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**Equipment Specified as Electrical and Electronic Waste**



## 2.0 Introduction

### 2.1 Description

The PhytoFlash is an *in situ* variable fluorescence system that can be used to determine the quantum efficiency of phytoplankton in both oligotrophic and mesotrophic environment. The PhytoFlash is distinct from other variable fluorescence instruments on the market in that it is the first solid-state instrument capable of variable fluorescence measurements on natural concentrations of phytoplankton. The solid-state platform allows for a much wider range of uses due to the small size, power efficiency, more stable components, and lower price point.

The PhytoFlash can be integrated into CTDs or multi-parameter systems, used in self contained mode in conjunction with the internal data logger and attachable battery or in flow-through mode for laboratory work and in underway monitoring mode. The variable fluorescence measurement is being used in an ever-growing list of applications, such as:

- Nutrient status of planktonic algae
- Detecting the onset of algal blooms
- Accurate measurement of algal biomass
- Monitoring algal community changes
- Measurement of non-photochemical quenching (laboratory mode)

The PhytoFlash technique utilizes 3 low intensity light emitting diodes (LEDs) to monitor the minimum fluorescence ( $F_o$ ) and 6 high intensity LEDs to saturate cells in the sample chamber and measure the maximum fluorescence ( $F_m$ ). The response curve from the saturating flash can be viewed in the laboratory mode. Unlike other variable fluorescence instruments, the PhytoFlash does not provide induction curve data and is not designed as a physiological instrument for laboratory use. Rather, it is designed as a simplified and robust variable fluorescence system for submersible and/or laboratory use.

The PhytoFlash can be purchased in the Blue (chlorophyll *a* specific) version. The Blue PhytoFlash uses blue excitation energy to excite the chlorophyll *a* molecule, specifically PhotoSystem II (PSII), which is the primary molecule used for light capture in most all chlorophyll *a* containing algal groups. The PhytoFlash units will estimate the photosynthetic efficiency of PSI or PSII depending on the unit purchased.

### 2.1 PhytoFlash Parameters

The PhytoFlash will provide the following parameters:



<b>F<sub>o</sub></b>	Minimum fluorescence
<b>F<sub>m</sub></b>	Maximum fluorescence
<b>F<sub>v</sub></b>	Variable fluorescence (F <sub>m</sub> -F <sub>o</sub> )
<b>F<sub>v</sub>/F<sub>m</sub> (yield)</b>	Maximum quantum yield of photochemistry
<b>Blank</b>	Calculated blank value used in calibration
<b>Response Curves</b>	Available during laboratory mode

## **2.2 Inspection and Setup**

The PhytoFlash shipment package consists of:

- PhytoFlash Fluorometer, configured and factory scaled:
  - Blue PhytoFlash – used for all chlorophyll *a* containing algal groups, (P/N: 2500-100) or titanium version (P/N: 2500-100-T)
  -

The titanium optical head was designed to withstand corrosion during long-term deployments or in highly corrosive environments. The body and connector of both the standard and titanium PhytoFlash models are designed with Delrin plastic.

- PhytoFlash User's Manual on CD, (P/N: 990-2500)
- Accessory Kit that includes an RS-232 interface cable with 12 Volt Power Supply and an Optical Cleaning Brush, (P/N: 2500-110)
- Dummy Plug, (P/N: 105-2570)

Optional Accessories include:

- Flow-through cap, (P/N: 2500-710)
- Shade cap, (P/N: 2500-510)
- Solid Standard, (P/N: 2500-900)
- Battery Pack Kit, (P/N: 2500-600)
- 24 inch Pigtail cable, (P/N: 2500-170)

Serial communication programs (i.e. HyperTerminal or ProComm Plus) are required for configuration and functional testing of PhytoFlash fluorometers.

## **3.0 Measurements with PhytoFlash**



### **3.1 Introduction to Measurement Modes**

The PhytoFlash contains a processor that runs the software for the system. All you have to do is to connect the PhytoFlash to a Windows based PC, open a serial emulator window with the appropriate communications settings, and turn on the PhytoFlash. The software will then appear in the serial emulator window (see section 3.2 for details).

The PhytoFlash has three Operating Modes: 1) Self Contained, 2) Integration - Digital (RS232) or Analog, and 3) Laboratory.

The **Self Contained Mode** is used in conjunction with the internal data logger and attachable battery pack or external power supply. In this mode the user can set data logging times and intervals (5 seconds - 600 minutes) and perform a functional test to ensure that the system is working properly.

The **Integration Mode** is used for integration of the PhytoFlash into multi-parameter systems such as CTDs. For Digital Integration, the multi-parameter system must be able to accept digital (RS-232) data and provide 8-30VDC. For Analog Integration, the multi-parameter system must be able to accept an analog signal output of 0-5V. These modes also allow the user to set instrument parameters and to perform functional tests.

The **Laboratory Mode** is intended for use with a computer and allows the user to view all data points during a run. This is the only mode where complete output data can be accessed. In the Self-Contained and Integrated Modes, only results of each saturation cycle are available (date/time, blank,  $F_o$ ,  $F_m$ ,  $F_v$ , and quantum efficiency). This mode allows the user to collect all data points that can then be transferred to data analysis software, such as MS Excel, where the saturation curve can be graphed and data analyzed.

### **3.2 Connecting the PhytoFlash to a PC Using HyperTerminal**

There is a 12-pin computer interface cable with a 12 V connection that will allow the PhytoFlash to interface with a PC.

1. Connect PhytoFlash (A) to the 12-pin interface cable (B)
2. Connect interface power source pin (B) to 12 V power supply (C)
3. There must be power supplied from the 12 V power supply in order to activate the PhytoFlash



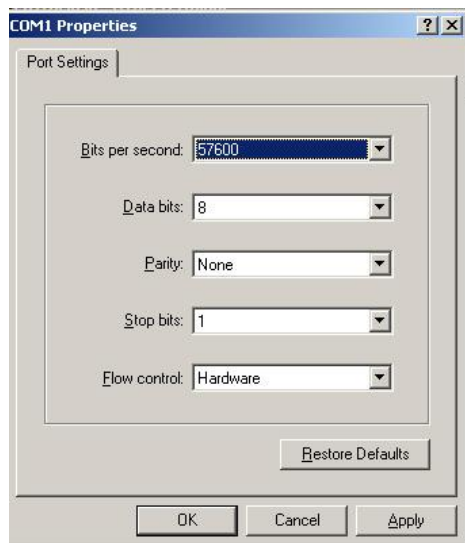
4. Connect the RS232 female plug to the 9 pin serial port on a PC



The PhytoFlash contains a processor chip that includes the software needed to interface with the PC. The following steps will guide you through the process of establishing communication between a PC and the PhytoFlash:

1. On a MS Windows computer, open the HyperTerminal program (Start→All Programs→Accessories→Communications→ HyperTerminal)
2. The **Connection Description** screen will appear. Name the connection description. This file will save the communication parameters for the PhytoFlash and can be used in the future to establish communication with the instrument quickly.
3. The **Connect To** screen will appear. Choose the appropriate communications port in the **Connect Using** window. Click **OK**.
4. The **Port Settings** screen will appear. Choose the following port settings;  
Bits per second: 57600  
Data bits: 8  
Parity: None  
Stop bits: 1  
Flow control: Hardware  
Click **Apply** and **OK**

(NOTE: Bits per second or Baud Rate can be adjusted to meet connection requirements; see page 8 for adjustment details)



5. The **HyperTerminal** window will appear. You are now ready to connect the PhytoFlash and plug in the AC power adapter. Once power is applied, the Phytoflash will start the initialization process. The initial PhytoFlash screen (Figure 1) will appear in the HyperTerminal window after a few moments.

### 3.3 Use of the PhytoFlash Software with a PC

#### 3.3.1 Home Screen

The PhytoFlash **Home Screen** (Figure 1) allows you to select from three Operating Modes: Self Contained Mode, Integration (Digital or Analog) Mode, or Laboratory Mode. It also allows the user to edit the **Setup Menu** designed for all modes.

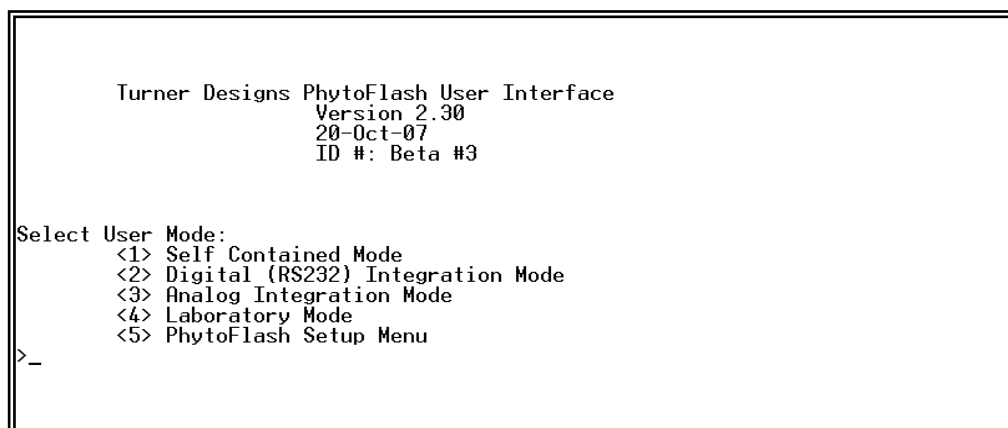


Figure 1



- To navigate through the menu, choose the appropriate number followed by pressing ENTER.

### **SETUP CONFIGURATION MENU**

The **PhytoFlash Setup Menu** (Figure 1, number **5**) is designed for all modes and allows you to:

- Set the time for the real-time clock on the PhytoFlash (HH:MM)
- Set the date (MM:DD:YYYY)
- Restore factory default settings
- Select Baud Rate
  - Baud Rate can be set by choosing Select Baud Rate (Figure 2, number **4**) and entering the number corresponding to the pre selected baud rates shown

(**NOTE:** Once a new Baud Rate has been chosen, the unit must be disconnected and then reconnected to HyperTerminal using the new Baud Rate setting to avoid program errors)



```
Select User Mode:
  <1> Self Contained Mode
  <2> Digital (RS232) Integration Mode
  <3> Analog Integration Mode
  <4> Laboratory Mode
  <5> PhytoFlash Setup Menu
>5

PhytoFlash Setup Menu

  <1> Current Time: 12:56
  <2> Current Date: Fri, Jan 12, 2007
  <3> Restore Self Contained Configurations to Factory Default settings
  <4> Select Baud Rate

  <ESC> Return to Previous Screen
>4
New baud rate (1 = 4800, 2=9600, 3=19200, 4=38400, 5=57600) >
```

Figure 2

### 3.3.2 Self Contained Mode

From the **Home Screen** (Figure 1), enter **1** to select the **Self Contained Mode**. This will bring you to the **Self Contained Setup Screen** (Figure 3). You are able to save custom settings for up to five different configurations.

```
PhytoFlash Self Contained Mode
Select Configuration:

  <1> Configuration #1
  <2> Configuration #2
  <3> Configuration #3
  <4> Configuration #4
  <5> Configuration #5
  <6> Download Data Logger Memory
  <7> Clear Data Logger Memory

  <ESC> Return to Previous Screen
  <E> Edit Configuration Name
```

Figure 3

Choose any default factory configurations (1-5) to proceed to the next screen. You can also re-name a configuration by typing **E** from this screen (Figure 3) to edit configuration names. Configuration names can be up to 15 characters long. After re-naming a configuration, access the **Self Contained Setup Screen** (Figure 3) by using



the **ESC** key. Then choose a configuration (1-5) and press enter. This will bring you to the **Self Contained Configuration Screen** (Figure 4).

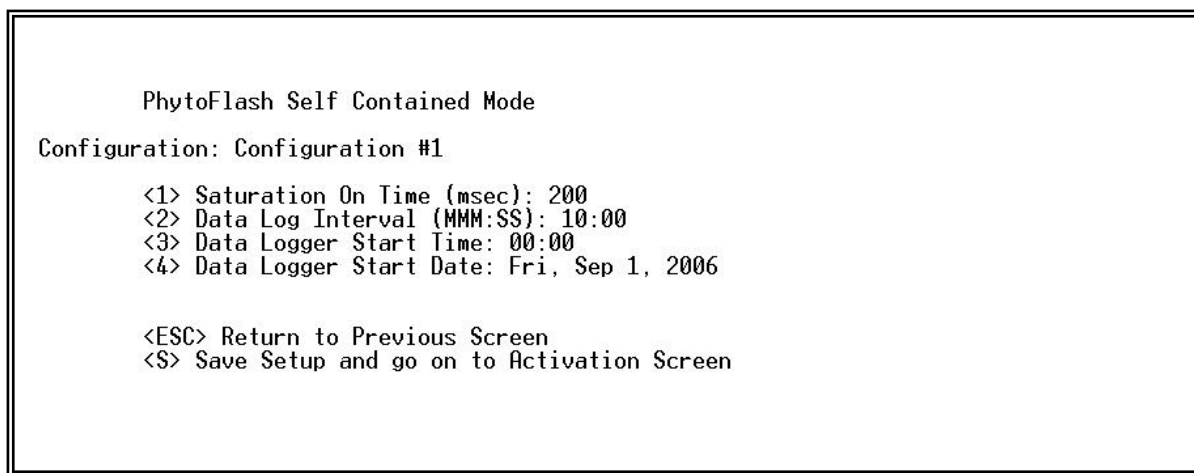


Figure 4

The **Self Contained Configuration Screen** allows you to:

- Select the Saturation LED On Time (200-10,000 msec)

**Note:** Users cannot set a saturation pulse that is equal to or within 4 seconds, of the data logging interval

- Set the Internal Data Logger Interval (5 seconds - 600 minutes) (MMM:SS)
- Set the Internal Data Logger Start Time (HH:MM)
- Set the Internal Data Logger Start Date (MM:DD:YYYY)
- Save the configuration and proceed to the **Self Contained Activation Screen**
- (Figure 5)

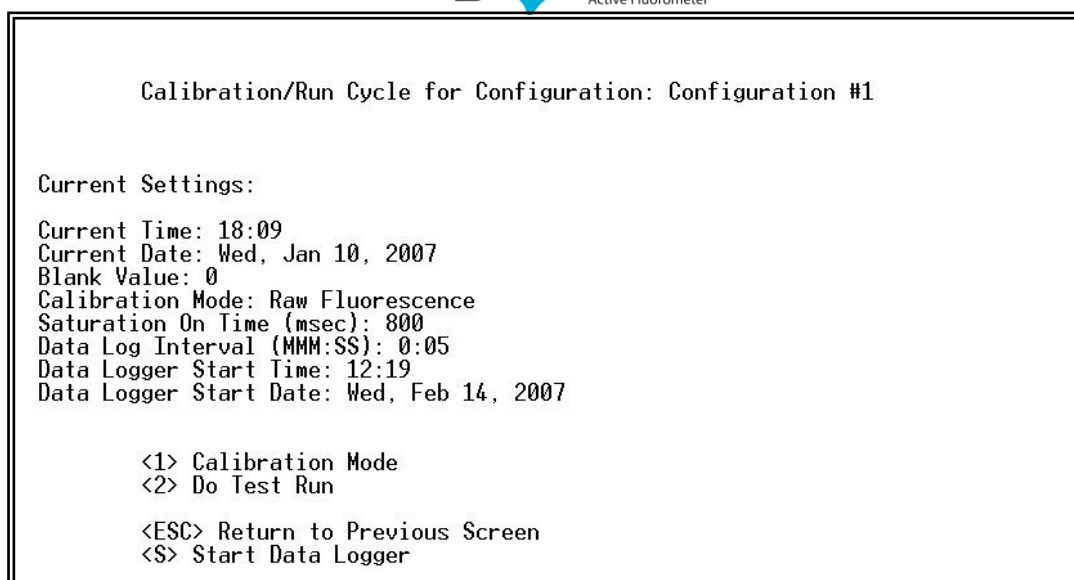


Figure 5

The **Self Contained Activation Screen** allows you to:

- View current settings
- Continue to Calibration Mode (See section 3.3.5)
- Conduct a Test Run
- Enable the Internal Data Logger

If you would like to conduct a **Test Run** before deploying, select **2** and press enter. This will trigger the PhytoFlash to run one saturation cycle and the calculated results will be displayed. [Note: To easily view this data, make sure that the capture text feature has been started, if you are using HyperTerminal (See section 3.3.6)]

If you would like to enable the data logger:

- Select **S** and press enter
- Disconnect the serial cable from the computer
- Disconnect the power currently being supplied to the PhytoFlash
- Connect battery or external power

**Note:** The PhytoFlash will begin to take readings at programmed time and date as soon as the battery pack or an external power source is supplied. If you've set the PhytoFlash to begin logging as soon as power has been supplied, you will experience a 30-second delay prior to logging.

#### ***3.3.2.1 Use of the PhytoFlash with the Battery Pack***

1. Connect PhytoFlash (A) to the 12-pin battery cable end (B)



2. Connect the 2-pin battery cable end (B) to the battery pack (C)
3. Secure the PhytoFlash and the battery pack to a solid mounting fixture



### 3.3.3 Integration Mode

The PhytoFlash can be integrated into multi-parameter systems, such as CTDs or a third party system, using Digital (RS232) or Analog Integration Modes depending on how the multi-parameter system is configured.

#### 3.3.3.1 Digital (RS232) Integration Mode

Digital (RS232) Integration Mode allows users to integrate the PhytoFlash into a third-party system, such as a CTD or external data logger that are able to accept digital (RS-232) data and provide 8-30VDC.

From the **Home Screen** (Figure 1), enter **2** to select Digital (RS232) Integration Mode and press enter (Figure 7).

The **Digital Integration Setup Screen** allows users to:

- View current settings
- Continue to Calibration Mode
- Set Saturation On Time (200-10,000 msec)
- Conduct a Test Run
- Start Integrated Readings



```
Digital (RS232) Integration Mode

Current Settings:
Blank Value: 0
Calibration Mode: Raw Fluorescence
Saturation On Time (msec): 200

<1> Calibration Mode
<2> Saturation On Time (msec): 200
<3> Do Test Run

<ESC> Return to Previous Screen
<S> Start Integrated Readings
```

Figure 7

If you would like to conduct a **Test Run** before deploying, select **3** and press enter. This will trigger the PhytoFlash to run one saturation cycle and the calculated results will be displayed.

**Note:** Before connecting the PhytoFlash to a third party system, users must enable Integrated Readings by selecting **S**. The PhytoFlash will begin to take readings about 30 seconds after an external power source is supplied.

### **Integration into a third-party system**

The sampling rate of the third-party system must be configured to send a signal to the PhytoFlash. It will send either an "M" character to the PhytoFlash, which will cause it to take another reading, or an ESC character, which will take it back to the Home Screen. All other characters will be ignored. If the user chooses to turn off the third-party power in between samples there is a 30 second warm up time for the PhytoFlash. Wiring diagrams can be found in Appendix D.

**Note:** We recommend continuous power to be supplied to the PhytoFlash from the third-party system.

#### **3.3.3.2 Analog Integration Mode**

Analog Integration Mode allows users to integrate the PhytoFlash into any third party systems that accepts 0-5V analog signal output. There are two streams of analog data available. Voltage representing the yield (Fv/Fm) parameter will automatically be provided. Users may chose either Fo or Fm as the second analog output during setup. Analog wiring diagram can be found in Appendix D.

From the **Home Screen** (Figure 1), enter **3** to select **Analog Integration Mode**.



From the **Analog Integration Mode Screen** (Figure 8) users can:

- View Current Settings
- Continue to Calibration Mode (see section 3.3.5)
- Set Saturation On Time (200-10000msec)
- Set the Second Analog Output (Off, Fo, or Fm)
- Conduct a Test Run
- Start Integrated Readings

```

      Analog Integration Mode
Current Settings:
Blank Value: 0
Calibration Mode: Raw Fluorescence
Saturation On Time (msec): 200

    <1> Calibration Mode
    <2> Saturation On Time (msec): 200
    <3> Analog Output: Off
    <4> Do Test Run

    <ESC> Return to Previous Screen
    <S> Start Integrated Readings

```

Figure 8

By selecting option **3** from the **Analog Integration Mode Screen** (Figure 8) users can select: Off, Fo, or Fm (Figure 9)

- If Off is selected, analog output will be disabled
- If **Fo** or **Fm** is selected, you will be prompted to set the full scale (5 volt) value for that parameter

Note: Be sure to set the Full Scale value of Fo or Fm to a value between 0-50000 (See Appendix E)

```

      Analog Integration Mode
Current Settings:
Blank Value: 0
Calibration Mode: Raw Fluorescence
Saturation On Time (msec): 800

    <1> Calibration Mode
    <2> Saturation On Time (msec): 800
    <3> Analog Output: Fo, 5 volts = 5000
    <4> Do Test Run

    <ESC> Return to Previous Screen
    <S> Start Integrated Readings
>3
Set the Analog Output to: 0=Off, 1=Fo, 2=Fm (0, 1, or 2) >1
What value of Fo will equal 5 volts? >

```

Figure 9



The Full Scale value is user determined. The Full Scale value selected will be equal to a voltage output of 5 volts DC. The maximum number the full scale can be set to is 50000. However, if more resolution is required, the Full Scale value can be defined to span a smaller range.

**Note:** For recommended Full Scale settings and analog calculations see Appendix E.

### **3.3.4 Laboratory Mode**

The **Laboratory Mode** is intended for use with a PC and allows the user to view the complete saturation cycle data. This is the only mode where all data points can be accessed and transferred to data analysis software, such as MS Excel, where the saturation curve can be graphed and data analyzed. In the Self Contained and Integrated Modes, only the calculated results of each saturation cycle are available (date/time, blank, Fo, Fm, Fv, and yield).

**Laboratory Mode** is also the only mode in which you can manipulate the Concentration Level Detector. There are three concentration level settings: AUTO, LOW, and HIGH. LOW represents low algal concentrations (~0 - 5 µg/l) and sets a higher sensitivity level while the HIGH represents high algal concentrations (~5 - 100 µg/l) and sets the PhytoFlash to a lower sensitivity level. AUTO allows the PhytoFlash to choose the best sensitivity to set depending on the intensity of the fluorescent signal being measured.

From the **Home Screen** (Figure 1), enter **4** to select Laboratory Mode. This will bring you to the **Laboratory Setup Screen** (Figure 10).

The **Laboratory Setup Screen** allows you to:

- View current settings
- Continue to Calibration Mode
- Set Saturation On Time (200-10,000 msec)
- Enable Output of Complete Curve Data
- Set Concentration Level
- Run a Sample

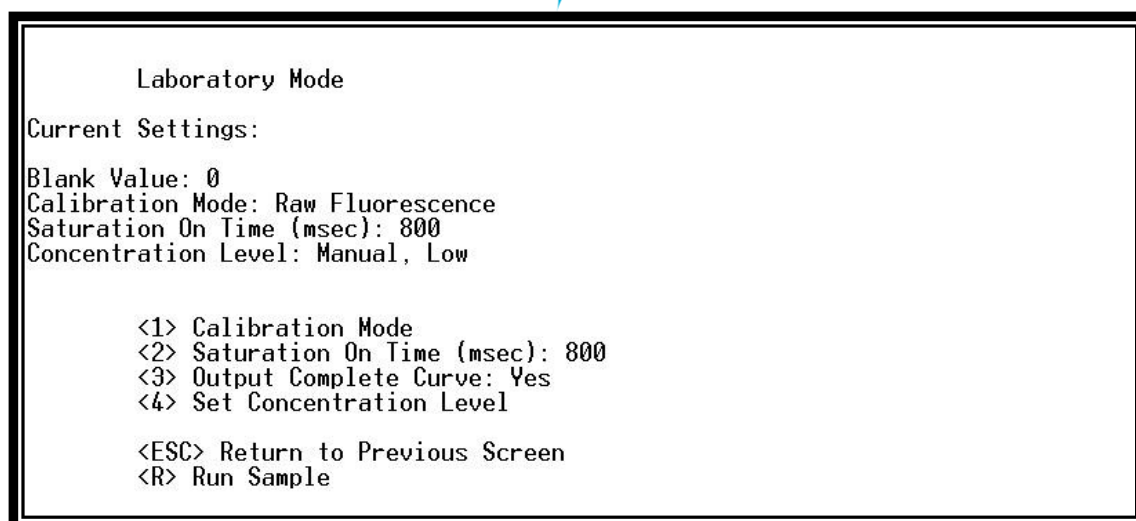


Figure 10

In order to see all data points in a curve, you will need to first **enable Output Complete Curve** by choosing **3** from the **Laboratory Setup Screen**. If you do not activate the **Output Complete Curve** mode, when you Run Sample you will only receive the calculated results.

Set Concentration Level (Figure 10, number **4**) should be used to optimize the resolution of the fluorescence data (Figure 11) [**Recommended setting is AUTO**]

The **Laboratory Concentration Level Screen** allows you to:

- Select **Auto** Detector
- Select **Low** Concentration
- Select **High** Concentration



```
Laboratory Mode
Current Settings:
Blank Value: 0
Calibration Mode: Raw Fluorescence
Saturation On Time (msec): 800
Concentration Level: Auto Detect

    <1> Calibration Mode
    <2> Saturation On Time (msec): 800
    <3> Output Complete Curve: Yes
    <4> Set Concentration Level

    <ESC> Return to Previous Screen
    <R> Run Sample
>4
Select Concentration Mode (A/M, Auto Detect/Manual) >M
Select Concentration Level (L/H, Low/High) >
```

Figure 11

If you expect your unknown samples to contain low concentrations [0-5 µg/L] of algae, you should run the saturation cycle on the **Low** Concentration Level. If you expect your unknown samples to contain high concentrations [5-100 µg/L] of algae, you should run the saturation cycle on the **High** Concentration Level. If the sample is too high for the **High** Concentration range, you will receive the following error message “ERROR: Input Data out of Range”.

### 3.3.5 Calibration Procedure

The user is able to calibrate the PhytoFlash in all modes if required.

The **Calibration Screen** (Figure 12) allows you to:

- Select Raw Fluorescence Mode
- Select Direct Concentration Mode
- Display current Direct Calibration Values



```
PhytoFlash Calibration Modes
Blank Value: 25
Calibration Mode: Raw Fluorescence
Last Calibration: 17:59, Thu, Feb 16, 2006

    <1> Select Raw Fluorescence Mode
    <2> Select Direct Concentration Mode
    <3> Display current Direct Concentration values

    <ESC> Return to Previous Screen
```

Figure 12

### 3.3.5.1 Raw Fluorescence Mode

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

If you would like to run the PhytoFlash in **Raw Fluorescence Mode** select **1** (Figure 12). Raw Fluorescence mode allows you to measure a blank sample by selecting **M** (Figure 13). In most cases it is advisable to run a blank sample to correct for any background signal associated with the sample. This can be accomplished by filtering a sample to remove all algal cells. Raw Fluorescence Mode also allows you to enter a value for the blank by selecting **E**. Users can enter 0 if they do not require blank subtraction.

```
PhytoFlash Calibration Modes
Blank Value: 30
Calibration Mode: Raw Fluorescence
Last Calibration: 18:01, Thu, Feb 16, 2006

    <1> Select Raw Fluorescence Mode
    <2> Select Direct Concentration Mode
    <3> Display current Direct Concentration values

    <ESC> Return to Previous Screen
>1
Do you want to Measure a Blank or Enter a Blank (M/E, Measure/Enter)? >M
Run Blank cycle (y/n)? >_
```

Figure 13



### 3.3.5.2 Direct Concentration Mode

Values reported in the **Direct Concentration Mode** are scaled to a predetermined standard value. It is recommended to determine extracted chlorophyll *a* of a typical algal sample on a laboratory fluorometer to determine the proper “standard” value. Refer to the Environmental Applications page on our website <https://www.turnerdesigns.com/parameters> for further instructions on chlorophyll *a* correlation.

If you would like to run the PhytoFlash in **Direct Concentration Mode** select **2** and press enter (Figure 14). You are able to use the **Existing** calibration values stored in the PhytoFlash (only **one** previous calibration will be stored) by selecting **E**, or perform a new calibration by selecting **N**.

```
PhytoFlash Calibration Modes
Blank Value: 800
Calibration Mode: Direct Concentration
Last Calibration: 18:06, Thu, Feb 16, 2006

    <1> Select Raw Fluorescence Mode
    <2> Select Direct Concentration Mode
    <3> Display current Direct Concentration values

    <ESC> Return to Previous Screen
>2
Use Existing calibration values or perform a New calibration (E/N, Existing/New)
? >N
Run Blank cycle (y/n)? >_
```

Figure 14

Direct Concentration Mode requires users to **Run a Blank Cycle** by selecting **y** (Figure 14). A blank can be obtained by filtering a sample to remove all algal cells.

The blank sample value will be stored and you will be prompted to **Run a Standard Test** with a known standard. You will then be prompted to enter a **Standard Value**. This value can be arbitrary (**i.e. 250**) or a close approximation to the total algal concentration. The blank value, displayed on the screen after calibration for Direct Concentration Mode will be scaled to the Standard Value selected.

**Calculation Example:**

Blank RFU = 50,  
Blank Subtracted Std. RFU = 500,  
Std. Value = 250,



$$\text{Displayed Blank Value} = \frac{\text{Standard Value (250)} * \text{Blank RFU (50)}}{\text{Blank Subtracted Standard RFU (500)}} = 25$$

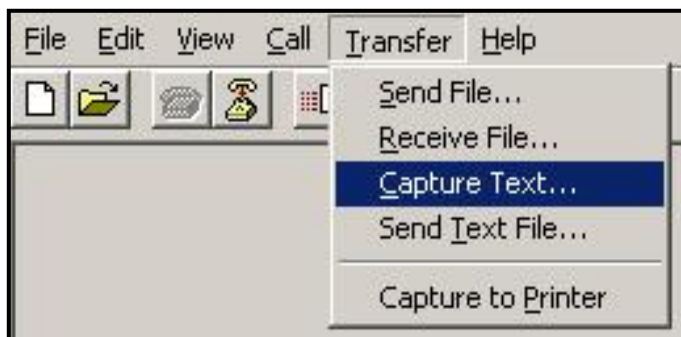
### 3.3.6 Downloading Data Using HyperTerminal

The PhytoFlash will store up to 10,000 points, however HyperTerminal cannot be used to analyze PhytoFlash logged data. In order to analyze logged data you must capture the data in the HyperTerminal window and save that data in **txt** format. That **txt** file can then be opened in a data viewing or analysis programs, such as MS Excel.

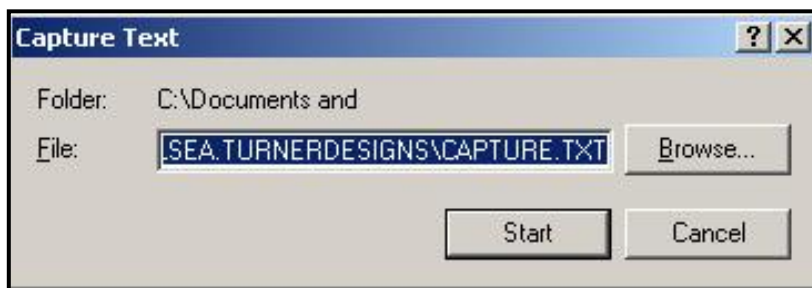
**The most important point to remember is that you must start to Capture Text before you run the PhytoFlash.**

To save data:

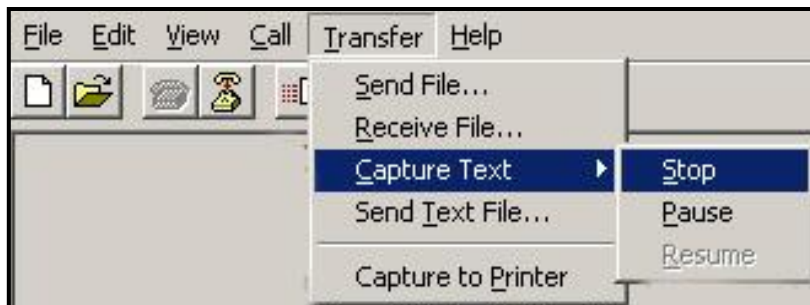
1. On the HyperTerminal window toolbar, choose Transfer and then Capture Text



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



3. You are now able to start running the PhytoFlash. You can view complete data, download the data logger, etc....
4. Once you are finished, you will need to return to the HyperTerminal window toolbar, choose **Transfer** and then **Capture Text** and select **Stop**. This will close the **.txt** file.



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

Figure 15 is an example of the data format during download.

Output Data Logger (y/n)? >y						
Configuration: GreenAlgae						
Date	Time	Fo	Fm	Blank	Fv	Yield
01/05/06	16:35:00	17	39	0	22	0.564
01/05/06	16:36:00	25	60	0	35	0.583
01/05/06	16:37:00	28	67	0	39	0.582
01/05/06	16:38:00	27	72	0	45	0.625
01/05/06	16:39:00	23	68	0	45	0.662

Figure 15



## 4.0 Recommended Measurement Practices

### 4.1 Theory of Operation

The PhytoFlash detection system is best described as a short-pulse, multiple turnover variable fluorescence system. The PhytoFlash has been designed to allow for real-time monitoring in remote locations. To accommodate this type of application, the PhytoFlash does not output complete data in the Self-Contained or Integration Modes but rather outputs only the calculated results from each run. Each data point includes the following information; **Date/Time/Fo/Fm/ Blank/Fv/Yield**

The measurement protocol is as follows:

Three low intensity LEDs, from here on referred to as the **Monitoring LEDs**, are used to measure the fluorescence response. The intensity of the Monitoring LEDs has been factory set and can not be adjusted. The excitation allows for even distribution of light throughout the entire sample volume and the intensity is low enough to prevent an increase in the photosynthetic rate. This can be evaluated in the Laboratory Mode.

**Fo:** When a sample cycle is activated, the Monitoring LEDs are turned on and 500 ms of warm-up time is provided before data is collected. This is followed by a collection of 20 data points to determine the minimum fluorescence (Fo) value. The calculated Fo is the average of the 20 data points minus the blank value.

**Fm:** The maximum fluorescence (Fm) protocol immediately follows the Fo protocol. Fm is caused by light saturation of all algal or cyanobacterial cells in the sample through the firing of the Saturation LED flash. The saturation array is comprised of six high intensity blue LEDs. The duration of the Saturation Flash can be customized from 200 – 10,000 ms, default is 200 ms. The Fm reading is the highest value during the Saturation Flash minus the blank value.

**Sample Cycle:** The sample cycle includes 20 data points prior to the saturation flash and 380 data points following. The data is collected at a rate of 230 Hz. This data can only be viewed by enabling “Output Complete Data” in the Laboratory Mode (See section 3.3.4).

**Fv:** Variable Fluorescence is  $F_m - F_o$

**Yield:** is the Quantum Yield calculated as  $F_v / F_m$ . The Quantum Yield is the efficiency of phytoplankton to photosynthesize.

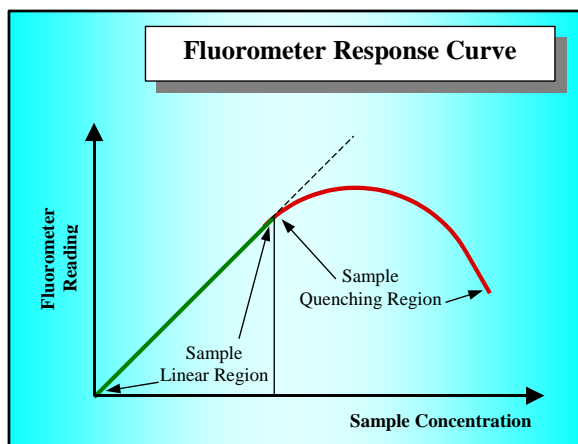
**Blank:** is the fluorescence signal associated with a sample containing no algal cells. Dissolved compounds that fluoresce, such as dissolved organic matter, degraded chlorophyll's (pheophytin), and/or particulate material that cause scattering of the excitation light may affect blank signals. The blank is automatically subtracted from  $F_o$  and  $F_m$ .

#### 4.2 Linear Range and Quenching

The linear range is the concentration range in which the PhytoFlash 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 (Figure 16). 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



**Figure 16**  
*Graph showing Linear and Quenching Regions of the sample's response*

the reading increases, the sample is beyond the linear range of the fluorophore.



### **4.3 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/parameters>



## 5.0 Care and Maintenance

### 5.1 Rinsing

The PhytoFlash should be rinsed or soaked in freshwater following each deployment, ideally until it is completely clean again.

### 5.2 Cleaning the Optics

The optical window should be visually inspected after each deployment following a soaking in fresh water. If cleaning is needed, use the Optical Cleaning Brush included in the Accessory Kit (P/N: 2500-110) to clean the window with soapy water.



**Note:** The PhytoFlash should not come in contact with any organic solvents (i.e. acetone, methanol) or strong acids and bases.



## 6.0 Warranty

### 6.1 Terms

Turner Designs warrants the PhytoFlash 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.

### 6.2 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](mailto: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).

### **6.3 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. 1<sup>st</sup> Street  
San Jose, CA 95112



**Equipment Specified as Electrical and Electronic Waste**



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## Appendix A

### *PhytoFlash Specifications*

#### **Blue PhytoFlash Optical Specifications**

Excitation Filter	440 nm
Emission Filter	680 nm
LED	Blue
Minimum Detection Limit	0.15 µg/l

#### **Electronic Specifications**

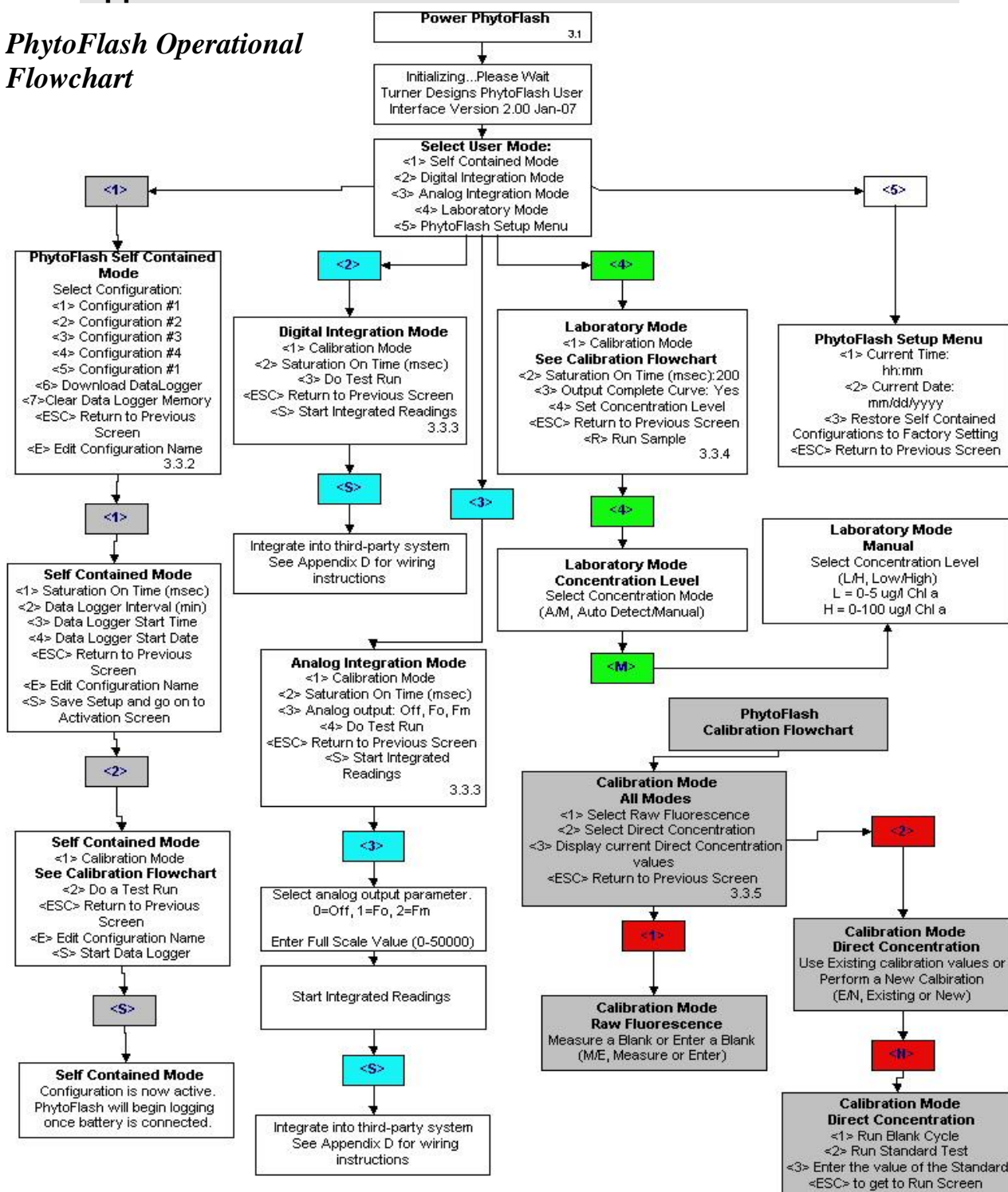
Input Voltage Range	8-30 VDC
Data Rate	360Hz
Saturating LED duration	200-10,000 ms, 200ms (default)
Data Format	RS-232 or Analog (0-5V Output)
Minimum Sampling Rate	5 seconds (minimum)

#### **Physical Dimensions**

Length	12 inches	30.5 cm
Width	3 inches	7.6 cm
Weight (in air)	3.25 pounds	1.47 kg
Weight (in water)	1.01 pounds	0.46 kg
Sample Volume	5.36 ml	
Standard Unit Materials	Delrin plastic, Aluminum optical head	
Titanium Unit Materials	Delrin plastic, Titanium optical head	

## Appendix B

### PhytoFlash Operational Flowchart



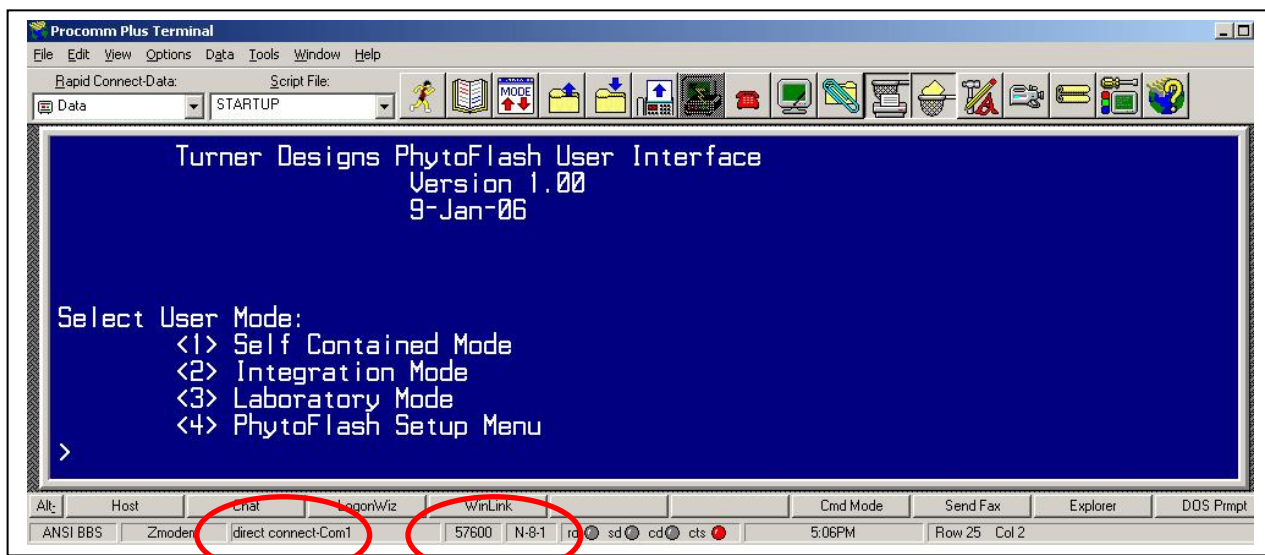


## Appendix C

### Connecting PhytoFlash to a PC with ProComm 4.8

The PhytoFlash contains a processor chip that contains the software needed to interface with the instrument. The following steps will guide you through the process of establishing communication between a PC and the PhytoFlash:

1. Open ProComm 4.8 on your PC
2. Connect the PhytoFlash to the interface cable



3. You can click the **Port Settings** on the bottom of the screen to edit.
4. Choose the following port settings;
  - Connect: **Com 1**
  - Bits per second: **57600 (Use the most recent Baud Rate setting)**
  - Parity: Data bits Stop bits: **N:8:1**
5. You are now ready to connect the PhytoFlash and plug in the AC power adapter. Once power is applied, the initial **Turner Designs PhytoFlash User Interface** window will appear in the ProComm window.
6. You are now able to operate the PhytoFlash by following the instructions in section **3.3 Use of the PhytoFlash with a PC**.

## Appendix D

### PhytoFlash Bulkhead Connector and Wiring Table

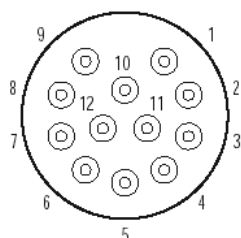
The PhytoFlash can be connected to any external data logger or CTD system that can accept RS-232 signal output and can provide 8-30V DC power for digital integration and any system that accepts Analog Output Signal of 0-5V.

An interface cable to connect to a CTD should be purchased through the CTD manufacturer. Please see Appendix F for additional information on interface and deployment cables.

A mounting bracket will be required in order to attach to a CTD system. In some cases the CTD manufacturer will supply the mounting bracket. If not, the user will need to make a custom bracket.

**Figure 17. Bulkhead Connector Pin Configuration:**

PhytoFlash Connector



Sub D Connector

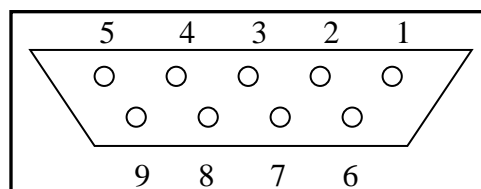


Table 1. Wire Guide.

Pinout #	Wire Color	PhytoFlash Function	RS232 Sub D Connector	Pinout Yield & (Fo or Fm)	Pinout Yield	Pinout Fo or Fm
1	Red	V BATT +		V Batt +	V Batt +	V Batt +
2	Black	VBATT -		V Batt -	V Batt -	V Batt -
3	Blue	RXD	2			
4	Brown	TXD	3			
5	Yellow	DTR & DSR	4 & 6			
6	Orange	RTS & CTS	7 & 8			
7	White	RS232-GND	5			
8	Purple	ANALOG_0 (Yield)		Yield	Yield	
9	Grey	ANALOG_1 (Fo or Fm)		Fo or Fm		Fo or Fm
10	Green	ANALOG_GND		Analog_GND	Analog_GND	Analog_GND
11	Red/White	DIGITAL_0				
12	Black/White	Digital_1				



## Appendix E

### Analog Output Settings and Calculations

#### Setting Full Scale Value for Fo or Fm

The Full Scale value is user determined. The Full Scale value selected will be equal to a voltage output of 5 volts DC. The maximum number the full scale can be set to is 50000. However, if more resolution is required, the Full Scale value can be defined to span a smaller range. The recommended Full Scale value settings (Figure 18) can be used as a guide to help set the PhytoFlash to your aquatic system

**Note:** the values in figure 18 will vary for different aquatic systems)

Recommended Full Scale Value Settings	
[Chl a] (ug/L)	Full Scale Value
0 - 1	600 - 1000
0 - 5	3000 - 5000
0 - 25	15000 - 25000
0 - 50	30000 - 50000
0 - 150	50000

Figure 18

**Calculating Yield:** Yield data will be displayed as voltages from 0-5V. There is no full scale associated with the yield. To obtain yield values users must divide recorded voltage by 5 volts.

$$\text{Yield} = \frac{(\text{x}) \text{ volts recorded for yield}}{5}$$

**Calculating Fo or Fm after calibrating in Raw Fluorescence Mode:** Fo or Fm will be displayed as voltages from 0-5V. To calculate the Relative Fluorescence Units measured by the PhytoFlash use the following equation:

$$\text{Fo or Fm} = \frac{(\text{x}) \text{ volts for parameter} * \text{Full scale value for parameter}}{5}$$

\* Blank subtracted value

**Calculating Fo or Fm after calibrating in Direct Concentration Mode:** After calibrating to Direct Concentration Mode, Fo or Fm will be displayed as voltages from 0-5V. To calculate the parameters measured by the PhytoFlash use the following equation:

$$\text{Fo or Fm} = \frac{(\text{x}) \text{ volts for parameter} * \text{Full scale value for parameter}}{5}$$

\* Blank corrected calibrated value



## Analog Mode Calculation Worksheet

### Raw Fluorescence Mode

Blank Value	Parameter Selected Fo or Fm	5 Volt Value	Voltage Recorded from Fo or Fm	Voltage Recorded from Yield	Raw (Fo or Fm) [F=(D*C)/5]	Actual Yield [G=E/5]
A	B	C	D	E	F	G
237	Fm	15000	3.6003	2.6275	10800.9	0.526
237	Fo	50000	0.379	3.056	3790	0.611

### Direct Concentration Mode

Blank Value	Standard Run Value	Assigned Standard Value (Arbitrary value)	Displayed Blank Value [D=(C/B)*A]	Parameter Selected Fo or Fm	5 Volt Value	Voltage Recorded from Fo or Fm	Voltage Recorded from Yield	Calibration Scaled Fo or Fm [I=(G*F)/5]	Raw Fo or Fm [J=(B/C)*I]	Actual Yield [K=H/5]
A	B	C	D	E	F	G	H	I	J	K
113	2655	2500	106	Fo	15000	1.9041	3.074	5712.3	6066	0.615
113	2655	2500	106	Fo	15000	0.1065	1.3423	319.5	339	0.268

### Definition of terms:

#### **Raw Fluorescence or Direct Concentration Mode**

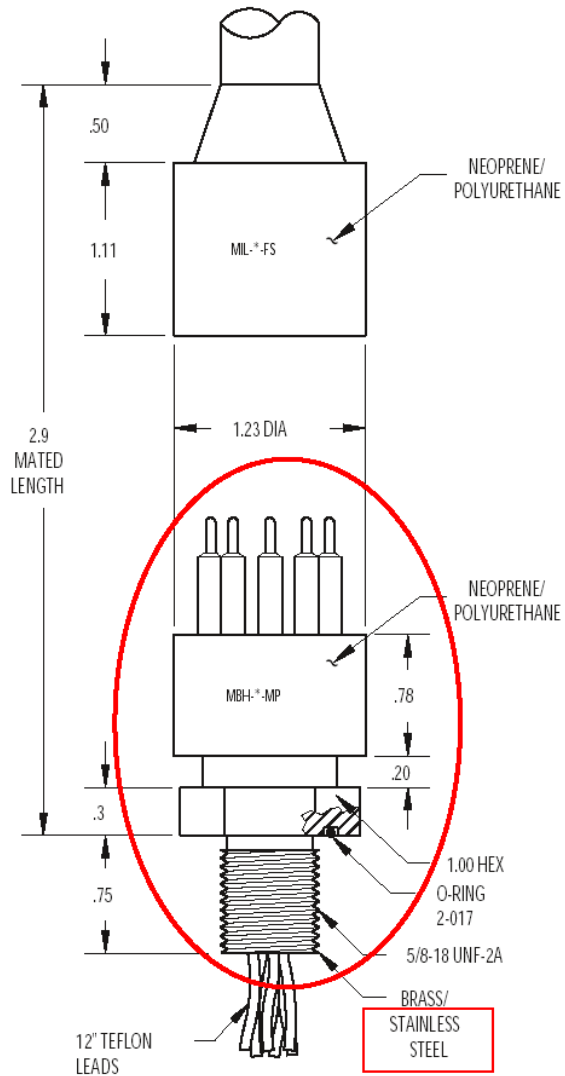
Blank Value = A value measured or entered for the blank sample  
5 Volt Value = 0-50000 full scale value setting for (Fo or Fm)  
Voltage Recorded = The analog output values given in 0-5V range for (Fo or Fm) and yield

#### **Direct Concentration Mode**

Standard Run Value = the value measured from a known Std.  
Assigned Std. Value = a value entered to calibrate the instrument to the known Std. (i.e. Calibration Value)  
Displayed Blank Value = calibration adjusted blank value  
Calibration Scaled (Fo or Fm) = calibrated Fo or Fm values

## Appendix F

### PhytoFlash Pigtail Cable and Connector Information



**Figure 18**

*Turner Designs offers a 24" length cable with 20 gauge colored leadwire that connects to the PhytoFlash 12-pin male connector with a locking sleeve (Part No: 105-2510).*



## Appendix G

### ***Request for Quote Template for Non-Standard Pigtail Cable Length/Connector for Use with PhytoFlash Fluorometers***

To request a quote from Impulse Enterprise for a custom pigtail cable/connector, follow these steps:

- I) Complete the 5 lines below between the asterisks (\*) with your specific information.
- II) Highlight the lines between the asterisks (\*) and copy to the PC clipboard.
- III) Paste the information into the “Comments” field on the Contact Us page on the Impulse web page at the following URL:  
<http://www.teledynemarine.com/impulse>
- IV. Complete the remaining fields of the Impulse Enterprise <Contact Us> e-mail form
- V. Click on “Send E-Mail”.

\*\*\*\*\*

Please quote for a custom pigtail cable/connector for use with Turner Designs PhytoFlash variable fluorometer to meet the following requirements:

1. Pigtail is required for integration with \_\_\_\_\_  
(Identify multi-parameter system as appropriate)
2. Cable Length (to include connectors)\_\_\_\_\_, (State units, feet, meters, etc.)
3. Required connector on non-PhytoFlash end of pigtail: \_\_\_\_\_  
(Provide sufficient information for Impulse Enterprise to request a quote for the required “host” end of cable)
4. Pin out from Host End to PhytoFlash end: \_\_\_\_\_  
or advise:
  - a. Supply Voltage (+) from Host is carried on pin \_\_\_\_\_
  - b. Supply Ground from Host is carried on pin \_\_\_\_\_Provide sufficient information for Impulse Enterprise to make the proper electrical hook-up between the two connector ends)
5. No. of Pigtails required\_\_\_\_\_

(Provide contact details if different from your information entered in the Impulse Enterprises fields on their “Contact Us” e-mail form.

.....

For assistance from Turner Designs, call 1 (877) 316-8049

For assistance from Impulse Enterprise, call 1 (800) 327-0971