TURNER DESIGNS
Reliable Instruments for an Unreliable World

Trilogy Laboratory Fluorometer

User's Manual

Version 1.3
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Turner Designs
1995 N. 1st Street
San Jose, CA 95112
Phone: (408) 749-0994
FAX: (408) 749-0998
www.turnerdesigns.com

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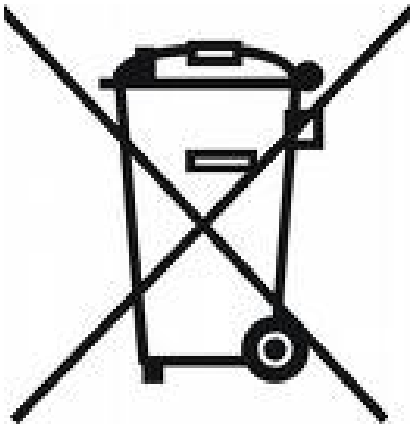


WASTE ELECTRICAL AND ELECTRONIC EQUIPMENT (WEEE) DIRECTIVE

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 are 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. Getting Started

1.1 Highlights of the Trilogy Fluorometer

1.2 Description

The Trilogy Laboratory Fluorometer is a multifunctional laboratory instrument that can be used for making fluorescence, absorbance, and turbidity measurements using the appropriate snap-in Optical Module. A color touch screen with simple menus makes for an intuitive user-friendly interface.

Fluorescence Modules are available for discrete sample measurements of various fluorescent materials including chlorophyll (*in vivo* and extracted), rhodamine, fluorescein, PTSA, cyanobacteria pigments, ammonium, CDOM/FDOM, optical brighteners, histamines, crude oil, hydrocarbons and other fluorescent compounds.

The Absorbance Module accepts interchangeable filter paddles so measurements can be made at different wavelengths in order to identify or place a sample in a particular class of compounds. The standard filter paddle wavelengths/bandwidths are: 560/10; 600/10 and 750/10 nm.

The Turbidity Module uses an Infrared (IR) LED with a wavelength of 850 nm as required for reference method: ISO 7027/DIN EN 27027, “Water Quality – Determination of Turbidity”. Using an Infrared LED allows turbidity to be measured at wavelengths that are not normally absorbed by organic matter, thereby reducing susceptibility to interference.

Optical Modules contain the necessary light source and filters for the relevant application.

1.3 Unpacking and Inspection

Upon receiving the Trilogy, please inspect it carefully and make certain all accessories are present. Refer to the checklist shipped with the instrument for order-specific items.

A typical Trilogy shipment includes:

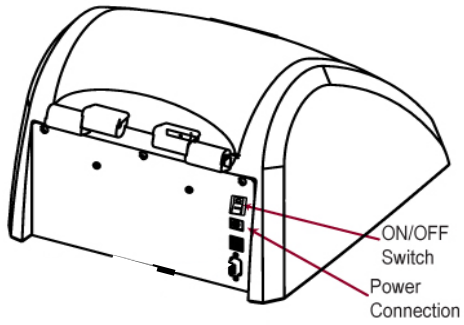
- Checklist
- Trilogy Laboratory Fluorometer
- USB Flash Drive: User’s Manual (pdf); Quick Start Guide (pdf); Spreadsheet Interface Software
- Solid Secondary Standard P/N 8000-952 (not intended for use with UV applications, absorbance or turbidity optical modules)
- Power Supply Kit
- RS-232 Cable
- Quick Start Guide (hardcopy)
- 12 mm Round Adaptor
- Cuvettes or Test Tubes if ordered.
- Optical Module(s) as ordered. ***Note: Refer to the Optical Specification Guide http://www.turnerdesigns.com/t2/doc/spec-guides/998-7281.pdf for details. Modules denoted with a P/N 7200-###W are for use with glass or quartz 10x10mm square cuvettes.***

1.4 Setup

Place the Trilogy Fluorometer on a flat, level surface. Allow at least 6 inches (16 cm) of clearance above the instrument to open and close the lid. Position the instrument so that the touch screen faces you.

Connect the power supply into the power connection of the instrument, see Figure 1, and plug it into a wall outlet. See Specifications for power requirements.

Figure 1:



1.5 Getting to the Home Screen

1. With the unit turned off, lift the lid and insert an optical module into position - see Figure 2. Press down on the module until you hear it snap into place. The module should be **flush** with the top of the Trilogy when it is properly seated. **Be sure to close the fluorometer lid so that the Trilogy power ON step will complete successfully.**

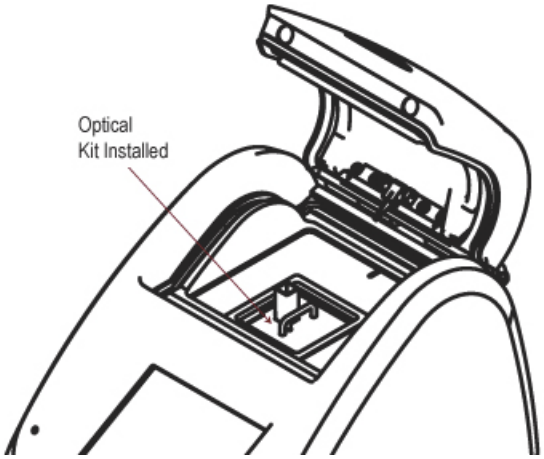
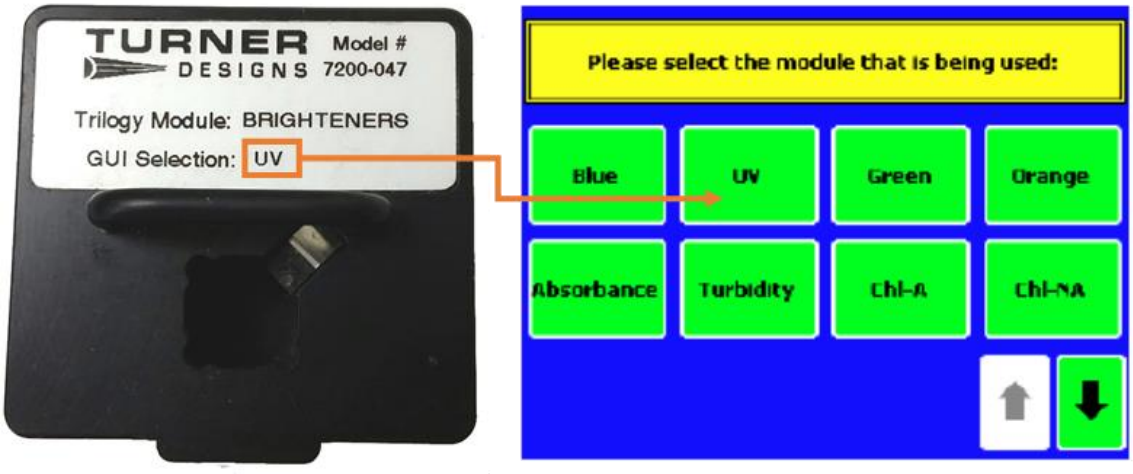


Figure 2: Showing Optical Kit installed in preparation for getting to the Home Screen.

2. Turn ON the On/Off switch located on the back of the Trilogy, see Figure 1. Verify the display becomes active, and shows the module selection screen - see Figure 3.

Figure 3: Module Selection



3. Select the module inserted and touch **"OK"** on the confirming screen.

4. When the Home Screen is displayed – see Figure 4, you are now ready to use the Trilogy in its Raw Mode (measurements are relative) or calibrate the Trilogy and the snapped-in Optical Application Module to make quantitative measurements.

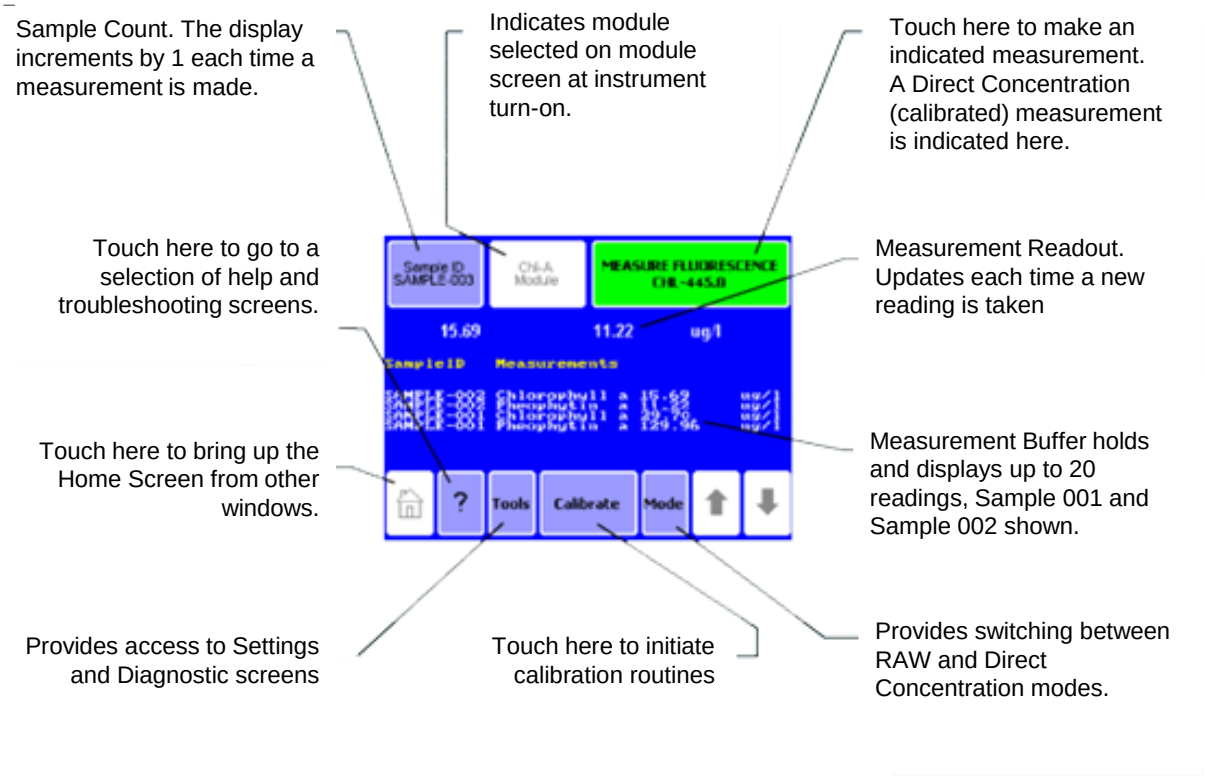


Figure 4: Trilogy Home Screen Display for Fluorescence Module Configuration.

1.6 Precautions

- **The Trilogy is intended for indoor use only.**
- **Wipe up spills immediately and avoid using wet fingers on the touch screen.**
- **The Trilogy contains sensitive optical components and precision-aligned mechanical assemblies. Avoid rough handling.**
- **Do not leave the lid open for extended periods of time.**
- **Turn OFF the Trilogy to change or install Optical Application Modules.**
- **The Trilogy should not be exposed to environments with high humidity - 75% RH (relative humidity) maximum.**

Note: After 20 minutes without activity or user stimulation, the Touch Screen hibernates. Lightly touch the screen to activate.

2. Operation

2.1 Fluorescence Optical Module

Installation

1. Power the Trilogy OFF
2. Grasp the handle of the Optical Application Module and align the kit with the sample compartment.
3. Press down firmly to lock the Optical Application Module in place. You should hear or feel a click indicating the module has snapped into place - see Figure 2.
4. Close the lid and power ON the Trilogy. Use the touch screen to identify the type of Optical Application Module installed – see Figure 3.

Removal

1. Power OFF the Trilogy before removing the Optical Application Module.
2. Grasp the handle and gently pull up to release it from the sample compartment.
3. Close the lid of the Trilogy.

2.1.1 Touch screen Basics (Fluorescence)

Home Screen
The "Home" screen appears after confirmation of the Optical Application Module. The "Home" screen provides information for the multiple functions of the Trilogy. From the "Home" screen, select "Calibrate," "Tools," "Mode," or "Help". The "Home" screen is also the measurement screen - see Figure 4.

2.1.2 Measuring Samples

There are two measurement modes available on the Trilogy when using the Fluorescence Module:

Raw Fluorescence Mode – No calibration required
Direct Concentration Mode – Calibration required - see Section 3.

Touch "**Mode**" on the Home Screen to select the measurement mode.

1. **Raw Fluorescence Mode**: The Raw Fluorescence Mode should be used for qualitative measurement and relative changes in fluorescence rather than absolute concentration values. Readings are displayed in Relative Fluorescence Units (RFU).

2. **Direct Concentration Mode**: The Direct Concentration mode makes absolute measurements based on a calibration - see Section 3 for the Calibration Procedure.

Different applications require different cuvettes. Refer to the **Optical Specification Guide** http://www.turnerdesigns.com/t2/doc/spec-guides/998-7281.pdf for specific application recommendations.

1. Turn ON the Trilogy
2. Open the lid of the Trilogy and insert the cuvette. Close the lid.
3. Touch "Sample ID" to name your sample (optional). Using the keypad, enter the sample name into the name field and touch "Save" to save the sample ID.
4. Touch "Measure Fluorescence" to make a measurement. The Trilogy will measure the sample for 6 seconds and report the average reading for the sample.

The Trilogy reports data on the "Home" screen and displays the results for the 20 most recent measurements. Use the arrow keys to scroll through the most recent measurements. The data automatically exports to a printer or PC when properly connected - see Section 5. Please note the Trilogy does not store more than 20 measurements at one time. If more than 20 readings are taken, the oldest reading will be overwritten. If you want to save additional measurements you must be connected to the computer. Measurements are not stored between power cycles.

2.1.3 Tools

Touch the “Tools” key to access "Settings."

Tools - Settings

View Cal Details

Touch "View Cal Details" to see information on the current calibration for Direct Concentration Mode. "View Cal Details" specifically provides information on the raw fluorescence for each standard and the blank as well as the unit of measure and the Optical Application Module.

Continuous Sampling

The Continuous Sampling feature enables repeat measurements at user-defined intervals.

1. Touch "Continuous Sampling" and turn the feature ON. Highlight the frequency of measurement and the number of total measurements. The maximum number of total measurements is 9999.
2. Touch "OK" to return to the "Home" screen.
3. Connect the Trilogy to a printer or a PC to collect the data. Touching the screen repeatedly causes an early-abort of Continuous Sampling measurements.

Measurement Tip:

On the Home Screen, touch **Settings** then touch the **Lid Start** key to turn the feature ON. When the Lid Start feature is ON, measurement begins as soon as the lid closes. The lid start feature allows for immediate measurement and eliminates the need to touch the "Measure" key. Also, the touch screen does not hibernate when Lid Start is ON.

Return to the **Lid Start** key under the **Settings** menu to turn the feature OFF.

2.1.4 Fluorescence Troubleshooting

Symptom	Possible Solution
Bad calibration error message	A bad calibration error message may occur if the blank is brighter than the standard. Compare the RFU values of the standard and the blank in the Raw mode.
Erratic reading	When direct fluorescence readings do not produce expected values, review the standard value entered during the calibration. The number of the standard value should correspond to the actual concentration of the standard.
Negative values	After calibration, the blank value is automatically subtracted from subsequent readings. A negative reading can occur if a sample reading is less than the blank or this could indicate that the module is not properly snapped into place. Press down on the module and listen for a click indicating the module is properly installed.
Low readings	Check the excitation and emission wavelengths of the analyte against the specifications of the Fluorescence Optical Application Module in use. Different analytes require different Optical Application Modules.
High background	A wet cuvette or spill could contaminate the cuvette holder and increase the background signal. Carefully clean the cuvette holder with a Q-tip and / or lens cleaner.

2.2 Absorbance Module

Installation

1. Power OFF the Trilogy.
2. Align the Absorbance Module with the sample compartment.
3. Press down to lock the Absorbance Module in place. You should hear or feel a click indicating the module has snapped into place - see Figure 2.
4. Close the lid and power ON the Trilogy. Select "Absorbance" from the list of options on the touch screen, press “OK” to confirm that the module is correct.
5. Install the filter paddle that corresponds to the wavelength of absorbance for the assay, see Figure 5.

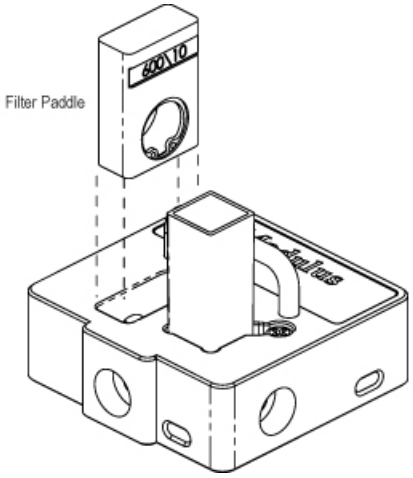


Figure 5.

Removal

1. Power OFF the Trilogy before removing the Absorbance Module.
2. Open the lid, grasp the handle and gently pull up to release it from the sample compartment.
3. Close the lid.

2.2.1 Touch Screen Basics (Absorbance)

Home Screen

The "Home" screen appears after confirming the absorbance meter operation by installing the Absorbance Module. From the "Home" screen, select "Calibrate," "Tools," "Mode" or "Help". The "Home" screen is also the measurement screen - see Figure 6.

Mode

Touch "Mode" to select the unit of measure for absorbance. The available options include Absorbance units (Ab) and Transmittance (%T).

The following formulas describe the method of the Trilogy Absorbance Module for measuring % transmittance and absorbance:

$$\%T = [(s-z) / (b-z)] * 100$$
$$Ab = 2 - \text{Log}_{10} * (\%T)$$

Where,
z = zero
b = baseline
s = signal

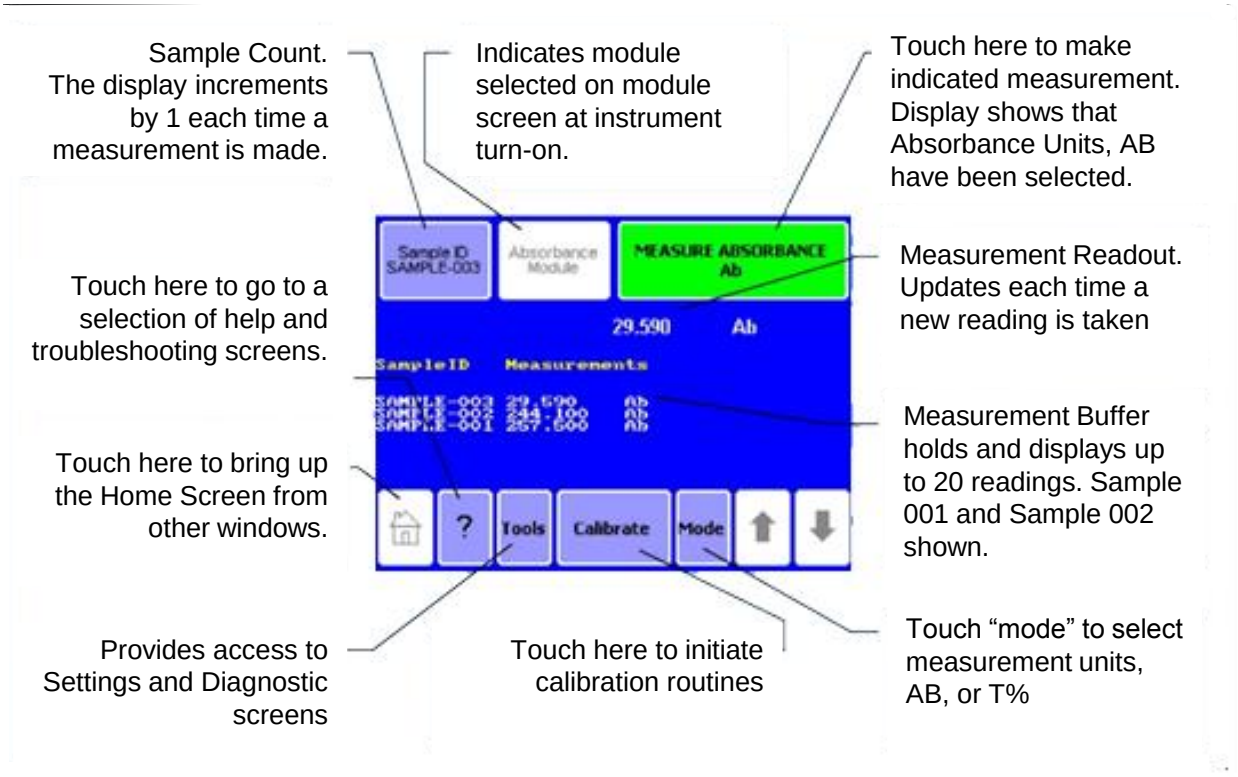


Figure 6: Home Screen When Using Absorbance Module

2.2.2 Measuring Samples

The Absorbance Module accommodates 10 x 10 mm methacrylate and polystyrene cuvettes as well as glass and quartz cuvettes (minimum 2 mL volume).

1. Turn ON the Trilogy.
2. Open the lid and insert the cuvette. Close the lid.
3. Touch "Sample ID" to name your sample (optional). Using the keypad, enter the sample name into the name field and touch "Save" to save the sample ID.
4. Touch "Measure Absorbance Ab" to make a measurement. The Trilogy will measure the sample for 6 seconds and report the average reading for the sample.

The Trilogy reports data on the "Home" screen and displays the results for the 20 most recent measurements. Use the arrow keys to scroll through the

most recent measurements. The data exports to a printer or PC if properly connected, see Section 5. Please note the Trilogy does not store more than 20 measurements at one time. If more than 20 readings are taken, the oldest reading will be overwritten. If you want to save additional measurements you must be connected to the computer. Measurements are not stored between power cycles.

2.2.3 Tools

Tools – Settings
Touch the "Tools" key to access "Settings."

View Cal Details
Touch "View Cal Details" to see information on the current calibration for the baseline and the zero.

Continuous Sampling
The Continuous Sampling feature enables repeat measurements at user-defined intervals.

1. Touch "Continuous Sampling" and turn the feature ON.
2. Highlight the frequency of measurement and the number of total measurements. The maximum number of total measurements is 9999.
3. Touch "OK" to return to the "Home" screen.
4. Connect the Trilogy to a printer or a PC to collect the data obtained during Continuous Sampling. Touching the screen repeatedly causes an early-abort of Continuous Sampling measurements.

Measurement Tip:

On the Home Screen, touch **Settings** then touch the **Lid Start** key to turn the feature ON. When the Lid Start feature is ON, measurement begins as soon as the lid closes. The lid start feature allows for immediate measurement and eliminates the need to touch the "Measure" key. Also, the touch screen does not hibernate when Lid Start is ON.

Return to the **Lid Start** key under the **Settings** menu to turn the feature OFF.

2.2.4 Absorbance Troubleshooting

Symptom	Possible Solution
Non-linear response	Many absorbance assays do not produce a linear response but instead produce a sigmoidal or pseudo-sigmoidal response. Refer to the Application Note for the assay for more information.
Low readings	Check the filter installed in the Absorbance Module and make sure it is the correct filter for the assay. View the Calibration details from the Tools menu
Bad Calibration Error Message	Install the proper filter and use the ultrapure water in a clean cuvette to update the zero. Check the Calibration details from the Tools menu.

2.3 Turbidity Optical Module

Installation

1. Power the Trilogy OFF
2. Open the lid, grasp the handle of the Optical Application Module and align the kit with the sample compartment.
3. Press down firmly to lock the Optical Application Module in place. You will hear or feel a click indicating the module has snapped into place - see Figure 2.
4. Close the lid and power ON the Trilogy. Use the touch screen to identify the type of Optical Application Module installed – see Figure 3.

Removal

1. Power OFF the Trilogy before removing the Optical Application Module.
2. Open the lid, grasp the handle and gently pull up to release it from the sample compartment.
3. Close the lid.

2.3.1 Touch Screen Basics (Turbidity)

Home Screen
The "Home" screen appears after confirmation of the Optical Application Module. The "Home" screen provides orientation for the multiple functions of the Trilogy. From the "Home" screen, select "Calibrate," "Tools," "Mode," or "Help". The "Home" screen is also the measurement screen – see Figure 7.

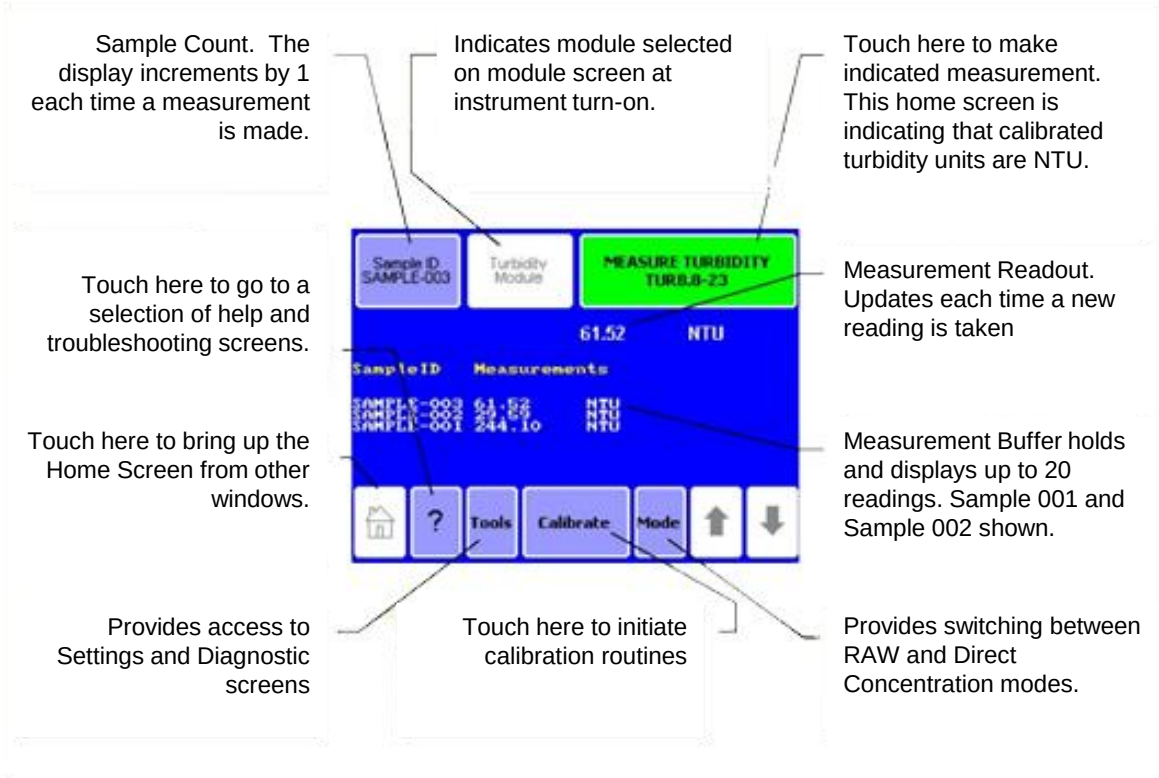


Figure 7: Home Screen When Using Turbidity Module

2.3.2 Measuring Samples

There are two measurement modes available on the Trilogy when using the Turbidity Module:

Raw Mode – No calibration required
Direct Concentration Mode – Calibration required – see Section 3.

Touch "**Mode**" on the Home Screen to select the measurement mode.

1. **Raw Mode**: The Raw Mode should be used for qualitative measurement and relative changes rather than absolute concentration values. Readings are displayed in Raw Turbidity Units (RFU).
2. **Direct Concentration Mode:** The Direct Concentration mode makes absolute measurements based on a calibration. Readings are displayed in Nephelometric Turbidity Units (NTU). See Section 3 for the Calibration Procedure.

Use polystyrene cuvettes for measuring turbidity.

1. Turn ON the Trilogy. Open the lid of the Trilogy and insert the cuvette. Close the lid.
2. Touch "Sample ID" to name your sample (optional). Using the keypad, enter the sample name into the name field and touch "Save" to save the sample ID.
3. Touch "Measure Turbidity" to make a measurement. The Trilogy will measure the sample for 6 seconds and report the average reading for the sample.

The Trilogy reports data on the "Home" screen and displays the results for the 20 most recent measurements. Use the arrow keys to scroll through the most recent measurements. The data automatically exports to a printer or PC when properly connected - see Section 5. Please note the Trilogy does not store more than 20 measurements at one time. If more than 20 readings are taken, the oldest reading will be overwritten. If you want to save additional measurements you must be connected to the computer. Measurements are not stored between power cycles.

2.3.3 Tools

Tools - Settings

View Cal Details

Touch "View Cal Details" to see information on the current calibration for Direct Concentration Mode. "View Cal Details" specifically provides information on the raw fluorescence for each standard and the blank as well as the unit of measure and the Optical Application Module.

Continuous Sampling

The Continuous Sampling feature enables repeat measurements at user-defined intervals.

1. Touch "Continuous Sampling" and turn the feature ON. Highlight the frequency of measurement and the number of total measurements. The maximum number of total measurements is 9999.
2. Touch "OK" to return to the "Home" screen.
3. Connect the Trilogy to a printer or a PC to collect the data. Touching the screen repeatedly causes an early-abort of Continuous Sampling measurements.

Measurement Tip:

On the Home Screen, touch **Settings** then touch the **Lid Start** key to turn the feature ON. When the Lid Start feature is ON, measurement begins as soon as the lid closes. The lid start feature allows for immediate measurement and eliminates the need to touch the "Measure" key. Also, the touch screen does not hibernate when Lid Start is ON.

Return to the **Lid Start** key under the **Settings** menu to turn the feature OFF.

2.3.4 Turbidity Troubleshooting

Symptom	Possible Solution
Trilogy readings do not agree with other Turbidity meters	Calibrate both meters with the same calibration standard solution. If meters still display significantly different readings, it may be that the second turbidity meter does not make an IR measurement and the sample contains interference colors.
The turbidity readings change each time a reading is taken	This is normal. Particles in a liquid sample do not remain in the same position and these position changes affect the scattering of the light and therefore the turbidity reading.
My turbidity readings seem to be different when I re-calibrated with a new primary standard.	Formazine standards form the basis of all turbidity measurements and they are very susceptible to aging. ISO 7027 recommendation specifies that the 4,000 NTU Formazine solution can be kept for only 4 weeks. For consistent readings calibrate with current standards.

3. Calibration Overview

3.1 Why Calibrate

When calibrated, the Trilogy converts the signal to concentration units in relation to the standard(s) used for the calibration. This saves you from having to perform calculations.

3.2 When to Calibrate

- Recalibrate if the ambient temperature changes by ± 10°C.
- Recalibrate after changing Optical Application Modules or if you make measurements on a new analyte.
- Verify the need to calibrate by reading a stable, known concentration standard immediately after calibration and again every few hours to see if readings have changed significantly. Recalibrate when the accuracy becomes unacceptable for your study.

3.3 Calibration Options - Fluorescence and Turbidity

There are two measurement modes available on the Trilogy when using either the Fluorescence or Turbidity Modules. See Section 3.13 for Absorbance Module Calibration Procedure.

Raw Fluorescence or Raw Turbidity Mode – No calibration required
Direct Concentration Mode – Calibration required

1. **Raw Fluorescence Mode**: The Raw Fluorescence or Raw Turbidity Mode should be used for qualitative measurement, relative changes in fluorescence or scatter rather than absolute concentration values. Readings are displayed in Relative Fluorescence Units (RFU).

2. **Direct Concentration Mode**: Direct Concentrations can be calibrated by using single or multi-point calibrations. In multi-point calibrations, up to five standards and a blank can be read generating a calibration curve for increased accuracy. The software uses these points to calculate direct concentrations. The Trilogy will display the actual concentration of your samples in units that were chosen during calibration.

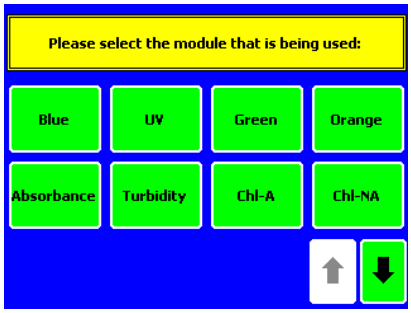
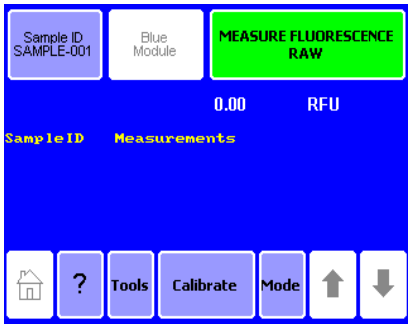
3.4 Calibration Procedures

1. **Raw Fluorescence Mode**: A calibration is not necessary to measure fluorescence with the Trilogy. Simply use the Raw Fluorescence Mode or Raw Turbidity Mode to obtain the fluorescence value of a sample in Relative Fluorescence Units (RFU). Use a standard curve to determine the concentration of the analyte in the samples. The Trilogy does not manipulate data while operating in the Raw Mode. It is not necessary to zero the Trilogy for use in the Raw Mode; however, a blank sample should be run to determine background fluorescence or scatter. A solid secondary standard may be used to verify instrument stability and function - see Section 3.10.

2. **Direct Calibration Mode**: The Direct Concentration Mode requires a calibration with one blank solution and at least one standard solution. The following procedure applies to the turbidity module and all the fluorescence modules with the exception of the Chl *a* Acidification and Non-Acidification modules. There are separate procedures for these two exceptions. The procedure requires the use of at least one calibration standard of a known concentration (Fluorescein, Rhodamine WT, etc.). Up to 5 standard solutions can be used for a multi-point calibration. Calibrations can be given a name and stored for future use.

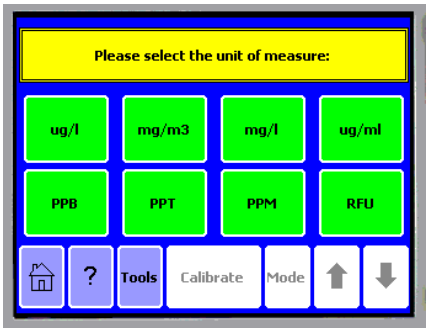
3.5 Direct Calibration Procedure – Fluorescence and Turbidity Modules, Single Point and Multi-Point Calibration.

See Sections 3.6 to 3.9 for procedures to calibrate the Chl *a* Acidification and Non-Acidification Modules.

Instructions	Sample Screen
a) Turn ON the Trilogy. Open the lid. Insert the correct module for testing, making sure you hear a click sound. Close the lid.	
b) Select the module/application to be calibrated and confirm by touching "**OK**". **Note: Refer to the module label to confirm which module is in use.**	
c) On the home screen, touch **"Calibrate"** to begin a calibration sequence.	

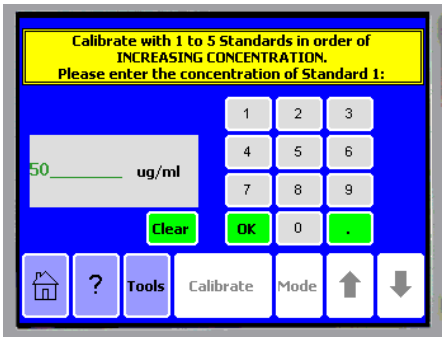
d) Select **“Run New Calibration”.**

e) Select the unit of measurement.



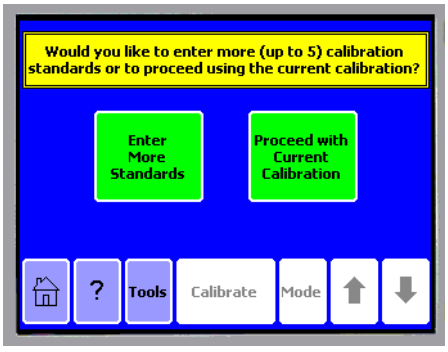
f) Open the lid and insert the calibration “blank” and touch **“OK”**. Close the lid.

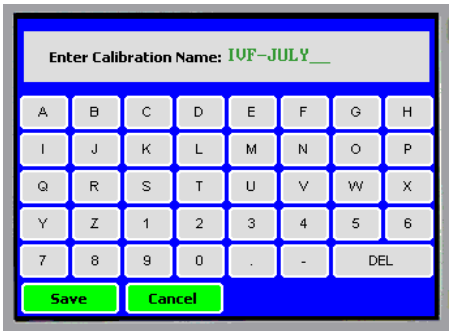
g) Enter the concentration for the first Standard. If doing multi-point calibrations, be sure to use Standards in order of increasing concentration. Press **“OK”** once the concentration of the first standard has been entered in. Then open the lid and remove the blank.



h) Follow the screen prompt indicating that the standard should be inserted, close lid and touch **“OK”**.

i) After the calibration is complete, either select **“Proceed with Current Calibration”** or select **“Enter More Standards”,** in which case, return to “g” above.



j) Name and Save the calibration for future use (optional).	
k) Subsequent readings in the Direct Concentration mode should reflect the actual concentration of the analyte in relation to the standards used for calibraiton.	
l) Confirm successful completion of the calibration by measuring the same Standard. The displayed concentration should equal the value used in step “g” above.	

3.6 Extracted Chlorophyll Measurements with the Chl *a* – Acidification Fluorescence Module

EPA Method 445.0 is a standard method for measuring extracted chlorophyll *a* and pheophytin *a* in marine and fresh water algae by fluorescence. It requires extraction with 90% acetone, measurements before and after acidification, and some fairly simple calculations to arrive at the chlorophyll *a* and pheophytin *a* concentrations, see Appendix D. Method 445.0 is detailed and straightforward. If high concentrations of pure chlorophyll *b* are present - see Section 3.9.

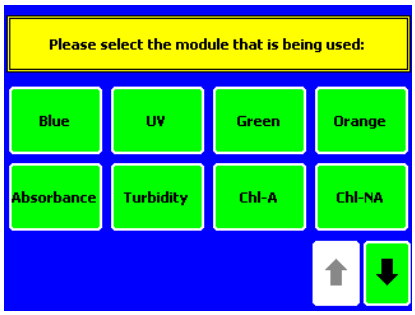
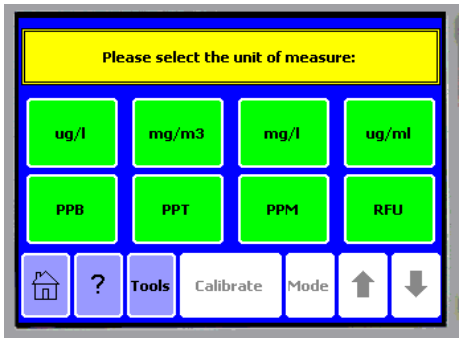
A known concentration of pure chlorophyll *a*, as a standard, is required at least the first time you calibrate your Trilogy. We recommend that you periodically check the calibration of your instrument with the Solid Secondary Standard or a known standard to verify stability. Liquid Primary Chlorophyll a P/N 10-850 and Solid Secondary standards P/N 8000-952 are available from Turner Designs.

3.7 Calibrating and Displaying Corrected Readings for the Chl *a* Acidification and Non-Acidification Modules

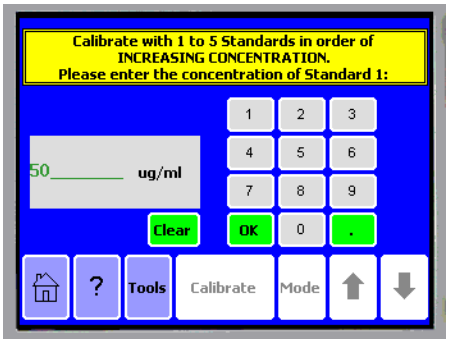
The Trilogy software can correct displayed chlorophyll readings for filtered and solvent volumes used during the calibration and measurement cycles. ***Note: If the volumes are unknown enter 1 for filtered volume and 1 for solvent volume and they will cancel out in the internal calculations.*** Either “**Chl A”** or “**Chl NA”** selections in the module selection screen ensure that the appropriate corrections are applied to measured data. This results in displayed readings that correspond to the actual sample concentration.

Note: As an alternative to the internal calibration and calculations, external calibration and calculations allow for more flexibility in interpretation of results, see Appendix D for formulas and Appendix E for examples.

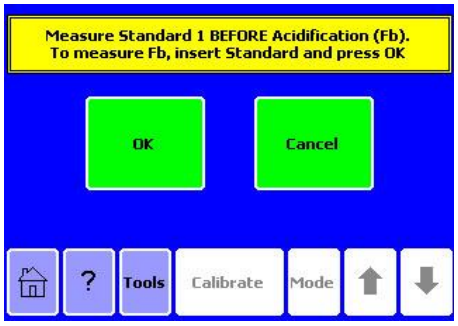
3.8 “Direct Calibration” Procedure – Extraction, Acidification, Single Point and Multi-Point

Instructions	Sample Screen
Turn ON the Trilogy. Open the lid. Insert the correct module for testing, making sure you hear a click sound. Close the lid.	
a) Touch “**Chl – A**” to select the Chlorophyll *a* Acidification module and confirm by touching “**OK**”.	
b) On the home screen, touch **"Calibrate"** to begin a calibration sequence.	
c) Select **“Run New Calibration”.**	
d) Select the unit of measurement. Press **“OK”**.	
e) Open lid, insert calibration “blank” – 90% acetone or solvent used for samples, close lid and touch **“OK”.**	

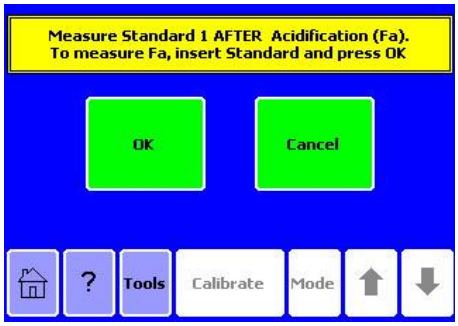
f) Enter the concentration for the first Standard. Then press **"OK".** If using the Turner Designs Chlorophyll *a* Standard, this will be the concentration data supplied with the Standard. If doing multi-point calibrations, be sure to use Standards in order of increasing concentration.



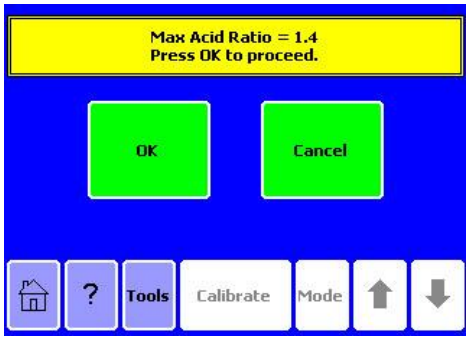
g) Open lid, remove blank and then follow the screen prompt indicating that the Standard before Acidification (Fb) should be inserted. Insert sample, close lid and touch **"OK"**.

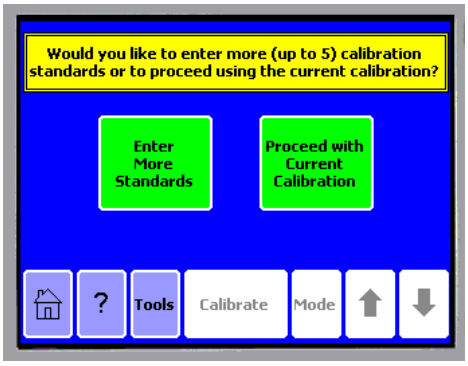
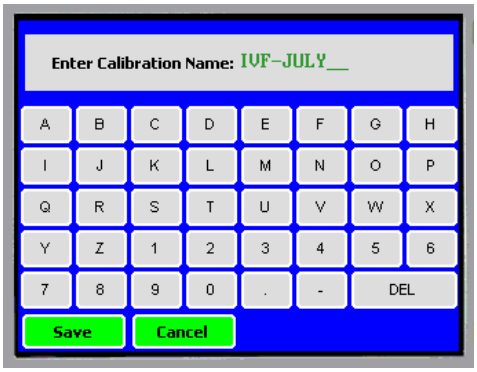


h) Open lid, remove the standard before Acidification (Fb). Acidify the standard, then insert the Standard after Acidification and touch **"OK"**. The (Fa) value will be measured and the ratio of the two readings will be displayed as seen in the next step.



i) If the ratio is within the required range, touch **"OK"**. The ratio will be stored in the Trilogy for use in the EPA Method 445.0 for measurement of chlorophyll *a* and pheophytin *a*.



j) After the calibration is complete, either select **"Proceed with Current Calibration"** or select **"Enter More Standards",** in which case, return to "g" above.	
k) Name and Save the calibration for future use (optional).	
l) It is recommended that the Solid Standard is now measured and the displayed value is noted to enable a quick calibration check prior to later use.	

3.9 Direct Calibration Procedure – Extraction, Non-Acidification, Single Point and Multi-Point

The Welschmeyer method is a simplified way to measure chlorophyll *a* without the need for acidification. It accurately measures chlorophyll *a* even in the presence of chlorophyll *b* and pheophytin *a*, however, you **cannot** obtain a pheophytin *a* measurement with this procedure. Using this method, you extract your samples according to EPA Method 445.0, but skip the acidification step.

You still need to calibrate the instrument the first time using a known concentration of pure chlorophyll *a* in 90% acetone.

The calibration procedure for the Chlorophyll Non-Acidification follows the same steps as for the Direct Calibration mode - see Section 3.5, however, the measurement procedure will prompt for the filtered and solvent volumes.
Note: If the volumes are unknown enter 1 for filtered volume and 1 for solvent volume and they will cancel out in the internal calculations.

3.10 *In vivo* Chlorophyll *a* Calibration

In vivo chlorophyll *a* analysis is the measurement of chlorophyll *a* fluorescence within a living cell. The advantage of this type of analysis is that it is quick and simple and does not require special sample preparation or extraction. It allows the user to measure a large number of samples in the field; however, without comparisons to extractive analysis, *in vivo* readings are qualitative in nature.

***In vivo* measurements using the RAW mode:**

1) Insert the *in vivo* chlorophyll *a* Trilogy module Turner Part Number 7200-043.
2) Turn on the Trilogy using the switch located on the back panel.
3) Select the "Blue" module. This is indicated on the module label under GUI selection.
4) Press "OK" after verifying that the module loaded matches your selection. The default mode loaded is "Raw Fluorescence".
5) Before measurements are made, it is advisable to filter a sample and measure the filtrate to obtain a blank or "Background" fluorescence reading for a given location.
6) Thoroughly mix the sample by inverting or shaking to prevent settling of algal cells, open the lid and quickly insert sample.
7) Close the lid and press the "Measure Fluorescence Raw" button.
8) Subsequent readings will indicate relative changes in concentration levels. These readings will be presented in Relative Fluorescence Units of measure (RFU).

3.11 Blank Subtracting

Blanks provide background fluorescence values of samples excluding the fluorophore of interest. Subtracting a blank sample from subsequent samples increases accuracy of fluorophore estimates. An accurate "blank" is typically a water sample that has been filtered through a GF/F or membrane filter in order to remove the algal cells, but to still retain additional dissolved components.

3.12 Using the Secondary Standard

This section describes how to use the Solid Secondary Standard, P/N 8000-952, with most of the Trilogy fluorescence modules. It cannot be used with CDOM/FDOM, Histamine, PTSA, OBA, Crude Oil, Hydcrocarbons, Absorbance or Turbidity modules. The two main benefits of using the Solid Secondary Standard are:

1) It can be used in place of a primary liquid standard once a correlation between a primary standard and the solid standard has been established.

2) It can be used to check the fluorometer stability and/or check for loss in sensitivity resulting from instrument/optical module problems.

The Solid Secondary Standard provides a very stable fluorescent signal. It has an adjustment screw so you can tune the Solid Standard to provide a signal to match a specific sample. It should be noted that each Solid Standard/Fluorometer relationship is unique. This means that a given Solid Standard cannot be used for identical readings on multiple fluorometers or modules.

3.12.1 Using the Solid Secondary Standard for *in vivo* Chlorophyll Applications

1. To establish a correlation between a known chlorophyll concentration and the fluorometer reading, measure a sample containing algae and note the fluorometer reading.
2. Insert the Solid Standard in the Optical Module and adjust the Solid Standard to produce the same reading on the fluorometer as in step 1 by turning the Secondary Standard adjustment screw. Clockwise produces a lower signal.
3. Perform a chlorophyll extraction using the Trilogy Laboratory Fluorometer, Spectrophotometer or HPLC to determine the actual chlorophyll *a* concentration in the sample[1]. This will provide the correlation between the solid standard and the actual chlorophyll *a* concentration.
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 Trilogy reading.

Figure 8: The *Adjustable Secondary Standard includes a fluorescent rod that provides an extremely stable signal.*

3.12.2 Using the Solid Secondary Standard for Dye Applications:

The Solid Secondary Standard accessory can also be used to check the fluorometer's stability for dye tracing applications.

1. To use the Solid Standard to establish a correlation between a known dye concentration and the fluorometer reading, measure a dye solution of known concentration, say 50 ppb, and note the Trilogy reading.
2. Place the Solid Standard in the Optical Module, and turn the adjustment screw to produce the same displayed concentration as in step 1. Turning the secondary standard adjustment screw clockwise reduces the displayed concentration.

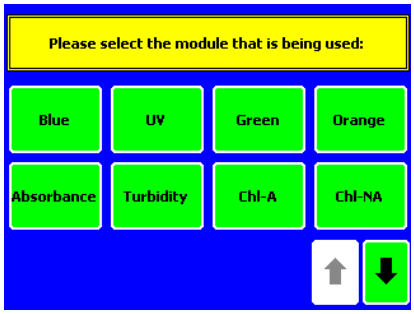
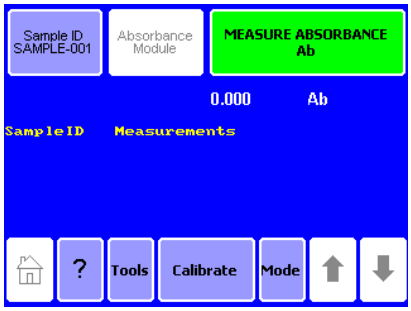
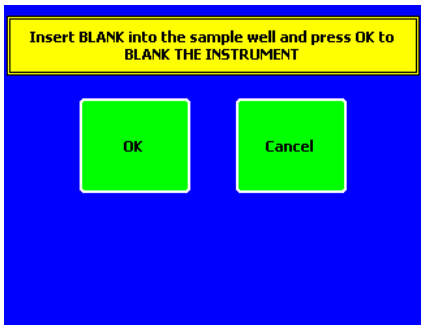
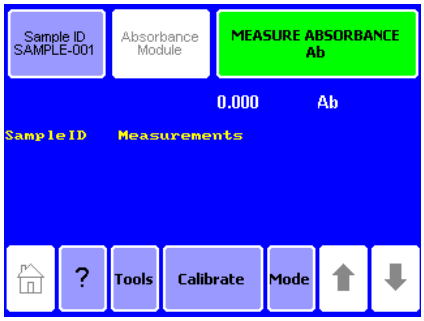
Comprehensive information on dye trace measurements can be found at the following Turner Designs URL:

http://www.turnerdesigns.com/customer-care/fluorometer-application-notes/fluorescent-dye-tracing-application-notes

3.13 Absorbance Module Calibration Procedure

Calibrate the Trilogy after powering up and after changing filters. For best results, calibrate the Trilogy and Absorbance Optical Module immediately before reading a series of samples. Comprehensive information on Absorbance measurements can be found at the following Turner Designs URL: http://www.turnerdesigns.com/customer-care/fluorometer-application-notes/absorbance-fluorometer-application-notes

[1] Information on doing a chlorophyll *a* extraction can be found on the Turner Designs web site at this URL: http://www.turnerdesigns.com/customer-care/fluorometer-application-notes/epa-method-445-fluorometer-application-notes

Instructions	Sample Screen
Turn ON the Trilogy. Open the lid. Insert the correct module for testing, making sure you hear a click sound. Close the lid.	
a) Touch "**Absorbance**" to select the Absorbance Application Module and calibration firmware and confirm by touching "**OK**".	
b) On the home screen, touch **"Calibrate"** to update the blank.	
c) Open lid, insert a sample cuvette containing your blank sample, close lid and touch **"OK"** to complete the calibration update.	
d) When the blanking is complete, the display will revert to the Absorbance Home/Measurement Screen.	

4. Touch Screen Basics

The touch screen provides a user-friendly method to operate the Trilogy. The touch screen is sensitive to the light pressure of a fingertip. It is not necessary to use a stylus. After 20 minutes without activity or user stimulation, the touch screen hibernates. Lightly touch the screen once to reactivate. To select a function, touch the button corresponding to the function once.

4.1 Tools

Touch the "Tools" key to access "Settings" and "Diagnostics."

4.2 Settings

Contrast

Touch the "Contrast" key to increase or decrease the brightness of the touch screen and enhance visibility. The arrows increase or decrease contrast. Touch the "Home" key to save the adjustment and return to the "Home" screen.

Reset

The "Reset" button restarts the Trilogy. Normal operation does not require this feature. The Reset feature erases data displayed on the "Home" screen.

Lid Start

Touch the "Lid Start" key to turn the feature ON. While the Lid Start feature is ON measurement begins as soon as the lid closes and the touch screen does not hibernate. The Lid Start feature allows for immediate measurement and eliminates the need to touch the "Measure" key. Return to the Lid Start key under the "Settings" menu to turn the feature OFF.

4.3 Diagnostics

Touch Screen Calibration

The "Diagnostics" menu contains a method for screen calibration. Although the touch screen is calibrated at the factory, it may need re-calibration over time. Follow the instructions on the screen for calibration. You will have the option to abort, reset to factory settings or accept the new calibration.

Device Configuration

The "Device Configuration" key contains useful information on firmware revisions and instrument setup.

4.4 General

Please note the Trilogy does not store more than 20 measurements at one time. Measurements are not stored between power cycles.

5. System Connections

Establish a connection between the Trilogy and a PC or a printer to export data. Connect the 9-pin RS-232 serial cable between the Trilogy and the PC or printer. The male 9-pin connector attaches to the Trilogy and the female connector attaches to the PC.

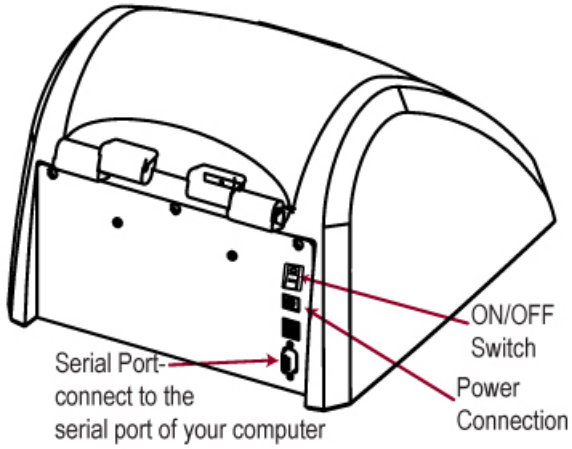


Figure 9:

5.1 Serial Output Functional Check

To perform a functional check on the Trilogy Serial Output, complete the above for the Trilogy and then complete the following steps. Note that the Serial Interface Software is not required.

1. Connect the Trilogy Serial Port to the PC Serial Port. If no serial port is available, connect to USB with a serial to USB adaptor such as P/N 2300-115.

2. To receive ASCII data from the Trilogy, open a HyperTerminal program or some other appropriate data logging software on the PC.

3. Open a connection in HyperTerminal with the following COM Port settings:

Baud Rate:	9600
Data Bits:	8
Parity:	None
Stop Bits:	1
Flow Control:	None

4. Make a measurement with the Trilogy.

5. At the completion of the measurement, the concentration value will be displayed on the Trilogy display window and also displayed in the HyperTerminal window.

This completes the Functional Check of the Trilogy Serial Output. If you successfully completed these steps, the Trilogy Serial Output is functioning. If you were not able to complete these steps, refer to the troubleshooting - see Section 6.3.

6. Spreadsheet Interface Software Installation

Install the Spreadsheet Interface Software (SIS) to send data to an Excel spreadsheet. The Spreadsheet Interface Software requires a PC loaded with Windows '98 or higher (tested up to Windows 10) an available serial port, and Excel. Insert the software flash drive into the USB on the PC to initiate the installation program. After the installation is complete, an icon for the SIS appears on the PC desktop and in the "Programs" menu.

Make sure no other program on the PC is already using COM 1.

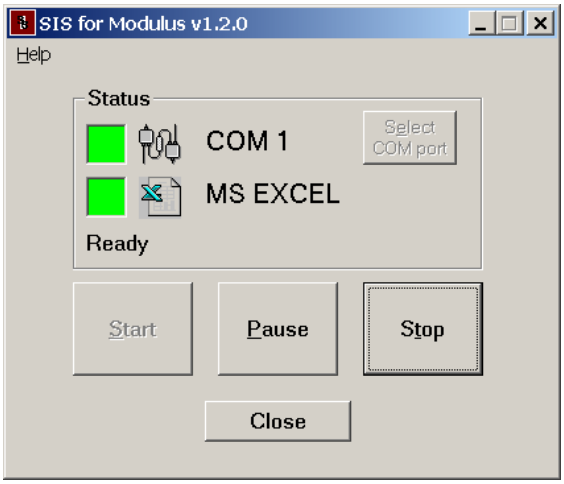


Figure 10:
Spreadsheet Interface Software status window showing communications are set up on COM 1 and that the data will be displayed in an EXCEL spreadsheet

6.1 Excel Spreadsheet Example

Turner Designs	
SAMPLE-001	426.73 RFU
SAMPLE-002	24.57 RFU
SAMPLE-003	35.49 RFU
SAMPLE-004	2.56 RFU

6.2 Viewing Calibration Data in Excel.

With the Trilogy connected to the Spreadsheet Interface Software, stored calibration data can be sent to Excel. From the touchscreen select “Calibrate” then “Use stored calibration”. Select the stored calibration to be sent to Excel and select “View Calibration Details”. This information will appear in the opened Excel file.

6.3 Spreadsheet Interface Software Troubleshooting

Symptom	Possible Cause	Possible Solution
Excel does not open	Excel is not installed on the PC.	Make sure Excel is installed on your PC.
	The software cannot find Excel.	Open Excel from the Programs Menu on the PC, then open the spreadsheet interface software.
Both green lights are on, but data does not appear in Excel	Wrong COM port selected.	Click **"STOP"** then click on the **"COM"** button to change the COM port.
	Trilogy is not connected to PC.	Check the RS-232 connection between the Trilogy and the PC.
New data does not report to Excel	There is an editing process occurring within an Excel cell.	Wait until all the data is collected before editing the Excel spreadsheet.
The software does not install	The PC allows only administrators to install new software.	Log in as Administrator for the PC and install the software or contact your IT support desk.
The software does not open	The software was not installed properly.	Log in as Administrator, remove the software and re-install.

7. Warranty and Obtaining Service

7.1 Warranty

Turner Designs warrants the Trilogy 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. Your instrument must be installed according to instructions in the User's Manual. 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.

7.2 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.

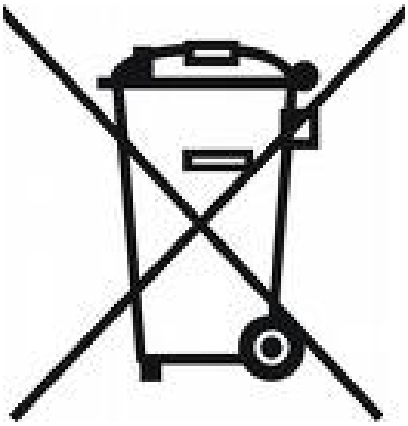
3. If proper performance is not obtained you will be issued a Return Materials Authorization number (RMA) to reference. Package the unit, write the RMA number on the outside of the shipping carton, and ship the instrument, prepaid, to Turner Designs. If the failure is covered under the warranty terms, 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).

7.3 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, Inc.
1995 N. 1st Street
San Jose, CA 95112



Appendix A Instrument Specifications

Sample Adaptors	Modules accommodate 10 x 10 mm square plastic cuvettes 12 mm round test tube adaptor P/N 016-0810 is required for 12 x 75 mm round tubes and 12 x 35 mm round vials. Modules denoted with a P/N 7200-###-**W** are for use with glass or quartz 10x10 mm square cuvettes.
Readout	Direct Concentration (µg/L, ppb, etc.) or Raw Fluorescence (RFU) One to Five point calibration with up to 18 calibrations stored
Light Source & Detector	LED and Photodiode
Blank	Reads and subtracts blank
Data Output	100% ASCII format through a 9 pin RS-232 serial cable at 9600 baud
PC Operating System (optional if connected to PC)	Windows 98 or later. Tested for use up to Windows 10
Power	100 to 240VAC Universal Power Supply included, Output 12VDC 0.84A Max
Dimensions	12.92”D x 10.44”W x 8.42”H (32.82 cm D x 26.52 cm W x 21.39 cm H)
Operating Temperature	60 – 105 °F (15 - 40 °C)
Weight	8.1 lbs (3.65 kg)
Humidity	75% RH maximum
Warranty	One year

Refer to the Optical Specification Guide http://www.turnerdesigns.com/t2/doc/spec-guides/998-7281.pdf for details about specific modules and applications.

Appendix B Principles of Fluorescence

Principles of Fluorescence

Fluorescence is a physical property of certain atoms and molecules. It is a molecule's ability to absorb light energy at one wavelength, then instantaneously re-emit light energy of another, usually longer, wavelength. Each compound that fluoresces has a characteristic excitation wavelength (the wavelength of light that it absorbs) and a characteristic emission wavelength (the wavelength of light that it emits when the molecules relax and return to their ground state). These excitation and emission wavelengths “or spectra” are often referred to as the compound's fluorescence signature. Figure 11 shows the key components of a filter fluorometer.

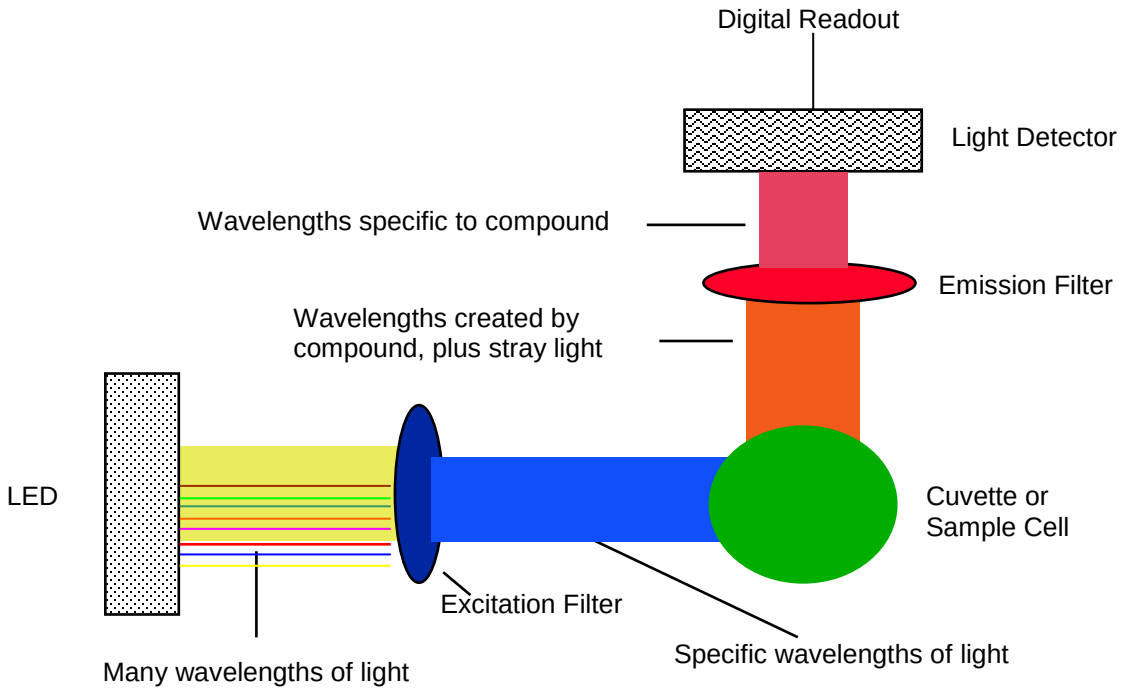


Figure 11:

LED: The light emitting diode provides the light energy that "excites" the compound of interest. The LED actually provides a broader range of light than that which excites the compound. This broad light range is illustrated by the "many wavelengths of light" shown in Figure 11.

Appendix C Linear Range, Quenching and Temperature Considerations

Linear Range and Quenching

The linear range is the concentration range in which the Trilogy output 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 non-linear 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 - see Figure 12. At even higher concentrations, the fluorescence signal will decrease even though the sample concentrations are continuing to increase. This effect is known as “signal quenching”.

Linearity may 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 of the fluorophore.

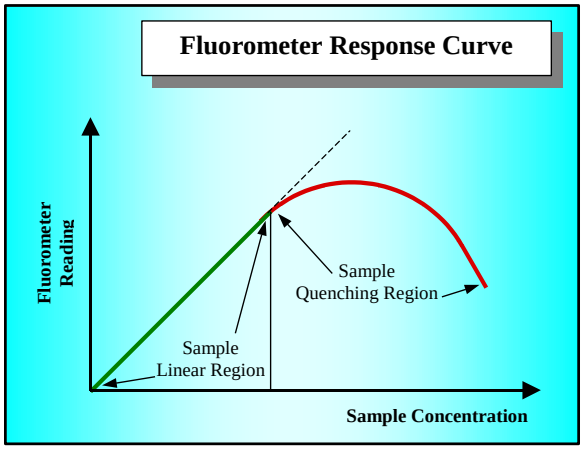


Figure 12:
Graph showing Linear and Quenching Regions of the sample’s response

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

Appendix D Chlorophyll *a* Acidification and Non-Acidification Calculations

When in direct concentration mode, the following calculations occur within the Trilogy and the screen values displayed are corrected chlorophyll *a* and pheophytin *a* values for the acidification method and the corrected chlorophyll values for the non-acidification method. Provided as a reference for external calibration as described in Section 3.7 Note.

Acidification Method

I. Variables stored during calibration phase of fluorometer

$C_{stand[1]}$ = Concentration of standard 1
F_{blank} = Fluorescence of Blank value
$F_{stand[1],B}$ = Fluorescence of standard 1 before acidification
$F_{stand[1],A}$ = Fluorescence of standard 1 after acidification
F_m = Acidification Ratio = $(F_{stand[1],B} - F_{blank}) / (F_{stand[1],A} - F_{blank})$

II. Variables required from the sample analysis phase

$F_{samp,B}$ = Fluorescence of sample before acidification
$F_{samp,A}$ = Fluorescence of sample after acidification
$V_{solvent}$ = Volume of solvent used to extract sample
V_{water} = Volume of water filtered

III. Interpolation equation used in end calculation of chlorophyll *a* and pheophytin *a* concentrations

$Interp_{,B} = C_{stand[1]} * (F_{samp,B} - F_{blank}) / (F_{stand[1],B} - F_{blank})$
$Interp_{,A} = C_{stand[1]} * (F_{samp,A} - F_{blank}) / (F_{stand[1],B} - F_{blank})$

IV. End calculation for corrected chlorophyll *a* and pheophytin *a*

Chlorophyll *a* concentration = $[F_m/(F_m-1)] * (Interp_{,B} - Interp_{,A}) * (V_{solvent}/ V_{water})$
Pheophytin *a* concentration = $[F_m/(F_m-1)] * [(F_m * Interp_{,A}) - Interp_{,B}] * (V_{solvent}/ V_{water})$

Non Acidification Method

I. End calculation for corrected chlorophyll *a*

Chlorophyll *a* concentration = $C_{stand[1]} * [(F_{samp} - F_{blank})/ (F_{stand[1]} - F_{blank})] * (V_{solvent}/ V_{water})$

Appendix E External Calibration for Extracted Chlorophyll *a* Examples

As an alternative to the internal calibration and calculations, external calibration and calculations allow for more flexibility in interpretation of results.

To do this, run a series of dilutions for a known standard on the Trilogy in RFU mode along with an acetone blank and record the values as shown below:

Calibration Data for Chlorophyll A	
Standard Conc. µg/L	Fluorometer Reading
2	3385.8
10	13841.3
50	67245.8
100	134545.7
200	273006.3
Blank	842.4

Fb = 3385.8	Fa =1936.1	Fm =2.33

Take a reading of samples:

Fluorometer Raw Readings				
Sample A	Pheophytin	a	981249.87	RFU
	Chlorophyll	a	588728.81	RFU

Establish:
Fb = fluorescence before acidification
Fa = fluorescence after acidification
Fm = acid ratio

Variables	values
Cstand[1] = 2 ug/L	2
Fblank = 842.4	842.4
Fstand[1],B = 3385.8	3385.8
Fstand[1],A =1936.1	1936.1
Fm =2.33	2.33
Vsolvent = 10 mL	10
Vwater = 500 mL	500
Fsamp,B = 981249.87	981249.9
Fsamp,A = 588728.81	588728.8

Complete calculations:

Fm = Acidification Ratio = (Fstand[1],B – Fblank) / (Fstand[1],A – Fblank)

= 2.33

Interp,B = Cstand[1] * (Fsamp,B - Fblank) / (Fstand[1],B - Fblank)

= 770.94

Interp,A = Cstand[1] * (Fsamp,A - Fblank) / (Fstand[1],B - Fblank)

= 462.28

Chlorophyll a concentration = [Fm/(Fm-1)] * (Interp,B - Interp,A) * (Vsolvent/ Vwater)