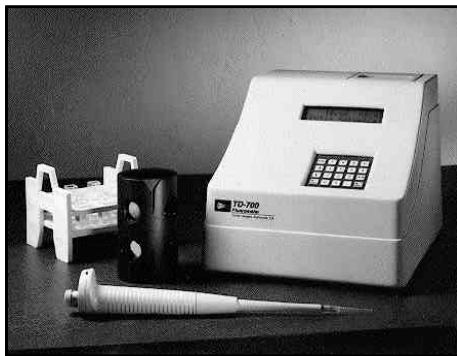


TD-700 Laboratory Fluorometer



Operating Manual

***Dated: 5/8/2002
Version 2.0***

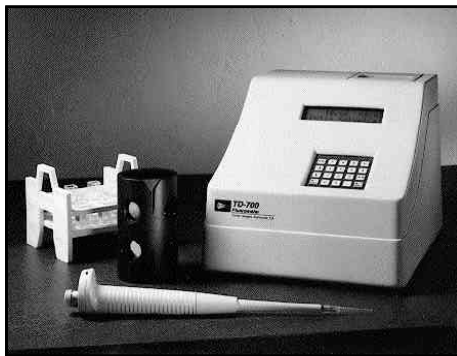
Part Number 7000-998



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***TD-700 Laboratory Fluorometer
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I. Introduction

A. Description

The TD-700 Fluorometer is a compact, laboratory fluorometer designed for discrete sample measurement of various fluorescent materials, including chlorophyll, rhodamine, fluorescein, histamine, vitamins, and other fluorescent compounds. When properly calibrated, TD-700 will electronically set the optimal sensitivity and range for your samples, and will readout the actual concentration of the compound when in the Direct Concentration mode. The TD-700 can be set up to detect the specific compound you want to measure simply by changing the lamp and optical filters.

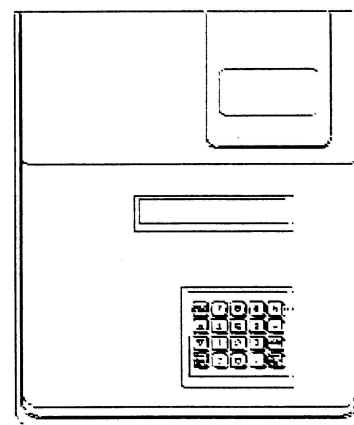
B. Inspection and Setup

Upon receiving your fluorometer, please inspect it carefully and make sure all accessories are present (refer to the packing list shipped with the instrument).

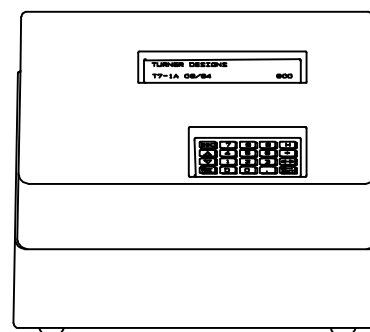
To get started (Refer to the Quick View Diagram, page 7):

1. Locate the instrument on a flat surface with the back of the instrument well ventilated (at least three inches from a wall).
2. Open the sample chamber lid located on the top of the unit.
3. Remove the Filter Cylinder by grasping the inside rim of the cylinder and pulling up.
4. Install the correct optical filters for your application. See Section III for details.
5. Check that the correct lamp is installed for your primary application. See Section IV for details.
6. Check to see that the sample adaptor is the correct one for your needs. Refer to Appendix 6 for a detailed explanation of TD-700 Sample Adaptors.
7. Reinsert the Filter Cylinder, lining up either the A, B, C or D application indicators on the cylinder with the silver alignment mark on the inside rim of the sample chamber. Replace the sample adaptor making sure that the adaptor is properly seated in the filter cylinder, and close the sample chamber lid.
8. Plug in the unit. **Be sure to use only the Turner Designs power supply provided with your instrument.** It is critical to use the power supply provided by Turner Designs to meet EMI requirements.
9. Turn on the power switch (rear of unit) and allow the unit to warm up for the countdown period (600 seconds; 10 minutes).

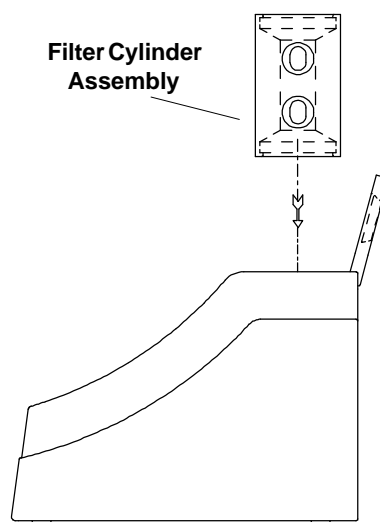
NOTE: For optimal instrument stability, leave the instrument turned on.



TD-700 Top View



TD-700 Front View



TD-700 Side View

C. General Precautions



Do not leave the instrument reading "OVER" for an extended period of time (several minutes). Instrument instability can result.

If you are using the quartz halogen lamp, do not touch the bulb. It may be hot. Also, oils from your hand will affect the lamp's transmission.

When using solvents or other chemicals, please refer to the appropriate Material Safety Data Sheet (MSDS) for information regarding handling.

When using potentially hazardous chemicals, please use closed-cap test tubes whenever possible and limit exposure. If closed cap test tubes are not available, Parafilm™ should be used to prevent the spillage of volatile or other potentially hazardous chemicals.

Chemicals should be used in accordance with local regulations and used in a well ventilated area.

Do not defeat the lamp interlock switch. UV light may cause permanent damage to your eyes.

D. Definition of Symbols

 Direct Current. 12 volts D.C.



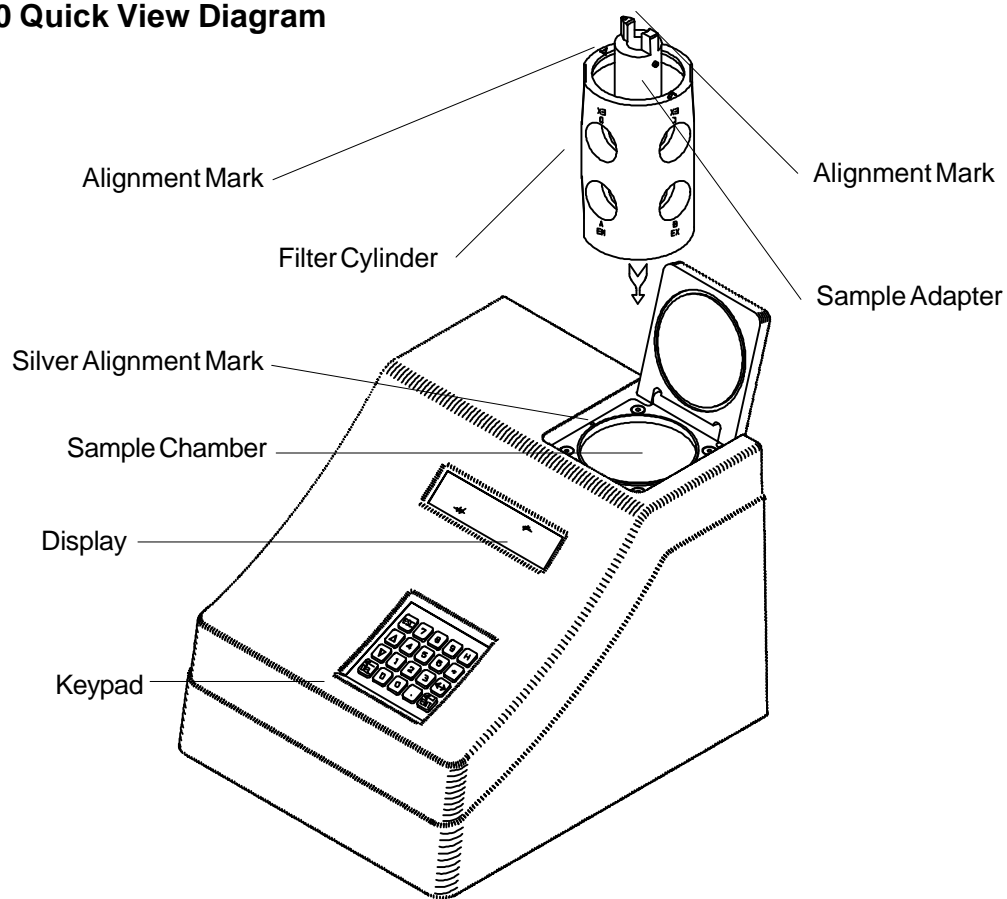
Easily touched higher temperature parts.



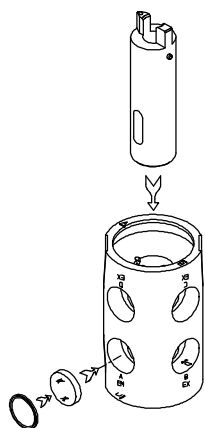
Caution. Read instruction manual and refer to warning text.

II. Hardware Overview

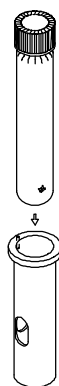
A. TD-700 Quick View Diagram



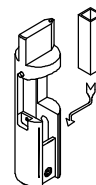
TD-700 Laboratory Fluorometer



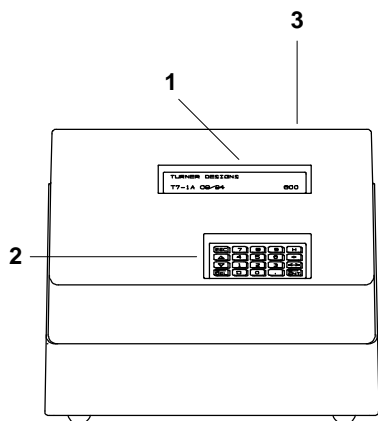
TD-700 Filter Cylinder and
13 mm Sample
Adapter



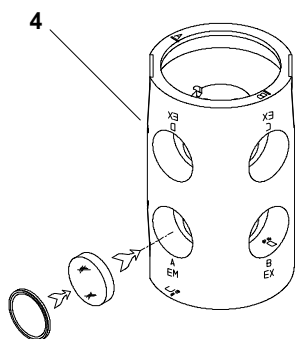
25 mm Round Sample
Adapter



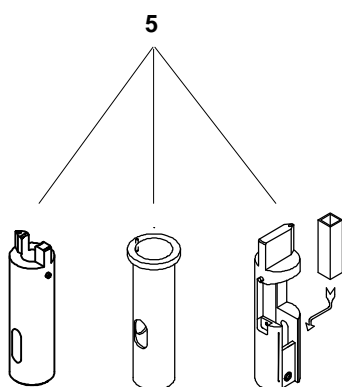
10 mm Square Cuvette
Adapter



TD-700 Front View



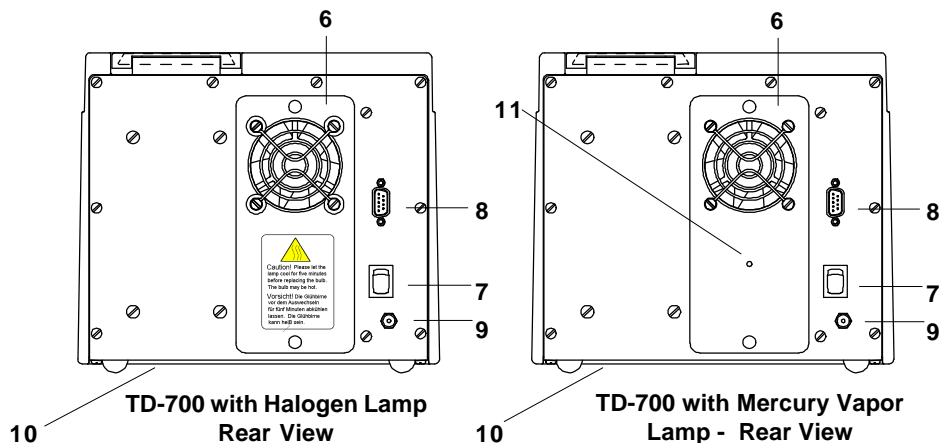
TD-700 Filter Cylinder Assembly



TD-700 Sample Adapters
13 mm, 25 mm, and 10 mm

B. TD-700 Controls

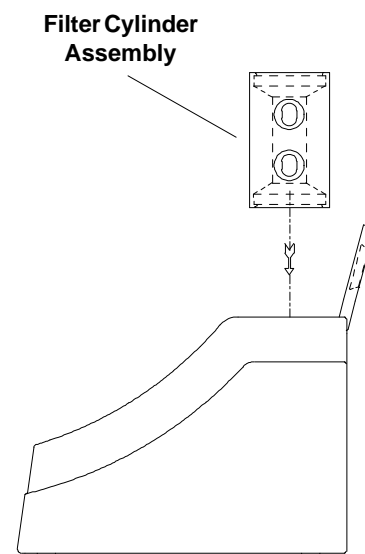
1. **Display** — Shows the screens and readout for samples. It is lit continuously when the unit is operating. Except during calibration, the contrast of the display can be adjusted on any screen by pressing the up or down arrow keys.
2. **Keypad** — The keypad is used to enter new values and to move through the software screens.
3. **Sample Chamber** — Samples are placed inside the chamber for reading. It houses the Filter Cylinder and the cuvette adaptor.
4. **Filter Cylinder** — The Filter Cylinder has openings for various optical filter combinations. It can be removed by grasping the inside rim of the cylinder and pulling it straight up.
5. **Sample Adapters** — Adapters are available for various test tube or cuvette sizes. The sizes used with the TD-700 are: 25 mm round, 13 mm round, 10 mm square, or 100µl minicell.
6. **Lamp Access Panel** — To access the lamp, remove the panel by pulling on the two black quick-release latches on the lamp access panel. The lamp provides excitation light at wavelengths specific to the compound to be measured.
7. **Power Switch** — ON/OFF switch.
8. **RS-232 Serial Port Connection** — RS-232 (DB9 female from the TD-700) serial port for connecting to a computer or printer.
9. **Power Plug Socket** — The power supply connects into the rear panel of the instrument.
10. **Drain Plug** — The TD-700 is equipped with a non-removable plug (located underneath the instrument) for drainage if there is a spill in the sample compartment.
11. **Lamp Viewport** — The mercury vapor lamp access panel has a lamp viewport that allows the user to observe whether the lamp is lit without opening the door.



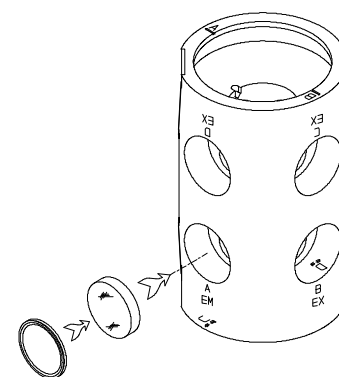
III. Optical Filter Installation and Removal

The TD-700 Fluorometer must have two different optical filters to operate correctly: an excitation filter and an emission filter. TD-700 filters are one-inch diameter, round, colored glass or highly-reflective glass. The filters are mounted in the Filter Cylinder using flexible rubber o-rings. The Filter Cylinder is accessed through the sample chamber. The Cylinder can hold four different excitation/emission filter combinations, and is labeled on one rim, "A" and "B"; and labeled on the opposite rim, "C" and "D". Each of these letters identifies a filter application set. Once the filter sets are installed properly in the cylinder, changing from one application to another requires a simple repositioning of the cylinder and possibly a lamp change. To remove or install optical filters in the Filter Cylinder:

- a. Remove any test tubes or cuvettes and sample adaptors from the instrument. Then, grasp the Filter Cylinder by the rim and pull straight up and out of the unit.
- b. Locate the filters (excitation and emission filters) for the application you want to run. Filters are marked on their rim with an identification number.
- c. The Filter Cylinder has eight openings for four different filter sets. For example, the openings for filter set A are marked "A/EX" and "A/EM"; filter set B is marked "B/EX" and "B/EM". Select the A, B, C, or D positions to install the filters.
- d. Find the openings for the filter set you want to remove. For example, if you want to install the filters in position B, then remove any filters from the B/EX and B/EM openings. To remove a filter, take out the flexible rubber o-ring holding the filter in place. Use a tool such as a plastic pen cap or plastic-nosed forceps to do this. Be careful not to scratch the surface of the filter. Place your hand over the opening and tilt the cylinder so the filter drops out into your hand. **Be careful, the filters are glass and may break if dropped.**
- e. Locate the excitation filter to be installed. Handle the filter on the edges so as to avoid leaving fingerprints on the filter, or wipe off the filter before installing. If the filter has one side that is "mirrored" or highly reflective, it should be installed so the mirrored side faces outward from the cylinder (toward the lamp). Insert it (mirrored side out, if any) in the opening marked with the set letter/"EX". Push the filter in so it rests flush with the back of the opening. Then reinsert the o-ring and press it in until it is flush against the filter.



TD-700 Side View



TD-700 Filter Cylinder Assembly

- f. Locate the emission filter to be installed. Handle the filter at the edges so as to avoid leaving fingerprints on the filter, or wipe off the filter before installing. If the filter has one side that is “mirrored” or highly reflective, it should be installed so the mirrored side faces toward the inside of the cylinder (toward the sample). Insert it (mirrored side in, if any) in the opening marked with the appropriate set letter “EM”. Push the filter in so it rests flush with the back of the opening. Then reinsert the o-ring and press in with your plastic pen cap until it is flush against the filter.

- g. Position the Filter Cylinder in the sample chamber so that the alignment mark for the filter set you are using is aligned with the silver alignment mark on the inside rim of the sample chamber.

IV. Lamp Installation and Removal

There are two types of lamps available for the TD-700: the Mercury Vapor and the Quartz Halogen

A. The Mercury Vapor Lamp

The low pressure mercury vapor lamp comes in various types depending on the application of interest. The average life of a mercury vapor lamp is 8000 hours. The lamp can be checked without removing the lamp access panel by viewing through the lamp view port. If the lamp access panel is removed, the power to the mercury lamp will be cut off. This safety feature will prevent the user from being exposed to U.V. light. **Do not defeat the interlock switch. U.V. light may cause permanent damage to your eyes.**



Check the lamp view port if:

1. The instrument is not responding, even though the unit is plugged in and the power is on.
2. The readings are very low, unstable, or drifting.

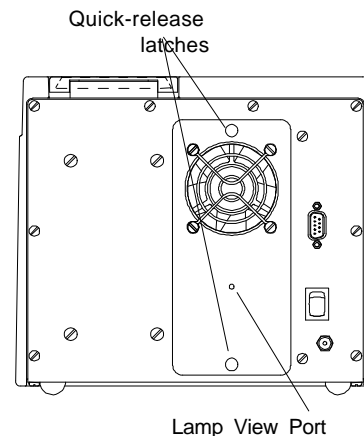
Note: After replacing a lamp, you must always recalibrate.

To replace the mercury vapor lamp:

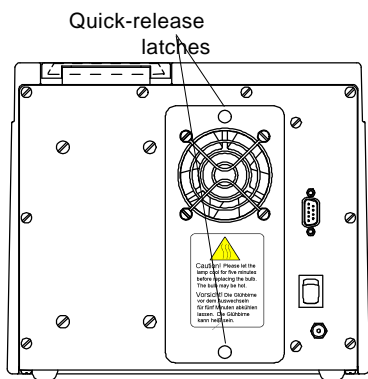
1. Unplug the instrument and remove the lamp access panel by pulling on the two black quick-release latches on the rear panel of the instrument.
2. Before replacing a lamp, make sure the current lamp is fully seated in both the upper and lower lamp sockets. **Be cautious when removing the lamp. It may be hot.**
3. To remove the lamp, grasp it carefully and turn it about 90 degrees until the prongs line up with the slots on the lamp sockets. Slide it out.

NOTE: To avoid breaking the lamp when replacing it, make sure it is fully seated! At the bottom lamp socket, a metal spring exerts pressure against the lamp; be sure this spring is not preventing proper seating before twisting lamp into place.

4. To install a new lamp, line up the lamp prongs with the slots on the lamp sockets, push the lamp in and turn it about 90 degrees so it is firmly seated. Make sure both end caps are properly seated or the lamp will not work!
5. Replace the lamp access panel and push in the black quick-release latches to snap the panel in place.
6. Turn on the power. Check the lamp view port to ensure the lamp is lit. Also make sure the fan is operating. If the fan is not operating, check the connector plug.



TD-700 with Mercury Vapor Lamp - Rear View



TD-700 with Halogen Lamp
Rear View



B. The Quartz Halogen Lamp

The average halogen lamp life is 2000 hours.

Check the lamp if:

1. The instrument is not responding, even though the unit is plugged in and the power is on
2. The readings are very low, unstable, or drifting.

To replace the quartz halogen lamp:

1. Turn off the instrument and remove the lamp access panel by pulling on the two black quick-release latches on the rear panel of the instrument.
2. Unplug the power jack that connects the lamp panel assembly to the instrument. The lamp access panel should now pull free of the instrument.
3. **DO NOT TOUCH THE LAMP BULB with an ungloved hand; the bulb may be hot, and/or the oils from your hand will affect the lamp's transmittance and cause incorrect readings.** If the bulb is burned out, grab the bulb with a tissue and pull it out of the assembly. Using the same tissue, insert a new bulb (orientation does not matter).
4. Plug the power jack back into the main instrument and replace the lamp access panel. Push in the black quick-release latches to snap the panel in place.
5. Turn on the power. Check to ensure the lamp is lit by viewing it through the fan opening. Also make sure the fan is operating. If the fan is not operating, check the connector plug.
6. You must recalibrate if you replace the lamp.

V. Instrument Parameters (Firmware)

Instrument parameters are set through the TD-700 firmware interface. Firmware screens are called up using the keypad. To see how these screens fit together, see the firmware flowchart on pages 16 & 17. For details about alarms and instrument diagnostics, see Appendix 3, Section A, and Section B.

A. Power-up Screen

When the unit is first turned on, a screen appears showing the title, software version, and a 600-second (10-minute) countdown. Once the 600-second countdown expires, the HOME screen will appear. The countdown allows the instrument to warm up adequately before measurements begin. For optimal stability and accuracy, it is recommended that you allow the instrument to complete the countdown period. For best results, leave the instrument turned on in your laboratory.

If you wish to bypass the countdown period, press <H>, <ESC>, or <ENT>. From the HOME screen, press <9> to return to the Power-up screen.

B. HOME Screen

After the countdown period, the HOME screen is displayed. The HOME screen is where samples are read and data is sent to the printer or computer. Press <H> to return to this screen from any screen (except during calibration).

From the HOME screen, several functions can be accessed.

- Press <D> to send data to a printer or computer.
- Press <*> to Discrete Sample Average (See Section X for details).
- Press <0> to Autozero or blank the instrument (See Section X for details).
- Press <8> for Diagnostic Screens.
- Press <7> for Data Stream.

C. Setup/Calibration Screen

The Setup/Calibration screen, accessed from the HOME screen by pressing <ENT>, is a screen where the user selects either to go to the calibration sequence or to review or change the setup parameters. The user may also press <D> from this screen to send the current calibration information to a printer or computer.

TURNER DESIGNS
 T7-1B 10/96 600

A. Power-up Screen

XXX ppm
 <ENT> -Setup & Cal

B. HOME Screen

1. Setup
 2. Calibration

C. Setup/Calibration Screen

Simple Mode

1. Mode

Raw Fluorescence

1. Mode
2. Cal Procedure

Direct Concentration

1. Mode	3. Units
2. Cal Procedure	

D. Setup Screen

This screen is used to choose the calibration procedure for the TD-700. -Simple, Multi-Optional Raw Fluorescence, Multi-Optional Direct Concentration. This screen will have 1 of 3 looks depending upon how the instrument is currently configured. (See figure to the left).

To access the Setup Screen, from the Home screen press <ENT> then <1>. Press the appropriate number to access the setup parameters.

1. Mode. This parameter allows the user to choose between Simple & Multi-Optional calibration modes. (See Section V E for more details).
2. Cal Procedure. This parameter allows the user to choose between Raw Fluorescence and Direct Concentration calibration procedures (See Section V E for more details).
3. Units. Used only in Multi-Optional - Direct Concentration mode only. This is to choose which units your standards and samples are measured in.

For all 3 of these parameters, use the <-> key to toggle between choices. When you have your choice selected, press <ESC> or <H>.

E. Calibration Screens

The instrument will initiate the calibration process when <2> of the keypad is pressed from the "Setup/Calibration" Screen. The firmware will guide the user through the process. Depending upon the calibration mode and procedure chosen in the Setup screens, the steps will be different. (See firmware flowchart on pages 16 & 17). Here is a description of the 3 different calibration procedures.

Simple: One point calibration

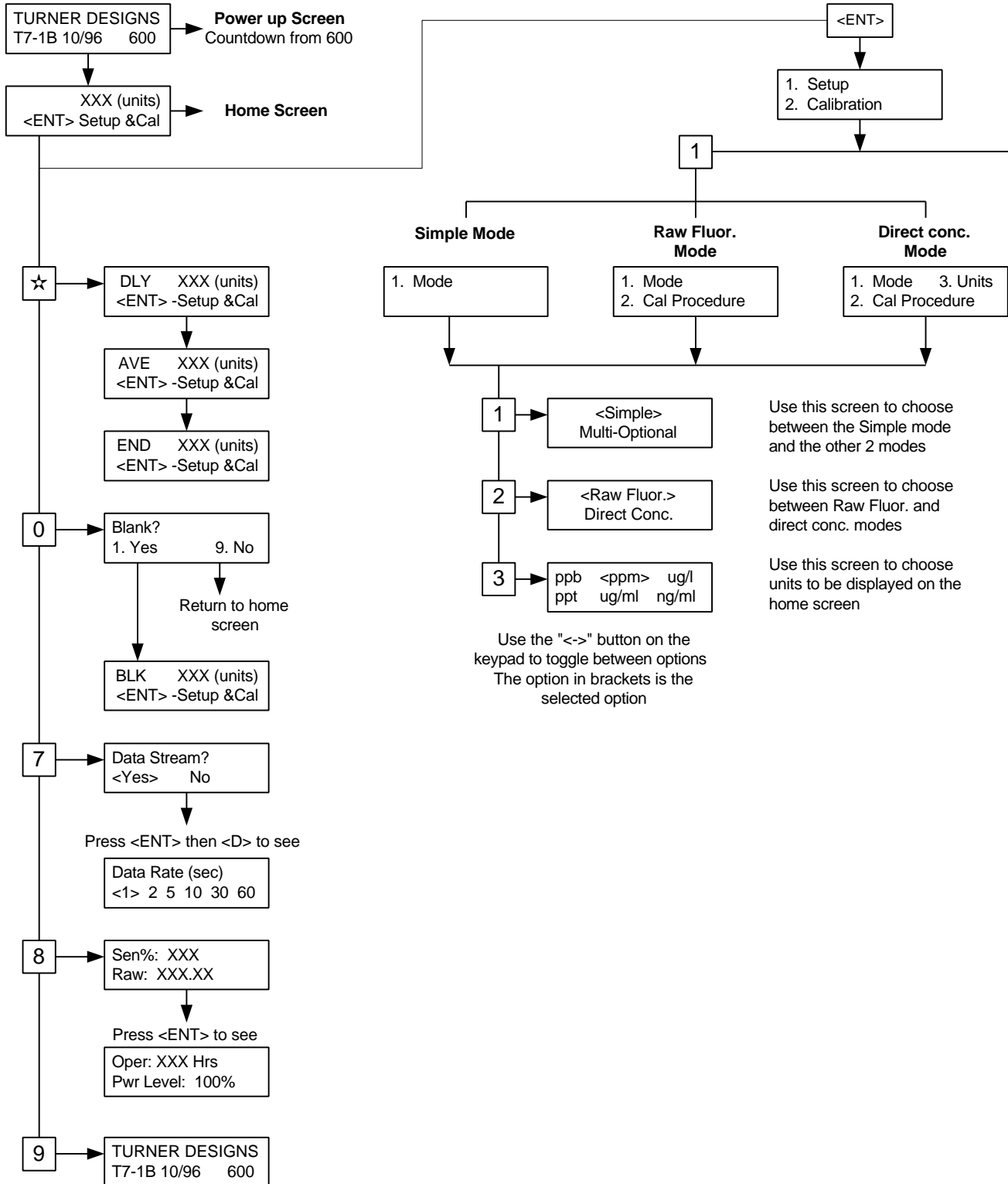
This calibration allows you to use one standard (or sample) and no blanks. The instrument gives the standard a relative value of 500 on a scale of 0-1000. The instrument uses a preprogrammed absolute zero as the 0 point.

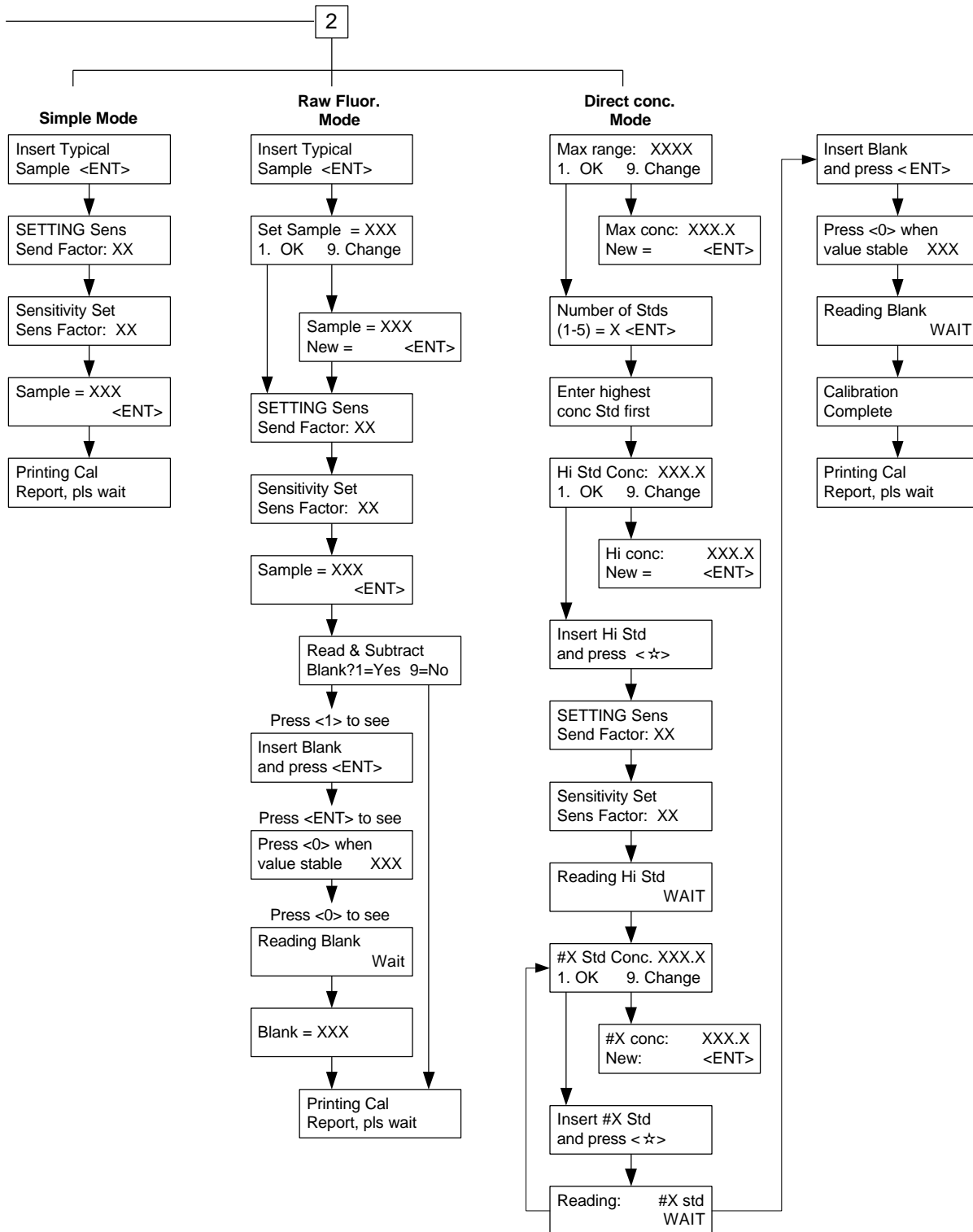
Multi-Optional/ Raw fluorescence: two point calibration (standard + blank)

This calibration allows you to use one standard and the option to blank subtract. The user can specify the magnitude in relative fluorescence units for the standard (on a scale of 0-1000). As an example, if you expect your standard is ~75% of the full range for the application (see application notes on our website for ranges), then call the standard 750 RFUs.

MultiOptional/Direct Concentration: Multi point calibration (up to 5 standards + blank)

This calibration allows you to use up to 5 different standards plus a blank for the calibration. You can choose the units of your standards, and define the magnitude of the maximum readable level. In the end, the output can be in direct concentration so no further calculations are needed.





Note: Calibration data will be stored even after power to the instrument is turned off.

Note that a separate calibration is stored for each of the three modes. For example, if you calibrate in the Simple Mode, this calibration remains in effect until you recalibrate in the Simple Mode. If you calibrate in the Multi-Optional Mode - Direct Concentration, this will not change the calibration in the Simple Mode. Similarly, a calibration in the Multi-Optional Mode - Direct Concentration will not affect a calibration in the Multi-Optional Mode - Raw Fluorescence.

Thus, in effect, the TD-700 can store three separate calibrations at the same time, one for each mode. This also means that when you change modes, as each mode is a separate function, you must calibrate in the mode you want to use.

VI. Calibration Overview

A. Why Calibrate?

The TD-700 calibration procedures set the instrument's sample range, (concentration range) and sensitivity based on a chosen fluorescent standard or sample. In addition, calibration in the Direct Concentration mode assigns a digital value to a known standard so that subsequent standards or samples can be easily referenced to the original. For example, during Direct Concentration calibration you can assign a value of 500 to a sample and know with certain confidence that a sample that reads 250 contains half as much analyte as the original sample (assuming that the blank reads zero).

B. When to Calibrate

- For greatest accuracy, calibrate before running a new batch of samples.
- Recalibrate if the ambient temperature changes by +/- 5°C.
- Recalibrate after changing lamps, filters, cuvette sizes, or the analyte you are measuring.
- 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.

C. TD-700 Calibration Options

There are three calibration procedures available on the TD-700:

- Simple Mode- Raw Fluorescence Calibration
 - Multi-Optional Mode- Raw Fluorescence Calibration
 - Multi-Optional Mode- Direct Concentration Calibration
1. **Simple Mode Calibration:** In the Simple Mode calibration procedure the instrument range is automatically set based on the typical standard or sample chosen. You cannot manually adjust the instrument range when calibrating in this mode. The chosen sample will be automatically set to 50% of the maximum value that can be accurately read by the instrument, (500 out of 1000). Also, there is no option to read and subtract a blank during this calibration procedure.
 2. **Multi-Optional Raw Fluorescence Mode:** In the Multi-Optional Mode Raw Fluorescence calibration you may manually adjust the instrument range by changing the default sample value of 500 to another number from 100 to 950. If you assign a value higher than 500 to your chosen sample, you will decrease the maximum sample concentration that can be read and increase the instrument's sensitivity and resolution. If you assign a value lower than 500, you will increase the maximum concentration that can be read and decrease the instrument's sensitivity and resolution.

3. **Direct Concentration Calibration:** Direct Concentration Calibration, only available in the Multi-Optional Mode, is a multi-point calibration in which up to five standards and a blank are read. The software uses these points to set the optimal instrument range and sensitivity, and to calculate the direct concentration of unknowns. In a multi-point calibration, the instrument generates a calibration curve for superior accuracy. The TD-700 will display the actual concentration of your samples. The display units are user-selectable and chosen in the setup menu.

Note: Fluorescence measurements are affected by factors such as temperature, linearity, and instrument drift. Before calibrating or running samples for the first time, we recommend reviewing Appendix 1.

Calibration: Simple Mode

1. Setup
2. Calibration

1. Mode

<Simple>
Multi-Optional

1. Setup
2. Calibration

Insert Typical
Sample <ENT>

SETTING Sens
Sens Factor: XX

Sensitivity Set
Sens Factor: XX

Sample = XXX
<ENT>

Blank: X.X
Cal Std: XXX.X

<1> - Abort Cal
<ESC> - Resume

VII. Calibration: Simple Mode

The Simple Mode calibration procedure is a single point calibration in which a standard is run in order to set the optimal range and sensitivity of the instrument. The chosen sample will be set automatically to one half of the maximum value that can be accurately read by the instrument. You cannot manually adjust the instrument range and sensitivity during this procedure. Also, there is no option to read and subtract a blank. If you do not require these capabilities, you may wish to use the Simple Mode calibration as it is expedient and straightforward.

1. To choose the Simple Mode, press <ENT> from the HOME screen, press <1> for Setup, then <1> again for Mode. Use << > to choose the Simple Mode. Press <ESC> twice to return to the Setup/Cal Screen.
2. Press <2> from the Setup/Cal Screen. The Simple Mode calibration sequence will appear.
3. Fill a clean test tube or cuvette with a sample that is about half the maximum concentration you wish to read. You do not need to know the exact concentration; you are using it to set the optimal instrument range and sensitivity. Wipe the outside of the test tube or cuvette dry, and insert it into the sample adaptor in the sample chamber.
4. The TD-700 will now set sensitivity, as indicated by the SENS FACTOR (sensitivity factor), so that the sample you inserted will read 500 (half of maximum) on the HOME screen.
5. Upon finishing the sensitivity adjustment, the screen prompts you to acknowledge the value set by pressing <ENT>. Then, calibration data will automatically printout to a printer or a computer and return you to the HOME screen. See Appendix 2 for details regarding the calibration printout.
6. Errors: After reading the sample, the TD-700 will automatically adjust to the optimal range for sample measurement. If the sample used is too concentrated or too dilute, the instrument may not be able to reach the target sensitivity. In these cases, a note message will appear indicating that, based on the calibration, the unit has reached its sensitivity maximum or minimum. By pressing <ENT> you tell the instrument to accept the maximum or minimum sensitivity value. It is recommended, however, that you adjust your sample concentrations to fall within range, and then recalibrate.
7. To view the last calibration data set, press <ENT> from the HOME screen, then <9> to view your blank and calibration standard value. Press <H> to return to the HOME screen.
8. To print the last calibration data set, press <ENT> from the HOME screen, then <D>. Press <H> to return to the HOME screen.
9. To abort the calibration, press <ESC> at any time during the calibration sequence. Press <1> to abort or <ESC> to resume.

Calibration: Multi-Optional Raw Fluorescence

1. Setup
2. Calibration

1. Mode
2. Cal Procedure

Simple
<Multi-Optional>

1. Mode
2. Cal Procedure

<Raw Fluor.>
Direct Conc.

1. Mode
2. Cal Procedure

1. Setup
2. Calibration

3. Insert Typical
Sample <ENT>

4. SETTING Sens
Sens Factor: XX

Sensitivity Set
Sens Factor: XX

Sample=XXX
<ENT>

Read & Subtract
Blank? 1=Yes 9=No

VIII. Calibration: Multi-Optional Mode - Raw Fluorescence

The Multi-Optional Mode - Raw Fluorescence calibration procedure is a single point calibration in which a standard and optional blank are run in order to set the optimal range and sensitivity of the instrument. The user chooses from a range of 100-950 on a scale of 1000, the relative magnitude of the standard. The user also has the option to subtract the blank signal from all the readings.

1. To choose the Multi-Optional - Raw Fluorescence Mode, press <ENT> from the HOME Screen, press <1> for Setup, then <1> again for Mode. Use << > to choose the Multi-Optional Mode. Press <ESC> to return to the previous screen, then press <2> to choose the calibration procedure. Use the << > key to choose "Raw Fluor." for the Raw Fluorescence calibration procedure. Press <ESC> twice to return to the Setup/Cal Screen.
2. To access the calibration sequence, press <2> from the Setup/Cal Screen. The Multi-Optional - Raw Fluorescence calibration sequence will appear.
3. Fill a clean test tube or cuvette with a sample that you wish to read. You do not need to know the exact concentration; you are using it to set the optimal instrument sensitivity and range. Wipe the outside of the test tube or cuvette dry, and insert it into the sample adaptor in the sample chamber. Press <ENT> to proceed to the next screen.
4. Default setting raw fluorescence reading is 800. If this is acceptable press <1> to continue. If you want to change this value, press <9>. Type in the new value (between 100 - 950) and press <ENT>. Press <1> to continue.
5. The TD-700 will now set its sensitivity, as indicated by the SENS FACTOR (sensitivity factor), based on the final sample value that you accepted. Once the sample is set, the TD-700 asks whether you want to run a blank. If you wish to have blank subtracted, press <1>. If not, press <9> and the calibration sequence will end here and you will be returned to the HOME screen. At this point, the calibration data will printout automatically to a printer or a computer. See Appendix 2 for details regarding the calibration printout.

6. If you choose to run a blank, fill a clean test tube or cuvette with the blank solution, wipe the outside of the test tube or cuvette dry, insert it into the sample adaptor in the sample chamber, and press <ENT>. Allow the reading to stabilize and press <0>. The unit will read the blank, then automatically return to the HOME screen. At this point, the calibration data will printout automatically to a printer or a computer. See Appendix 2 for details regarding the calibration printout.
7. Errors: After reading the sample, the TD-700 will automatically adjust to the optimal range for sample measurement. If the sample used is too concentrated or too dilute, the instrument may not be able to reach the target sensitivity. In these cases, an error message will appear indicating that, based on the calibration, the unit has reached its sensitivity maximum or minimum. By pressing <ENT> you tell the instrument to accept the maximum or minimum sensitivity value. It is recommended, however, that you adjust your sample concentrations to fall within range, and then recalibrate.
8. To view the last calibration data set , press <ENT> from the HOME screen, then <9> to view your blank and calibrating standard value. Press <H> to return to the HOME screen.
9. To print the last calibration data set, press <ENT> from the HOME screen, then <D>. Press <H> to return to the HOME screen.
10. To abort the calibration, press <ESC> at any time during the calibration sequence. Press <1> to abort or <ESC> to resume.

Insert Blank
and press <ENT>

6.

Press <0> when
value stable XXX

Reading Blank
WAIT

Blank = XXX

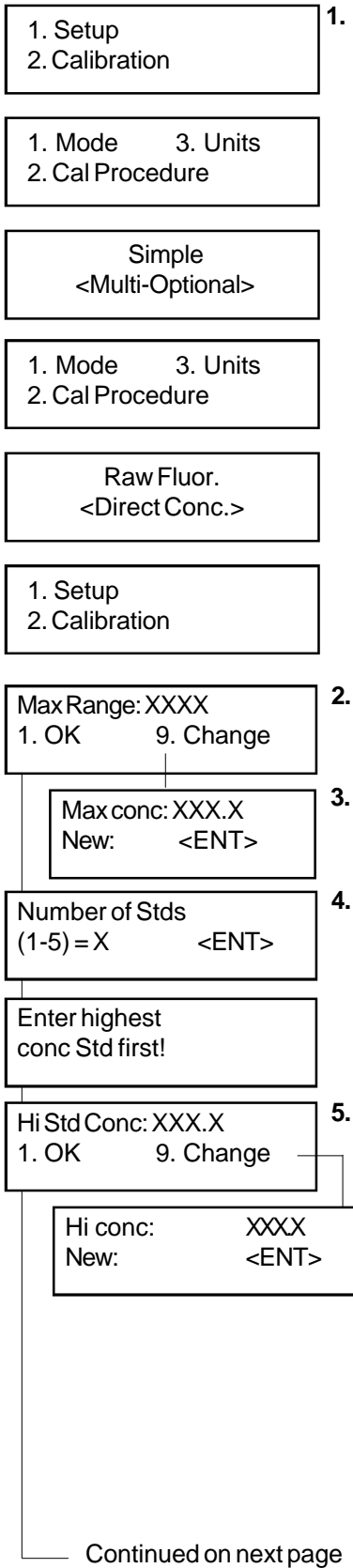
Blank: X.X
Cal Std: XXX.X

8.

<1> - Abort Cal
<ESC> - Resume

10.

**Calibration: Multi-Optional
Direct Concentration**



IX. Calibration: Multi-Optional Mode - Direct Concentration

The Multi-Optional Mode - Direct Concentration calibration procedure is a multi-point calibration in which one to five standards plus a blank are run in order to determine the concentration of an unknown. This procedure will also set the optimal range and sensitivity of the instrument. The Direct Concentration procedure should be used if you require superior accuracy and wish to determine the actual concentration of an unknown. The TD-700 will display actual concentration in the units chosen during setup.

A. To Calibrate

1. To choose the Multi-Optional - Direct Concentration Mode, press <ENT> from the HOME Screen, press <1> for Setup, then <1> again for Mode. Use <<> to choose the Multi-Optional Mode.

Press <ESC> to return to the previous screen, then press <2> to choose the calibration procedure. Use the <<> key to choose "Direct Conc" for the Direct Concentration calibration procedure.

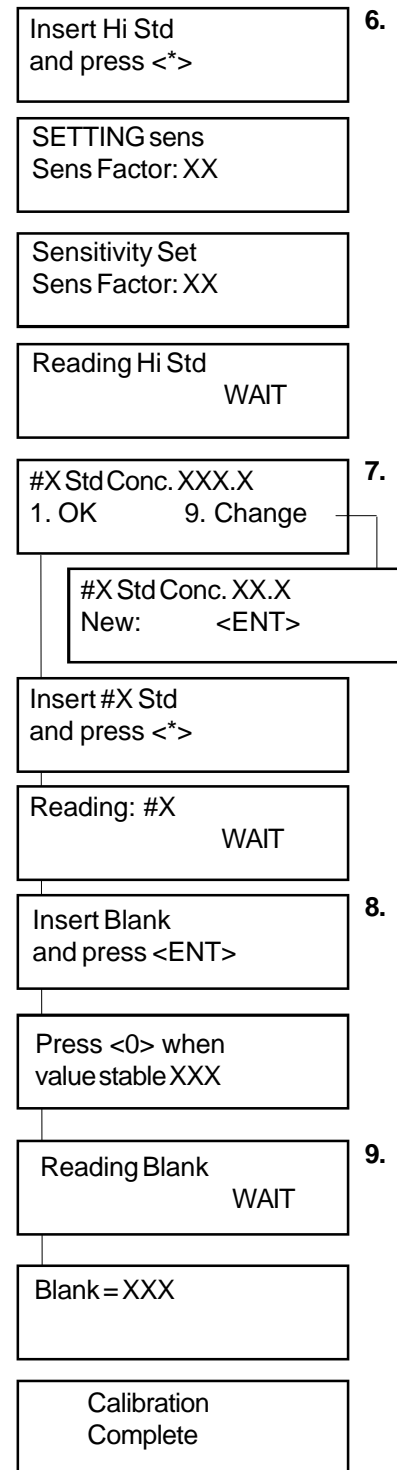
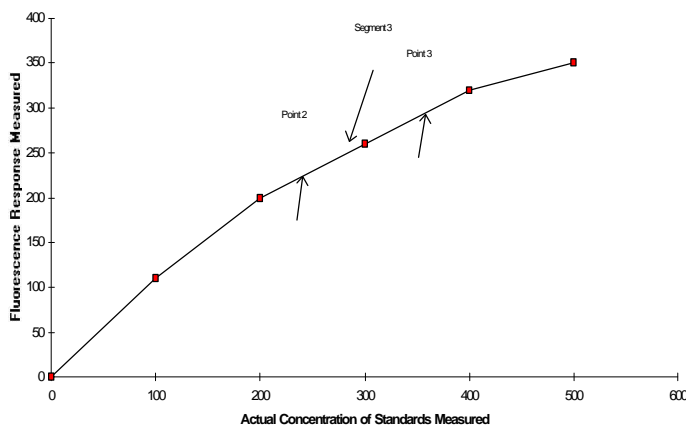
Press <ESC> to return to the previous screen, then press <3> to choose the units of measure. Use the <<> key to choose the required unit of measure. Press <ESC> twice to return to the Setup/Cal Screen.
2. To access the calibration sequence, press <2> from the Setup/Cal Screen. The Direct Concentration calibration sequence will appear.
3. When the TD-700 calls for the maximum range (the maximum concentration you want to read), press <1> to accept the current value or <9> to enter a new value. Range for reentry: 1 - 1000.
4. Key in the number of standards you will be using (1 to 5 concentrations). Press <ENT>.
5. When the TD-700 calls for the "HiStd Conc," press <1> to accept the current value and advance to the next screen. Or press <9> to change the value. Key in the actual concentration of the highest concentration Standard (Hi Std) you are using and press <ENT>. The numerical range is 0.1 to 1000. If you are using only 1 standard, IT MUST be within the linear range for accurate results (see Appendix 1 for a discussion of linear range).

6. Fill a clean test tube or cuvette with the Hi Std (highest concentration standard you are using). Wipe the outside of the cuvette dry and insert it into the sample adaptor in the sample chamber. Press <*>. The unit will adjust sensitivity (as shown by the SENS FACTOR), to the level appropriate for that standard then read the standard.
7. If you are using only 1 standard, the TD-700 will prompt you to insert the Blank. If you are using 2 or more standards, the unit will prompt you to enter the actual concentration of the second standard (#2 Std, as in step 5) and to insert the second standard (#2 Std, as in step 6), then the third (#3 Std), etc. Use a clean, rinsed test tube or cuvette and insert the next standard; press <*>; then <ENT> when finished.
8. When all the standards have been run, the TD-700 will prompt you to insert the Blank. Fill a clean test tube or cuvette with the Blank, wipe the outside dry, and insert it into the sample adaptor in the sample chamber. Press <ENT>.
9. Wait for the Blank reading to stabilize, then press <0>. The TD-700 will read the Blank, then automatically return to the HOME screen. Calibration data will printout automatically if connected to a printer or computer. (See Appendix 2 for details regarding the calibration data printout.)

B. How Sample Concentrations will be Calculated

As you have read, the Direct Concentration calibration procedure accepts multiple standards and one blank. The concentration vs. fluorescence plot of both the standard and the blank will result in something close to a straight line (assuming the standards are within the linear range of the assay and instrument). If you draw a straight line from point to point on the calibration plot and record the slope of the line between each point, you can use these slope values to calculate to sample concentrations. For example, the graph below displays a plot of five standards and a blank. Drawing a line between each point will give you five different linear segments. If the fluorescence of a sample reads between point 2 and point 3, the slope of the segment that connects point 2 and point 3 (segment 3) is used to calculate the direct concentration of this sample.

The following equation would be used to calculate sample concentration



of a sample that had a fluorescence between points 2 and 3:

$$=(F_s - F_2) / m_3 + C_2$$

Where:

- F_s is the fluorescence of the sample
- F_2 is the fluorescence of standard #2
- m_3 is the slope of the line between points 2 and 3.
- C_2 is the concentration of standard #2.

C. To View the Last Calibration

From the HOME Screen, press <ENT> to reach the Setup/Cal screen. From the Setup/Cal screen, press <D> to send the current calibration information to a printer or computer. Press <ESC> to return to the HOME Screen.

D. To Abort the Calibration

To abort the calibration, press <ESC> at any time during the calibration sequence. Press <1> to abort or <ESC> to resume.

<1> - Abort Cal <ESC> - Resume

X. Reading Samples

A. Reading Samples

1. Fill a clean test tube or cuvette your with sample, wipe the outside dry, and insert it into the sample adaptor in the sample chamber. Close the lid.
2. Record the sample reading.

You have three different options for recording sample readings:

a. Discrete Sample Averaging to Printer or Computer

Discrete Sample Averaging averages the reading of a sample for 12 seconds. This feature minimizes operator guesswork when reading samples, provides greater accuracy, and reduces error from temperature effects. Inserting a sample and pressing <*> from the HOME screen initiates the following sequence:

7 second delay period for the signal to stabilize
12 second averaging period
5 second display of readout

The words DLY, AVG, and END appear on the upper left of the LCD display and alert the user to the instrument's current status. After the sequence is complete, the display reads "END", the reading will freeze for 5 seconds, and the readout will automatically be sent to your computer or printer. The letters "(disc.)" will appear next to the reading on the printout to indicate that the discrete sample averaging feature was used on this measurement.

b. Read Samples from Display

Your second option is to read samples directly from the display without using the Discrete Sample Averaging function. Allow the reading to stabilize, then press <D> to send the reading to a computer or printer or write down the result.

c. Data Stream

Instantaneous readings are reported out to a printer or computer at a preset interval. See Section X C for further details.

3. Rinse the cuvette 3 times, preferably with the sample you wish to measure. Repeat steps 1 through 3 until all samples are read.

Note: Do not leave highly concentrated samples in the instrument for an extended period of time. Unstable readings may result.

DLY	XXX (units)
<ENT> -Setup & Cal	

AVG	XXX (units)
<ENT> -Setup & Cal	

END	XXX (units)
<ENT> -Setup & Cal	

**HOME Screen during
Discrete Sample Averaging
sequence**

B. Resetting Blank to Zero

This feature allows you to reset the blank to zero from the HOME screen when in the Multi-Optional Mode. Although running a calibration is the best method to minimize sample error due to instrument drift, a quick and easy way to reduce the effects of dark current, and blank fluorescence, is to "autozero" or "reset the blank to zero" from the HOME screen. However, be sure to run at least one calibration before using this feature. Resetting the blank to zero from the HOME screen is quite useful if you wish to read multiple sets of blank and relative sample readings, and do not wish to recalibrate. When resetting the blank to zero, you are replacing the last blank setting with the new blank setting. Again, this procedure will not compensate for errors in sample readings due to instrument drift. **For maximum accuracy, you should recalibrate.**

1. Insert a blank, then press <0> from the HOME screen.
2. Press <9> to abort the procedure or <1> to Reset Blank to Zero. Press <1> and without further prompting, the instrument will blank to zero.
3. If the reading for the blank exceeds the maximum, the unit will display an error message: "Blank level too high." If this message should appear, evaluate your sample to ensure that you are reading the blank and not another type of sample. Once you have verified that you are reading the blank, try the procedure again. If the message persists, recalibrate the instrument.

C. Data Stream

This feature allows you to output data directly to a printer or a computer at regular intervals. Only the value seen on the LCD screen will be printed. No index or raw fluorescence value will be printed.

1. Press <7> from the HOME screen.
2. Press <↔> to toggle between <Yes> and <No> (turns data stream on or off).

When the data stream feature is on, the data rate can be changed. The number selected is the time interval between data output.

1. Press <D> from the HOME screen when the data stream feature is on.
2. Use the <↔> key to toggle between numbers.

```
Blank?
1. Yes   9. No
```

```
BLK XXX ppm
<ENT> -Setup & Cal
```

```
Data Stream?
Yes <No>
```

```
Data Rate (sec)
<1>  2   5  10  30  60
```

APPENDIX 1

Before Calibration or Reading Samples

A. Materials Needed

- 1 or more cuvettes (25 mm test tubes; 13 mm test tubes or 10 mm square cuvettes).
- A Blank (sample water or liquid containing none of the fluorescent material) (except when using the instrument in Simple Mode).
- Standard(s) (1 to 5 solutions of varying but known concentrations) for the Direct Concentration Calibration Procedure; OR, for the Simple mode, one standard that is approximately half the level of the highest sample you are likely to read (used to set the optimal sensitivity and range of the TD-700).
- Samples to be read.

B. When Handling Samples

1. Take care not to spill samples into the sample chamber. Wipe up any spills promptly. See Appendix 9, Section A, for details on maintenance.
2. Rinse the test tube or cuvette 3 times between samples (preferably with the sample to be read). The TD-700 is very sensitive and even small amounts of material from a previous sample may result in errors. Complete rinsing is especially important if you are using the same test tube or cuvette for samples and blank.
3. Fill the test tube or cuvette at least 75% full; significant error will result if it is not full enough.
4. The cuvette **MUST BE DRY** on the outside when taking readings. Moisture on the outside will result in error.

C. Linear Range and “Quenching”

The linear range is the concentration range in which the readout of the TD-700 is directly proportional to the analyte concentration. 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.

Beyond the linear range (above the upper concentration limit), the fluorescence readings rise at a decreasing rate and a calibration curve is necessary for accurate readings. At even higher concentrations, readings will begin to decrease even though the sample concentration is increasing. This effect is known as "concentration quenching".

For example, Rhodamine WT fluorescent dye provides linear readings from about 10 parts per trillion to approximately 0.1 ppm. Using the 25 x 150 mm Sample Adaptor, linearity should be checked when measuring concentrations of dye higher than 0.1 ppm.

At dye concentrations below 0.1 ppm, a single-point calibration (one standard and a blank) may be used to calibrate the TD-700. For concentrations between 0.1 and 0.5 ppm, use a multi-point calibration with 3 to 5 different standard concentrations. Above 0.5 ppm, “concentration quenching” occurs, and samples should be diluted into the linear range before taking readings. Or, you can switch to a smaller size test tube or cuvette (smaller pathlength) and increase the linear range.

Linearity may be checked by diluting a sample 1:1 or some other convenient ratio. If it is linear, the reading will decrease in direct proportion to the dilution. If the reading goes up (“concentration quenching”), you are beyond the range for a calibration curve. If the reading does not decrease in direct proportion to the dilution, you are in the range for a calibration curve, but still beyond the linear range.

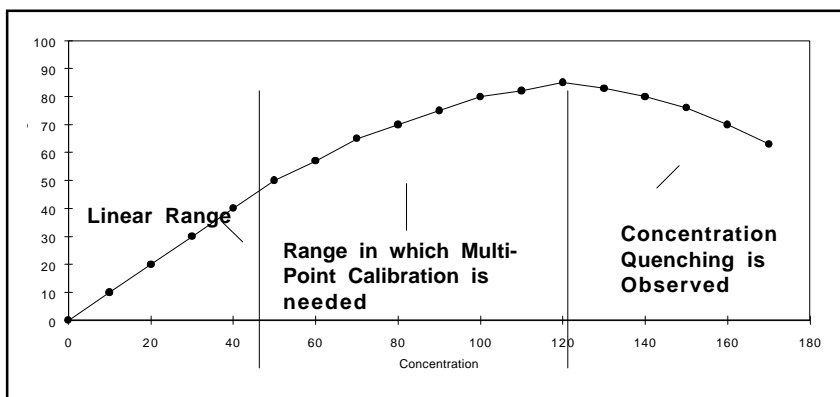


Figure 1. Linearity, Calibration Curve, and Quenching

D. Temperature Considerations

Fluorescence is temperature sensitive. As the temperature of the sample increases the fluorescence decreases. For greatest accuracy, read blank, standard, and samples at the same temperature. (The TD-700 Fluorometer does not compensate for temperature changes.) NOTE: The unit’s lamp will heat samples if left in the instrument. Thus it is recommended that you use the instrument’s Discrete Sample Averaging feature to ensure that samples are read after they have been in the instrument for the same period of time and are at approximately the same temperature.

E. Positioning Samples

Sample cuvettes often will give slightly different measurements depending upon their orientation in the sample adaptor. This is due to defects in the shape of the test tube that are not visible to the human eye. We recommend that the test tube be marked at the top and positioned in the adaptor the same way each time to minimize error.

F. Data Quality

The TD-700 is only as accurate as the standards that are used to calibrate it. This is why it is important to take care when preparing standards, samples, and blanks. One should follow good laboratory practices when preparing these standards.

APPENDIX 2

The Calibration Printout

A. Printout Capability with a Printer or Computer

To use this function with a printer:

1. Connect the TD-700 to a serial printer via the RS-232 port on the back of the instrument. If you ordered a printer from Turner Designs, the appropriate serial cable is included.
2. Press <D> or <*> from the HOME screen and the readout currently displayed will be sent to the printer.

To use this function with a computer:

1. Connect the TD-700 to your computer via the RS-232 port on the back of the instrument. Use the DB9 serial cable included with your instrument.
2. The TD-700 signal is in ASCII format so you can use various communications programs to import data to your computer. On your computer, run the communications program you have chosen. For a step-by-step procedure using Microsoft Windows^a terminal, see Appendix 5.
3. Press <D> or <*> from the HOME screen and the readout currently displayed will be sent to the computer with an index number.

B. Sample Reading with a Printer or Computer

When <D> or <*> is pressed from the HOME Screen, the TD-700 sends out an index marker (1 - 999), the concentration readout of the sample, and the Raw Fluorescence (all in ASCII format). Note that the column headings will appear only before the first index, and the index will begin again from "1" every time you calibrate or turn off the instrument. An example of the indexing feature is illustrated by the left column in the sample printout below.

In the Direct Concentration Calibration Procedure (**for example only**):

Samples:	Conc.	Raw Fluor.
1	32.01	42.80
2	16.00	21.40
3	8.002	10.70

For the Simple and Raw Fluorescence modes (**for example only**):

Samples:	Raw Fluor.
1	42.80
2	21.40
3	10.70

C. Calibration Printout Using the Direct Concentration Calibration Procedure:

(Example only! Your numbers will vary.)

Press <D> from the Setup/Cal screen to send the calibration information to the printer or computer.

Mode: Multi-Optional
 Cal. Procedure: Direct Conc.
 Units: ppb
 Sensitivity Level: 48

Current Calibration
 Entered Max Range: 220.00

Calibration Standard Summary:

Std.	Conc.	Raw Fluor.
BLK	0	0
Std.3	1.50	6.706
Std.2	6.20	29.09
Hi Std.	50.00	227.9

Calibration Curve Data:

Conc. Range	Fluor. Range	Slope
0(BLK) - 1.500	0.00 - 6.710	0.223
1.500 - 6.200	6.710 - 29.09	0.210
6.200 - 50.00	29.09 - 227.9	0.220

Slope %RSD: 3.127

An Explanation of the Printout

The first four lines of the printout display the TD-700's current parameters: the mode, the cal procedure, the units, and the instrument sensitivity level. The sensitivity level (a number between 1 and 100) is a measure of the photomultiplier voltage. Next is the calibration that is stored in the unit's memory. The maximum concentration range entered during the calibration sequence is displayed here. Following this is the Calibration Standard Summary which displays each standard, its concentration, and its corresponding raw fluorescence value. Concentration and raw fluorescence values are organized in the Calibration Curve Data table so that the slope of the line between each data point is displayed. The percent relative standard deviation (%RSD) is displayed below this. The % RSD is an indicator of how well each of the slope values correlate to one another. A consistent slope (a slope that is almost the same for all data points) will result in a low %RSD. A nonlinear set of data will result in a high %RSD. The %RSD can be thought of as an overall indicator of the data quality. A %RSD of less than 25% is considered by many to be acceptable while a %RSD of less than 5 indicates excellent linearity. Both the individual slopes and average slope will change depending on the numbers input during calibration. There is no "best" slope value.

Note that during calibration, the maximum you can enter for the Max Range is 1000. However, the actual maximum achieved after calibration may be higher, depending upon the reading for the Blank and for the High Standard. This is a normal condition; the instrument will set to the best sensitivity to obtain the Max Range you specified.

D. Calibration Printout in the Multi-Optional Raw Fluorescence Mode:

(Example only! Your numbers will vary.)

Press <D> from the Setup/Cal screen to send the calibration information to the printer or computer.

Mode: Multi-Optional
Cal. Procedure: Raw Fluor.
Units: fsu**
Sensitivity Level: 48

RAW Fluor. Sample + Blank = 500.0
RAW Fluor. Blk = 123.0
Adj. Fluor. Sample = 377.0
Adj. Fluor. Blank = 0.000

An Explanation of the Printout

The first four lines of the printout display the TD-700's current parameters: the mode, the cal procedure, the units, and the instrument sensitivity level. Following this is the calibration stored in the unit's memory. The calibration stored consists of the raw and adjusted sample and blank value (if applicable), expressed in raw fluorescence units.

E. Calibration Printout Using the Simple Mode: (Example only! Your numbers will vary.)

Press <D> from the Setup/Cal screen to send the calibration information to the printer or computer.

Mode: Simple
Cal. Procedure: Raw Fluor.
Units: fsu**
Sensitivity Level: 48

RAW Fluor. Sample = 500.0

An Explanation of the Printout

The first four lines of the printout display the TD-700's current parameters: the mode, the cal procedure, the units, and the instrument sensitivity level. Following this is the calibration stored in the unit's memory. The calibration stored consists of the sample value only, expressed in raw fluorescence units.

** fsu = Fluorescent Signal Units, the designated units for Raw Fluorescence readings.

APPENDIX 3 Alarms and Diagnostics

A. Alarm Screen

There are alarms built into the TD-700 to warn of an internal instrument malfunction or low power.

Table 1. Alarms

<u>Alarm</u>	<u>Delay</u>	<u>Alarm Condition</u>	<u>Normal</u>
High Voltage (HV Bad)	3 min.	>1000 or <25 V	150 to 850
Low Power (Low PWR)	1 min.	<40%	100%

```
ALM    XXX ppm
<ENT> -Setup & Cal
```

```
HV Bad
```

An alarm will be activated if the abnormal condition is in effect for a specified delay period. If an alarm is activated, "ALM" will blink on the HOME screen.

To see what alarm is active, from the home screen press <ESC>. For example, if the high voltage (HV) is outside the specified range you will see "HV Bad". If the HV alarm is triggered (HV Bad is seen when <ESC> is pressed from the HOME screen), contact Turner Designs.

If the low power alarm is triggered, check the power at the source and the power supply.

Refer to the Diagnostic Screens (next section) for definitions.

B. Diagnostic Screens

There are diagnostic screens which show the status of internal fluorometer functions. To access these screens, from the HOME screen, press <8>, then <ENT> to see the next screen in the sequence.

```
Sen %: XX
Raw:   XXX
```

```
Oper: XXX Hrs
Power level: 100%
```

Definitions:

Sen %. Sen % is also referred to as **Sens Factor** in the software and **Sensitivity Level** on the printout. All three terms indicate the fluorometer's sensitivity on a scale of 1 to 100. The Sen % is directly proportional to the voltage on the photomultiplier tube. The higher the number, the greater the photomultiplier tube voltage, the greater the sensitivity (and typically, the greater the "noise") of the readings.

Raw. The “raw” fluorescence signal output from the instrument’s light detector (the photomultiplier tube). This is the output the TD-700 uses (in conjunction with the actual value entered for the standards, and the fluorescence signal for standards and blank) to arrive at the readout on the HOME screen. It can be used to diagnose problems with the unit.

NOTE: To see the raw fluorescence for the standard(s) and the blank as set during the last calibration, from the Calibration & Setup menu, press <D> to print or send to a computer.

NOTE: If you calibrated in the Simple Mode or Multi-Optional Mode “Raw Fluor.” Calibration Procedure, to see the raw fluorescence for the standard and the blank as set during the last calibration, from the Calibration & Setup menu, press <9> and they will appear on the display.

Operation Hour (Oper). Shows how many hours the instrument has been operating.

Power level. When at 100%, indicates power supply is functioning correctly.

APPENDIX 4 Troubleshooting

<u>Symptom</u>	<u>Solution</u>	<u>Section To See</u>
Power ON but readings stay at zero	Check the lamp; change if necessary. Make sure the sample lid is closed tightly.	Section IV
Power switch ON, but no power	Make sure unit is plugged in securely; check power from the wall socket; Make sure power supply is functioning.	
Display reads OVER	Sample reading is too high for the instrument to read at the current sensitivity level. Dilute samples to get an on-scale reading. Or recalibrate and reduce sensitivity.	Appendix 8, H.
Display reads negative (-)	Reading for Blank is less than zero due to drift; reading for samples are less than Blank. Reset Blank to Zero or recalibrate.	Appendix 8, H.
Drifting readings	The instrument is open loop and readings will drift over a period of time; recalibrate to cure.	Section VII Section VIII Section IX
Erratic readings	Instrument readings will fluctuate somewhat due to instrument noise, especially at high sensitivity and when reading low concentrations. If readings are very erratic, check the lamp and optical filters for damage/freckling.	Section III Section IV
Low readings; not enough resolution	Sensitivity was not adjusted adequately during calibration; recalibrate. Or, make sure the proper lamp and filters are being used.	Section VII Section VIII Section IX Section III Section IV

APPENDIX 5

Data Collection

You can use various communications software programs to import data to your computer. The signal is sent from the TD-700 in ASCII format.

1. Connect the 9-pin connector that extends from the TD-700 to the serial communications port of your computer (COM1 or COM2) using a 9-pin serial cable. You may need a 9-to-25 pin serial adaptor. Obtain this at your local computer store.
2. In Window's OS, locate the HyperTerminal program in the Accessories directory and double-click on the icon to open it. If operating in MAC OS, use a comparable terminal program to capture the ASCII data.
3. Give the New Connection a name such as TD700.
4. In the Phone Number window, choose Direct to COM 1 or Direct to COM 2 depending on which COM Port that the instrument is connected to. (This can be the hardest part of the procedure. Different computer manufacturers use different conventions.)
5. Set the Port Settings to the following parameters : Bits per second: 9600, Data Bits: 8, Parity: none, Stop Bits: 1, Flow Control: Xon/Xoff.
6. To store the data to a file, choose the Transfer menu and select Capture. Give the captured file a name.
7. Stop the Capture when you are done by selecting the Transfer menu, and then by selecting Capture/Stop.
8. Access your data using Excel, Word, or other programs by opening the file you saved in step 6.

APPENDIX 6

Sample and Cuvette Adapters

Test tube and cuvette size, apertures, and the sample cell path length all affect the sensitivity of the instrument. In general, the greater the diameter of the test tube or cuvette (the greater the path length), the more sensitive the readings and the lower the linear range.

The smaller the window and path length, the less sensitive the readings.

You must recalibrate the instrument if you change cuvette adapters, or if you change path lengths or attenuators.

A. Installing and Removing Sample Adapters

Available for the TD-700 are a variety of sample adapter to cater to the sample size needs of researchers. These are :

-25 mm test tube adapter
(P/N 7000-982, standard with TD-700 model 7000-000)

-13 mm test tube adapter
(P/N 7000-981, standard with TD-700 model 7000-000)

-10x10 mm square cuvette adapter
(PN 7000-988, standard with TD-700 model 7000-009)

-Minicell adapter
(P/N 7000-951, fits inside 10x10 cuvettes adapter
P/N 7000-009)

To install/use adapters

1. Open the lid to the sample chamber.
2. Align pin or point arrow on the adapter to the silver dot on the TD-700.
3. Slide the sample adaptor inside the sample chamber and push down until it is fully seated in the chamber.

To remove sample adapter:

1. Open the lid to the sample chamber.
2. Remove the test tube.
3. Grasp the sample adaptor by the rim and pull straight up.

B. Minicell Adapter

The minicell cuvettes allows for very small volumes (75-250 μ l) to be analyzed.

To install

1. Slide minicell adapter into the 10x10 mm cuvette adapter, where a normal cuvette would sit.
2. Insert the borosilicate glass minicell into the adapter.
3. Insert into the TD-700 as described earlier.

APPENDIX 7

Glossary

Accuracy	The degree to which a measured result approximates the true value of the quantity being measured. This value is usually expressed as a percent.
Analyte	The substance you wish to measure.
Attenuator	A part used to cut down the amount of light that enters and exits the sample. Can be used to adjust the sensitivity and dynamic range of your analysis.
Blank	Sample water that contains none of the fluorescent material to be measured. Blank is used during calibration to set the instrument to zero.
Calibration	Setting the sensitivity of the instrument to the standard and "zeroing" the instrument to subtract blank.
Calibration Curve	See Linearity. By using multiple standards during calibration in the Direct Concentration calibration procedure, a calibration curve will be obtained, which will provide accurate readings even when the readings are nonlinear.
Dark Current	A small amount of current flows in a photomultiplier tube even when the tube is operated in a completely dark state. This output is called dark current and the magnitude is greatly dependent upon the amount of voltage applied to the photomultiplier tube (i.e. the greater the Sensitivity Factor, the larger the dark current).
Detection Limit	May be used to refer to either the minimum or maximum concentration that can be read. Limits are specific to the fluorescent material, filters, lamp, and sample or cuvette adaptor used.
Direct Concentration	Refers to the actual concentration of the substance being read. "Direct Concentration" is a user-selected TD-700 calibration procedure which, after calibration, provides the actual concentration of samples in the units selected by the user.
Discrete Sample Averaging	A feature of the TD-700 which allows a sample reading to be averaged over a set period of time, which minimizes operator guesswork, temperature effects, and inconsistent sample handling. When <*> is pressed from the HOME screen, after a 7-second delay, the signal will be averaged for 12 seconds, and the average displayed for 5 seconds.

Drift	The change in measurement of the same sample over time.
Dynamic Range (maximum range)	The range of concentrations that can be read by the TD-700 at the current level of sensitivity. Since zero is frequently assumed to be the minimum, dynamic range in this manual often refers to the maximum concentration that can be read. The user can select the desired maximum (Max Range) during calibration in the Direct Concentration calibration procedure.
Fluorescence	The TD-700 Fluorometer measures the concentration of various analytes in samples of interest via fluorescence. A fluorescent molecule has the ability to absorb light at one wavelength and almost instantly emit light at a new and longer wavelength. A fluorescent molecule has a unique “fluorescence signature,” which allows one substance to be distinguished from another. In a fluorometer, light from a lamp is passed through an excitation filter that transmits light of a wavelength range specific to the sample compound being measured. The light passes through the sample, which emits light proportional to the concentration of the fluorescent material present and proportional to the intensity of the exciting light. The emitted light is passed through an emission filter that selects for the appropriate wavelength range, and the resultant light is then detected by a photomultiplier tube.
Gain	An indicator of the sensitivity of the instrument usually expressed in powers of 10.
Linear Range	The concentration range in which standards correlate with a straight line. Usually expressed as a concentration maximum such as 100 ppb.
Noise	The amount of fluctuation in the instrument's measurement due to the instrument or detector itself.
Optical Path Length	The length of sample through which light passes. This is defined by the cuvette size and adaptor used.
Quenching	A phenomenon which occurs when the fluorescence measurements decrease even though analyte concentration is increasing.
Raw Fluorescence	Refers to the “relative” fluorescence of a substance being read, rather than the actual concentration. “Raw Fluorescence” is a user-selected TD-700 calibration procedure which, after calibration, provides the “fluorescence intensity” of samples relative to the standard and to each other.

Resolution

Resolution is related to sensitivity, but refers to the "detail" which can be read. When the instrument sensitivity level is high, lower concentrations can be read with ease (high resolution) but higher concentrations can not be read on-scale. When the sensitivity is low, higher concentrations can be read, but the detail is not as good at low concentrations (low resolution).

**Sens Factor
Sen %
Sensitivity Level**

These three terms are synonymous and are used to indicate the TD-700 fluorometer's sensitivity on a scale of 1 to 100. Sens Factor is the term used in the software during calibration, Sens % can be found on the diagnostic screen, and sensitivity level is the term used on the printout. The Sensitivity Factor is directly proportional to the voltage on the photomultiplier tube. The higher the Sensitivity Factor, the greater the sensitivity (and typically, the greater the "noise") of the readings.

Sensitivity

The basic operating level of the instrument. It is related to the detection limits, the dynamic range, and the resolution. The electronic sensitivity is set during calibration using the keypad. Sensitivity can also be adjusted mechanically using different cuvette sizes.

APPENDIX 8

Error Messages and Notes

During calibration, the TD-700 will display an error message if incorrect values are entered or if the readings may result in an inadequate calibration.

A. Invalid Input

This screen will appear when you enter a value that is outside the range for the parameter. For example, if you enter a number greater than 1000 for the Max range, you will see "Invalid Input."

B. Error1

The concentration entered for the standard (Std) is greater than the maximum concentration entered for the Maximum Range. Press <ENT> and enter a lower concentration for the Std. OR press <ESC>, then <1> to abort the calibration; use a lower concentration standard or enter a higher value for the Maximum Range.

C. Note 1

If the sensitivity exceeds the maximum before reaching the target sensitivity, a note message will appear. The unit will automatically adjust sensitivity to the maximum. If sensitivity falls below the minimum before reaching the target sensitivity, the unit will automatically adjust sensitivity to the minimum. Note that this may change the Max range concentration you entered.

D. Error3

The **concentration entered** for #2-5 Std is greater than the concentration entered for the highest Std. Press <ENT> and enter a lower concentration for the Std. OR press <ESC>, then <1> to abort the calibration. Begin again using the highest concentration standard first.

E. Error4

Fluorescence reading for #2-5 Std is higher than the reading for the highest standard. Press <ESC> to abort the calibration; rerun using the highest concentration standard first.

F. Error5

Fluorescence reading for blank is higher than the **reading** for one or more of the standards. Press <ENT> to rerun blank; make sure blank is OK and rerun. OR, press <ESC> to abort the calibration; calibrate again using standards more concentrated than blank.

A. Invalid input
<ENT> to reenter

B. Error1: Std conc
> max range

C. Note1: MAX (or MIN) sens
Press <ENT>

D. Error3: Std conc
ent. >Hi Std

E. Error4: #X Std
reads >Hi Std

F. Error5: Blk reads
> Std #X

G. Error6: Bad Calibration

G. Error6

Calibration is bad. This may occur if the actual value you entered for a standard does not match its fluorescence reading in comparison with one or more of the other standards. For example, you put in a standard you say has an actual value of 10 and it reads 150, and you put in another standard you say has an actual concentration of 5 and it reads 200. Press <ESC>, then <1> to abort the calibration; calibrate again using properly diluted and labeled standards.

H. HOME Screen Displays OVER, or Negative Numbers

1. OVER

If the HOME screen displays "OVER" (and the lid is closed), this means that the sample reading is too high for the instrument's light detector to read at the current level of sensitivity, i.e., sensitivity is set too high (raw fluorescence reading >1000).

If you see OVER, check the reading in the Diagnostics sequence. If it is >1000, make sure the proper filters are installed. Examine the filters for damage: freckling of mirrored surfaces, cracks, etc.

If you are reading samples of high concentration, you can dilute them until you get an on-scale reading. Or, if you wish to reduce sensitivity, calibrate the unit again. If you are in the Direct Concentration calibration procedure, choose a higher Max range conc. or a more concentrated "high" standard. If you are using the Raw Fluor. Calibration Procedure, use a more concentrated standard and/or chose a lower value for the sample setting if you are in the Multi-Optional Mode.

NOTE: On rare occasions, you might see "-OVER". This means that the raw fluorescence reading is less than -1000. This might happen if you calibrated with an extremely high Blank. Do not leave the instrument reading "OVER" for an extended period of time (several minutes).

2. Negative (-) numbers

If the HOME screen is displaying a negative number, it means that the sample is reading less concentrated than the Blank. This might occur in a case where you calibrated with a high Blank; or it might occur if the Blank is reading negative due to instrument drift. To cure, if you are in the Raw Fluorescence Calibration Procedure, you can use the Reset Blank to Zero function. Or, you can recalibrate.

I. Error: Blank level too high

I. Blank Level Error

If the blank level is too high when the user tries to re-blank the instrument from the HOME screen, this error may occur. Most likely this error is a result of using the wrong solution to blank with. To remedy, use the correct blank solution. If the error persists, recalibrate.

APPENDIX 9

Maintenance, Warranty, & Service

A. Maintenance

If possible, do not spill liquids into the sample chamber. However, there is a drain plug on the bottom of the instrument underneath the cuvette adaptor. If there is a spill:

1. Unplug the instrument.
2. Remove the test tube or cuvette, the sample adaptor, and Filter Cylinder from the instrument. Completely dry all of these items and the optical filters.
3. Wipe up any moisture inside the sample chamber.
4. Plug in the unit and turn on the power. Allow it to run for a few hours until completely dry inside.

Periodically wipe off the outside of the instrument with a damp cloth. Do not use solvents or abrasive cleaners to clean the TD-700.

B. Warranty

Turner Designs warrants the TD-700 Fluorometer and accessories to be free from defects in materials and workmanship under normal use and service for a period of one year from the time of initial purchase, with the following restrictions:

1. The instrument and accessories must be installed, powered, and operated in compliance with the directions in this TD-700 Laboratory Fluorometer Operating Manual and directions accompanying the accessories.
2. Damage incurred in shipping is not covered.
3. Damage resulting from measurement of samples found to be incompatible with the materials used in the sample system is not covered.
4. Damage resulting from contact with corrosive materials or atmosphere is not covered.
5. Damage from sea water and other moderately corrosive materials that are not promptly removed from the instrument is not covered.
6. Damage caused by modification of the instrument by the customer is not covered.
7. Failure of limited life parts, such as lamps, is not covered.

C. Obtaining Service

Warranty Service

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

1. Write or call the Turner Designs service department and describe as precisely as possible the nature of the problem.
2. Carry out minor adjustments or tests as suggested by the Service Department.
3. If proper performance is not obtained, ship the instrument, prepaid, to Turner Designs, with a statement of shipping charges. The instrument will be repaired and returned free of charge, along with a check to cover shipping charges, for all customers in the contiguous continental United States.

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

NOTE! Under no conditions should the instrument or accessories be returned without notice. Prior correspondence is needed:

- a. To ensure that the problem is not a trivial one, easily handled in your laboratory, with consequent savings to everyone.
- b. To specifically determine the nature of the problem, so that repair can be rapid, with particular attention paid to the defect you have noted.

Out-of-Warranty Service

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

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

Address for Shipment:

Turner Designs
845 W. Maude Ave.
Sunnyvale, CA 94085

APPENDIX 10

Specifications and Features

Sample Adaptors:	25 mm sample adaptor accommodates 25 x 150 mm test tubes, a 13 mm sample adaptor for 13 x 100 mm test tubes, and an adaptor for 10 x 10 x 45 mm square cuvettes. A Range Extending Adaptor with variable apertures for 10 x 10 x 45 mm square cuvettes is also available.
Sensitivity:	Less than 20 picograms/ml FITC.
Power:	External power supply, 100-240 VAC +/- 10% of the nominal voltage, Max. 30 watts.
Dimensions:	23.5 cm W x 28 cm D x 21 cm H, (9.25" W x 11" D x 8.25" H).
Weight:	5.9 Kg (13 lbs).
Operating Temperature:	41 - 104°F; 5 - 40°C.
Display:	16 x 2 character LCD; (3.86" x .86"; 9.8 cm x 2.18 cm).
Keypad:	4 x 5 keys (3" x 2.7"; 7.62 cm x 6.86 cm).
Data Output:	100% ASCII format through a 9-pin RS-232 serial cable at 9600 baud.
Filter Cylinder:	Accommodates eight, 1-inch round filters, (four excitation and four emission).
Software:	Menu-driven microprocessor-controlled.
Detector:	Factory-installed photomultiplier tube. Standard: 300 - 650 nm. Optional Red Sensitive: 185 - 870 nm.
Lamp:	Quartz Halogen Lamp (20 watts; Lamp Life = 2000 hours) or Low Pressure Mercury Vapor Lamp (4 watts; Lamp Life = 8000 hours).
Printer:	Optional Seiko™ Printer (16.0 cm W x 17.0 cm D x 6.7 cm H).
Material:	UL-approved polyurethane. Sheet metal back and base.
Readout:	Direct Concentration or Raw Fluorescence.
Environmental Conditions:	Indoor use only. Altitude specification: 0-2000m. Transient Overvoltages according to INSTALLATION CATEGORY II. Pollution Degree 1 and 2 in accordance with IEC 664.

Maximum Relative Humidity:	75% for temperatures up to 31°C, decreasing linearly to 50% relative humidity at 40°C.
Calibration:	Multi-point Calibration for Direct Concentration Measurement or Single Point Raw Fluorescence Calibration.
Operating Mode:	"Simple" for the inexperienced user; or "Multi-Optional" for the advanced user.
Blank:	Reads and subtracts Blank.
Discrete Sample Averaging:	Sample readings can be averaged to improve accuracy (7-second delay; 12-second signal averaging; 5-second display readout).
Warranty:	One-year warranty.
Approvals:	CE, TUV, and UL.

APPENDIX 11

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 2 shows the key components of a filter fluorometer.

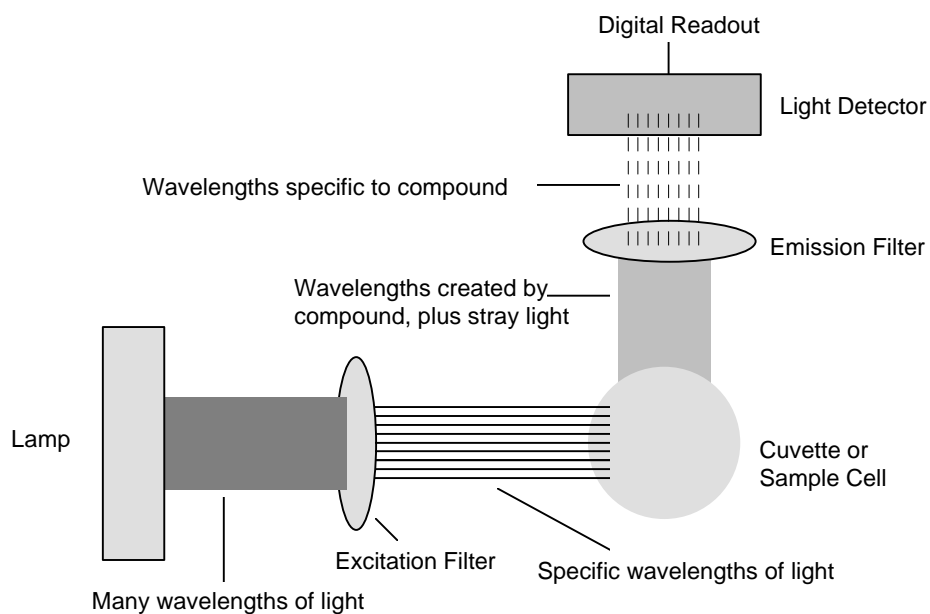


Figure 2: Principles of Filter Fluorometry

Lamp: The lamp or light source provides the light energy that "excites" the compound of interest. The lamp 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 2.

Excitation Filter: The excitation filter is used to screen out the wavelengths of light not specific to the compound you are measuring. This filter allows a smaller band of light energy to pass through and excite the sample solution.

Cuvette: The cuvette or sample cell contains the sample of interest. The cuvette material must allow the compound's absorption and emission light energy to pass through (for example, compounds that fluoresce below 300 nm require a quartz cuvette). The emission light energy will be emitted from the sample solution at a right angle to the incident light energy. The size of the sample cell will also effect your measurement. The greater the diameter of the cuvette, the lower the concentration that can be read.

Emission Filter: Stray light and any background material fluorescence will also be emitted from the sample solution. The emission filter is chosen to screen out these components allowing only wavelengths of light specific to the compound to pass through.

Light Detector: The TD-700 light detector is a photomultiplier tube. The light allowed to pass through the emission filter, (the wavelengths of light specific to the compound) are detected by the photomultiplier tube. The light intensity, which is directly proportional to the compounds' concentration, is then registered as a digital readout.

APPENDIX 12

Instructions for Using the Solid Standard with the TD-700 Fluorometer

1. Calibrate your instrument with a known standard as you usually do per sections VII - IX.
2. Remove calibration standard and any cuvette adaptors from instrument.
3. Facing the instrument, place the solid standard in the filter cylinder with the letter "L" (etched on top of standard holder) on your left hand side. Standards are marked with the letter "L" to designate low concentration and "H" to designate high concentration.
4. Press <*> to read value. Record this number on the label located at the bottom of the standard as the low concentration, "L" .
5. Now pull standard out of filter cylinder, rotate standard 180 degrees, and place in filter cylinder again, so that the letter "H" is on your left hand side.
6. Press <*> to read value. Record this number on the label located at the bottom of the standard as the high concentration, "H".
7. Remove solid standard and replace any cuvette adaptors that were removed in step 2.
8. Record instrument settings on TD-700 Calibration Record with Solid Standard sheet that has been included for future reference.
9. Proceed with analyzing your samples.
10. You may use your solid standard at any time to check the stability of the instrument. Simply remove any cuvette adaptors and insert your standard. Press <*> to read. The value should be similar to what was previously obtained in steps 4 and 6 above.
11. Also, you may use it to calibrate on future days, using the values obtained in steps 4 and 6 above. For example, if the values obtained for "L" and "H" were 50 and 100 respectively, you can use both to calibrate your instrument on another day. When calibrating, enter in "100" for your first standard concentration "H" and "50" for your second concentration "L": If calibrating with only one of the concentrations, enter in the corresponding value (50 or 100) depending on whether "L" or "H" is on your left side when facing the instrument.

TD-700 Calibration Record with Solid Standard

Date	Instr. S/N	Calibration Std Conc(s)	Units	Mode (Simple or Multi)	Cal Procedure (Raw or Direct)	Maximum Range	"L" Reading	"H" Reading