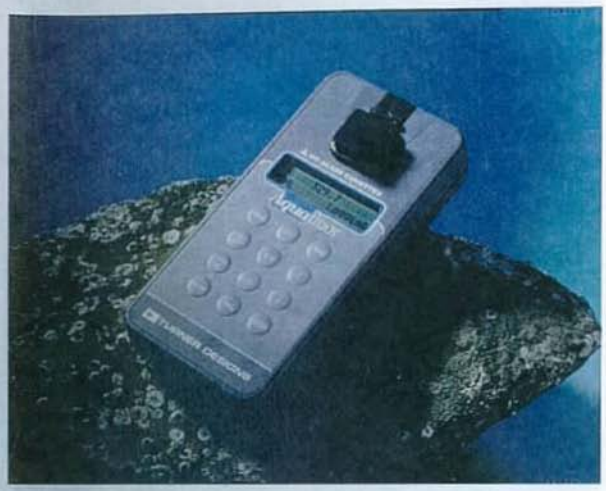
*Aqua*fluor™

Handheld Fluorometer and Turbidimeter



User's Manual

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P/N 998-0851

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1. Introduction

1.1 Description

The *Aqua*fluor™ is a dual-channel mini fluorometer designed for quick, easy and accurate fluorescence and turbidity measurements. When properly calibrated with a standard of known concentration, the *Aqua*fluor™ displays the actual concentration of the compound.

1.2 Inspection and Setup

1.2.1 Inspection

Upon receiving your instrument, please inspect everything carefully and make sure all accessories are present. All shipments include:

- The *Aqua*fluor™
- The User's Manual
- 4 AAA batteries
- 4 Polystyrene cuvettes
- Storage Pouch

1.2.2 Setup

Before the *Aqua*fluor™ can be used, the supplied batteries must be installed.

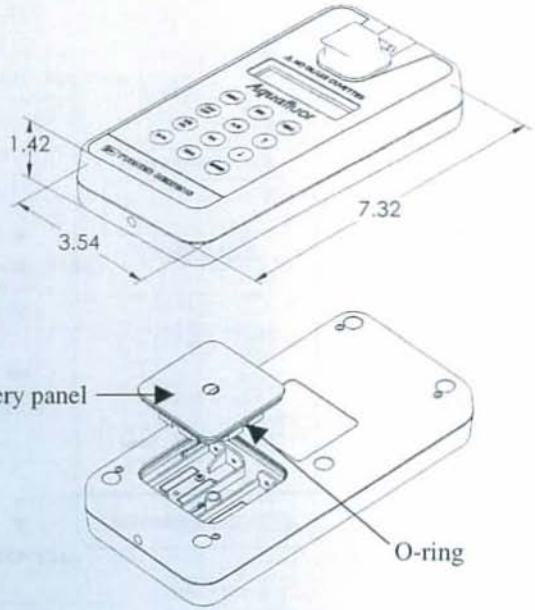
1. On the backside of the instrument, loosen the screw and remove the battery panel (see Section 2 for diagram).
2. Install the 4 AAA batteries into the appropriate spaces.
3. Replace the battery panel and tighten the screw. The panel has an o-ring, which creates a watertight seal. The battery panel may be difficult to install if there is no

lubrication on the o-ring. Use a silicon based o-ring grease to lubricate the o-ring if necessary.

1.3 General Information and Precautions

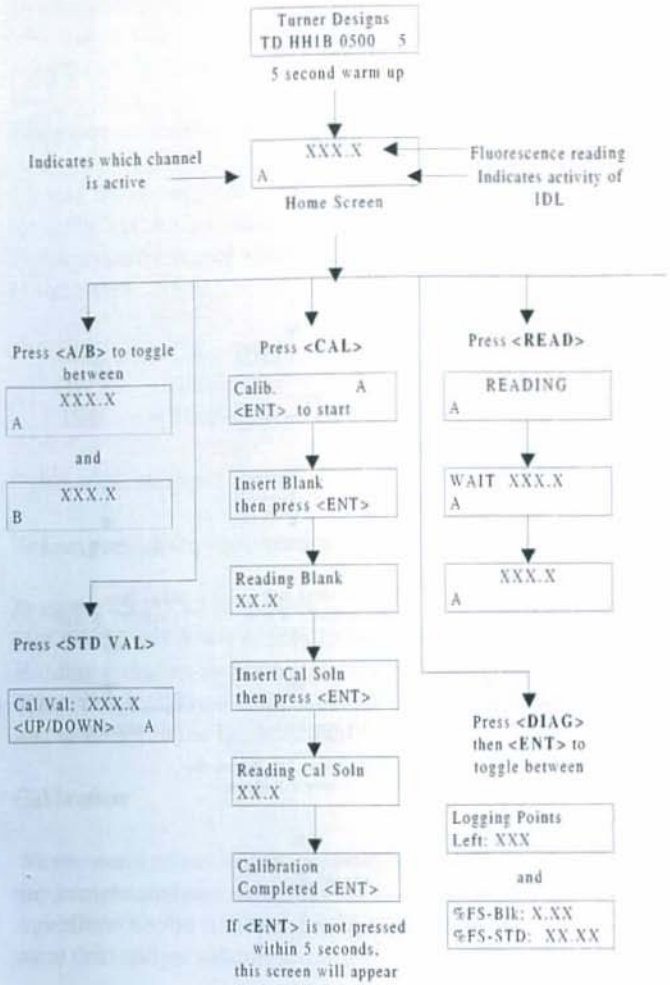
- The sample compartment cannot accept glass or quartz cuvettes.
- A minimum volume of 2mls in a 10x10 cuvette is required for best results.
- Avoid having any air bubbles in your sample. They can significantly affect the fluorescent reading.
- For best results measuring low turbidities, use good polystyrene cuvettes (P/N 7000-957). See Section 4.4 for further information.

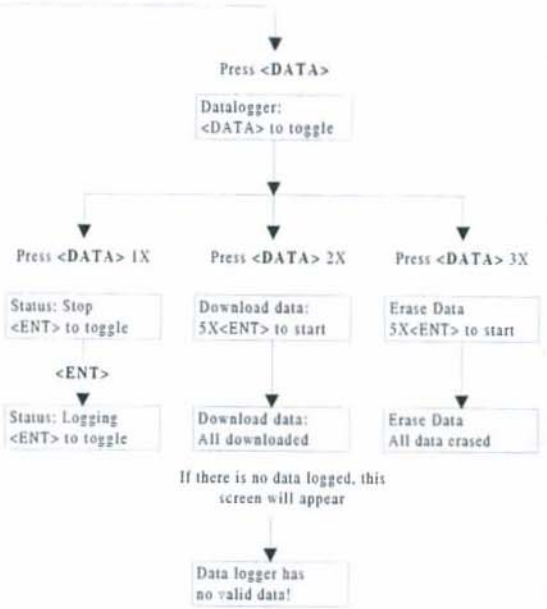
2. Quick View Diagrams



3. Instrument Parameters

3.1 Firmware Flowchart





3.2 Instrument Power Up

To turn on the *Aqua*fluor™, press the <ON/OFF> button. The instrument takes 5 seconds to warm up. After the warm up, the *Aqua*fluor™ is ready for operation.

3.3 Fluorescence or Turbidity Channel

Choose the appropriate channel for your analysis. To do this, press the <A/B> button to toggle between the 2 channels. The activated channel will be displayed in the lower left corner of the Home screen.

CHL = Chlorophyll
RWT = Rhodamine WT
TRB = Turbidity

3.4 Calibration Standard Value

Before performing a calibration, set the value of your standard.

1. Press the <STD VAL> button.
2. Use the up and down arrows to adjust the standard value. Holding either arrow button down will activate faster scrolling.
3. When finished, Press <ESC> or <ENT> to accept the value and to return to the Home screen.

3.5 Calibration

We recommend that you always calibrate before performing any sample analysis. After the initial calibration, the *Aqua*fluor's solid standard can be used to check for instrument drift and recalibration.

1. Press the <CAL> button.
2. Press <ENT> to start the calibration.
3. Insert your blank and press <ENT>. The *Aqua*fluor™ will average the fluorescence for 10 seconds.
4. Insert the calibration standard and press <ENT>.
5. Press <ENT> when the calibration is complete to accept the calibration. If <ENT> is not pressed within 10 seconds, you will be asked if you want to abort the calibration. Press the up or down arrow to abort or accept the calibration respectively.

If at anytime during steps 1-4 you want to stop the calibration, press <ESC>. This will return you to the Home screen and will default the instrument to the previous calibration.

3.6 Sample Analysis

1. Insert your sample.
2. Press either <READ> button. The instrument will autorange, then measure and average the fluorescence signal over a 5-second interval.
3. The result will be displayed at the top and center of the Home screen.
4. The top left corner will display "WAIT" for 5 seconds. Once "WAIT" disappears, another sample reading can be performed.

3.7 Internal Data Logging (IDL)

This is an optional feature. If this feature has been purchased, your *Aqua*fluor™ can log up to 1000 data points. The DATA screens control logging, downloading and erasing the data. For further information, see Appendix B.

3.7.1 Activate Data Logging

1. Press the <DATA> button 2 times.
2. Press <ENT> to toggle between logging and stop statuses.
3. Press <ESC> when finished to return to the Home screen.

3.7.2 Download Data

1. Connect the *Aqua*fluor™ to the serial port of your computer.
2. Open the Turner Designs Interface Software. See Appendix B for computer requirements and installation.
3. Press the <DATA> button 3 times.
4. Press <ENT> 5 times to start the data download.
5. Press <ESC> when finished to return to the Home screen.

3.7.3 Erase Data

1. Press the <DATA> button 4 times.
2. Press <ENT> 5 times to erase all logged data.
3. Press <ESC> when finished to return to the Home screen.

3.8 Diagnostic Information

1. Press <DIAG> to access the diagnostic screens.
2. The first screen shows the number of data points available for internal data logging.
3. Press <ENT> to toggle to the %FS (Full Scale) values from the calibration blank and standard.
4. Press <ESC> when finished to return to the Home screen.

4. General Considerations for Analysis

4.1 Handling Samples

1. Take care not to spill samples into the sample chamber. Wipe up any spills promptly.

2. The *Aqua*fluor™ is very sensitive and even small amounts of material from a previous sample may result in errors. Use a clean cuvette for all readings. Thorough and proper cleaning of cuvettes between sample readings is essential, and is especially important if you are using the same cuvette for samples and blank.

3. Fill the cuvette at least 50% full (2mls). Significant error can result if the cuvette does not contain this minimum volume.

4. The cuvette MUST BE DRY on the outside when taking readings. Moisture and condensation on the outside can result in error.

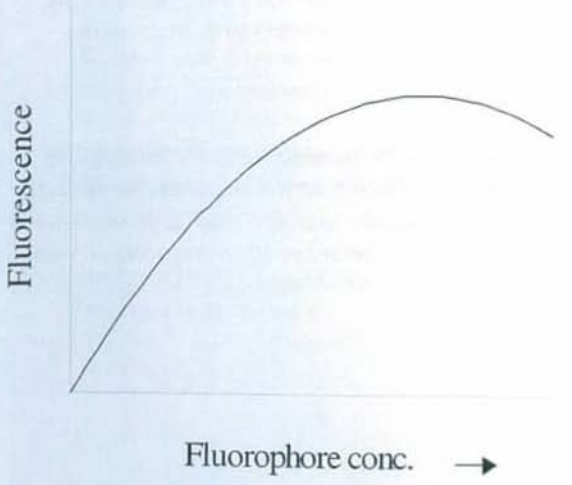
5. Minute bubbles in samples will cause drifting readings. Take care not to introduce bubbles into samples. Slight tapping with your finger on the outside cuvette wall will often help dissipate bubbles.

4.2 Linear Range and Quenching

The linear range is the concentration range in which the readout of the *Aqua*fluor™ is directly proportional to the concentration of the fluorophore. The linear range begins with the smallest detectable concentration and spans to an upper limit (concentration) that is dependent upon: the properties of the fluorescent material, the filters used, and the path length.

A nonlinear relationship is seen at very high concentrations where the fluorescence signal does not increase at a constant rate in comparison to the change in concentration. At even higher concentrations, fluorescence signal will decrease even though the sample concentrations are greater. This effect is known as "signal quenching".

Linearity may be checked by diluting a sample 1:1 or some other convenient ratio (be sure to use your matrix blank for the dilutions). If the sample is within 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 of your fluorophore.



4.3 Temperature Considerations

Fluorescence is temperature sensitive. As the temperature of the sample increases, the fluorescence decreases. For greatest accuracy, read blank, standard, and samples at the same temperature.

4.4 Positioning Samples

For low concentration samples, cuvettes often will give slightly different measurements depending upon their orientation in the sample compartment. This is due to defects in the shape of the cuvette that are not visible to the human eye. We recommend that the cuvette be marked at the top and positioned in the sample compartment the same way each time to minimize error.

We have found that turbidity is particularly sensitive to this factor. We recommend for best results, using high quality polystyrene cuvettes (P/N 7000-957) which showed little orientation and cuvette to cuvette variation in testing.

4.5 Data Quality

The *Aqua*fluor™ is only as accurate as the standards that are used to calibrate it. This is why it is important to take care when preparing standards, samples, and blank. One should follow good laboratory practices when preparing all solutions and reagents.

5. Warranty

5.1 Terms

Turner Designs warrants the *Aqua*fluor™ Fluorometer and accessories to be free from defects in materials and workmanship under normal use and service for a period of one year from the time of initial purchase, with the following restrictions:

1. The instrument and accessories must be installed, powered, and operated in compliance with the directions in this *Aqua*fluor™ User's Manual and directions accompanying the accessories.

2. Damage incurred in shipping is not covered.

3. Damage resulting from measurement of samples found to be incompatible with the materials used in the sample system is not covered.

4. Damage resulting from contact with corrosive materials or atmosphere is not covered.

5. Damage from seawater and other moderately corrosive materials that are not promptly removed from the instrument are not covered.

6. Damage caused by modification of the instrument by the customer is not covered.

5.2 Warranty Service

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

1. Write or call the Turner Designs service department and describe as precisely as possible the nature of the problem.

2. Carry out minor adjustments or tests as suggested by the Service Department.

3. If proper performance is not obtained, ship the instrument, prepaid, to Turner Designs, with a statement of shipping charges. The instrument will be repaired and returned free of charge, along with a check to cover shipping charges, for all customers in the contiguous continental United States.

For customers outside of the contiguous continental United States, and who have purchased our equipment from one of our authorized distributors, contact the distributor. If you have purchased direct, contact us. We will repair the instrument at no charge, but we will not pay for shipment, documentation, etc. These charges will be billed at cost.

NOTE! Under no conditions should the instrument or accessories be returned without notice. Prior correspondence is 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 that repair can be rapid, with particular attention paid to the defect you have noted.

5.3 Out-of-Warranty Service

Proceed exactly as for Warranty Service, above. If our service department can assist you by phone or correspondence, we will be glad to, at no charge.

Repair service will be billed on a basis of time and materials. A complete statement of time spent and materials used will be supplied. Shipment to Turner Designs should be prepaid. Your bill will include return shipment freight charges.

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

Appendix A: Instrument Specifications

A1. General Specifications

Specification	Description
Size	1.75" x 3.5" x 7.25" (4.45cm x 8.9cm x 18.4cm)
Weight	13.9oz (0.4kg)
Dynamic range	3 orders of magnitude
Resolution	12 bits
LCD Display	2 x 16 characters
Case	Meets IP 67 Standard; dustproof and waterproof
Temperature	41-104 °F; 5-40 °C
Detector	Photodiodes: measurement capability from 300-1000nm
Calibration Type	Single-point and blank
Alarms	Low battery, circuit failure, High blank
Cuvette Type	10mm x 10mm plastic
Warm Up Time	5 seconds
Automatic Power Down	After 90 seconds of inactivity

A2. Optical and Application Specifications

	Chlorophyll Channel	Rhodamine Channel	Turbidity Channel
Light Source	Blue LED	Green LED	Green LED
Excitation Optics	460±20nm	540±20nm	515±10nm
Emission Optics	>665nm	>570nm	515±10nm
Limit of Detection	0.25ug/l	0.4ppb	0.5NTU
Max range	> 800 ppb	>300ppb	>150 NTU
Temperature coefficients	1.4%/°C Linear	0.026/°C Exponential	N/A

Appendix B: Internal Datalogging

B1. Shipping Checklist

Instruments with internal data logging purchased will also receive in their shipment:

- Interface cable
- Turner Designs Spreadsheet Interface Software (2 disks)

Both of these items are necessary for retrieving the stored data in the *Aqua*fluor™.

B2. Hardware Requirements

- PC with Windows 95 or later
- MS Excel 5.0 or later
- At least 1 available serial port

B3. Installation

1. Exit all Windows programs.
2. Insert Disk 1 and run the setup program.
3. The setup wizard will install the necessary files. You will be prompted for Disk 2 when necessary.
4. When the setup is complete, an icon named "_TD2" will be found in the "Programs" menu.
5. Restart your computer.

B4. Connecting

1. Using the provided cable, connect the 9 pin adapter of the cable into the available serial port of your computer.

1. Plug the opposite end of the cable into the base of the *Aqua*fluor™.
2. Open MS Excel 5.0 or later.
3. Open the TD2 spreadsheet interface software.
4. Click on the box to the right of the COM port icon to select the appropriate COM port. This is usually COM port 2.
5. Click on "Start". The program will open an Excel spreadsheet for data transfer. The boxes left of the COM port and MS Excel should both be green.
6. Follow the directions from Section 3.7 for collecting and downloading data from the *Aqua*fluor™. Data will automatically appear in the excel spreadsheet. BE SURE to save this data BEFORE closing the TD software.

B5. Real Time Data Transfer

Data can also be transferred directly to the computer after each reading. To do so:

1. Stop data logging (see 3.7.1)
2. Follow steps 1-6 of B4 to crate the connection between the *Aqua*fluor™ and your computer.
3. Insert a sample and press the <READ> button. The results will automatically transfer to the active Excel spreadsheet.

B.6 IDL Troubleshooting

Difficulties can arise when parameters are set incorrectly or connections with the cable are not tight enough. Here are some common problems.

1. Box to the left of the COM port is red. This means that the COM port is not available. Causes:
   a. Another instrument or program (such as palm pilot/ hot sync) could be occupying the port, making it unavailable. Make sure to close all programs of this type before downloading data
   b. The port selected is incorrect. Follow step 4 of connecting to choose another COM port.

2. All lights are green, but no data transferred, even though the instrument says "All data downloaded".
   a. The connection between the instrument and the computer is bad. Check and tighten the cable connections. Make sure both ends of the cable are plugged in tightly.

Appendix C: *In Vivo* Chlorophyll

The detection of *in vivo* chlorophyll *a* is by nature, a qualitative measurement. Physiological, environmental, morphological, and temporal factors all contribute to the variation between the *in vivo* signal and the actual chlorophyll *a* concentration of a sample. Physiological effects stem from the change in fluorescence per unit chlorophyll of cells at varying physiological states. On a basic level, an 'unhealthy' cell will fluoresce more than a 'healthy' cell due to the light energy absorbed is channeled into photosynthesis. However, in natural assemblages of phytoplankton, there is normally a mix of species at varying degrees of health, thus averaging out the physiological effect.

Environmental effects derive from mainly two factors: light and temperature. The light history of an algal population will affect fluorescence of living cells. Cells in a darker environment will fluoresce more per unit chlorophyll than cells in a well lit zone of the water column. One way of reducing the effects of light is to "dark adapt" your sample before analyzing it. Temperature effects are discussed in section 4.3 of the manual. For best sample analysis, all samples and calibration solutions should be measured at the same temperature.

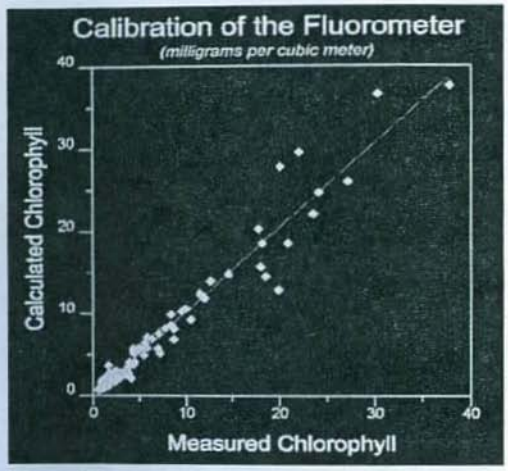
Temporal/Spatial effects are mainly due to differences in quantum efficiency and cell size between different species of phytoplankton and photosynthetic bacteria.

Interfering compounds in natural waters derive from several sources. The most common interfering compounds include pheophytins, chlorophyll *b* and *c*, dissolved organic matter and fluorometer. Optical filters with a wider bandpass will

be susceptible to more interference than filters with a narrower bandpass.

In spite of these concerns, this does not mean that actual chlorophyll concentrations cannot be extrapolated from the *in vivo* data. A simple way of correlating *in vivo* data to actual chlorophyll concentrations is accomplished by periodically collecting "grab" samples for chlorophyll extraction. Several samples should be collected within each niche or environment.

At the time of collection, the *in vivo* value must be noted. Once the chlorophyll concentration has been determined through extraction, the concentration should be correlated with the corresponding *in vivo* value (see Graph C1)



Graph C1

For detailed information on chlorophyll analysis, please see the reference list below or visit the Turner Designs webpage at www.fluorometer.com