# **Combination Fluorometer–Scattering Sensor**

*ECO FLbb-AP2*

## **User's Guide**

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

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## ECO Sensor Warranty

This unit is guaranteed against defects in materials and workmanship for one year from the original date of purchase. Warranty is void if the factory determines the unit was subjected to abuse or neglect beyond the normal wear and tear of field deployment, or in the event the pressure housing has been opened by the customer.

To return the instrument, contact WET Labs for a Return Merchandise Authorization (RMA) and ship in the original container. WET Labs is not responsible for damage to instruments during the return shipment to the factory. WET Labs will supply all replacement parts and labor and pay for return via 3<sup>rd</sup> day air shipping in honoring this warranty.

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## Return Policy for Instruments with Anti-fouling Treatment

WET Labs cannot accept instruments for servicing or repair that are treated with anti-fouling compound(s). This includes but is not limited to tri-butyl tin (TBT), marine anti-fouling paint, ablative coatings, etc.

Please ensure any anti-fouling treatment has been removed prior to returning instruments to WET Labs for service or repair.

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## Shipping Requirements

1. Please retain the original ruggedized shipping case. It meets stringent shipping and insurance requirements, and protects your meter.
  2. Service and repair work cannot be guaranteed unless the meter is shipped in its original case.
  3. Clearly mark the RMA number on the outside of your case and on all packing lists.
  4. Return instruments using 3<sup>rd</sup> day air shipping or better: do **not** ship via ground.
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Electrical equipment marked with this symbol may not be disposed of in European public disposal systems. In conformity with EU Directive 2002/96/EC (as amended by 2003/108/EC), European users of electrical equipment must return old or end-of-life equipment to the manufacturer for disposal at no charge to the user.

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## 1. Specifications

Mechanical		Electrical	
<i>Diameter</i>	6.3 cm (instrument only)	<i>Digital output resolution</i>	12 bit
<i>Length</i>	14.9 cm	<i>RS-232 output</i>	19200 baud
<i>Weight in air</i>	0.578 kg	<i>Connector</i>	IE55-1206-BCR
<i>Weight in water</i>	0.143 kg	<i>Input</i>	7–15 VDC
<i>Pressure housing</i>	ABS, epoxy, HDPE, 6061 aluminum (hard anodized)	<i>Data output rate</i>	1 Hz (user settable to 4 Hz)
		<i>Operating current, typ.</i>	35 mA
		<i>Operating current, max</i>	50 mA

Optical		Environmental	
<i>Backscattering wavelength</i>	700 nm	<i>Temperature range<sup>2</sup></i>	- 2 to 30 deg C
<i>Backscattering sensitivity<sup>1</sup></i>	0.0015 m <sup>-1</sup>	<i>Depth rating</i>	2000 m
<i>Backscattering range<sup>1</sup></i>	0–3 m <sup>-1</sup>		
<i>Fluorometer EX/EM</i>	470/695 nm		
<i>Chlorophyll sensitivity</i>	0.015 µg/l		
<i>Fluorescence range</i>	0 –30 µg/l		
<i>Linearity</i>	99 % R <sup>2</sup>		

<sup>1</sup>Backscattering specifications are given in beam  $c_p$  (m<sup>-1</sup>) based on the regression of the response of the instrument relative to the beam  $c_p$  measured at the coincident wavelength using an ac-s spectrophotometer. Scale factors for backscattering incorporate the target weighting function and the solid angle subtended.

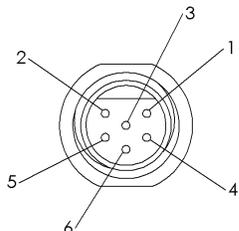
<sup>2</sup>The water temperature range through which the instruments are tested. The -2 deg C minimum covers all natural waters on earth. Please contact WET Labs for testing to higher temperatures.

## 2. ECO FLbb-AP2 Meter Components

The following subsections describe the bulkhead connectors and items delivered with the ECO sensor.

### 2.1 FLbb-AP2 Connector

ECO FLbb-AP2 meters use an IE55-1206-BCR six-socket bulkhead connector. The pin functions for this connector are shown below.

	<b>Pin</b>	<b>Function</b>
	1	Ground
	2	RS-232 (RX)
	3	Reserved
	4	V in
	5	RS-232 (TX)
6	Reserved	

## 2.2 Delivered Items

The standard ECO delivery consists of the following:

- the instrument itself
- protective cover for optics
- this user's guide
- instrument-specific calibration sheet
- ABS white plastic mounting bracket
- Fluorescent stick for bench testing.

## 3. Theory of Operation

The *Environmental Characterization Optics (ECO)* combination fluorometer and backscattering sensor allows the user to measure chlorophyll fluorescence at 470 nm and backscattering at 700 nm within the same volume.

The fluorometer allows the user to monitor chlorophyll concentration by directly measuring the amount of chlorophyll-*a* fluorescence emission from a given sample volume of water. Chlorophyll, when excited by the presence of an external light source, absorbs light in certain regions of the visible spectrum and re-emits a small portion of this light as fluorescence at longer wavelengths. A blue LED (centered at 470 nm and modulated at 1 kHz) provides the excitation source. The blue light from the sources enters the water volume at an angle of approximately 55–60 degrees with respect to the end face of the unit. Fluoresced light is received by a detector positioned where the acceptance angle forms a 140-degree intersection with the source beam. A red interference filter is used to discriminate against the scattered blue excitation light. The red fluorescence emitted is synchronously detected by a silicon photodiode.

Scattering is measured simultaneously by detecting the scattered light from a 700 nm LED at 140 degrees to the same detector used for fluorescence. The scattering measurement is performed at the same 140 degree angle as the chlorophyll fluorescence.

## 4. Instrument Operation

### Operating environment notice

*ECO* sensors are precision instruments. Data transfers should be performed away from harsh environments such as strong electric fields or electrostatic discharge sources. If used in a such an environment, there is a chance that the sensor may experience disturbances that will temporarily stop data transfers. In that event, move the sensor to a location further from the electric field or ESD source. Reset the sensor by momentarily turning its power supply off, then on.

### 4.1 Initial Checkout

Supplied from the factory, *ECOs* are configured to begin continuously sampling upon power-on.

Connect the instrument to a power supply; turn it on. Light should emanate from the meter.

### 4.2 Upkeep and Maintenance

After each cast or exposure of the instrument to natural water, flush with clean fresh water, paying careful attention to the sensor face. Use soapy water to cut any grease or oil accumulation. Gently wipe clean with a soft cloth. The sensor face is composed of ABS plastic and optical epoxy and can easily be damaged or scratched.

### **WARNING!**

**Do not use acetone or other solvents to clean the sensor.**

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## 5. Data Analysis

Data from the *ECO* fluorometer and scattering sensor represents raw output. Applying linear scaling constants, this data can be expressed in meaningful forms of chlorophyll fluorescence and backscattering.

### 5.1 Chlorophyll Scale Factor

The scale factor is factory-calculated by obtaining a consistent output of a solution with a known concentration, then subtracting the meter's dark counts. The scale factor, dark counts, and other characterization values are given on the instrument's characterization sheet.

For chlorophyll, WET Labs uses the chlorophyll equivalent concentration (CEC) as the signal output using a fluorescent proxy.

Scale Factor =  $xx \mu\text{g/l} \div (\text{Chl Equivalent Concentration} - \text{dark counts})$

For example:  $25 \div (3198 - 71) = 0.0080$ .

The scale factor is then applied to the output signal to provide the direct conversion of the output signal to chlorophyll concentration. WET Labs supplies a scale factor on the

instrument-specific calibration sheet that ships with each meter. While this constant can be used to obtain approximate values, field calibration is highly recommended.

## 5.2 Chlorophyll Response

Digital chlorophyll data is processed using linear scaling is linear. Obtaining a “calibrated” output simply involves subtracting a digital offset value from output when measuring a sample of interest and multiplying the difference by the instrument scaling factor.

$$[\text{Chl}]_{\text{sample}} = (C_{\text{output}} - C_{\text{dc}}) * \text{Scale Factor}$$

where

$[\text{Chl}]_{\text{sample}}$  = concentration of a chlorophyll sample of interest ( $\mu\text{g/l}$ )

$C_{\text{output}}$  = output when measuring a sample of interest (counts)

$C_{\text{dc}}$  = dark counts, the measured signal output of meter in clean water with black tape over the detector

Scale factor = multiplier in  $\mu\text{g/l}/\text{counts}$ .

## 5.3 Data Corrections

*Attenuation coupling*—For the population of photons scattered within the remote sample volume in front of the sensor face, there is attenuation along the path from the light source to the sample volume to the detector. This results in the scattering measurements being underestimates of the true volume scattering in the hydrosol. Corrected volume scattering coefficients can be obtained by accounting for the effect of attenuation along an average pathlength. This average pathlength was numerically solved in the weighting function determinations developed by Dr. Ron Zaneveld that are used in the calibration procedures.

Since the calibration of the BB uses microspherical scatterers, the component of attenuation that can be attributed to scattering is incorporated into the scaling factor, i.e., the calibration itself. Thus, only absorption of the incident beam needs to be included in the correction.

The dependence on absorption,  $a$ , is determined as follows, where the measured scattering function at a given value of  $a$ ,  $\beta_{\text{meas}}(\text{angle}, a)$ , is corrected to the value for  $a = 0 \text{ m}^{-1}$ ,  $\beta_{\text{corr}}(117^\circ, a=0)$ :

$$\beta_{\text{corr}}(117^\circ, a=0) = \beta_{\text{meas}}(117^\circ, a) \exp(0.0391a)$$

Absorption can be measured with an ac-9 device. For each ECO-BB wavelength, the matching absorption coefficient must be used from the ac-9. Because the ECO-BB incorporates short pathlengths and relatively small scattering volumes in its measurements, this attenuation error is typically small, about 4 percent at  $a = 1 \text{ m}^{-1}$ .

## 5.4 Derived Parameters

### 5.4.1 Volume Scattering of Particles

The corrected volume scattering of particles,  $\beta(117^\circ, \lambda)$  values represent total volume scattering, i.e., scattering from particles and molecular scattering from water. To obtain

the volume scattering of particles only, subtract the volume scattering of water,  $\beta_w(117^\circ, \lambda)$ :

$$\beta_p(117^\circ, \lambda) = \beta(117^\circ, \lambda) - \beta_w(117^\circ, \lambda)$$

where  $\beta_w(117^\circ, \lambda)$  is obtained from the relationship (from Morel 1974):

$$\beta_w(\theta, \lambda) = 1.38(\lambda/500\text{nm})^{-4.32}(1+0.3S/37)10^{-4} (1+\cos^2\theta(1-\delta)/(1+\delta))\text{m}^{-1}\text{sr}^{-1}, \delta=0.09$$

where S is salinity.

For total scattering of pure water,

$$b_w(\lambda) = 0.0022533 (\lambda/500\text{nm})^{-4.23}.$$

For total scattering of seawater (35–39 ppt),

$$b_{sw}(\lambda) = 0.0029308 (\lambda/500\text{nm})^{-4.24}.$$

For backscattering by water, divide  $b_w$  or  $b_{sw}$  by 2. The units for the b coefficients are ( $10^{-4} \text{ m}^{-1}$ ).

#### 5.4.2 Backscattering Coefficients

Particulate backscattering coefficients,  $b_{bp}(\lambda)$  with units of  $\text{m}^{-1}$ , can be determined through estimation from the single measurement of  $\beta_p(117^\circ, \lambda)$  using an X factor:

$$b_{bp} = 2\pi X \beta_p(117^\circ)$$

From measurements of the volume scattering function with high angular resolution in a diversity of water types, Boss and Pegau (2001) have determined X to be **1.1** (Boss, E., and S. Pegau, 2001). The relationship of scattering in an angle in the back direction to the backscattering coefficient, *Applied Optics*). This factor estimates  $b_{bp}$  with an estimated uncertainty of 4 percent. The conversion can be used for  $\beta(117^\circ)$  measurements made at any visible wavelength.

To compute total backscattering coefficients,  $b_b(\lambda)$  with units of  $\text{m}^{-1}$ , the backscattering from pure water,  $b_{bw}(\lambda)$  (see Table above), needs to be added to  $b_{bp}(\lambda)$ :

$$b_b(\lambda) = b_{bp}(\lambda) + b_{bw}(\lambda).$$

## 6. Characterization and Testing

ECO FLbb-AP2 is configured for a chlorophyll measurement range of 0.0–30  $\mu\text{g/l}$ . The scattering sensor's measurement range is 0–3  $\text{m}^{-1}$ . Gain selection is done at WET Labs by setting several gain settings inside the instrument, and running a dilution series to determine the zero offset and to ensure the dynamic range covers the measurement range of interest. The dilution series also establishes the linearity of the instrument's response. As is the case with other fluorometers, a detailed characterization must be performed to determine the actual zero point and scale factor for your particular use.

The tests below ensure the meter's performance.

1. **Dark counts:** The meter's baseline reading in the absence of source light is the dark count value. This is determined by measuring the signal output of the meter in clean, de-ionized water with black tape over the detector.

2. **Pressure:** To ensure the integrity of the housing and seals, *ECO*s are subjected to a wet hyperbaric test before final testing. The testing chamber applies a water pressure of at least 40 PSI.
3. **Mechanical Stability:** Before final testing, the *ECO* meters are subjected to a mechanical stability test. This involves subjecting the unit to mild vibration and shock. Proper instrument functionality is verified afterwards.
4. **Electronic Stability:** This value is computed by collecting a sample once every second for twelve hours or more. After the data is collected, the standard deviation of this set is calculated and divided by the number of hours the test ran. The stability value must be less than 2 counts/hour.
5. **Noise:** Noise is computed from a standard deviation over 60 samples. These samples are collected at one-second intervals for one minute. A standard deviation is then performed on the 60 samples, and the result is the published noise on the calibration form. The calculated noise must be below 2 counts.
6. **Voltage and Current Range Verification:** To verify the *ECO* operates over the entire specified voltage range (7–15 V), a voltage test is performed at 7 and 15V, and the current draw and operation is observed. The current must remain constant at both 7 and 15V.

## 7. Terminal Communications

*ECO* sensors can be controlled from a terminal emulator or customer-supplied interface software. This section outlines hardware requirements and low-level interface commands for this type of operation.

### 7.1 Interface Specifications

- baud rate: 19200
- data bits: 8
- parity: none
- stop bits: 1
- flow control: none

### 7.2 Command List

Command	Parameters passed	Description
!!!!	none	Stops data collection; allows user to input setup parameters. Note that if the meter is in a sleep state, the power must be turned off for a minute, then powered on while the “!” key is held down for several seconds.
\$ave	single number, 1 to 65535	Number of measurements for each reported value
\$mnu	none	Prints the menu
\$pkt	single number, 0 to 65535	Number of individual measurements in each packet
\$rls	none	Reloads settings from flash
\$run	none	Executes the current settings

## 8. Sample Output File



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<b>N/U</b>	<b>Chl Sig</b>	<b>NU</b>	<b>BB Sig</b>	<b>Therm</b>
470	54	700	51	550
470	53	700	51	549
470	54	700	52	550
470	54	700	52	550
470	55	700	52	550
470	53	700	51	550

*Note: Firmware v. 4.04 has 470 in column 1 (3.01 uses 695).*

## **WET Labs WEEE Policy**

In accordance with Directive 2002/96/EC and the Council of 27 January 2003, WET Labs policy regarding the collection and management of Waste Electrical and Electronic Equipment (WEEE) is published here and is available at [www.wetlabs.com](http://www.wetlabs.com).

A core component of our corporate vision is to accept responsibility for preserving our environment and we embrace the opportunity to work with our customers and the EU to reduce the environmental impact resulting from the continuous improvement of our products.

### **WEEE Return Process**

To meet the requirements of the WEEE Directive, WET Labs has instituted a product end-of-life take back program. To arrange return for an end-of-life WEEE product:

1. Contact WET Labs Customer Service
  - By phone: 1-541-929-5650
  - By email: [support@wetlabs.com](mailto:support@wetlabs.com)

WET Labs will provide:

WEEE RMA number

Shipping account number, method, and address

2. Package and ship the WEEE back to WET Labs

WEEE will be processed in accordance with WET Labs' equipment end-of-life recycling plan.

## Revision History

<b>Revision</b>	<b>Date</b>	<b>Revision Description</b>	<b>Originator</b>
A	5/7/09	New Document (DCR 667)	M. Johnson
B	9/14/11	Add WEEE Statement (DCN 775)	H. Van Zee
C	6/4/12	Add ESD operating notice (DCN 798)	J. Pearson, H. Van Zee