TURNER DESIGNS
Reliable Instruments for an Unreliable World


# Trilogy Laboratory Fluorometer

# User's Manual

Version 1.7
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P/N 998-7210

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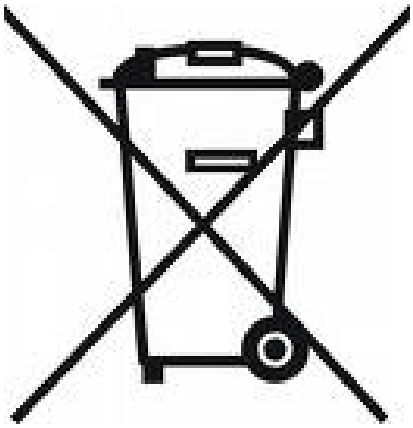


**WASTE ELECTRICAL AND ELECTRONIC EQUIPMENT (WEEE) DIRECTIVE**

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.
There are two versions of the Trilogy:

Model 7200-000 with S/N 720XXXXXX
Model 7200-002 with S/N 721XXXXXX - adds USB 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 including markers used for DNA/RNA bioassays.

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 desired 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:

- Trilogy Laboratory Fluorometer
- Solid Secondary Standard P/N 8000-952. **Not intended for use with UV applications, absorbance, or turbidity optical modules.**
- Power Supply Kit P/N 7200-941
- USB Data Cable P/N 021-7202 for use with Model 7200-002
  ***Note: For users with Model 7200-000 USB Data Cable P/N 021-7202 will not work. You must use RS-232 Cable P/N 021-0700.***
- Quick Start Guide (hardcopy)
- 12 mm Round Adaptor P/N 016-0810
- USB drive with Trilogy Software and USB Driver
- Cuvettes or Test Tubes if ordered
- Optical Module(s) as ordered. ***Note: Refer to the Optical Specification Guide for details. Modules denoted with a P/N 7200-###W are for use with square glass or quartz 10x10mm 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. Plug the power supply into the power connection port of the instrument, see Figure 1, and plug it into a wall outlet. See Specifications for power requirements.

**Figure 1:**

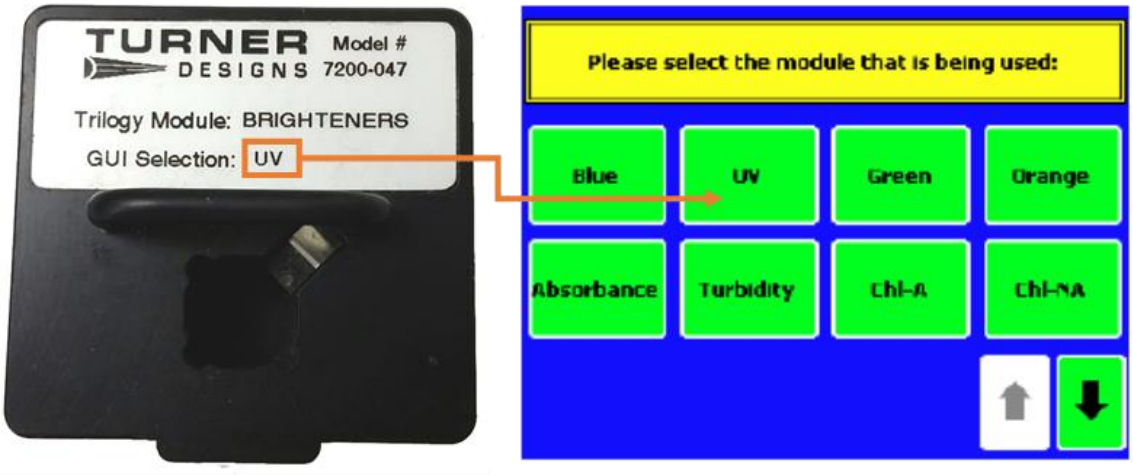
1.5 Getting to the Home Screen

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 Module 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.

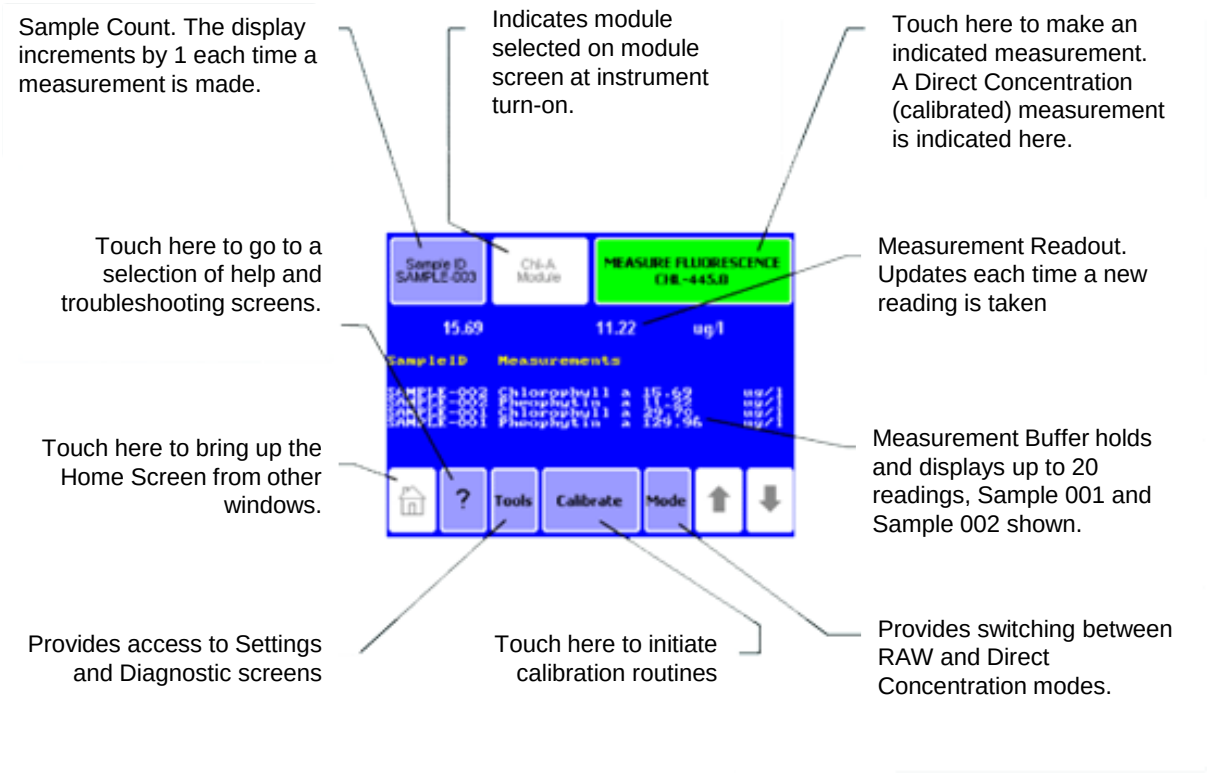



**Figure 4:** Trilogy Home Screen Display for Fluorescence Module Configuration.

## 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 looking at relative changes in fluorescence rather than absolute concentration estimates. 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*** for specific application recommendations.

1. Power 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 display the average reading on the screen.

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 a computer and using Trilogy Software. 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 calibration information for the selected application. "View Cal Details" specifically provides raw fluorescence for each standard and the blank as well as the unit of measure for the Optical Application Module selected.

**Continuous Sampling**

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

1. Touch "Continuous Sampling" and touch the OFF button in the top right hand corner of the display to turn the feature ON. Touch the numerical values to the right of the “Frequency, meas/sec” title to highlight it and use the up/down arrow keys to set a desired interval. Then touch the numerical value to the right of “Total number of measurements” to highlight it and use the up/down arrow keys to set a desired value. For Model 7200-002 the maximum number of total measurements is 9999. To fast scroll, touch and hold the up/down arrow keys.

***NOTE: Although the Model 7200-000 screen indicates 9999 measurements, the maximum number of total measurements is 1000.***

2. Touch "OK" to return to the "Home" screen.
3. Connect the Trilogy to a printer or a PC to collect data. Opening the

lid while Continuous Sampling is underway will halt Continuous Sampling and reset the sampling queue.

**Measurement Tip:**

On the Home Screen, touch **Tools**, then **Settings**, then touch the **Lid Start** selection 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** selection under the **Settings** menu and touch it again 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, touch “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.

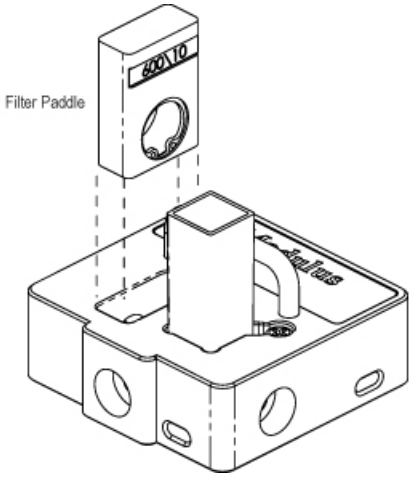



**Figure 5.**

**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 selecting Absorbance from the GUI selection screen and confirming the Absorbance Module is installed. 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 Percent 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

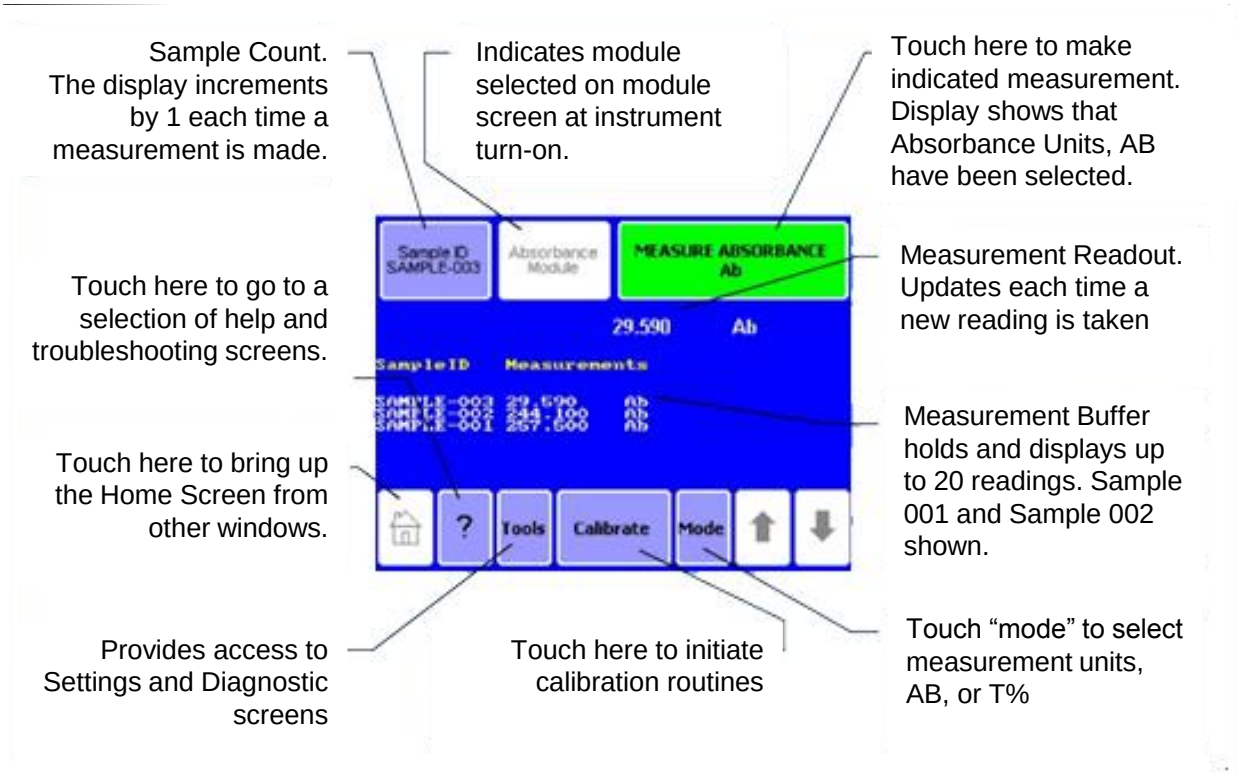



**Figure 6:** Home Screen When Using Absorbance Module

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. Power 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 display the average reading on the screen.

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 a computer and using Trilogy Software. 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 touch the OFF button in the top right hand corner of the display to turn the feature ON. Touch the numerical values to the right of the “Frequency, meas/sec” title to highlight it and use the up/down arrow keys to set a desired value. Then touch the numerical value to the right of “Total number of measurements” to highlight it and use the up/down arrow keys to set a desired value. For Model 7200-002 the maximum number of total measurements is 9999. To fast scroll, touch and hold the up/down arrow keys.

***NOTE: Although the Model 7200-000 screen indicates 9999 measurements, the maximum number of total measurements is 1000.***

2. Touch "OK" to return to the "Home" screen.
3. Connect the Trilogy to a printer or a PC to collect data. Opening the lid while Continuous Sampling is underway will halt Continuous Sampling and reset the sampling queue.

**Measurement Tip:**

On the Home Screen, touch **Tools**, then **Settings**, then touch the **Lid Start** selection 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** selection under the **Settings** menu and touch it again 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.

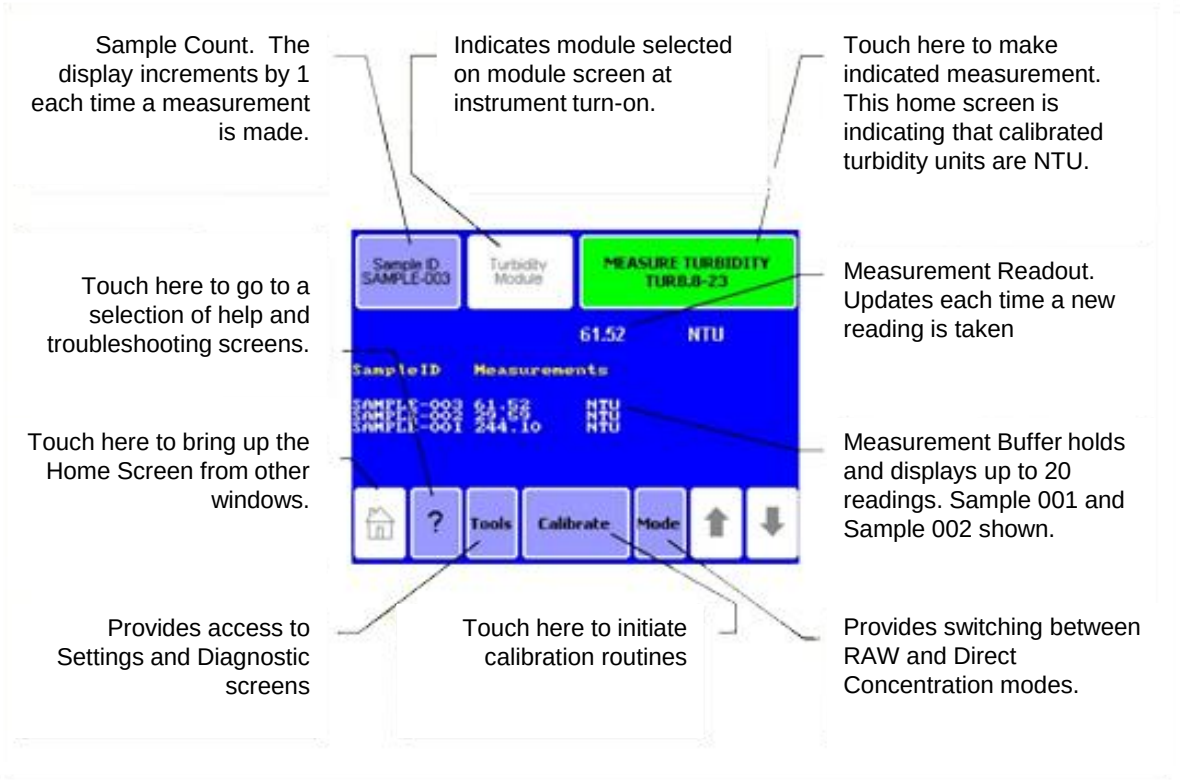



**Figure 7**: Home Screen When Using Turbidity Module

### 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.

If a calibration has been stored, touch "**Mode**" on the Home Screen to select the measurement mode.

1. **Raw Mode**: The Raw Mode should be used for qualitative measurement looking at relative changes rather than absolute concentration estimates. 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 touch the OFF button in the top right hand corner of the display to turn the feature ON. Touch the numerical values to the right of the “Frequency, meas/sec” title to highlight it and use the up/down arrow keys to set a desired value. Then touch the numerical value to the right of “Total number of measurements” to highlight it and use the up/down arrow keys to set a desired value. For Model 7200-002 the maximum number of total measurements is 9999. To fast scroll, touch and hold the up/down arrow keys.

***NOTE: Although the Model 7200-000 screen indicates 9999 measurements, the maximum number of total measurements is 1000.***

2. Touch "OK" to return to the "Home" screen.
3. Connect the Trilogy to a printer or a PC to collect data. Opening the lid while Continuous Sampling is underway will halt Continuous Sampling and reset the sampling queue.

**Measurement Tip:**

On the Home Screen, touch **Tools**, then **Settings**, then touch the **Lid Start** selection 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** selection under the **Settings** menu and touch it again 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 uses stored calibration values to automatically convert fluorescence responses to concentration estimates; this saves you from having to manually calculate concentration estimates using RFU.

### 3.2 When to Calibrate

- Recalibrate if the ambient temperature changes by ± 10°C.
- Recalibrate after changing Optical Application Modules or if you plan on measuring a fluorophore that is different than the fluorophore used to calibrate.
- 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 for 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 measurements, looking at relative changes in fluorescence or scatter rather than absolute concentration estimates. Readings are displayed in Relative Fluorescence Units (RFU).

2. **Direct Concentration Mode**: The Trilogy can be calibrated using a single or multi-point calibration. In multi-point calibrations, up to five standards and a blank can be read for increased accuracy when estimating concentrations. The software uses these points to generate a calibration curve. 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 fluorophore in the sample. 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 named and stored for future use.

3.5 Turner Designs offers liquid dye standards which can be used for Direct Calibration Procedure on the Trilogy converting relative fluorescence to concentration estimates. The response from various dye standards is correlated with actual fluorophore concentrations for many applications. Simply choose the appropriate standard for the application, if it is listed below, and calibrate the Trilogy using the following calibration instructions to obtain fluorophore concentration estimates.

| Application | Module Part Number | Liquid Calibration Standard Concentration (ppb) | Part Number | Fluorophore Concentration | Units of Measure | NOTES |
|---|---|---|---|---|---|---|
| Chl *in vivo* | 7200-043 | RWT 400 ppb | 6500-120 | 45 | µg/L | |
| Phycocyanin | 7200-044-W | RWT 200 ppb | 6500-020 | 2000 | ppb | |
| Phycoerythrin | 7200-042 and 7200-042-W | RWT 100 ppb | *See Notes | 1012 | ppb | Purchase 6500-020 and dilute to 50% with deionized water |

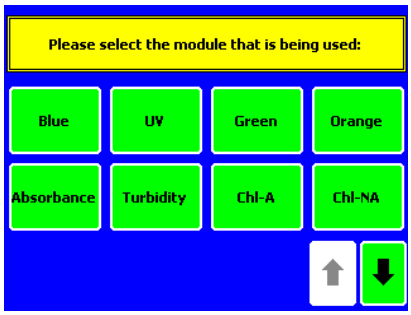
3.6 Direct Calibration Procedure – Fluorescence and Turbidity Modules, Single Point and Multi-Point Calibration.

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

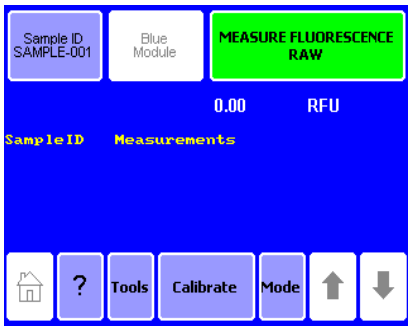
| Instructions | Sample Screen |
|---|---|
| a) Open the lid. Insert the module that will be used for testing, making sure you hear a click indicating the module has properly seated. Close the lid and power ON the Trilogy. | |

b) Select the application associated with the module that was snapped in and confirm by touching “**OK**”.

**Note: Refer to the module label to confirm module identification.**

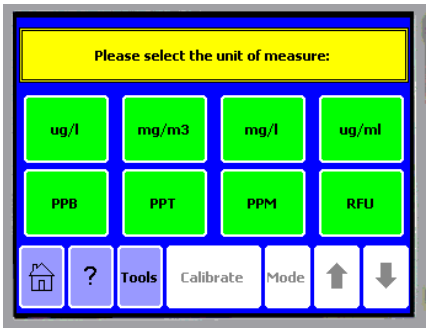



c) On the home screen, touch **"Calibrate"** to begin a calibration sequence.



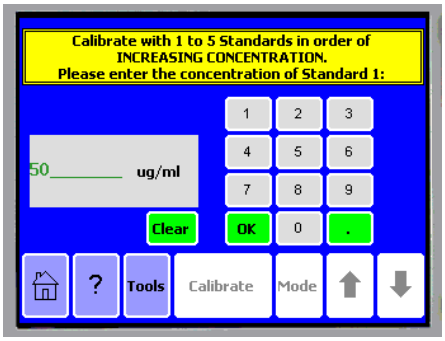

d) Select **“Run New Calibration”.**

e) Select the unit of measurement.



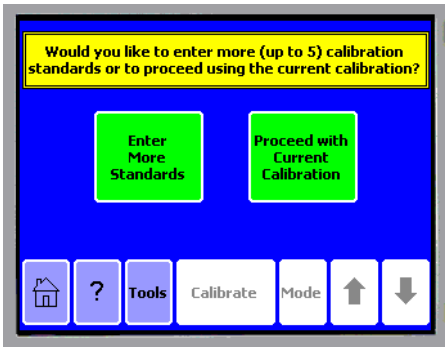

f) Open the lid and insert the blank sample. Close the lid and touch **“OK”**.

g) After the blank has been measured, open the lid and remove the blank. Enter the concentration for the first Standard and touch **"OK"** (Note: if doing multi-point calibrations, be sure to use Standards in order of increasing concentration).

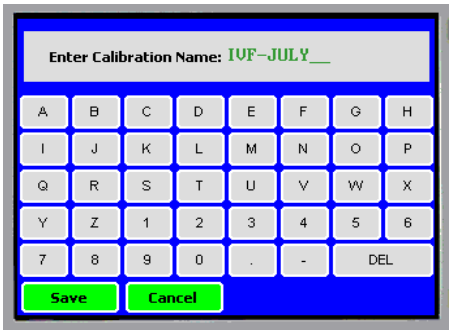



h) Insert the standard, close the lid, and touch **"OK"**.

i) After the standard has been measured, either select **"Proceed with Current Calibration"** and go to step "j" below or **"Enter More Standards"** and return to step "g" above.




j) Name and Save the calibration for future use.




k) Subsequent readings in the Direct Concentration mode should reflect the actual concentration of the fluorophore in relation to the standards used for calibration.

l) Confirm successful completion of the calibration by measuring the same standard used to calibrate the Trilogy. The displayed concentration should equal the value used in step "g" above.

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

EPA Method 445.0 is a standard method for quantification of extracted chlorophyll *a* and pheophytin *a* in marine and fresh water algae using fluorescence. It requires solvents for extracting chlorophyll from cells, measuring fluorescence before and after acidification, and some fairly simple calculations to arrive at the chlorophyll *a* and pheophytin *a* concentrations, see Appendix D. If high concentrations of pure chlorophyll *b* are present - see Section 3.8.

A known concentration of pure chlorophyll *a*, as a standard, is required to calibrate the Chl – A (Acidification) module. We recommend that you perform an external calibration, see Appendix D & E, using this module so that you can have a detailed analysis of your results. It is recommended that users periodically check the stability of their instrument/module using the Solid Secondary Standard or a standard of known concentration. **If using the Solid Secondary Standard, record all readings in RFU mode.** Liquid Primary Chlorophyll *a* P/N 10-850 and Solid Secondary standards P/N 8000-952 are available from Turner Designs. We also offer an Excel spreadsheet calculator on our website www.turnerdesigns.com that will facilitate external calibrations for the Chlorophyll a Acidification module.

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

The Trilogy can calculate environmental chlorophyll estimates using filtered and solvent volumes. The environmental chlorophyll concentration of a sample is the *in situ* chlorophyll concentration (i.e. the chlorophyll concentration prior to collecting and processing the water sample). Simply input the volume of water filtered and the volume of solvent used to automatically calculate the environmental chlorophyll concentration.

If the *in vitro* chlorophyll concentration (i.e. the extracted or the concentration of chlorophyll in the test tube) is desired, enter a 1 for filtered volume and 1 for solvent volume when prompted and the in vitro chlorophyll concentration will be displayed.

***Note: You can calculate the in situ concentration using in vitro estimates by multiplying by the solvent volume and dividing by the volume filtered, see Appendix D for formulas and Appendix E for an example.***

***Note: As an alternative to the internal calibration, external calibration allows for a more detailed analysis of results, see Appendix D for formulas and Appendix E for an example.***

## 3.9 "Direct Calibration" Procedure – Extraction, Non-Acidification, Single Point and Multi-Point (Welschmeyer Method)

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*

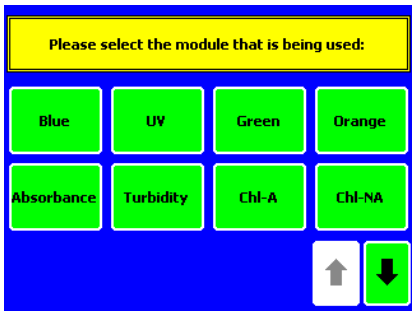
measurement using this procedure. Extract your samples according to EPA Method 445.0, but skip the acidification step.

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

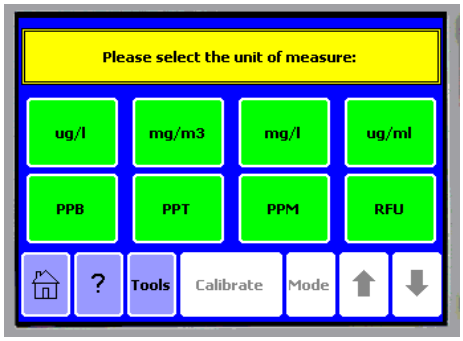
The calibration procedure for the Chlorophyll Non-Acidification follows the same steps as for the Direct Calibration mode - see Section 3.5, however, the measurement procedure will prompt for the filtered and solvent volumes.

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

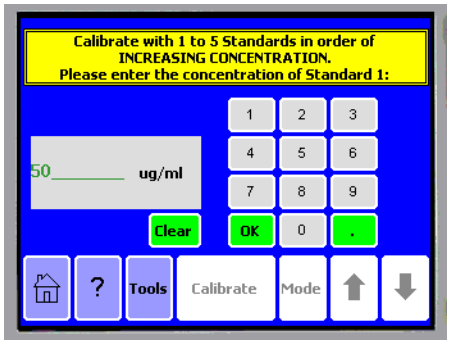
| Instructions | Sample Screen |
|---|---|
| Open the lid. Insert the **Chl – NA** module that will be used for testing, making sure you hear a click indicating the module has properly seated. Close the lid and power ON the Trilogy. | |
| a) Touch "**Chl – NA**" to select the Chlorophyll *a* Non-Acidification 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 and touch **“OK”**.




e) Open the lid and insert the blank sample. Close the lid and touch **“OK”**.

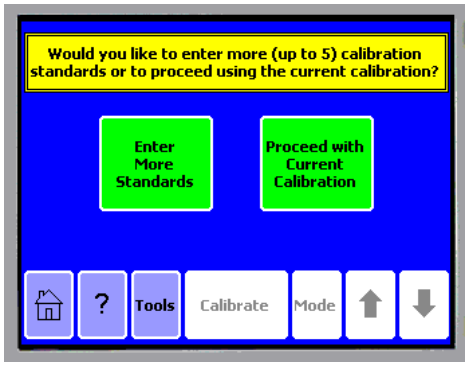
f) After the blank has been measured, open the lid and remove the blank. Enter the concentration for the first Standard and touch **“OK”** (Note: if doing multi-point calibrations, be sure to use Standards in order of increasing concentration).




g) Insert the standard into the module’s sample compartment, close the lid, and touch “**OK**”

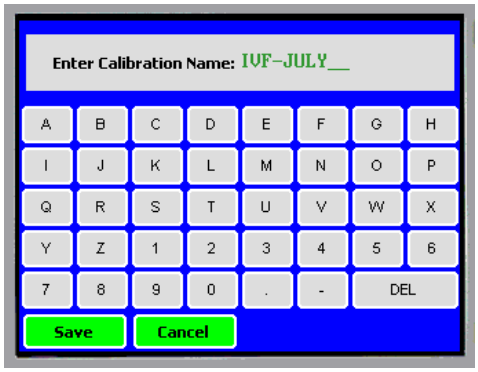
h) After the standard has been measured you will be prompted to either enter more standards or proceed with the current standard.

**NOTE:** for multiple standard, choose “**Enter More Standards**” and repeat steps f – h of this procedure until all standards have been measured.



i) When all the standards have been measured, touch "**Proceed with Current Calibration**"

j) Name and Save the calibration for future use (optional).




k) 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.

3.11 *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 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).

## 3.12 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.

## 3.13 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 UV, Absorbance or Turbidity modules. The two main benefits of using the Solid Secondary Standard are:

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

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.

### 3.13.1 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. Determine the 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.

[1] Information on doing a chlorophyll *a* extraction can be found on the EPA web site at this URL: https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NERL&dirEntryId=309417

3.13.2 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.

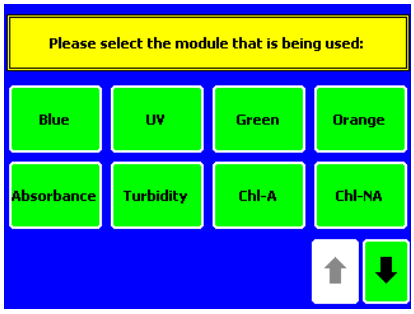
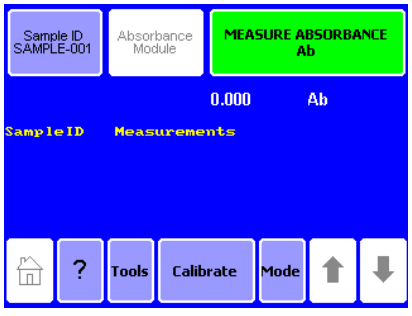
1. To use the Solid Standard to establish a correlation between a known dye concentration and the fluorometer reading, measure a dye solution of known concentration, say 50 ppb, and note the Trilogy reading.
2. Place the Solid Standard in the Optical Module, and turn the adjustment screw to produce the 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:

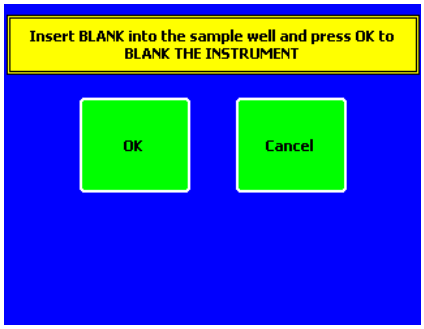
https://www.turnerdesigns.com/dye-fluorometer

3.14 Absorbance Module Calibration Procedure

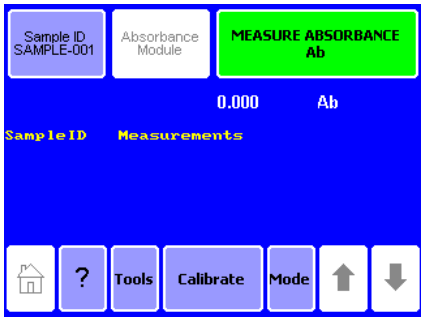
Calibrate the Trilogy after powering up and after changing filters. For best results, calibrate the Trilogy and Absorbance Optical Module immediately before reading a series of samples. Comprehensive information on Absorbance measurements can be found at the following Turner Designs URL: https://www.turnerdesigns.com/absorbance-fluorometer

| Instructions | Sample Screen |
|---|---|
| Turn ON the Trilogy. Open the lid. Insert the **Absorbance** module making sure you hear a click sound. Close the lid. | |
| a) Touch "**Absorbance**" to select the Absorbance Application Module and confirm by touching "**OK**". |  |
| b) On the home screen, touch **"Calibrate"** to blank the instrument. |  |

c) Open lid, insert a sample cuvette containing your blank sample, close lid and touch **“OK”** to complete the calibration.




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 display more than 20 measurements at one time. Measurements are not stored between power cycles.

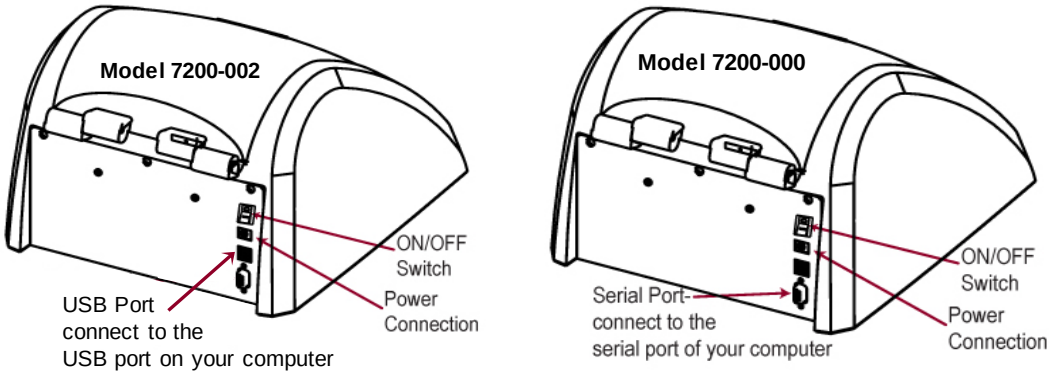
## 5. System Connections

Establish a connection between the Trilogy and a PC to export data.

5.1 Connecting the Trilogy to a PC via USB cable
The Trilogy Laboratory Fluorometer Model 7200-002 can be connected to a PC for streaming data directly into Excel spreadsheets. To connect via USB you will need:

- Microsoft Windows PC
- Microsoft Excel
- Trilogy Fluorometer P/N 7200-002
- Trilogy power supply
- Trilogy Software
- Trilogy USB Drivers
- Standard USB printer cable

***Note: For users with Model 7200-000 USB Data Cable P/N 021-7202 will not work. You must use RS-232 Cable P/N 021-0700.***
For Model 7200-000 connect RS-232 Cable P/N 021-0700 between the Trilogy and the PC and skip to Step 6.1.




5.2 Follow these simple instructions to install the USB driver for connecting the Trilogy to your PC:

1. Download the necessary driver folder from the flash-drive or https://www.turnerdesigns.com/trilogy-dowloads
2. The downloaded folder will be titled 'Trilogy USB Driver' and may be a *.zip folder ***Note: For *.zip folders, right-click on the folder and select extract to extract the contents.***
3. The folder will contain a file titled 'runme'
4. Double-click on 'runme' to start driver installation on the PC
5. Click 'Yes' to allow the installer to continue
6. Click 'Next' on the installation wizard's window
7. Click 'Install' to trust the source of the installation
8. Wait while the drivers are installed - this may take several minutes.
9. Once the drivers are installed, click 'Finish'

## 6. Spreadsheet Interface Software Installation

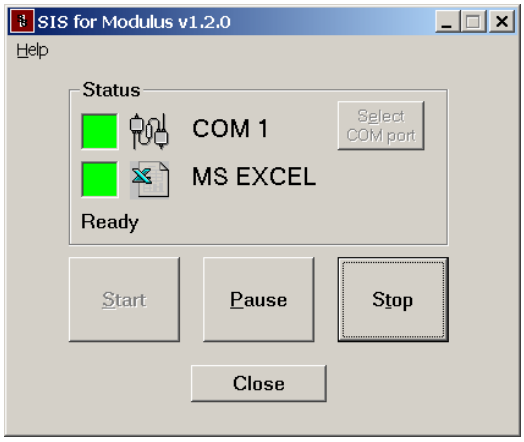
6.1 Trilogy Software Installation

1. Download the Software folder from the flash-drive or https://www.turnerdesigns.com/trilogy-dowloads
2. Click the 'setup.exe file' link.
3. Open the downloaded folder and double-click the 'setup' file.
4. Click 'Yes' to allow the installation to continue.
5. Click 'Next' on the installation wizard's window, then click 'Next'.
    a. Optional: Set a user name and organization and select who to install the application for.
6. If you want to skip step 5a, click 'Next' to change the destination folder indicating where the software will be installed.
7. Click 'Next', then 'Install' to install Trilogy Software.
8. Click 'Finish' when install has completed.
9. A Trilogy Software icon will be added to your desktop.

6.2 Connecting the Trilogy to your PC via USB

1. Connect the USB cable to your Trilogy.
   ***Note: USB port is on the Trilogy's back panel.***
2. Connect the other end of your USB cable to an available USB port on your PC.
3. Connect the power supply to the Trilogy.
   ***Note: Power port is on the Trilogy's back panel.***
4. Plug the power supply into an AC power source.
5. Use the ON/OFF switch to power up the Trilogy.
   ***Note: The lower right-hand corner of the PC's display will indicate a new hardware device (Trilogy) was detected and drivers are being located; this may take several minutes.***
6. Once drivers have been located and installed for the Trilogy, a message will be displayed in the lower right-hand corner of the PC's display indicating what COM port that was assigned.
   ***Note: You will need to know what COM port was assigned for the USB connection; if you missed the notification, you can go to your PC's device manager and open 'Ports' to find the COM port setting.***
7. Locate the Trilogy Software icon on your desktop and double-click it to open the software.
8. In the top right-hand corner of the window, click the select COM port button and set the COM port to the COM port assigned for your USB connection.
   ***Note: COM port set must be from 1 to 16.***
9. Click 'OK', then click 'Start'.

10. Both ‘MS EXCEL’ and ‘COM’ should show green indicator lights and an Excel spreadsheet should automatically open.
    a. If you don’t get green indicator lights for either ‘MS EXCEL’ or ‘COM’, you must restart your PC and repeat steps 7 – 10 of this procedure




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

11. Now all your measurements will be streamed and dumped into the Excel spreadsheet.
    ***Note: make sure to continually save the spreadsheet as there is no backup if data are deleted.***

6.3 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 |

6.4 Viewing Calibration Data in Excel.

***NOTE: Model 7200-002 does not have this feature at this time.***

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.

6.5 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 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. |
| USB cable will not install or connect | USB driver not installed properly | Log in as Administrator, remove the driver and re-install |
| | 32-bit computer | Connect to the computer using RS-232 Cable P/N 021-0700 |

## 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.

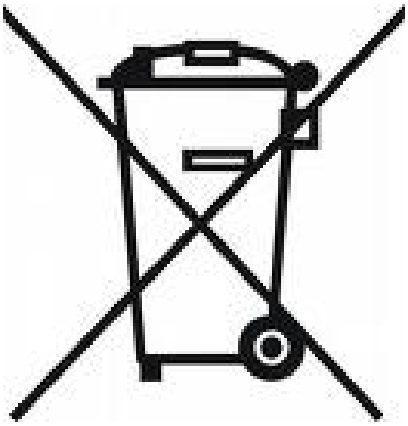
3. If proper performance is not obtained you will be issued a Return Materials Authorization number (RMA) to reference. Package the unit, write the RMA number on the outside of the shipping carton, and ship the instrument, prepaid, to Turner Designs. If the failure is covered under the warranty terms, 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 95112-4220



| | |
|---|---|
| Sample Adaptors | Modules accommodate 10 x 10 mm square plastic cuvettes<br><br>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.<br><br>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)<br><br>One to Five point calibration with up to 18 calibrations stored |
| Light Source & Detector | LED and Photodiode |
| Blank | Reads and subtracts blank |
| Data Output | • Model 7200-002 ASCII format via USB<br><br>• Model 7200-000 ASCII format via RS-232 serial cable at 9600 baud |
| PC Operating System (optional if connected to PC) | Windows 8 (x64 bit) or higher |
| 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<br>(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 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.

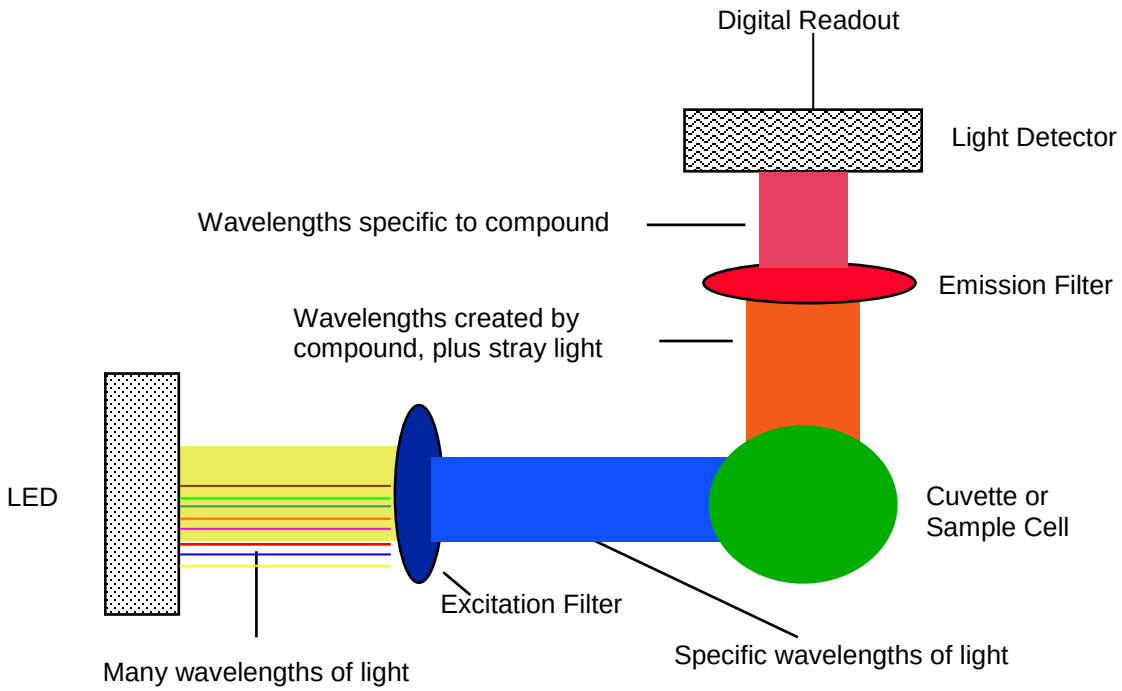



**Figure 11:**

**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.

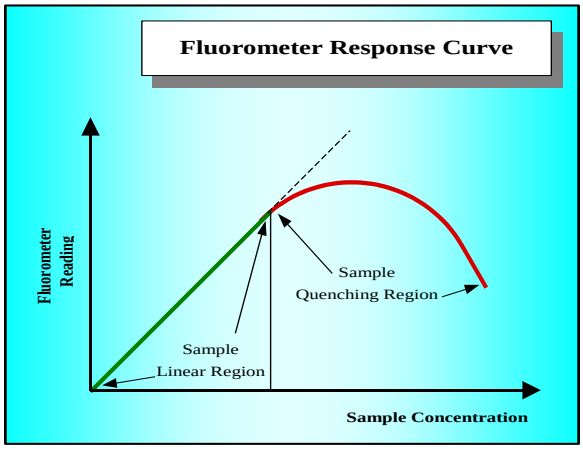



**Figure 12:**
Graph showing Linear and Quenching Regions of the sample’s response

### 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:

*https://www.turnerdesigns.com/chlorophyll-fluorometer*

## 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 Example for Chlorophyll *a* Acidification Module

As an alternative to the internal calibration, external calibration allows for a more detailed analysis 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 RFU | Blank Subtracted Reading RFUB |
| 0 | 975.6 | 0 |
| 2 | 3385.8 | 2410.2 |
| 10 | 13841.3 | 12865.7 |
| 50 | 67245.8 | 66270.2 |
| 100 | 134545.7 | 133570.1 |
| 200 | 273006.3 | 272030.7 |

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

| Before Acid RFUB | After Acid RFUB | Acid Ratio |
|---|---|---|
| Fb = 2410.2 | Fa = 1270.5 | Fm = 1.897 |

Take a reading of samples:

| Sample | RFU |
|---|---|
| Chl *a* | 40543.55 |
| Pheo *a* | 24571.84 |

| **Variables** | **Values** |
|---|---|
| Cstand[1] | 2 |
| Fblank | 975.6 |
| Fstand[1],B | 3385.8 |
| Fstand[1],A | 2246.1 |
| Fm | 1.897 |
| Vsolvent | 10 |
| Vwater | 500 |
| Fsamp,B | 40543.55 |
| Fsamp,A | 24571.84 |

Complete calculations:

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

**= 32.83**

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

**= 19.58**

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

**= 0.56**

Pheophytin *a* (µg/L) = [Fm / **(**Fm-1**)**] * [**(**Fm * Interp,A**)** – Interp,B] * [Vsolvent / Vwater]

**= 0.18**