TURNER DESIGNS
Reliable Instruments for an Unreliable World

C3 Submersible Fluorometer

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C3 Submersible Fluorometer

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C3 Submersible Fluorometer

1.0 Introduction

The C3 Submersible Fluorometer is manufactured according to user's optical specifications with one, two, or three optical sensors ranging in wavelengths from ultra violet to infrared. Each optical sensor is designed with a static excitation and emission filter. The C3 Submersible Fluorometer comes with a factory installed temperature probe and can be configured with a pressure sensor for depth measurements up to 600 meters and/or a wiper motor to minimize biofouling during extended or short-term deployments. The C3 Submersible Fluorometer Windows™ based user interface allows for easy calibration of each sensor, digital data reporting, data logging, file downloading, and digital export of data.

Available Optical Sensors:

- Turbidity
- *In Vivo* Chlorophyll a
- Blue Green Algae (Phycoerythrin)
- Blue Green Algae (Phycocyanin)
- CDOM
- Crude Oil
- Fluorescein ***(See important note on page 25)***
- Rhodamine Dye
- Optical Brighteners
- Custom Optics

2.0 Inspection and Setup

2.1 Instrument Checklist

The C3 Submersible Fluorometer package (P/N: 2300-000) includes:

- C3 Submersible Fluorometer
- Factory installed temperature sensor
- Interface cable for PC with 12V power supply
- CD includes C-Soft software, user's manual, and quick start guide

Temperature Sensor

12V power supply

Computer interface cable

C3 Submersible Fluorometer

Optional Accessories:

- Pressure sensor (P/N: 2300-360) *Factory installed*
- C3 Mechanical Wiper (P/N: 2300-450) *Factory installed*
  Note: The Pressure sensor and mechanical wiper can only be installed during the production of the unit
- Replaceable Brush (P/N: 2200-460)
- Submersible Battery Kit includes charger (P/N: 2200-601)
- C3 Submersible Battery Bracket (P/N: 2300-603)
- Flow Cap (P/N: 2300-700)
- Shade Cap (P/N: 2300-500)
- Shade Cap Weight (P/N: 2300-510)
- Booster Kit (P/N: 2200-900)
  Note: Booster Kits are required for cable lengths greater than 5 meters.
- Extended Cables:
  - 10 meters (P/N: 105-2595)
  - 25 meters (P/N: 105-2596)
  - 50 meters (P/N: 105-2597)
  - Custom lengths available upon request
- Secondary solid standard (P/N: 2300-900) for multiple sensors
  Note: Solid Standards are not available for turbidity or ultraviolet sensors

Mechanical Wiper

Submersible Battery

C3 Submersible Fluorometer

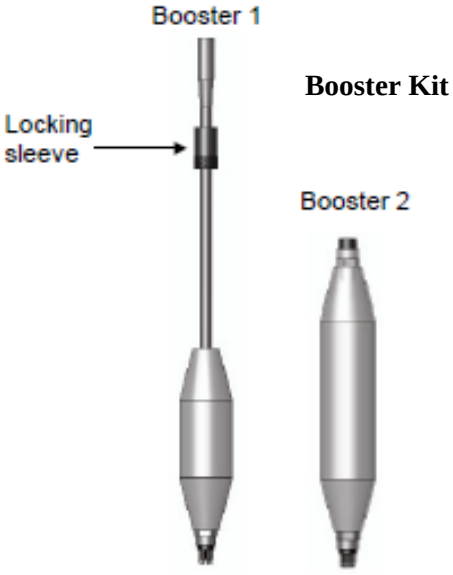
Battery Bracket

Solid Standard Insert

Secondary Solid Standard

Flow Cap

Shade Cap



Booster Kit

Identification

Sensors labeled 1, 2, or 3 adjacent to the optics on the face of the C3 Submersible Fluorometer correspond to channels 1, 2, and 3 in the C3 Windows Software.
Note: The channel labels can be modified in the C-Soft software.

Light emitting diode color table.

Application	Light Source Color
Chlorophyll a	Blue
Turbidity	Infra Red (No Color)
Phycocyanin	Yellow
Phycoerythrin	Green
Fluorescein	Blue ***(See important note on page 25)***
Rhodamine	Green
CDOM	Ultra Violet (No Color)*
Crude Oil	Ultra Violet (No Color)*
Optical Brighteners	Ultra Violet (No Color)*
****Do not look directly onto the optics. Ultraviolet light can be damaging to the eyes.***	

3.0 C-Soft Windows™ User Interface

The C-Soft Windows™ based user interface allows for intuitive calibration, data logging set up, and file downloading.

3.1 Software Installation and PC Requirements

3.1.1 Minimum PC Requirements

- 133MHz microprocessor
- 600 X 800 VGA resolution
- Microsoft Windows™ 98
- 32 MB RAM

3.1.2 Software Installation

1. Exit all Windows™ programs
2. Insert the C-Soft CD (also located on the Turner Designs Software web page, http://www.turnerdesigns.com/t2/sw/main.html).
3. Open the C-Soft software folder and double click on the setup icon.
4. Double click on the “setup.exe” icon to begin installation.

5. The software will automatically be installed in the PC's (C:/Program Files/C-Soft) folder unless user selects alternate location.
6. After restarting the PC double click on the "C-Soft" icon located on the desktop.

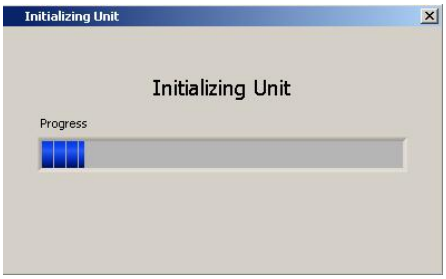
C-Soft Note: You may receive a warning message asking if you want to unblock messages, select "Unblock"

3.1.3 PC Software Interface

1. Connect the 9-pin serial end of the interface cable to the serial port on the back of the PC.

   Note: If your computer only has USB ports we recommend purchasing a USB to serial adapter cable. (See Turner Designs Support web page for recommendations)

2. Connect the 8-pin female end of the interface cable to the 8-pin male connector on the C3 Submersible Fluorometer.
3. Connect the interface cable's 12V pin to the 12V power supply.
4. Plug the 12V power supply into a power source.
5. Wait for initialization process to complete.



The connection icon, located on the upper right of the screen, will turn green if connected.
**Note: If the connection icon does not turn green disconnect and reconnect power.*

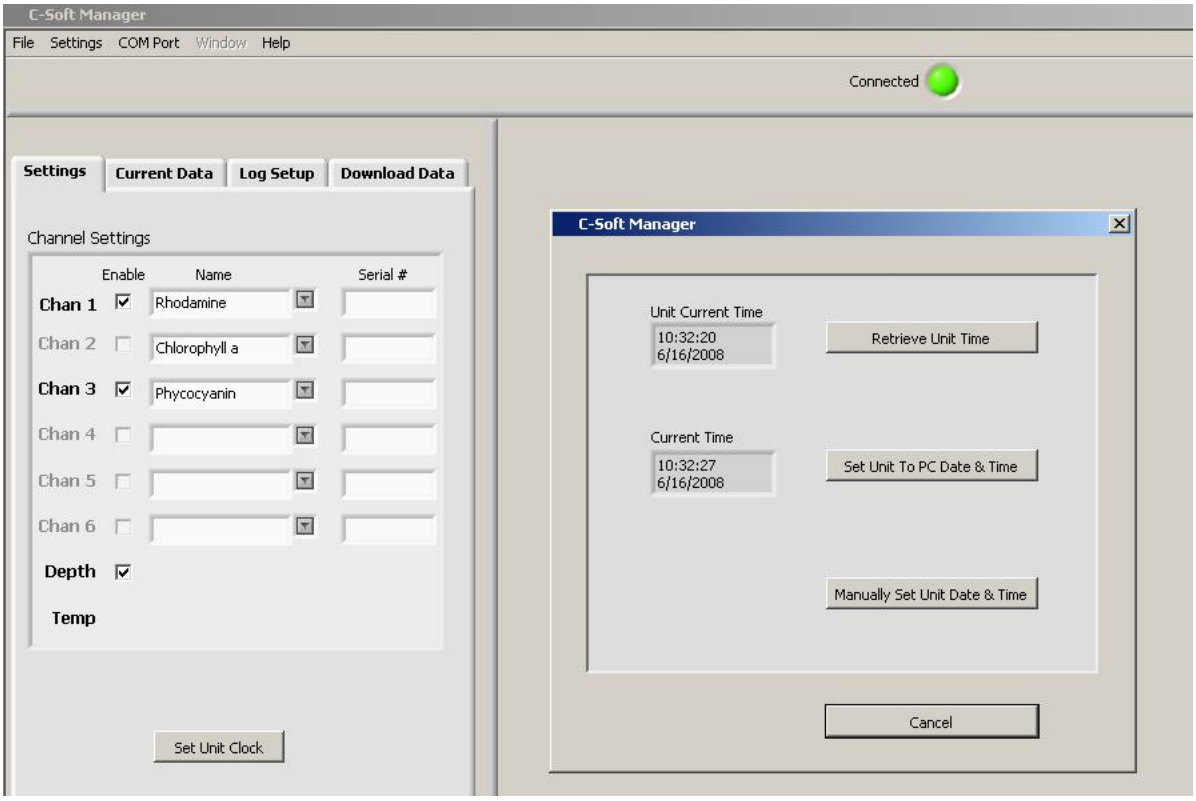
3.2 Software Operation

3.2.1 Settings Screen

The Settings Screen allows users to set the time and date, identify each optical sensor, update serial numbers, and enable/disable sensors.

- Users can manually set the date and time or choose to synchronize the C3 Submersible Fluorometer to the PC date and time.

- Each channel (1-3) in the Settings Screen has a location associated with it on the C3 Submersible Fluorometer that is numerically labeled (1-3), respectively. Channels 4-6 are designated for the C6 Multi-Sensor Platform.
- Click on the box adjacent to the channel to enable channel operation. If there is no sensor installed users will be unable to select that channel.
- Channels can be renamed using the dropdown selections or by manually entering a name.
- The serial number boxes are designed for the C6 Multi-Sensor Platform.
- Settings will automatically be saved after exiting the Settings screen.



3.2.2 Current Data and Calibration Screen

The Current Data Screen allows users to view real-time data acquisition, in both tabular and waveform formats, as well as conduct calibrations for each channel.

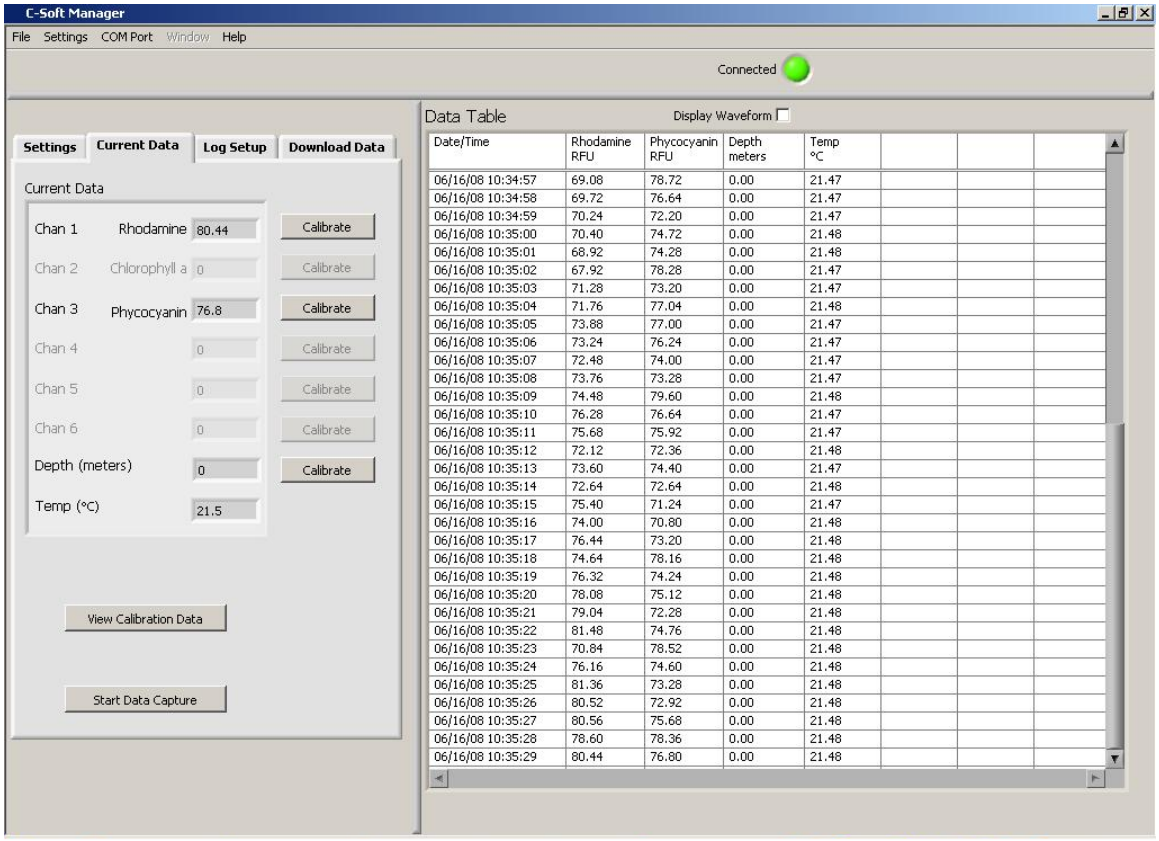
Current Data

- Channel names are displayed but cannot be changed in the Current Data screen.
- Real-time values are displayed next to each channel and in the adjacent Data Table.
- A calibration button is available for each channel and the depth sensor.

- Real-time data can be saved by selecting the “Start Data Capture” button. Users will be prompted to enter a file name and file location. Data files will be saved in (*.csv) format.

Tabular Screen

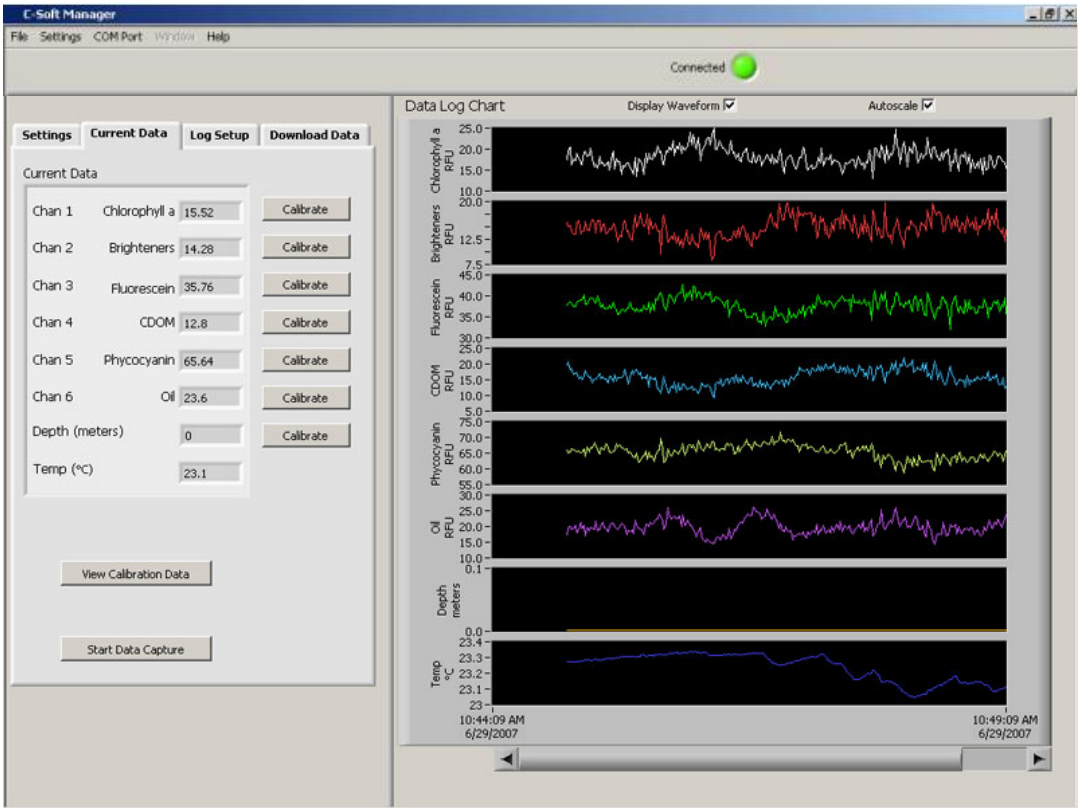
- Real-time data including time, date, temperature, and units are continuously scrolling on the tabular screen for enabled channels.
- Channel names will appear in the associated column headings.
- If units are not selected during calibration, a relative fluorescence units (RFU) label will appear.



Waveform Screen Users can graphically view real-time data for each sensor by clicking the “Display Waveform” box.

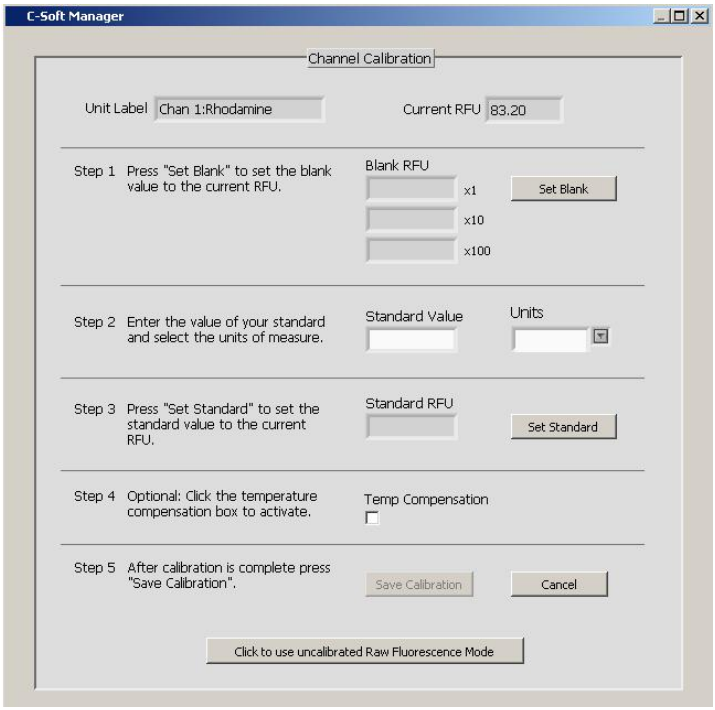
- Channel names and units will be displayed on the Y-axis.
- Time and date will be displayed on the X-axis.
- Clicking the “Autoscale” box will automatically scale graphs.
- Data can be saved during waveform mode by clicking “Start Data Capture”.

C3 Submersible Fluorometer



Calibration Screen

Users can calibrate sensors in either the direct concentration mode, raw fluorescence mode, or blank subtracted raw fluorescence mode.



Direct Concentration Mode
Values reported in the Direct Concentration Mode are scaled to a predetermined standard value and blank subtracted. The equation used to calculate concentrations after calibrating to Direct Concentration Mode is:

$$\frac{\text{Standard Value (units)}}{\text{Standard RFU}} \times \text{RFU Response} = \text{Concentration (units)}$$

Raw Fluorescence Mode Blank Subtracted

Values in the Raw Fluorescence Blank Subtracted Mode are noted as RFUB. It is important to note that these values are blank subtracted but are not scaled to a standard; they are **relative** values.

Raw Fluorescence Mode

Values in the Raw Fluorescence Mode are referred to as "Relative Fluorescence Units" (RFU). It is important to note that these values are not blank subtracted or scaled to a standard; they are **relative** values.

Direct Concentration Calibration

Following steps 1-5 will ensure values are reported as blank subtracted Concentrations in units of choice. See Appendix B for recommended practices.

Step 1: Blanking

- Place the C3 Submersible Fluorometer in a blank solution.

  Note: A blank solution is a solution without the fluorophore of interest (i.e. de-ionized water, artificial seawater, or filtered seawater)

  Note: Ensure the Optical head is free of air bubbles

- Wait until "Current RFU" readings have stabilized then select the "Set Blank" button to set the blank value.
- Future measurements for this sensor will be blank subtracted based on the blank response recorded during calibration.

Step 2: Standard Value and Units

- Place the C3 Submersible Fluorometer in a standard solution or use a secondary solid standard (see Appendix D for more information on solid standards).

  Note: Not all sensors are designed with solid standards

- Enter the known concentration value of the standard solution in the "Standard Value" box. If the concentration is not known, enter an arbitrary value (i.e. 100).

- Future measurements will be calculated based on the correlated response to the standard value.
- Enter manually or select desired units from the dropdown menu.

Note: Do not choose or enter "RFUB" if Direct Concentration Mode is used

Step 3: Set Standard

- When readings in "Current RFU" box have stabilized, select the "Set Standard" button.

Step 4: Optional Temperature Compensation

Temperature compensation is optional and allows the user to automatically compensate to the factory installed temperature sensor. Temperature compensation is available for Chlorophyll *a* and Rhodamine sensors only. Temperature is displayed in degree Celsius.

- Click on the "Temperature Compensation" box.
- Select either the Chlorophyll *a* or Rhodamine from the dropdown menu.
- Data will now be corrected for temperature fluctuations.

The temperature compensation coefficients for each fluorophore are listed in the table below.

Fluorophore	**Coefficient**	
Rhodamine	0.026 per degree C	Exponential
Chlorophyll *a*	1.4% per degree C	Linear

Step 5: Save Calibration

- After calibration is complete, select "Save Calibration".
- Data for the selected sensor will now be calibrated.

Raw Fluorescence Calibration – Blank Subtracted

Following steps 1-5 will ensure values are reported as Blank subtracted Relative Fluorescence Units (RFUB). See Appendix B for recommended practices.

Step 1: Blanking

- Place the C3 Submersible Fluorometer in a blank solution.

Note: A blank solution is a solution without the fluorophore of interest (i.e. de-ionized water, artificial seawater, or filtered seawater)

Note: Ensure the optical head is free of air bubbles

- Wait until “Current RFU” readings have stabilized then select the “Set Blank” button to set the blank value.
- Future measurements for this sensor will be blank subtracted based on the blank response recorded during calibration.

Step 2: Selecting Units

- Select RFUB from the units’ dropdown menu.
  Note: Do not enter a value in the standard value box

Step 3: Skip and go to Step 4

Step 4: Optional Temperature Compensation – Follow same steps under the Direct Calibration procedure

Step 5: Save Calibration

- After calibration is complete select “Save Calibration”.
- Data for the selected sensor will now be in relative fluorescence units and blank subtracted.

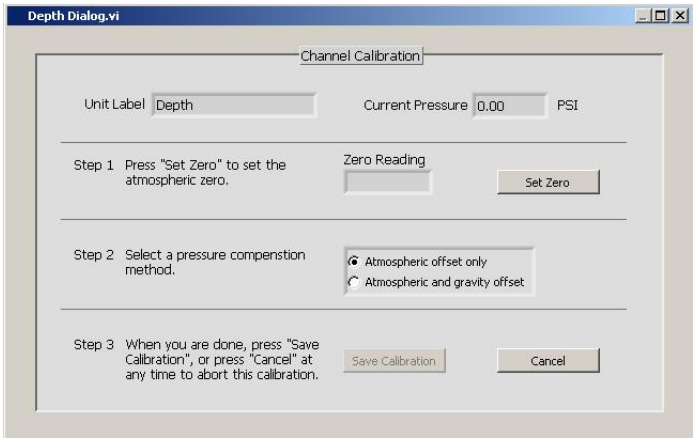
Raw Fluorescence Calibration

In the Current Data screen select the “calibrate” button next to a specific channel. At the bottom of the Calibration Screen, click to use “Uncalibrated Raw Fluorescence Mode”. Data for that channel will be uncalibrated and reported as “Relative Fluorescence Units” (RFU).

Depth Calibration (Optional Accessory)

Depth is displayed in meters only. There are two calibration options for depth:

1. The first uses only the atmospheric pressure as an offset. This is suitable for any shallow application, including most fresh water applications.
2. The second compensates for gravity variations with latitude, as designed from the UNESCO Technical Papers in Marine Science #44. This method assumes 0°C and 35PSU salinity.



Atmospheric Offset

Step 1 (Method 1):

- Select the "Calibrate" button for depth in the "Current Data" screen.
- Pressure in PSI will be displayed in the "Current Pressure" window.
- After "Current Pressure" readings are stable at desired location select "Set Zero".

Step 2 (Method 1):

- Select "Atmospheric offset only".

Step 3 (Method 1):

- Select "Save Calibration" button.
- Future depth values will be scaled to the offset.

Atmospheric and Gravity Offset

Step 1 (Method 2):

- Select the "Calibrate" button for depth in the "Current Data" screen.
- Pressure in PSI will be displayed in the "Current Pressure" window.
- After "Current Pressure" readings are stable, at desired location, select "Set Zero".

Step 2 (Method 2):

- Select "Atmospheric and gravity offset".
- Enter the latitude.

Step 3 (Method 2):

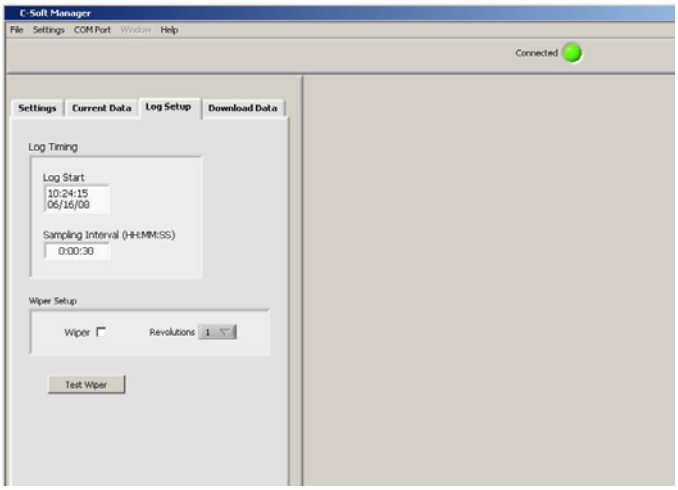
- Select "Save Calibration" button.
- Future depth values will be scaled to the offset.

3.2.3 Log Setup Screen

The Log Setup screen allows the user to configure a logging session, activate the mechanical wiper, and enable logging. C3 Submersible Fluorometer memory allows for a maximum of 480,000 data points.

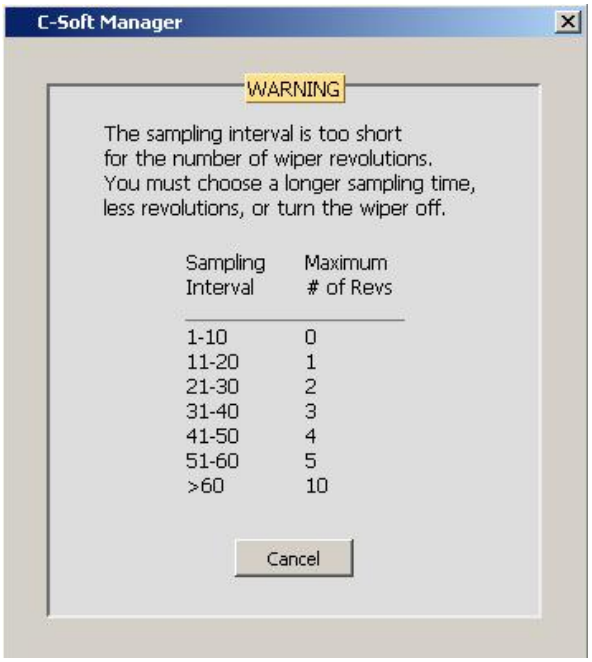
Enter a log start and stop date (MM/DD/YYYY) and time (HH:MM:SS).

- Enter the sampling interval (HH:MM:SS). The minimum sampling interval is 1 second.



- If the mechanical wiper is installed click the “Wiper” box to enable.
- Select the number of revolutions from the dropdown box (1-10 revolutions). All sensors will be wiped at specified revolutions before each measurement.
  Note: If users select revolutions that exceed sampling interval a warning message will be displayed

  Note: Do not click the Wiper Box to enable wiper if the wiper is not installed



- After log set up is complete select “Enable Datalog”.
- A prompt will appear asking if user is ready to enable logging, select “Yes”.
  Note: If data logging is set to start immediately, there will be a minimum delay of 100 seconds before startup after power has been supplied

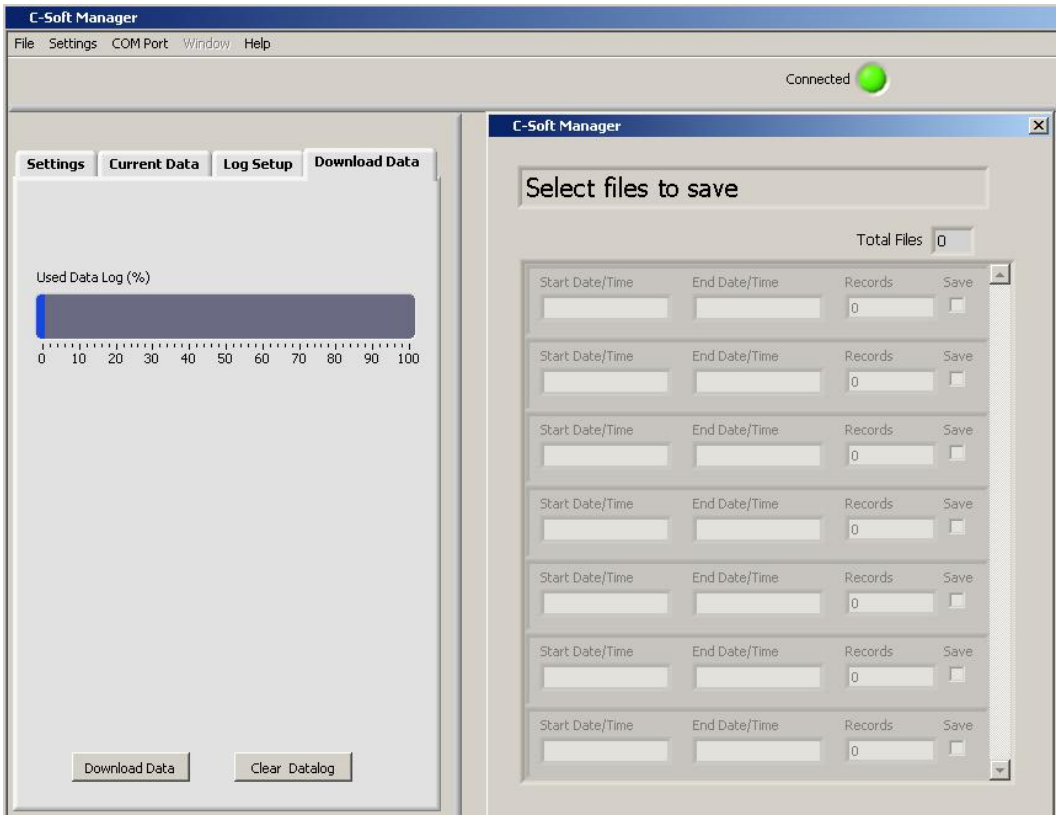
WARNING! As soon as data logging is enabled users will be unable to conduct calibrations or view current data. This should be the last step before the C3 Submersible Fluorometer is deployed.

3.2.4 Downloading and File Management

Users can download new and exisiting files saved in the C3 Submersible Fluorometer memory.

- Select Download Data.
  Note: This step may take up to 10 minutes depending on file size
- When download has completed choose the files to be saved by clicking the “Save” box.
- Users will be prompted to choose a folder location where data will be saved.

- Files will be saved in .csv format.
- Select “Clear Datalog” to erase existing data from memory.
- Users are able to view data in the “Current Data” window if desired.



3.2.5 Integrating the C3 Submersible Fluorometer to a Third Party Platform

The C3 Submersible Fluorometer can be digitally integrated into a third party platform including a CTD or datalogger.

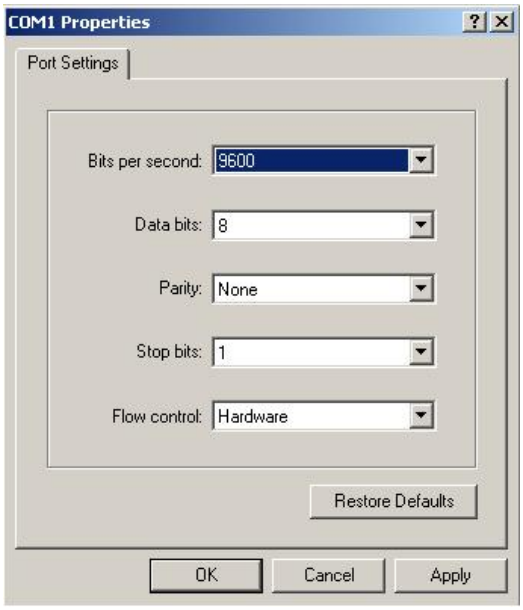
After the instrument has been calibrated proceed to Log Setup (Section 3.2.3). After the session has been initiated the C-Soft software will automatically shut down. Users should communicate with the C3 Submersible Fluorometer via HyperTerminal serial terminal emulator.

1. On an MS Windows computer, open the HyperTerminal program (Start→All Programs→Accessories→Communications→ HyperTerminal)

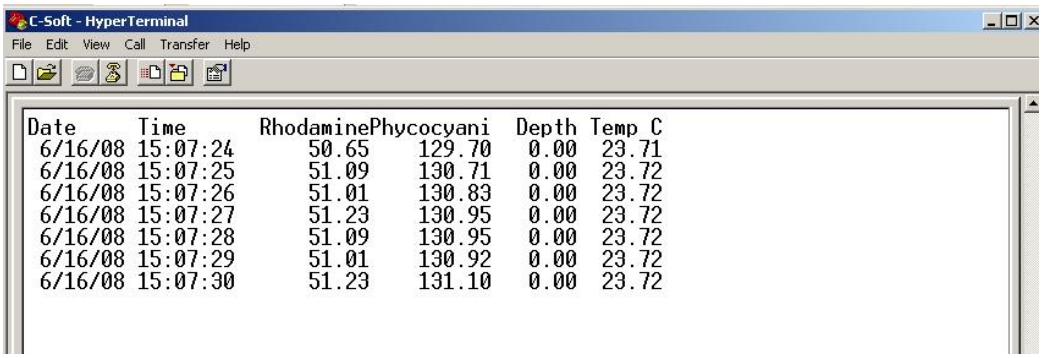
2. The **Connection Description** screen will appear. Name the connection description. This file will save the communication parameters for the C3 Submersible Fluorometer and can be used in the future to establish communication with the instrument quickly.

3. The **Connect To** screen will appear. Choose the appropriate communications port in the **Connect Using** window. Click **OK**.

4. The **Port Settings** screen will appear. Choose the following port settings;
   Bits per second: 9600
   Data bits: 8
   Parity: None
   Stop bits: 1
   Flow control: Hardware
   Click **Apply** and **OK**

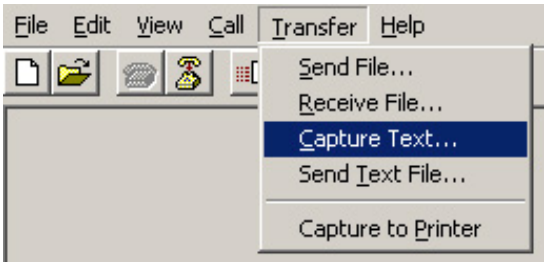


5. Next, the **HyperTerminal** window will appear. You are now ready to connect the C3 Submersible Fluorometer to a power source. Once power is applied, the initial C3 Submersible Fluorometer screen will appear in the HyperTerminal window after 1 ½ minutes or at the predetermined log start time.

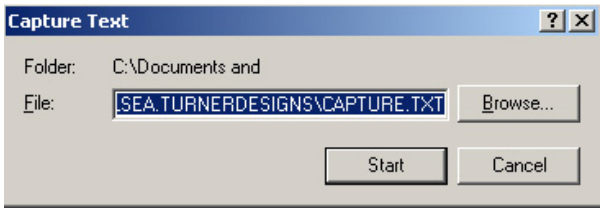


Most applications will integrate streaming data. Below are instructions on how to save streaming data on a PC.

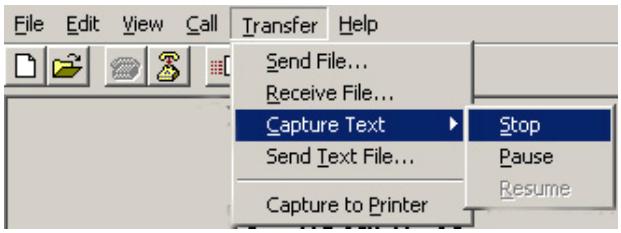
To save data:
1. On the HyperTerminal window toolbar, choose Transfer and then Capture Text



2. When you click on Capture Text, a window will appear asking you to name and save the .txt file to the location of interest.



3. Once you are finished, you will need to return to the HyperTerminal window toolbar, choose **Transfer** and then **Capture Text** and select **Stop.** This will close the **.txt** file.



4. Now open MS Excel or other data analysis software and open the **.txt** file that you created in HyperTerminal. Choose ALL FILES from the file type field.

4.0 Deployment & Accessories

4.1 Connecting the Submersible Battery Pack

The Battery Pack comes with a charger. There is also an optional 12-inch cable (P/N: 105-2590) that is not needed if the optional battery bracket is installed. The battery is fully charged after 8 hours.

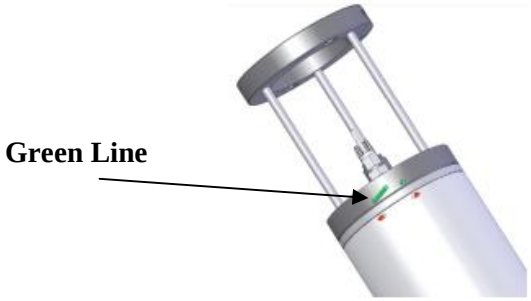
- After data logging is enabled attach the battery to the C3 Submersible Fluorometer with the 12-inch cable or via battery bracket.
- Secure C3 Submersible Fluorometer and battery to a fixed structure.
- Data logging will begin at specified time and date.

Note: The battery will allow for over 85 days of logging at 15-minute intervals.

4.2 Battery Bracket Installation

The optional battery bracket was designed for easy self-contained compact deployment of the C3 Submersible Fluorometer.

1. Align the stainless steel pin on the battery bracket with the associated pinhole on the C3 Submersible Fluorometer.
2. Apply pressure until the battery bracket is firmly attached the C3.
3. Thread the green plastic support line through the holes on the side of the bracket.



4. The line should be flush with the ends of the support line holes on the bracket.
5. Slip the battery through the bracket and line up the 8-pin end of the battery with the port on the C3.
6. Apply pressure until the battery is firmly attached to the C3.
7. An Allen wrench can be used to tighten the bracket at the end of the battery.

4.3 Installing the Mechanical Wiper

The C3 Submersible Fluorometer's mechanical wiper is an optional accessory that minimizes bio fouling during extended deployments. The wiper uses non-destructive brush material to clean the optics allowing for accurate readings under extreme bio fouling conditions. Users are able to select the number of wiper rotations before sampling.
Note: The wiper motor can not be retrofitted to a C3 Submersible Fluorometer. The wiper motor must be ordered with instrument and factory installed at the time of purchase.

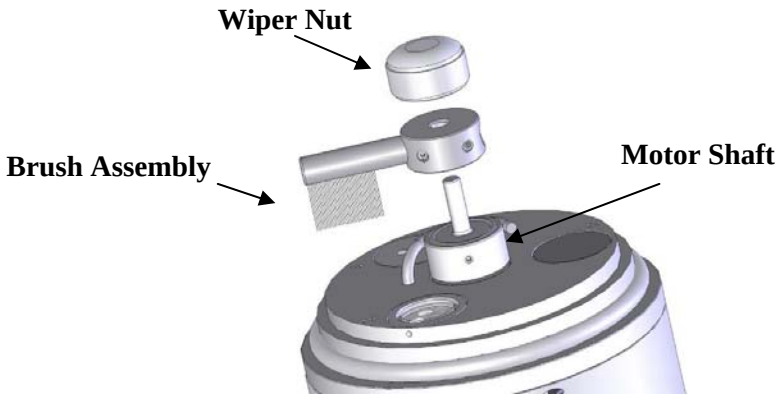
Benefits

- Limits debris and bio fouling organisms from interfering with readings
- Easy brush replacement design
- Adjustable wiping revolutions

Installation

1. Place the brush assembly on the motor shaft of the C3 Submersible.
2. Center the wiper brush between two of the sensors.
3. Hand tighten the wiper nut clockwise to lock the brush assembly into position. Make sure not to over tighten.
4. Open the C-Soft Windows software (See section 3.0).

In the Log Setup screen click the "Test Wiper" button to **home** the wiper motor.
The mechanical wiper will make 1-2 revolutions setting the wiper motor shaft to its home position.



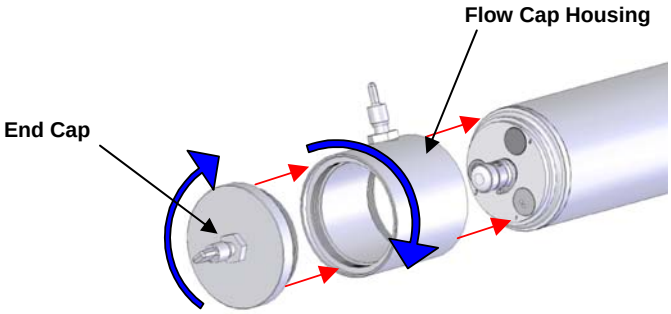
5. Readjust the wiper brush position by loosening the wiper nut and manually rotating the wiper if necessary.
6. Tighten the wiper nut once the wiper brush repositioned.
7. Click the "Test Wiper" button to ensure wiper will home to that position.

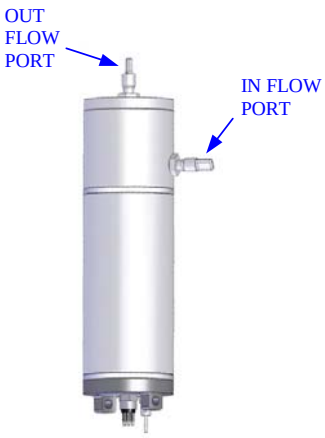
4.4 Installing the Flow Cap

The Flow Cap for the C3 Submersible Fluorometer was designed to enable flow through mode and can be configured with other instruments with online flow through systems. The flow cap eliminates the possibility of ambient light interference and it can be used as a calibration cup for holding standard solutions when calibrating optical sensors. The flow cap also offers protection for the optical head during deployment or transportation and will help limit bio fouling. The Flow Cap can be installed on C3 Submersible Fluorometers with or without the mechanical wiper.

Installation

1. Turning clockwise, fully hand tighten the Flow Cap housing onto the C3 Submersible Fluorometer. The port located on the Flow Cap Housing should be closest to the optical head.
2. Turning clockwise, hand tighten the End Cap onto the Flow Cap Housing until it is fully tightened.

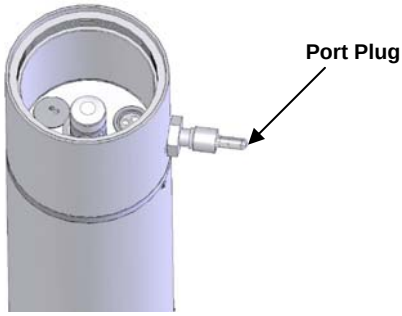




Note: Turner Designs recommends the following configuration for the Flow Cap. For optimal performance, position the instrument vertically with the sensor head facing upward to expel any air from the system that might cause skewed readings.

Using the Flow Cap as a Calibration Cup

1. Remove the End Cap.
2. Use a port plug to plug the port located on the Flow Cap Housing.
3. Position the unit vertically with optical head facing upward (see diagram to the right).
4. In this position the Flow Cap Housing can be used to hold standard solutions for calibrating the C3 sensors.



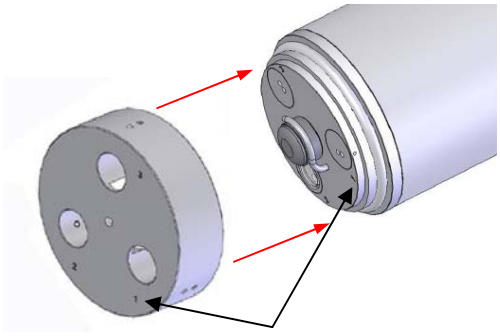
4.5 Using the Secondary Solid Standard

The C3 Secondary Solid Standard is designed with a specific filter that provides a stable fluorescent signal that is adjustable. The solid standard can be used in place of a primary liquid standard once a correlation between a primary standard and the solid standard has

been established. It can also be used to check the stability of the instrument, and/or check for loss in sensitivity resulting from the growth of bio-fouling organisms on the sensor optics.

Installing the Secondary Standard

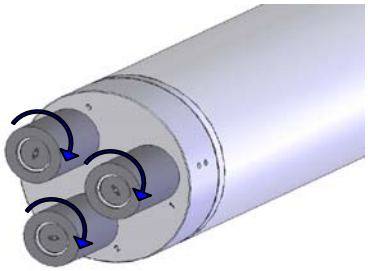
1. Align Solid Standard Cap numbers with C3 sensor head numbers, then snap the Solid Standard Cap in place.



Solid Standard Cap

2. Slightly rotate cap until it is set into position. ***Note: you will feel/hear a click when cap is positioned.***

3. Insert up to 3 Solid Standards into the desired optical port position of the Solid Standard Cap.



4. Rotate the Solid Standards until they set into position.

5. If there is a mechanical wiper it must be removed before using the solid standard.

Individual Solid Standard

1. To adjust each solid standard, use the green screwdriver to unscrew the locking nut as far as it will go.

2. To change the signal level, insert the blade of the green screwdriver through the hole in the locking nut. Rotate it until it engages with the adjustment screw that is beneath the locking nut.

3. The signal can be adjusted to desired reading by turning the screw. Turning the screw clockwise will move the filter closer to the optical head causing an increase in signal.

4. Once the desired reading has been obtained, the locking nut should be screwed down so that the adjustment screw is held firmly in place.

Note: The response of every solid standard is unique. Therefore, a new correlation must be determined for every sensor. Use the "ID" space on the Secondary Standard's label to uniquely identify each Standard.

Important Note Regarding Use of Fluorescein Sensor

The Fluorescein sensor, when enabled with other sensors for simultaneous data collection, will produce unreliable results. Therefore, the Fluorescein sensor should not be used for simultaneous data collection with other sensors.

5.0 Maintenance and Warranty

5.1 Maintenance

5.1.1 Rinsing

The C3 Submersible Fluorometer should be rinsed or soaked in freshwater following each deployment.

5.1.2 Care for the bulkhead connector

Install the 8-pin female end plug to the bulkhead between uses. A light coat of Silicone based grease should be used on the rubber of the male pins of the bulkhead to aid in sealing.

5.2 Warranty Service

To obtain service during the warranty period, the owner shall take the following steps:

1. Write, email or call the Turner Designs Technical Support department and describe as precisely as possible the nature of the problem.

   Phone: 1 (877) 316-8049

   Email: support@turnerdesigns.com

2. Carry out any adjustments or tests as suggested by the Technical Support Department.

3. If proper performance is not obtained you will be issued a Return Authorization number (RMA) to reference. Package the unit and ship the instrument, prepaid, to Turner Designs. If the failure is covered under the warranty terms, the instrument will be repaired and returned free of charge, for all customers in the contiguous continental United States.

   For customers outside of the contiguous continental United States who have purchased equipment from one of our authorized distributors, contact the distributor. If you have purchased directly, contact us. We will repair the instrument at no charge. Charges for shipment, documentation, etc. will be billed at cost.
   Note: The instrument or accessories should not be returned without first contacting Turner Designs.

   Prior correspondence including an RMA number are needed:

   A. To ensure that the problem is not a trivial one, easily handled in your laboratory, with consequent savings to everyone.

   B. To specifically determine the nature of the problem so repair can be rapid, with particular attention paid to the defect you have noted.

5.2.1 Out-of-Warranty Service

Follow steps for Warranty Service as listed above. If our Technical Support department can assist you by phone or correspondence, we will be glad to, at no charge.

Repair service will be billed on a fixed price basis, plus any applicable duties and/or taxes. Shipment to Turner Designs should be prepaid. Your bill will include return shipment freight charges.

Address for Shipment:
Turner Designs, Inc.
845 W. Maude Ave.
Sunnyvale, CA 94085

Appendix A. C3 Submersible Fluorometer Specifications

C3 Submersible Fluorometer	
Weight in Air	1.64 kg (3.6 lbs)
Length	23 cm (9.1 in.)
Diameter	10 cm (3.9 in.)
Material	Delrin
Temperature	-2 to 50 degrees C
Temperature Accuracy	0.5 degree C
Temperature Resolution	0.1
Depth	600 meters
External Power	8 to 30 VDC
Signal Output	Digital ASCII
Interface	RS232 (USB Adaptor)
Maximum Sampling Rate	1 second
Nonvolatile Memory	480,000 Data points
Power Draw - Sleep Mode	< 1mAmp
Power Draw - Max	1 Watt

Submersible Battery Pack	
Battery	Lithium-Ion
Weight in Air	0.75 kg (1.65 lbs)
Weight in Water	0.22 kg (0.50 lbs)
Length	13.46 cm (5.3 in.)
Diameter	7 cm (2.75 in.)
Material	Delrin
Temperature	-2 to 50 degrees C
Depth	600 meters
Capacity	2,200 mAH
Voltage Output	14.8V nominal
Protection Features	Protects against: overcharge, overheating, short-circuiting

Appendix B. C3 Submersible Fluorometer Operating Instructions

Identification

Sensors are labeled 1, 2 or 3 adjacent to the optics on the face of the instrument. Each channel is factory set in the C3 Windows Software.

**Note: The channel labels can be modified in the C-Soft software.*

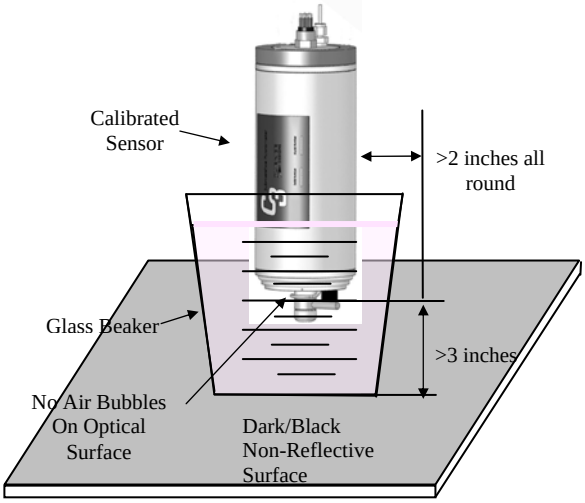
Light Emitting Diode Color Table

Application	Light Source Color
Chlorophyll a	Blue
Turbidity	Infra Red (No Color)
Phycocyanin	Yellow
Phycoerythrin	Green
Fluorescein	Blue
Rhodamine	Green
CDOM	Ultra Violet (No Color)*
Crude Oil	Ultra Violet (No Color)*
Optical Brighteners	Ultra Violet (No Color)*
Do not look directly onto the optics. Ultra Violet light can be damaging to the eyes.	

Recommended Measurement Practices

The following steps will improve the accuracy and repeatability of your measurements, especially at low concentration levels:

1. Use a **Glass Container** for your standards and/or samples. (Avoid plastic beakers – plastic may fluoresce and might interfere with measurements).
2. Place the glass beaker on a **non-reflective surface**, preferably black.
3. Ensure that the sensor is **more than 3 inches above the bottom** of the glass beaker.
4. Ensure that the sensor is in the center of the glass beaker, and has **more than 2 inches clearance** between the sensor and the inside surface of the beaker.
5. Check that the optical surface of the sensor is **free of air bubbles**.

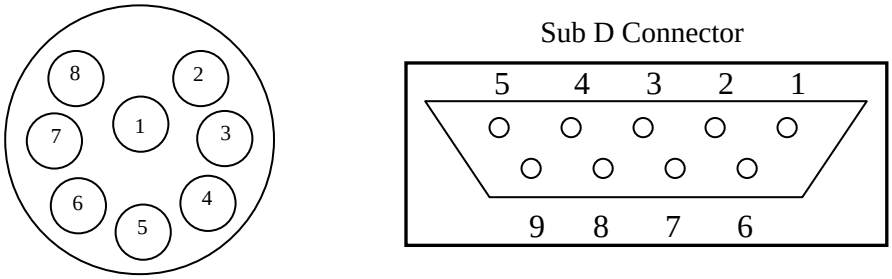


Appendix C. C3 Submersible Fluorometer Wiring Guide

The C3 Submersible Fluorometer outputs digital data in two formats: 1) Digital data that can only be read by C3 Submersible Fluorometer's software, 2) Digital data in ASCII format that can be read by larger multi parameter systems such as CTD's.

An 8-pin impulse cable provides two end connections: 1) a 9-pin RS232 serial port for connection to a PC or laptop computer and 2) a 12V port for supplying power to the unit.

C3 Submersible Fluorometer bulkhead and serial port connectors



Wire Guide

Pin Out	*Color*	*C3 Function*	*Corresponding Sub D Connector Pin*
1	Black	V BATT (+)	Power Connector Cable–Center Pin (+)
2	White	V BATT (-)	Power Connector Cable–Housing (-)
3	Red	GND	PIN 5
4	Green	RX	PIN 2
5	Blue	TX	PIN 3
6	Brown	DTR & DSR	PIN 4, 6
7	Yellow	RTS & CTS	PIN 7, 8
8	Orange	N/A	N/A

**Power ground and V Batt (-) are not common.*

Appendix D. Secondary Standard - *In Vivo* Chlorophyll *a* & Rhodamine Dye Applications

Use of the Solid Secondary Standard for *In Vivo* Chlorophyll Applications:

1. To establish a correlation between a known chlorophyll *a* concentration and the fluorescence output voltage immerse the sensor in a sample containing algae and note sensor value.
2. Dry off the optics on the C3 Submersible Fluorometer, attach the Solid Standard, and adjust the Solid Standard to produce the same output value from the sensor as in step 1, (turning the Secondary Standard adjustment screw clockwise produces a lower signal).
3. Next, perform a chlorophyll extraction using a Laboratory Fluorometer, Spectrophotometer or HPLC to determine the actual chlorophyll *a* concentration in the sample.
4. Now, at any time, the Solid Standard can be used to check/establish a new correlation between a known equivalent concentration and the current C3 chlorophyll a sensor output voltage.

Use of the Solid Secondary Standard for Dye Tracing Applications:

The Solid Secondary Standard accessory can also be used to check the fluorescence stability for making dye concentration measurements. If necessary, the Solid Standard can be used to establish a new correlation value without the need to use a calibration solution each time.

1. To use the Solid Standard to establish a correlation between a known dye concentration and the fluorescence output value, immerse the sensor in a dye solution of known concentration, say 50 ppb, and note the sensor value.
2. Dry off the optical end of the C3 Submersible Fluorometer, attach the Solid, and adjust to produce the same value from the sensor as in step 1, (turning the secondary standard adjustment screw clockwise produces a lower output).
3. Now, at any time, the Secondary Standard can be used to check/establish a new correlation between a known equivalent concentration and the current C3 rhodamine sensor output value.
4. Comprehensive information on dye trace measurements can be found at the following Turner Designs URL:
   http://www.turnerdesigns.com/t2/doc/appnotes/main.html#fluorescent

   Note: There are no Solid Secondary Standards available for the Turbidity or UV optical sensors.

Linear Range and Quenching

The linear range is the concentration range in which the C3 sensor output is directly proportional to the concentration of the signal. The linear range begins with the smallest detectable concentration and spans to an upper limit (concentration) that is dependent upon the properties of the material, filters used, and path length.

A non-linear relationship is seen at very high concentrations where the signal does not increase at a constant rate in comparison to the change in concentration, see figure below.

At even higher concentrations, the signal will decrease even though the sample concentrations are continuing to increase. This effect is known as “signal quenching”.

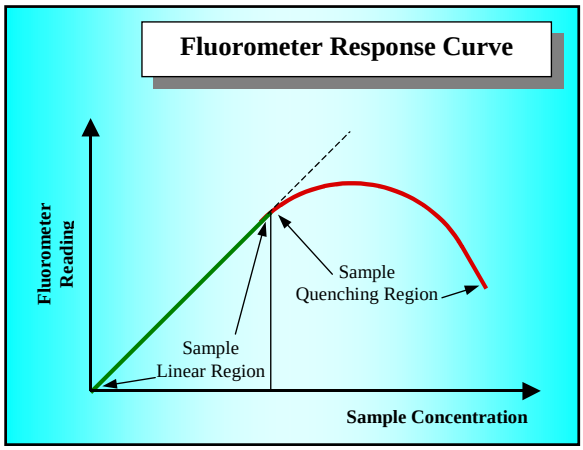
Linearity can be checked by diluting a sample 1:1 or some other convenient ratio. If the sample is still in the linear range, the reading will decrease in direct proportion to the dilution. If the reading does not decrease in direct proportion to the dilution or if the reading increases, the sample is beyond the linear range.

Temperature Considerations

Fluorescence is temperature sensitive. As the temperature of the sample increases, the fluorescence decreases. For greatest accuracy, record the sample temperature and correct the sensor output for changes in temperature.

For further information on how temperature, light, water quality and the physiological state of the algal cells can all affect the measurement of chlorophyll *a*, please refer to the application section of Turner Designs’ web site at the following URL:

http://www.turnerdesigns.com/esupport/understanding.html



Graph showing Linear and Quenching Regions of the sample’s response