

CyanoFluor™

Handheld HAB Indicator

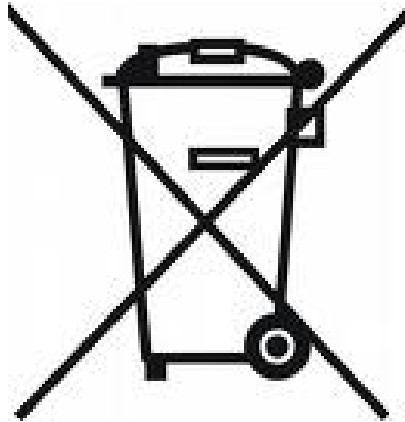


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WASTE ELECTRICAL AND ELECTRONIC EQUIPMENT (WEEE) DIRECTIVE

Turner Designs is in the business of designing and selling products that benefit the well-being of our environment. Accordingly, we are concerned with preserving the surroundings wherever our instruments are used and happy to work with customers by complying with the WEEE Directive to reduce the environmental impact resulting from the use of our products.

WEEE Return Process:

To arrange the return of an end-of-life product, proceed as follows:

If you purchased your instrument through a Turner Designs Distributor please contact your local representative. They will instruct you where to return the end-of-life product.

If you purchased your instrument directly from Turner Designs please contact Turner Designs Customer Service

By Phone: 1-408-212-4041 or Toll Free: (877) 316.8049

By Email: Customer Service at support@turnerdesigns.com

Turner Designs will provide a WEEE RMA Number, a Shipping Account Number, and a Ship to Address. Package and ship the product back to Turner Designs.

The product will be dealt with per Turner Designs' end-of-life recycling program in an environmentally friendly way.

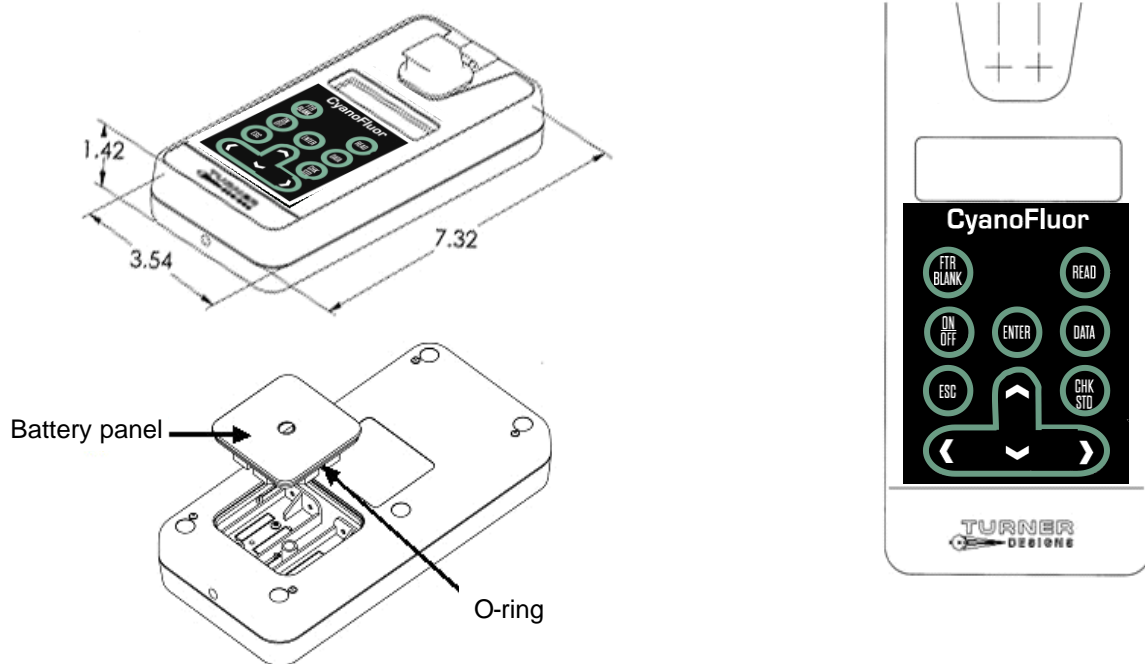
1 Introduction

1.1 Description

CyanoFluor™ Handheld HAB Indicator is configured for *in vivo* fluorescence detection from chlorophyll (Chl) and phycocyanin (PC) pigments in natural water samples. CyanoFluor calculates and reports a PC to Chl ratio that estimates the relative abundance of cyanobacteria, algae that are known to cause Harmful Algal Blooms (HABs). Monitoring PC to Chl ratios over time will indicate whether environmental conditions are changing, favoring cyanobacterial production, potentially leading to cyanobacterial blooms. This monitoring effort can be used to predict the onset of HABs and alert users so the proper protocols can be implemented to prevent or reduce harmful bloom effects. CyanoFluor provides raw fluorescence readings that can also be used for determining algal abundances and reporting µg/L chlorophyll estimates. CyanoFluor is factory calibrated; no calibration standards or tools are required or necessary to calibrate this instrument. Solid state optoelectronics ensure the instrument will remain stable over time.

1.2 Quick View Diagrams

CyanoFluor uses four standard or rechargeable AAA user-replaceable batteries.



1.3 Inspection

Upon receiving your instrument, please inspect everything carefully and make sure all accessories listed below are present. CyanoFluor comes in either a Field Package or Lab Package.

The Field Package 8700-000 ships in a carrying case including:

- CyanoFluor inside a storage pouch (batteries are installed)
- Calibration Check Standard
- 2 - Glass 10mm x 10mm Square Cuvettes
- 10 – Filter Capsules
- 60cc Plastic Syringe
- Data Download Cable
- Kim Wipes
- Laminated Quick Start Guide (QSG)
- USB Flash Drive with User's Manual, QSG, and Download Utilities Software

The Lab Package 8700-001 includes:

- CyanoFluor inside a storage pouch (batteries are installed)
- 1 - Glass 10mm x 10mm Square Cuvette
- 10 – Filter Capsules
- Data Download cable
- Laminated Quick Start Guide (QSG)
- USB Flash Drive with User's Manual, QSG, and Download Utilities Software

Replacement Accessories available for purchase:

- 10 each Filter Capsules P/N 8700-560
- 1 each Glass 10mm x 10mm Square Cuvette P/N 7000-955
- CyanoFluor Calibration Check Standard P/N 8700-951
- Data Download Cable P/N 021-0850
- Storage Pouch P/N 142-8000

1.4 General Information, Precautions, and Cleaning

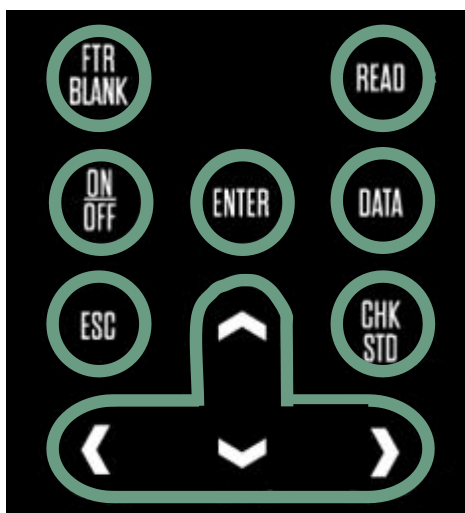
- **CyanoFluor is factory calibrated; no calibration is necessary.**
- The plastic battery tab at the back of the CyanoFluor must be removed before use. Grasp it firmly and pull gently, removing it to activate the batteries.



- Use caution around solvents, they will degrade the plastic case of the CyanoFluor.
- If a sample is accidentally spilled inside the sample compartment, invert the CyanoFluor to drain out the excess liquid. Then wipe the inside area dry with a clean soft towel or tissue followed by a quick wipe using non-abrasive material, such as Kim Wipes.
- If extra cleaning is needed, use a mild detergent to dampen a towel for cleaning.
- Although the CyanoFluor floats, do not submerge it in water.
- Do not expose the CyanoFluor to temperatures outside the specified range of 5 to 40 °C or damage may occur to the unit that will not be covered under warranty.

2 Instrument Operation and Calibration Check

2.1 Button Identification and Function



FTR BLANK – is used to correct for interference from Dissolved Organic Materials (DOM) in systems that have a high DOM concentration. See section 2.4 for details.

READ – is used to measure a sample; procedure detailed in section 2.5.

ON/OFF – turns the instrument ON or OFF.

ENTER – allows users to proceed to the next screen/menu.

DATA – when the DATA button is pressed users can choose between either sending/clearing data or setting date/time. Use the $\uparrow\downarrow$ keys to choose between the two options; a marker will indicate the selection made.

If SEND/CLEAR DATA is selected, press ENTER and a submenu will display. Users can choose, using $\uparrow\downarrow$ keys, to either send data to a connected computer or clear logged data; a marker will indicate the selection made.

- If *SEND DATA* is selected and ENTER is pressed, the instrument will send all logged data out as an ASCII file - see section 2.8.
- If *CLEAR DATA* is selected, the following message will display; “ENTER TO CONFIRM CLEAR DATA”; when ENTER is pressed all logged data will be deleted.

If SET DATE/TIME is selected, press ENTER and date and time will display on the screen as (MM/DD/YYYY) and (hh:mm). Use the $\uparrow\downarrow$ keys to select either parameter; a marker will indicate selection.

- If MM/DD/YYYY is selected, press ENTER and you will be allowed to adjust the month, date, and year using the $\uparrow\downarrow\leftarrow\rightarrow$ keys. Press ENTER to save the set date.

If ESC is pressed prior to saving the set date, the screen is exited and nothing is saved. Date is logged per sample.

- If *hh:mm* is selected, press ENTER and you will be allowed to adjust the hour and minutes using the $\uparrow\downarrow\leftarrow\rightarrow$ keys. Press ENTER to save the set time. If ESC is pressed prior to saving the set time, the screen is exited and nothing is saved. Time is logged per sample.

ESC – exits to the previous or home screen.

CHK STD – is used to determine calibration/instrument stability. “Cal. Check Passed” or “Cal. Check Failed” will be displayed after measuring the CyanoFluor Calibration Check Standard, using the CHK STD button, indicating whether the CyanoFluor’s calibration has changed. The Cal Check Standard P/N 8700-951 is required for this check, see section 2.3 for details.

2.2 Instrument Power Up

Press the ON/OFF button to power on the CyanoFluor. After a 5 second warm up, the CyanoFluor is ready for operation. Pressing the ON/OFF button again will turn the unit off or if left idle on the HOME screen for 3 minutes the unit will automatically turn off to save battery power.

Note: CyanoFluor will NOT automatically turn off if left idle while running a sample or if on any screen other than the Home Screen.

HOME SCREEN

The home screen displays the unit’s current date, time, CyanoFluor firmware version and remaining battery power as a percentage. These parameters will be displayed after powering the instrument on and warm up has completed as well as every time the ESC button is pressed to exit to the home screen.

MM/DD/YYYY	HH:MM
FW1.00	Batt: 100%

Battery power is checked whenever users turn the instrument on using the ON/OFF button. After the 5 second countdown,

- If the battery power is < 20%, the following warning message will display “**Battery <20%! Press <ENTER>**” you can continue making measurements after pressing ENTER, but it is recommended you replace the batteries as soon as possible.
- If the battery power is < 10%, the following message will display “**Battery Low Pwr! Replace Battery!**” you will not be able to make any measurements until batteries are replaced.

2.3 Calibration Check

CyanoFluor is factory calibrated. Users can check calibration stability using the Cal Check Standard P/N 8700-951.

Cal Check Standard

The Cal Check Standard P/N 8700-951 is a sealed glass 10x10 mm cuvette containing a specific, non-toxic fluorophore. **Do not open the Cal Check Standard.** If the seal around the Cal Check Standard's cap is broken or removed, the fluorophore contained within the cuvette may begin to degrade rendering the standard useless. Take care not to scratch the glass cuvette as that may change the standard's response resulting in "Failed" for the Calibration Check. Avoid extreme temperatures as the freezing or heating of the Cal Check Standard may change the standard's response resulting in "Failed" for the Calibration Check. The Cal Check Standard should be stored at room temperature (21 – 27 °C).

Procedure for Calibration Check

To check calibration stability:

NOTE: CyanoFluor should be on a flat surface when calibrating or reading standards and samples.

- 1) Carefully remove the Cal Check Standard from its packaging and inspect the standard. If the glass surfaces are not clean, use a Kim Wipe to wipe the sides of the cuvette before inserting it into the sample compartment; wipe away any moisture and/or fingerprints.
- 2) Insert the standard into the CyanoFluor's sample compartment and leave the CyanoFluor lid open.
- 3) Press the ON/OFF button to turn the instrument on and wait for the 5-second countdown.
- 4) Press the CHK STD button; results will be displayed as "Passed" or "Failed".
- 5) If results displayed "Passed", the instrument's calibration has not changed, remove the Cal Check Standard and return it to its original packaging to avoid damaging or breaking the cuvette.
- 6) If results displayed "Failed", you may need to clean the sample well.
 - i. Wipe the windows in the sample compartment with a clean dry end of a Q-tip to lightly wipe away any debris or residue from moisture that may be interfering with the measurement.
 - ii. Rerun the Calibration Check Standard 5 times while on a flat surface.
 - iii. If the Calibration Check passes 3 out of 5 times, the instrument is ready for use.
 - iv. If the Calibration Check fails 3 out of 5 times, repeat the cleaning procedure.
 - v. Rerun the Calibration Check Standard 5 times while on a flat surface.
 - vi. If the Calibration Check passes 3 out of 5 times, the instrument is ready for use.
 - vii. If the Calibration Check fails 3 out of 5 times, contact Turner Designs.
- 7) Remove the Cal Check Standard from the sample compartment and return it to its original packaging to avoid damaging or breaking the cuvette.

2.4 Filtrate Blank

The Filtrate Blank (FTR Blank) is used to correct for interference from Dissolved Organic Materials (DOM) in systems that have a high DOM concentration. It is recommended that users have FTR Blank activated at all times to correct for background fluorescence, even in low DOM environments. To activate the FTR Blank correction:

- 1) Press the ON/OFF button to turn the instrument ON and wait for the 5 second countdown.
- 2) Press the FTR Blank button, then select <ON> using the left arrow key.
- 3) Press ENTER and you'll return to the HOME screen.
- 4) With the FTR Blank ON, you will be prompted to run a filtrate after every measurement to correct for interference from DOM.

To deactivate the FTR Blank simply run through steps 1 – 3, but select <OFF> and press ENTER. When FTR Blank is OFF, you will not be prompted to run a filtrate blank after each measurement and the PC and CHL Filtrate Blank will be logged as "NA".

2.5 Sample Analysis Procedure

To make a measurement:

- 1) Take up a minimum of 40ccs of sample using a 60cc syringe.
- 2) Rinse the glass cuvette at least three times with the sample in the syringe.
- 3) Fill the rinsed cuvette $\frac{3}{4}$ full with sample from the syringe.
- 4) Use a Kim Wipe to wipe the sides of the cuvette to wipe away any moisture and/or fingerprints.
- 5) Press the ON/OFF button to turn the instrument on and wait for the 5-second countdown.
- 6) Press the READ button; you will be prompted to insert your sample and press read again.
- 7) Making sure not to spill any sample, insert the cuvette into the sample compartment
- 8) Press READ; the screen will display Measuring Please Wait...

NOTE: If FTR BLANK is ON, you will be prompted to insert a filtrate blank. See section 2.6 "Filtering Samples for DOM Correction", step 1.

- 9) Results will be displayed as:

Time (24 hour clock; HH:MM)	→	PC:CHL	1.5211	← PC to Chl Ratio
		20:39	#0001	← Sample Number (0000 – 1000)

- 10) Press the down arrow key to display Raw Fluorescence Values (RFU) for both PC & CHL channels:

PC(RFU)	125.00	← PC Channel Raw Sample RFU
CHL(RFU)	82.18	← CHL Channel Raw Sample RFU

- 11) Press the down arrow key again to display FTR Blank values for both PC & CHL channels if FTR Blank was ON and a filtrate blank was measured

PBLK FILT:	25.00	←	PC Channel Filtrate Blank RFU
CBLK FILT:	12.00	←	CHL Channel Filtrate Blank RFU

2.6 Filtering Samples for DOM Correction

NOTE: See Sample Analysis Procedure section 2.5 before proceeding with Filtering Samples for DOM Correction.

If FTR BLANK is ON, follow the procedure below to correct for background fluorescence from interference materials such as DOM.

- 1) Discard the sample in the cuvette.
- 2) Locate the filter capsules.
- 3) Attach one of the filter capsules to the end of the 60cc syringe with the sample.
- 4) Rinse the glass cuvette at least three times with the filtrate.
- 5) Fill the rinsed cuvette $\frac{3}{4}$ full with filtrate.
- 6) Use a Kim Wipe to wipe the sides of the cuvette to wipe away any moisture and/or fingerprints.
- 7) Making sure not to spill any filtrate, insert the cuvette into the sample compartment.
- 8) Press READ; the screen will display Measuring Please Wait...

NOTE: See Sample Analysis Procedure section 2.5 step 9 for result displays.

2.7 Viewing Data

Data can be viewed on the CyanoFluor's display as well as downloaded to a computer. To view data from the HOME screen, use the ←→ arrow keys to scroll to a desired sample, sample number will be displayed in the bottom right corner of the screen. Press the down arrow key to view raw fluorescence data for that sample.

2.8 Downloading Data

The following parameters are recorded in the order specified below for a single measurement and can be downloaded to a computer after they have been logged:

Date	MM/DD/YYYY
Time	hh:mm:ss
Serial Number	87 XXXXX
Sample Number	1 - 1000
PC:CHL Ratio	0 -50, "ND"
PC RFU	0 - 160,000, "ND"
CHL RFU	0 - 160,000, "ND"
PC Filtrate Blank	0 - 50,000, "NA"
CHL Filtrate Blank	0 - 50,000, "NA"

Note: "ND" indicates a Non Detect, meaning the sample is well below the lower detection limit of the instrument. "NA" means not applicable for that measurement.

To download data from the CyanoFluor:

- 1) Install the Download Utilities Software included on the USB thumb drive.
- 2) Plug the download data cable's stereo jack in the CyanoFluor data port.
- 3) Plug the download data cable's USB plug into your computer.
- 4) Wait for the download data cable's driver to automatically install.
- 5) Begin the Download Utilities Software.
- 6) Manually enter the COM port assigned to the download data cable. The COM port setting can be viewed and/or changed in the computer's Device Manager window.
- 7) Click the Browse button and select or create a folder in a desired directory and enter a filename.
- 8) Click Save and then click the Start button on the software's screen.
- 9) Press the ON/OFF button to turn the CyanoFluor on and wait for the 5-second countdown.
- 10) Press the CyanoFluor keypad's DATA button; use the $\uparrow\downarrow$ arrow keys to select SEND/CLEAR DATA.
- 11) Press ENTER and use the $\uparrow\downarrow$ arrow keys to select SEND DATA.
- 12) Press ENTER and wait for data to be sent.
- 13) The Download Utilities Software will indicate when download has completed, then click Stop.

It is recommended that you confirm data are saved to your computer by navigating to the saved file and opening the file to view data before clearing data from the CyanoFluor.

3 Sample Analysis Guidelines

- Take care not to spill samples into the sample chamber. Wipe up any spills promptly.
- The cuvette **MUST BE DRY** on the outside when taking readings. Any moisture or condensation on the outside of the cuvette can affect the reading.
- Fill the cuvette with at least 3.5 mL volume (~ $\frac{3}{4}$ full).
- Use the same cuvette for your samples, it is very important that you thoroughly rinse the cuvette between samples. CyanoFluor is very sensitive, therefore cross contamination between subsequent samples will skew results. Three rinses with the sample intended for measurement will help flush out any residual and decrease the chance for cross contamination.
- Do not use a cuvette cap as it may cause the cuvette to not seat properly in the sample compartment.
- When handling the cuvette, hold it near the top.
- Any bubbles in the sample will affect the readings. Take care not to introduce bubbles into samples. Remove any bubbles by lightly tapping with your finger on the outside cuvette wall or cover the top of the cuvette and tilt the sample to help dissipate bubbles.
- Wait at least 3 seconds between consecutive measurements to allow the optics to return to normal state.
- All samples analyzed should be within the specified concentration range 0 – 100 $\mu\text{g/L}$; Samples outside of that range should be diluted with artificial seawater or source water filtrate to reduce inner filter and quenching effects.

4 Measurement Parameters

4.1 Phycocyanin (PC) to Chlorophyll (CHL) Ratio Table

PC to CHL ratios indicate what portion of the total phytoplankton population is comprised of PC-containing algae (cyanobacteria). PC changes proportionally with the abundance of cyanobacteria and can be tracked using fluorescence of PC. When cyanobacteria are not present in water the fluorescence from PC is very low yielding ratios less than 0.1; when cyanobacteria make up 50% of the total phytoplankton population the PC:CHL ratio will be 0.5 or greater. The table below is a quick guide that displays empirically determined PC:CHL ratios using lab cultures. The species of cyanobacteria used to generate these data was *Cyanothece ATCC5201*. Other algae used were *Dunaliella salina*, *Tetraselmis sp.*, and *Rhodomonas salina*. This table is simply a guide; PC:CHL ratios will vary depending on algal species, light history, physiology, etc. It is important to note and monitor the rate of change for the PC:CHL ratio over time to determine relative changes in the abundance of cyanobacteria group. As the PC:CHL ratio increases so does the chance that a harmful algal bloom will occur.

Cyanobacteria portion	PC:CHL
100% cyanobacteria	> 1.00
75% cyanobacteria	0.75
50% cyanobacteria	0.50
25% cyanobacteria	0.25
< 10% cyanobacteria	< 0.15

4.2 Turbidity Effects

Suspended sediments may decrease accuracy in measurements. Coastal marine environments typically have sediment loads between 5 and 10 NTU equivalents. High sediment loads typically around 25 NTU equivalents will have minimal to no impact on the measurements (estimates). At really high sediment loads (>>25 NTU), consider settling samples prior to measuring as you will see effects especially at low algal abundances.

Always check PC and CHL RFU values and compare with blank RFU values to determine whether there is enough response from both channels to give a good estimate. As a general rule of thumb, response for at least one channel (PC or CHL) should be more than 3x the blank RFU values.

4.3 DOM Effects

Dissolved organic materials will affect the chlorophyll channel. If working in areas where DOM is high, it is recommended that you turn on your filtrate blank (section 2.4) and use the filtrate correction method described in the Quick Start Guide or Section 2.5/2.6 of this manual. This will correct for any interference or error in estimate due to a high background fluorescence which may be caused by dissolved organic materials.

4.4 External Calibrations to Track Algal Abundance

Algal abundance, or other concentration estimates such as $\mu\text{g/L}$, can be calculated using RFU values via external calibration. All that is required for an external calibration is a standard or sample that has a known concentration and your CyanoFluor. To externally calibrate the CyanoFluor using natural water samples:

- 1) Take a grab sample from your aquatic habitat
- 2) Measure the sample using the CyanoFluor and record the RFU value for chlorophyll
- 3) Use extraction methods (EPA method 445.0) to determine the actual chlorophyll $\mu\text{g/L}$ concentration of the water sample measured
- 4) Create a conversion factor that converts RFU to $\mu\text{g/L}$

Now all chlorophyll RFU values can be converted to $\mu\text{g/L}$ concentration estimates using the conversion factor created from the grab sample analyzed.

This kind of a correlation can also be established to convert PC RFU values to part per billion (ppb) estimates using a PC extraction method or PC pigment which may be ordered from www.prozyme.com and diluted in water to specific concentrations.

5 Warranty

5.1 Terms

Turner Designs warrants the CyanoFluor Handheld HAB Indicator and accessories to be free from defects in materials and workmanship under normal use and service for a period of 12 months from the date of shipment from Turner Designs, with the following restrictions:

- Turner Designs is not responsible for replacing parts damaged by accident or neglect. Damage from corrosion is not covered. Damage caused by customer modification of the instrument is not covered.
- This warranty covers only Turner Designs products and is not extended to equipment used with our products. We are not responsible for incidental or consequential damages, except in those states where this limitation is not allowed. This warranty gives you specific legal rights and you may have other rights which vary from state to state.
- Damage incurred in shipping is not covered.

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). Package the unit, write the RMA number on the outside of the shipping carton, 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.

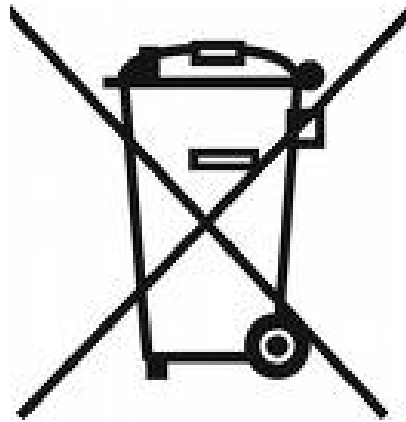
For customers outside of the contiguous continental United States who purchased equipment from one of our authorized distributors, contact the distributor. If you purchased directly, contact us. We will repair the instrument at no charge. Customer pays for shipping duties and documentation to Turner Designs. Turner Designs pays for return shipment. Custom duties, taxes and fees are the responsibility of the customer.

5.3 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
1995 N. 1st Street
San Jose, CA 95112



Appendix A: Specifications

CyanoFluor	
Sensitivity	0.3 µg/L
Linear Range	0 – 100 µg/L
Warm Up Time	5 seconds
Power	4 AAA standard or rechargeable batteries
Auto Power Off	After 3 minutes of inactivity on the HOME screen <u>only</u>
Light Source	LED
Detector	Photodiode
LCD Display	2 x 16 characters
Resolution	12 bits
Case	Meets IP 67 Standard; dustproof and waterproof
Temperature	41-104 °F; 5-40 °C
Internal Memory	1,000 records
Data Output Format	ASCII
Weight	0.87 lbs (0.4kg)
Size	1.75" x 3.5" x 7.25"(4.45cm x 8.9cm x 18.4cm)

Appendix B: Warning Messages

Memory Warning Messages

CyanoFluor's memory starts at 100% allowing for a maximum of 1,000 data points to be logged internally. If the memory is more than 90% full and the READ button is pressed, the following message will display:

"Warning: Memory > 90% Full"

Indicating that you have reached more than 90% of the total memory allowed. You will be allowed to continue sampling, but it is highly recommended you stop measuring samples at this point, download data to your PC, and clear the data log.

When memory is full, the instrument will display:

"Memory Full"

When READ is pressed you will not be allowed to analyze samples until data have been downloaded and cleared from memory.

Battery Warning Messages

Battery power is checked whenever users turn the instrument on using the ON/OFF button. After the 5 second countdown,

- If the battery power is < 20%, the following warning message will display "**Battery <20%! Press <ENTER>**" you can continue making measurements after pressing ENTER, but it is recommended you replace the batteries as soon as possible.
- If the battery power is < 10%, the following message will display "**Battery Low Pwr! Replace Battery!**" You will not be able to make any measurements until batteries are replaced.