TD-360 Mini-Fluorometer

Operating Manual



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TD-360 Mini-Fluorometer Operating Manual

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I. Introduction

A. Description

The TD-360 Mini-Fluorometer is a variable-wavelength filter fluorometer designed for quick, easy, and accurate fluorescence measurements. When properly calibrated with a standard of known concentration, the TD-360 displays the actual concentration of the compound. The TD-360 can be configured to detect different compounds simply by changing the light source 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, next page):

- 1. Position the instrument on a flat surface with the back of the instrument well ventilated (at least three inches from the wall).
- 2. Open the sample chamber lid by pressing the black bar at the front of the unit.
- 3. The optical filters are installed in a black holder inside the sample chamber. The holder is keyed for installation in the correct orientation. To remove the holder, simply lift it straight up. To change filters, change to a new holder with filters installed.
- 4. The light source is either a UV Fluorescent Light Source or a Blue LED Light Source or Green LED Light Source. To verify that the correct light source is installed for your application, look at the bottom of the instrument and read the markings on the bottom of the light source.
- 5. Plug in the unit. <u>Be sure to use only the Turner Designs power supply provided with your instru-</u> <u>ment</u>. Electronic emissions testing has been performed with this power supply only.
- 6. Turn on the power switch (rear of unit) and allow the unit to warm up for the countdown period (600 seconds; 10 minutes).

C. General Precautions

Wipe up spills immediately. See Appendix 5.

Unplug instrument before changing the light source. See Section IV.

II. Hardware Overview

TD-360 Quick View Diagram





B. TD-360 Functions

- 1. **Display.** Shows the screens and readout of samples. It is lit continuously when the unit is operating.
- 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 also houses the combination filter holder and cuvette holder.
- 4. Filter Holder/Cuvette Adaptor. The Filter Holder holds the excitation and emission filters, and a 10 mm square cuvette, 100 μ l minicell holder, or 9 μ l microcell adaptor.

The holder is keyed for installation in the correct orientation. The holder can be removed by pulling it straight up.

To change to a different optical filter set, simply install a new *holder* with correct filters already installed. See Section III.

- 5. Sample Cuvettes. The TD-360 holds 10 mm square cuvettes. Cuvettes come in various volumes, made of various materials, depending upon your application. A $100 \ \mu$ l minicell holder or $9 \ \mu$ l microcell adaptor is available.
- 6. Light Source Module Access. The light source provides excitation light at wavelengths specific to the compound to be measured. The light source is encased in a module. To replace the light source, simply replace the module. See Section IV.
- 7. Lid Release Bar. Push to open sample chamber.
- 8. Power Switch. ON/OFF switch is located on the back panel of the instrument.
- 9. RS-232 Serial Port Connection. RS-232 (DB9 female from the TD-360) serial port for connecting to a computer or printer.
- **10.** Power Plug Socket. The power supply connects into the rear panel of the instrument.

ALWAYS UNPLUG THE UNIT BEFORE REMOVING AND INSTALLING A LIGHT SOURCE.



III. Optical Filter Holder Installation and Removal

The TD-360 Mini-Fluorometer must have two different optical filters to operate correctly: an excitation filter and an emission filter. TD-360 filters are 1/2-inch diameter, round, colored glass or highly-reflective glass. The filters are mounted in the Filter Holder.

To change from one filter set to another, simply change to a new Filter Holder with the correct filters already installed (refer to the Quick View Diagram).

To change the Filter Holder:

1. Turn off the power.

- 2. Open the lid to the Sample Chamber by pressing the black bar on the top front of the instrument. Remove any cuvette present.
- 3. Grasp the Filter Holder and pull it straight up.
- Locate the correct replacement Filter Holder (correct filters already installed in the holder) for your application (see the Optical Guide).
 NOTE: The UV light source does NOT have an emission filter.
- 5. Orient the Holder inside the Sample Chamber filter holder opening (Holder is keyed for correct installation). Slide the new Filter Holder into the opening, making sure it is seated at the bottom of the filter opening.

NOTE: After replacing the Filter Holder, you must always recalibrate.

AFTER REPLACING THE FILTER HOLDER, YOU MUST ALWAYS RECALIBRATE!

IV. Light Source Installation and Removal

The light source provides excitation light at wavelengths specific to the compound to be measured. The TD-360 light source is encased in a module, which makes removal and installation simple. The module also prevents the possibility of injury from UV light. The light source is a UV Fluorescent Light Source, a Blue LED Light Source, or a Green LED Light Source.

Checking the light source:

If the light source is not working, the instrument will not function properly. Check the light source if:

1. "ALARM" is flashing on the lower right-hand corner of the HOME screen. Press <ESC> to see:

Bad Lamp or Bad Ref. Circuit

This means that the light source is bad or that there is a problem with the reference circuit. Change to a new light source. If this does not solve the problem, contact Turner Designs.

2. It is possible after some years of use, that the light source may appear to be on, but still need changing. If the instrument readings are low, unstable, or drifting, try a new light source.

If this does not correct the problem <u>and</u> you have verified the accuracy of your standard, blank, and any preparation of samples, then contact Turner Designs.

To remove and install the light source:

- 1. Turn off and UNPLUG the instrument.
- 2. REMOVE CUVETTE from the instrument's Sample Chamber.
- 3. Close the Sample Chamber lid.
- 4. Turn the instrument over so the bottom is facing up.
- 5. Remove the module by loosening the two screws on either end of the module. Then pull the module straight up and out.
- 6. Locate the new light source to be installed (see the Optical Guide).

- 7. Orient it so the "notched" end containing the "circuitry" is toward the back panel of the instrument and will make contact with the instrument's circuitry (see figure below). Slide the module in. Tighten the two screws to hold it in place (DO NOT OVERTIGHTEN).
- 8. Turn on the power. The "ALARM" should disappear from the HOME screen. Also, make sure the fan is operating. If the fan is not operating, check the connector plug.

NOTE: After replacing a light source, you must always recalibrate.



V. Instrument Parameters (Software) for TD-360 with serial # < 36-0403

Instrument parameters are set through the TD-360 software interface. Software screens are called up using the keypad. To see how the software screens fit together, see the Screens Flow Chart, page 4. For details about alarms, warnings, and instrument diagnostics, see Appendix 3.

A. Power-up Screen

When the unit is first turned on, a TURNER DESIGNS screen appears showing the software version and date. A 600-second (10-minute) countdown begins. After 10 seconds, "Warming Up" replaces the software version. Once the 600-second countdown is complete, 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.

If you wish to bypass the countdown period, press <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. It also displays the User number (1 - 5) of the current calibration settings (see Section D below).

Press <ESC> to return to this screen from other screens. Press <•> and <6> to adjust the LCD contrast.

All instrument screens are accessed through the HOME screen.

C. DATA Screen

From the DATA screen, accessed from the HOME screen by pressing <DATA>, the user can select two modes of data output (when connected to a computer or printer).

Press <1> to select Disc (Discrete), when a single reading is desired. After an averaging period, a Sample # and a reading will be sent to a printer or computer when the lid is opened and closed or <GO> is pressed. For example:

Sample #	Concentration
1	183.4

Press <2> to select Cont (Continuous), when a data stream is desired. Output for "Cont" mode is once per second. HOME screen reading will change once per second. When connected to a printer or computer, a data point will be sent out automatically once per second. For example:

184.3 184.4 184.4 184.7

D. SAVE/RECALL Screens

This set of screens allows five different calibrations to be saved and recalled.

To access the SAVE/RECALL screen, from the HOME screen press <SAVE/RECALL>. To save the current calibration as User 1 - 5, press <1>. A screen will appear asking you to "Save as User." Enter a number 1 - 5 to identify the user. Press <ENT>.

To Recall a calibration setting for a specific user, press <SAVE/RECALL>. Press <2> and a screen will appear asking you to "Recall User." Enter the user number 1 - 5 you wish to recall. Press <ENT>. On the HOME screen, the User number entered will appear in the left-hand corner as "User X". The calibration settings recalled will be the calibration last saved under that user number.

E. MENU Screens

When MENU is pressed from the HOME screen, the index can be reset, units can be selected for display purposes, and diagnostic screens can be accessed.

- 1. Reset Index. Data is logged (in the discrete mode only) with an index number 1 999. To reset the index to 1, press <1>.
- 2. Units. This screen allows you to select a "unit" designation for display on the HOME screen. <u>The</u> readout accurately reflects concentration in units only when properly calibrated with a standard of known concentration.

Unit options: 1. μ g/ml; 2. ng/ml; 3. pg/ml; 4. none Default unit designation: none. Press <2>, then a number 1 - 4 to select the desired units for display.

 Diagnostics (Diag). Press <3> and the instrument will automatically cycle through three diagnostic screens (3 seconds per screen). Diagnostic screens report information about the current calibration settings.

See Appendix 3 for an explanation of the diagnostic screens.

V. Instrument Parameters (Software) for TD-360 with serial # ≥ 36-0403

Instrument parameters are set through the TD-360 software interface. Software screens are called up using the keypad. To see how the software screens fit together, see the Screens Flow Chart, page 4. For details about alarms, warnings, and instrument diagnostics, see Appendix 3.

A. Power-up Screen

When the unit is first turned on, a TURNER DESIGNS screen appears showing the software version and date. A 600-second (10-minute) countdown begins. After 10 seconds, "Warming Up" replaces the software version. Once the 600-second countdown is complete, 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.

If you wish to bypass the countdown period, press <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. It also displays the User number (1 - 5) of the current calibration settings (see Section D below).

Press <ESC> to return to this screen from other screens. Press <•> and <6> to adjust the LCD contrast.

All instrument screens are accessed through the HOME screen.

C. DATA Screen

From the DATA screen, accessed from the HOME screen by pressing <DATA>, the user can select two modes of data output (when connected to a computer or printer).

Press <1> to select Disc (Discrete), when a single reading is desired. After an averaging period, a Sample # and a reading will be sent to a printer or computer when the lid is closed and <GO> is pressed. For example:

Sample #	Concentration
1	183.4

Press <2> to select Cont (Continuous), when a data stream is desired. Output for "Cont" mode is once per second. HOME screen reading will change once per second. When connected to a printer or computer, a data point will be sent out automatically once per second. For example:

184.3 184.4 184.4

184.7

D. SAVE/RECALL Screens

This set of screens allows five different calibrations to be saved and recalled.

To access the SAVE/RECALL screen, from the HOME screen press <SAVE/RECALL>. To save the current calibration as User 1 - 5, press <1>. A screen will appear asking you to "Save as User." Enter a number 1 - 5 to identify the user. Press <ENT>.

To Recall a calibration setting for a specific user, press <SAVE/RECALL>. Press <2> and a screen will appear asking you to "Recall User." Enter the user number 1 - 5 you wish to recall. Press <ENT>. On the HOME screen, the User number entered will appear in the left-hand corner as "User X". The calibration settings recalled will be the calibration last saved under that user number.

E. MENU Screens

When MENU is pressed from the HOME screen, the index can be reset, units can be selected for display purposes, and diagnostic screens can be accessed.

- 1. Reset Index. Data is logged (in the discrete mode only) with an index number 1 999. To reset the index to 1, press <1>.
- 2. Units. This screen allows you to select a "unit" designation for display on the HOME screen. <u>The</u> readout accurately reflects concentration in units only when properly calibrated with a standard of known concentration.

Unit options: 1. μ g/ml; 2. ng/ml; 3. pg/ml; 4. none Default unit designation: none. Press <2>, then a number 1 - 4 to select the desired units for display.

 Diagnostics (Diag). Press <3> and the instrument will automatically cycle through three diagnostic screens (3 seconds per screen). Diagnostic screens report information about the current calibration settings.

See Appendix 3 for an explanation of the diagnostic screens.

VII. Calibration

A. Why Calibrate?

The TD-360 calibration procedure sets the sensitivity based on a chosen fluorescent standard or sample. If the standard is a known concentration, when properly calibrated, the TD-360 will display the actual concentration of samples read.

B. When to Calibrate

- Calibrate if the ambient temperature changes by +/- 5°C.
- Calibrate after changing light source, filter holders, or 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.

C. Run Blank (Zero the Instrument)

Press <ESC> to abort the blank run.

- 1. Prepare a blank solution (matrix solution that contains little or none of the analyte to be measured).
- 2. Fill a clean cuvette with the blank solution and wipe the outside of the cuvette dry.
- 3. Open the Sample Chamber lid and insert the cuvette. Close the lid.
- 4. Press <BLANK>. The screen will display "Reading Blank," with a rotating timer for 10 seconds as the instrument reads blank.
- 5. Screen will display "BLANK offset: X.X % FS" for five seconds.

Then it will display: "Save Blk Data? <1> Yes <9> No."

Press <1> or <9>. If you press <1> to save, Blank data will be saved under the current User number (1 - 5).

D. Run Standard

Always run a blank before running the standard.

- 1. Prepare a standard solution, a known concentration or dilution of the analyte to be measured.
- 2. Fill a clean cuvette with the standard solution and wipe the outside of the cuvette dry.
- 3. Open the Sample Chamber lid and insert the cuvette. Close the lid.
- 4. Press <CAL>. If you have not blanked in the last two minutes, the screen will display "Always Blank Before Cal".

- 5. Screen will prompt you to enter the concentration of the standard. Enter the actual or "relative" concentration of the standard and press <ENT>.
 - 6. Screen will display "Reading Standard," with a rotating timer for 10 seconds as the instrument reads the standard.
 - 7. Screen will display: "Save Cal. Data? <1> Yes <9> No."

Press <1> or <9>. If you press <1> to save, calibration data will be saved under the current User number (1 - 5).

Calibration data will print out automatically if connected to a printer or computer. (See Appendix 2 for details regarding the calibration data printout.)

E. To Print the Current Calibration Data

If the TD-360 is connected to a printer or computer, the current calibration data may be manually printed. (See Appendix 2 for details regarding the calibration data printout.)

From the HOME screen, press <MENU>, then <DATA>. The current calibration for the User number (1 - 5) displayed will be printed or sent to the computer.

F. To Abort the Calibration

To abort the calibration, press <ESC> at any time during the calibration sequence.

VIII. Reading Samples for TD-360 with serial # < 36-0403

1. Fill a clean cuvette with sample, wipe the outside dry, and insert it into the sample adaptor in the sample chamber. Close the lid. In the discrete mode, from the HOME screen, the unit will automatically take a reading whenever the lid is opened, then closed. You may also press <GO> to obtain a reading.

In the continuous mode, the unit will output a reading to the HOME screen once per second.

2. Record the sample reading.

You have two options for recording sample readings:

a. Recording to a Computer or Printer

If connected, readings will automatically be sent to a computer or printer. In the discrete (Disc) mode, a sample # and concentration will be sent after the averaging period when the lid is closed or <GO> is pressed.

In the continuous (Cont) mode, a reading will be sent out once per second.

b. Read Samples from Display

Your second option is to read samples directly from the display. Close the lid or press <GO>, wait while the reading is averaged (rotating "timer"), then record the reading.

3. Repeat steps 1 and 2 until all samples are read.

IX. Reading Samples for TD-360 with serial $\# \ge 36-0403$

1. Fill a clean cuvette with sample, wipe the outside dry, and insert it into the sample adaptor in the sample chamber. Close the lid. In the discrete mode, from the HOME screen, press <GO> to obtain a reading.

In the continuous mode, the unit will output a reading to the HOME screen once per second.

2. Record the sample reading.

You have two options for recording sample readings:

a. Recording to a Computer or Printer

If connected, readings will automatically be sent to a computer or printer. In the discrete (Disc) mode, a sample # and concentration will be sent after the averaging period when the lid is closed and <GO> is pressed.

In the continuous (Cont) mode, a reading will be sent out once per second.

b. Read Samples from Display

Your second option is to read samples directly from the display. Close the lid and press <GO>, wait while the reading is averaged (rotating "timer"), then record the reading.

3. Repeat steps 1 and 2 until all samples are read.

APPENDIX 1 Before Calibration or Reading Samples

A. Materials Needed

1. One or more 10 mm square cuvettes. The TD-360 holds a single 10 x 10 mm square cuvette, made of various materials, depending on the application. Please consult ordering information for the TD-360 (available from the manufacturer). The following sample cuvettes are available:

Part Number	<u>Material</u>	<u>Volume</u>
7000-955, -956, -957, -959	Glass, Quartz, Plastic	1 - 3.5 ml
3600-931 mini-cell	Glass	70 -250 µl
7000-952 microcell	Glass, Quartz	3 - 9 µl

Note: The glass and quartz cuvettes (7000-955 and 7000-956) have a larger outside dimension than the plastic cuvettes (7000-957 and 7000-959). As a result, the glass and quartz cuvettes fit more snugly in the sample compartment which results in better detection limits.

- 2. Blank. Sample matrix that does not contain the analyte of interest.
- 3. Standard(s). At least one solution of known concentration of the analyte of interest is needed.
- 4. Samples to be read.
- 5. Application notes describing preparation of blank, standards, and samples are available for many protocols. Contact Turner Designs.

B. When Handling Samples

- 1. Take care not to spill samples into the sample chamber. Wipe up any spills promptly. See Appendix 5, Section A, for details on maintenance.
- 2. The TD-360 is very sensitive and even small amounts of material from a previous sample may result in errors. Use a clean cuvette for all readings. Thorough and proper cleaning of cuvettes between sample readings is essential, and is especially important if you are using the same cuvette for samples and blank.
- 3. Fill the cuvette at least 50% full; significant error will result if it is not filled enough.
- 4. The cuvette MUST BE DRY on the outside when taking readings. Moisture (condensation) on the outside will result in error.
- 5. Minute bubbles in samples will cause drifting readings. Take care not to introduce bubbles into samples. Slight tapping with your finger on the outside cuvette wall will often help dissipate bubbles.

C. Linear Range and Quenching

The linear range is the concentration range in which the readout of the TD-360 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 multi-point calibration curve is necessary for accurate readings. At even higher concentrations, readings will begin to decrease even though the sample concentrations are increasing. This effect is known as "concentration quenching".

Linearity may be checked by diluting a sample 1:1 or some other convenient ratio (be sure to use your reagent blank for the dilutions). 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.

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. NOTE: The unit's light source will heat samples if left in the instrument. Thus, it is recommended that readings for all samples be taken after they have been in the instrument for the same period of time.

E. Positioning Samples

For low concentration samples, 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 cuvette that are not visible to the human eye. We recommend that the cuvette be marked at the top (use a non-fluorescent material for marking) and positioned in the adaptor the same way each time to minimize error.

F. Data Quality

The TD-360 is only as accurate as the standards that are used to calibrate it. This is why it is important to take care when preparing standards, samples, and blank. One should follow good laboratory practices when preparing all solutions and reagents.

APPENDIX 3 Using the TD-360 with a Printer or Computer for TD-360 with serial # < 36-0403

A. Printout Capability with a Printer or Computer

To use with a printer:

- 1. Connect the TD-360 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. After calibration, calibration data will printout automatically.
- 3. To print out calibration data manually, press <MENU> from the HOME screen, then <DATA>.
- 4. When reading samples in the discrete (Disc) mode, after an averaging period, a Sample # and a reading will be sent to a printer when the lid is opened and closed from the HOME screen <u>or</u> <GO> is pressed. For example (numbers are for example only):

Sample #	Concentration
1	183.4
2	182.7
3	184.1
4	183.8

- 5. In the continuous (Cont) mode, a data point will be sent out automatically once per second. For example (numbers are for example only):
 - 184.3 184.4 184.4
 - 184.7

To use with a computer:

- 1. Connect the TD-360 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-360 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 WindowsTM terminal or a Macintosh computer, see Appendix 4.
- 3. After calibration, calibration data will printout automatically.
- 4. To send calibration data manually, press <MENU> from the HOME screen, then <DATA>.

5. When reading samples in the discrete (Disc) mode, after an averaging period, a Sample # and a reading will be sent to a printer when the lid is opened and closed from the HOME screen <u>or</u> <GO> is pressed. For example (numbers are for example only):

Sample #	Concentration
1	183.4
2	182.7
3	184.1
4	183.8

6. In the continuous (Cont) mode, a data point will be sent out automatically once per second. For example (numbers are for example only):

184.3 184.4 184.4 184.7

B. Calibration Printout

(Example only! Your numbers will vary.)

Calibration data is sent out automatically after calibration. To print out manually, press <MENU> from the HOME screen, then <DATA>. The current User calibration data will print out:

Turner Designs: TD360 Fluorometer Ver. T3-1A 08/97

Units: none Output: Discrete Calibration Stored as : User #1 Cal. Standard Concentration: 500.0 Blank Offset %: 1.1 Max. Readable Concentration: 4078

The two lines display instrument name and software version.

The next four lines of the printout display the TD-360's current parameters: the units chosen (see MENU screen), the data output chosen (discrete or continuous), the User number for the current calibration, and the concentration of the standard as entered during calibration.

Blank Offset % is the fluorescence intensity of the blank as compared to the maximum readable concentration, expressed as a percentage. The lower the number, the better the detection limits the user can expect. Any number below 5.0% is acceptable.

Max. Readable Concentration is the highest concentration that the instrument can read as currently calibrated (taking into account the concentration of the standard and fluorescence intensity of the blank).

NOTE: Blank offset %, Cal Standard Concentration, and maximum readable concentration can be viewed by pressing <MENU> from the HOME screen, then <3> Diag.

APPENDIX 3 Using the TD-360 with a Printer or Computer for TD-360 with serial # > 36-0403

A. Printout Capability with a Printer or Computer

To use with a printer:

- 1. Connect the TD-360 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. After calibration, calibration data will printout automatically.
- 3. To print out calibration data manually, press <MENU> from the HOME screen, then <DATA>.
- 4. When reading samples in the discrete (Disc) mode, after an averaging period, a Sample # and a reading will be sent to a printer when the lid is closed from the HOME screen and <GO> is pressed. For example (numbers are for example only):

Sample #	Concentration
1	183.4
2	182.7
3	184.1
4	183.8

- 5. In the continuous (Cont) mode, a data point will be sent out automatically once per second. For example (numbers are for example only):
 - 184.3
 - 184.4
 - 184.4
 - 184.7

To use with a computer:

- 1. Connect the TD-360 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-360 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 WindowsTM terminal or a Macintosh computer, see Appendix 4.
- 3. After calibration, calibration data will printout automatically.
- 4. To send calibration data manually, press <MENU> from the HOME screen, then <DATA>.

5. When reading samples in the discrete (Disc) mode, after an averaging period, a Sample # and a reading will be sent to a printer when the lid is closed from the HOME screen and <GO> is pressed. For example (numbers are for example only):

Sample #	Concentration
1	183.4
2	182.7
3	184.1
4	183.8

6. In the continuous (Cont) mode, a data point will be sent out automatically once per second. For example (numbers are for example only):

184.3 184.4 184.4 184.7

B. Calibration Printout

(Example only! Your numbers will vary.)

Calibration data is sent out automatically after calibration. To print out manually, press <MENU> from the HOME screen, then <DATA>. The current User calibration data will print out:

Turner Designs: TD360 Fluorometer Ver. T3-1A 08/97

Units: none Output: Discrete Calibration Stored as : User #1 Cal. Standard Concentration: 500.0 Blank Offset %: 1.1 Max. Readable Concentration: 4078

The two lines display instrument name and software version.

The next four lines of the printout display the TD-360's current parameters: the units chosen (see MENU screen), the data output chosen (discrete or continuous), the User number for the current calibration, and the concentration of the standard as entered during calibration.

Blank Offset % is the fluorescence intensity of the blank as compared to the maximum readable concentration, expressed as a percentage. The lower the number, the better the detection limits the user can expect. Any number below 5.0% is acceptable.

Max. Readable Concentration is the highest concentration that the instrument can read as currently calibrated (taking into account the concentration of the standard and fluorescence intensity of the blank).

NOTE: Blank offset %, Cal Standard Concentration, and maximum readable concentration can be viewed by pressing <MENU> from the HOME screen, then <3> Diag.

APPENDIX 4 Alarms, Diagnostics, and Error Messages

A. Alarm Screen

There is an alarm to warn you of problems with the light source or reference circuitry. If the alarm is active, "ALARM" will appear on the lower right hand corner of the HOME screen. Press <ESC> and see:

Bad	Lamp or
Bad	Ref. Circuit

This means that the light source is bad or that there is a problem with the reference circuitry. Change to a new light source. If this does not cure the problem, contact Turner Designs.

B. Diagnostic Screens

There are diagnostic screens which help in troubleshooting and verifying a valid calibration. To access these screens, press <MENU> from the HOME screen, then <3> to see the diagnostic screens. The unit will automatically (3-second interval) cycle through three screens:

Oper Hrs: XX XXX.X (units) XXX.X (units)
--

Definitions:

Blank %FS (full scale) is the fluorescence intensity of the blank as compared to the maximum readable concentration (full scale), expressed as a percentage. The lower the number, the better the detection limits the user can expect. Any number below 5.0% is acceptable.

Oper Hrs is the amount of hours the instrument has been in operation since purchase.

Cal Std Conc is the concentration of the standard as entered during calibration. The units selected under <MENU>, item #2 will also be displayed. NOTE: Units are for display only. They reflect actual units only when the instrument has been calibrated with a standard of known concentration of those units.

Max. Readable Concentration is the highest concentration that the instrument can read as currently calibrated (taking into account the concentration of the standard and fluorescence intensity of the blank).

C. Error Message Screens

The TD-360 will display an error message if incorrect values are entered or if the readings may result in an inadequate calibration.

1. 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 9999 for the Cal Std, you will see "Invalid Input."

2. Standard < Blank (Std<Blank)

During Calibration, this indicates that fluorescence reading for the standard is lower than the reading for the Blank solution. Most likely, this results from using the wrong standard (or dilution) and/or Blank solution. Press <ESC> to abort the calibration; check the standard and Blank solutions; calibrate again using a standard that is more concentrated than the Blank. Rerun the Blank if you suspect the Blank solution was contaminated.

3. Blank Too High

During Blank run, reading for Blank is too high for the instrument to zero. Most likely this error is a result of using the wrong Blank solution. Press <ESC> to abort. To remedy, use the correct Blank solution. Rerun Blank.

- 4. HOME Screen Displays OVER or Negative Numbers
 - a. 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.

If you are reading samples of high concentration, you can dilute them until you get an on-scale reading. Or, if you cannot dilute them, calibrate the unit again using a higher concentration standard.

If the problem continues, verify that the proper filters and light source are installed; that the filters and o-rings are properly seated in the holder; and that the holder is properly seated in the sample compartment. Examine the filters for damage: freckling of mirrored surfaces, cracks, etc.

b. Negative (-) numbers

If the HOME screen is displaying a negative number, it means that the sample is reading less concentrated than the Blank as set during the last blank run. This might occur in a case where you calibrated with a high Blank or with a contaminated Blank. To correct, recalibrate after verifying that Blank solution is uncontaminated and properly prepared.

If it is the Blank that reads negative (but still close to zero), it is probably simply that the temperature of the Blank has increased. Allow Blank to return to temperature during Blank run.

APPENDIX 5 Data Collection Using a PC or Macintosh Computer

You can use various communications software programs to import data to your computer. The signal is sent from the TD-360 in ASCII format. You may use the Turner Designs Data Collection Software (P/N 10-AU-4000), should you require a DOS-based program. Complete instructions are included with the software.

A. To use Microsoft Windows[™] Terminal to import data from the TD-360:

- Connect the 9-pin connector that extends from the TD-360 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. Run your Microsoft WindowsTM program. Once in Windows, locate the Terminal program in the accessories directory and double-click on the icon to open it.
- 3. Click on the "Setting" option in the menu bar and select "Communications" option to configure your COM port to accept the input from the TD-360.
- 4. Set the "Connector" to COM1 or COM2 (depending on your computer). Set the "Baud Rate" to 9600. The other options should be set correctly. However, you should check that the "Data Bits" is set to 8, the "Parity" is set to None, the "Flow Control" is set to Xon/Xoff, and the "Stop Bits" is set to 1. Click on "OK". If the correct COM port is selected, there will be no error message given by Windows. If it is not, the message will be, "Terminal Error The selected COM port is either not supported or is being used by another device. Select another port." If this message is given, the TD-360 is not connected to the correct COM port, or there is a conflict with another device such as a mouse. Resolve this issue before continuing. If there is no message after clicking on "OK", you are connected to the TD-360.
- 5. Save this configuration using the "Save" command under the File menu. Use a file name that you will remember such as TD360.TRM
- 6. Create a file that you will send the data to by selecting "Receive Text File" under the Transfers menu. Give the file a name.
- Operate the TD-360. When you press <D> or <*> (Discrete Sample Averaging) from the HOME screen, you should notice data appearing on the computer screen. This data is being sent to the file you have created.
- 8. When you are finished operating the TD-360, select the "Stop" command under the Transfers menu, save your file (now has data), and exit the Terminal software.
- 9. To manipulate, plot or review your data, open ExcelTM, WordTM, etc., and open the file you created in step 6.

B. Data acquisition using Windows '95

The TD-360 outputs RS-232 serial ASCII data, which can be captured through the serial port of any computer with Windows. In particular, data can be logged using the HyperTerminal program that is standard with any Windows '95[™] software.

- 1. Open HyperTerminal.
- 2. Make a New Connection.
- 3. Give the New Connection a name.
- Choose the COM Port that the instrument is connected to. (This can be the hardest part of the procedure. Different computer manufacturers use different conventions. On most computers, it is either COM 1 or COM 2.)
- 5. Set the following parameters : Baud Rate: 9600 Data Bits: 8 Parity: none Stop Bits: 1 Flow Control: Xon/Xoff
- 6. Save the Connection.
- 7. To store the data to a file, choose the Transfer menu and select Capture. Give the captured file a name.
- 8. Acquire the data.
- 9. Stop the Capture when you are finished by selecting the Transfer menu and then by selecting Capture/ Stop.
- 10. Access and manipulate the data using ExcelTM, WordTM, or other programs.

C. To use a Macintosh computer to import data from the TD-360:

The serial data output function on Turner Designs' instruments is fully compatible with a Macintosh computer. Turner Designs instruments send out an ASCII signal, which is readable by most standard Macintoshcompatible programs.

You will need the appropriate cables and adapter to establish the connection. If you do not already have the required cables, Turner Designs offers a Macintosh Cable Kit (P/N 7000-940).

Refer to Turner Designs Operating Manual for instructions about data output settings on instrument.

Data Capture Using a Macintosh Desktop Computer

- Connect your computer to the RS-232 port on the instrument with the cables included in the kit. Computer ⇒ round 8 pin-25 pin cable ⇒ 25 pin/9 pin adapter ⇒ RS-232 cable (came with instrument) ⇒ instrument.
- 2. On your computer, open the communications program you have chosen to use (Z modem, Microphone II, Clarisworks, any terminal program, etc.). For details on operating your communications program, refer to your program manual.

NOTE: If there is no appropriate communications software on your computer you can download Z-modem from most shareware sites on the Internet.

- 3. Access apple menu \Rightarrow control panels \Rightarrow communications
- 4. Once in communications, set baud rate = 9600, data bits = 8, stop bits = 1.
- 5. When in the discrete (Disc) mode, data will be sent automatically after the lid is closed or <GO> is pressed. Press <DATA>, then <1> to select Disc.

Data transfer will begin automatically, once per second, if in the continuous (Cont) mode. Press <DATA>, then <2> to select Cont.

6. When data transfer is complete, either transfer data to a spreadsheet program or use cut and paste functions.

Data Capture Using a Macintosh Laptop Computer

Because laptop computers have only one serial port, establishing a connection with a Turner Designs instrument can be a more complex process compared to use of a desktop. However, once the connection has been established and setup noted, the process is identical to the desktop procedure.

When starting computer, hold down the "Shift" key to turn off all extensions. This will free up any programs that could be occupying the serial port. However, some of the extensions may be needed for data transfer and therefore each laptop user must go through a process of trial and error to find the essential extensions. (NOTE: Every computer varies in extensions and therefore must be handled on a case by case basis)

Once the computer is booted up with extensions off, follow desktop instructions 1-5.

If no data appears in window:

- 1. Access apple menu \Rightarrow control panels \Rightarrow extensions manager
- 2. Once in extensions manager, you must check various extensions that may affect the serial port connection and restart computer with chosen extensions to determine if data transfer is initiated (examples: apple share, apple modem, serial arbitrator, and other serial devices). NOTE: When you restart computer DO NOT hold down shift key, so chosen extensions will be activated.

NOTE: Examples of extensions that will not affect serial port connections include; quick time, apple guide, printers, etc.). Also, the apple talk program must be disabled to establish a connection. To do this, apple menu \Rightarrow chooser \Rightarrow apple talk \Rightarrow disable.

3. Once data transfer is established, save settings or note which extensions are on/off for ease in future data collection.

APPENDIX 6 Maintenance, Warranty, & Service

A. Maintenance

If possible, do not spill liquids into the sample chamber. If there is a spill:

- 1. Unplug the instrument.
- 2. Remove the cuvette and filter holder 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-360.

B. Warranty

Turner Designs warrants the TD-360 Mini-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-360 Mini-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 light sources, 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.

YOU MUST INCLUDE AN RMA NUMBER ON ALL EQUIPMENT RETURNS.

3. If proper performance is not obtained, YOU MUST OBTAIN AN RMA number BE-FORE shipping the instrument to Turner Designs. After obtaining an RMA number, pack the instrument well; insure it; write the RMA # on the outside of the carton and ship it to Turner Designs prepaid. The instrument will be repaired and returned free of charge for all customers in the United States. We will pay for return shipment and include a check to reimburse you for the cost of surface shipment to us.

If you purchased directly from Turner Designs outside of the United States, contact Turner Designs. We will repair the instrument at no charge. We cannot, however, pay shipping, duties, or documentation costs outside the continental United States.

For customers outside of the United States, who have purchased our equipment from one of our authorized distributors, contact the distributor.

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. Our service department is happy to assist you by phone or correspondence 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. Your bill will include any shipment freight charges.

Address for Shipment:

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

APPENDIX 7 Specifications

- Sensitivity: 500 picograms/mL using PicoGreen and 5 nanograms/mL using Hoechst 33258 in a standard square cuvette.
- Optical Filter Holder: Accommodates one pair of 1/2" round optical filters.
- Detector: Factory installed photodiode. Wavelength range: 300-1100 nm
- Light Source: Low Pressure Mercury Vapor Lamp. Wavelength range 350-400 nm, peak 365 nm.
 - Optional: Blue LED peak wavelength 460 nm. Green LED peak wavelength 525 nm.
- Sample Compartment: Accommodates 10 x 10 x 45 mm square cuvettes. Optional 100 μ l and 9 μ l microcell.
- Readout: Direct Concentration
- Calibration: Single-Point Calibration
- Blank: Reads and subtracts Blank.
- Discrete Sample Averaging: Sample readings are averaged to improve accuracy.
- Kinetics Software: Outputs once every second. User toggles feature on or off.
- Data Output: 100% ASCII format through a 9-pin RS-232 serial cable at 9600 baud.
- Operating Temperature: 60 95°F; 15 36°C.
- Weight: 2.8 kg (6.1 lb.)
- Dimensions: 15.2 cm W x 31.5 cm D x 9.9 cm H (6.0" W x 12.4" D x 3.9"H)
- Power: 12 Volt External Power Supply, 100-240 VAC, ±10% of the nominal voltage, 50/60 Hz, Max. 25 Watts.
- Warranty: One-year warranty.
- Approvals: CE
- Environmental Conditions: Indoor use only. Altitude specification 0-2000 m. Transient over voltages according to INSTALLATION CATEGORY II. Pollution degree 1 and 2 in accordance with IEC664.
- Maximum Relative Humidity: 75% for temperatures up to 31°C, decreasing linearity to 50% relative humidity at 40°C.

APPENDIX 8 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.



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 the figure.

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-360 light detector is a photodiode. The light allowed to pass through the emission filter (the wavelengths of light specific to the compound) are detected by the photodiode. The light intensity, which is directly proportional to the compounds' concentration, is then registered as a digital readout.