The design and manufacture of water quality and research instruments

MODEL 10 FLUOROMETER USER'S MANUAL

OCTOBER 1990

Part Number 10-070

TURNER DESIGNS
920 W. Maude Avenue • Sunnyvale, CA 94086
(408) 749-0994 • FAX (408) 749-0998

Model 10 Series Fluorometer User's Manual

TABLE OF CONTENTS

Section One

INTRODUCTION

1.1 GETTING STARTED

Congratulations on the purchase of your new fluorometer! With proper care, it should give you years, possibly decades, of reliable service. If, at any time, you need help with your instrument, just call us. We're here to help you.

Please fill out and return the warranty sheet at the beginning of this manual. Timely return of this information will ensure that you receive prompt notice of improvements and other pertinent details.

How to Use This Manual. We suggest you review Sections One through Four of this manual before adjusting any controls. You will not damage the instrument by adjusting the controls, but it is probable that the controls are set pretty much where you will want them. If you readjust before reading the instructions, you may incur unnecessary frustration. These Sections present all the information you need to get up and running.

If you are familiar with fluorescence studies and wish to begin immediate operation, Sections 3.3 and 3.4 contain instructions for calibration and operation of the instrument.

Supplementary information is found in the appendices. Take a moment right now to glance through the appendices, so you will know where to turn for specific information.

1.2 DO YOU HAVE EVERYTHING?

Make sure you have everything you ordered by checking your shipment against the packing list. In the unlikely event something is missing, call us at once so we can help resolve the discrepancy.

You should have received:

- Turner Designs Model 10 Series Fluorometer
- Model 10 Series Fluorometer User's Manual (this manual)

- A power cord. One end has a three-prong plug for plugging into an AC power source or alligator clips for DC attachment, and the other end has a screw-in connector for attachment to the fluorometer's power/telemetry connector.
- A box of fuses, marked 3AG 3 AMP SLO-BLO
- A box of fuses, marked 3AB 1/2 AMP SLO-BLO

Unless you said you did not want the High-Volume-Continuous-Flow Cuvette System, there are also:

- A short piece of precision bore glass tubing -- your spare cuvette.
- An Allen wrench (to tighten sample fittings)
- A bag with four O-rings in some extra lubricant.
- A jar with ten packets of desiccant. Keep the jar sealed until needed. (Refer to Appendix 6.4.5.)

If you ordered anything else, it is in the smaller box. Instructions for any accessories ordered are included with the accessory.

For your convenience, an Information Sheet (on the following page) has been provided so you can document your fluorometer and the accessories ordered. By completing it, your manual will become specific to your fluorometer, making reorder and troubleshooting easier. Details about your instrument's set-up (it lists things installed by Turner Designs before shipment) can be found in the Instrument Set-Up Form in the front of this manual.

Turner Designs MODEL 10 FLUOROMETER

INFORMATION SHEET

FLUOROMETER MODEL #10-_____ S/N: _____

PURCHASED ON INVOICE #_____ DATED: _____

USER:_____

POWER SOURCE: ___115 Volt AC ___230 Volt AC ___12 Volt DC

PURCHASED POWER CABLE #10-_____

LOCATION OF INSTRUMENT (i.e., Lab, Field, Building #, Site, etc.)

Accessories Purchased with the Instrument: Installed on/in Instrument?

10-_____ _____ _____
10-_____ _____ _____
10-_____ _____ _____
10-_____ _____ _____
10-_____ _____ _____
10-_____ _____ _____
10-_____ _____ _____
10-_____ _____ _____

Accessories Purchased After Initial Instrument Purchase:

Date

10-_____ _____ _____
10-_____ _____ _____
10-_____ _____ _____
10-_____ _____ _____
10-_____ _____ _____
10-_____ _____ _____
10-_____ _____ _____
10-_____ _____ _____

Section Two

FLUOROMETER CONTROLS & OPERATING CONDITIONS

2.1 FLUOROMETER CONTROLS & INDICATORS

(See Appendix 6.1 for a discussion of operating principles.)

The Model 10 Fluorometer has been designed so that no internal controls will require setting during normal operation of the instrument. All operating controls are on the front panel. (Refer to Figure 1.) Note their locations, but do not make any adjustments just yet.

No matter what combination of control settings you choose, it is impossible to damage the instrument.

NOTE 1: If you remove the cover (14 screws) from the sample compartment, first be sure the power is off, because **UV LIGHT FROM THE LAMP WILL CAUSE DAMAGE TO YOUR EYES EVEN THOUGH NOTHING WILL BE FELT IMMEDIATELY. If the lamp is viewed without eye protection, it will cause a sunburn to the eyes, which will be experienced as itching several hours later.**

NOTE 2: If the instrument has been used before, you may wish to preserve calibration. This can be done by noting the position of the lamp before removal. (See Appendix 6.7.1.)

The operating controls and their functions are:

#1 **Sensitivity Knob.** This knob, the Sensitivity Fine Adjustment, and the Sensitivity Knob Lock Screw are located on the lower right side of the sample compartment casting. (See Figure 1.) The Sensitivity Knob sets the basic sensitivity of the instrument to both sample and blank. The knob is a coarse adjustment; the Fine Adjustment can be used for precise adjustment, but normally is used only during tuneup of the range multipliers. (See Section 3.5.2.)

Turning the knob counterclockwise decreases the sensitivity of the instrument; turning it clockwise increases sensitivity. Note that the knob can only be turned about 90 degrees. It is a very responsive

adjustment. However, it is not necessary to do fine adjustment with it. This is normally done with the Span Control, described later.

#2 **X1-X100 Switch.** This switch is located on the upper right side of the sample compartment casting. Its positions are indicated on the front panel. (See Figure 1.) By flipping this switch, the sensitivity is changed 100-fold. When in the X1 position, the sensitivity of the instrument is indicated by the range light lit; when in the X100 position, the sensitivity is 100 times what is indicated by the range light lit.

Blank suppression is not affected by turning this switch.

#3 **Ranges.** There are four ranges that, in conjunction with the X1-X100 Switch, determine the sensitivity of the instrument. By convention, like the X1-X100 Switch, these ranges are <u>sensitivity</u> multipliers with the values: Min Sens, X3.16, X10, and X31.6. (Thus, Min Sens is the least sensitive range but is useful for reading high concentrations of the analyte being measured. X31.6 is the most sensitive range and is useful for reading low concentrations of the analyte of interest.)

When a range light is lit, it indicates that the instrument is operating in that range.

The range multipliers are adjusted at the factory for your main application. If you change applications, the multipliers will no longer be exactly the stated value. Internal adjustments need to be made, or a correction factor determined. (See Section 3.5.2.)

#4 **Step (Range) Switch.** If you depress the Step Switch there will be an audible "click" and the instrument will change ranges. The current range is indicated by the range light that is lit.

If you depress and hold the STEP button, it will continue to advance until you release it. There are <u>four</u> positions that change only in one direction. Thus, if a reading goes off-scale (and it is just one range too far), holding the button down until you hear <u>four</u> clicks will be the fastest way to get where you wish to go.

The Auto-Man Switch, described below, also controls ranging.

#5 **Auto-Man (Range) Switch.** Autoranging is an internal function of the instrument that helps keep the

instrument on the optimum range without operator intervention.

In the Man position this "range" change can occur only by depressing the Step Switch, described above.

The Step Switch is operational when the Auto-Man Switch is in the Auto position. It is sometimes desirable to override the rather slow (four seconds per step) autoranging. For example, if the instrument goes off-scale on the X31.6 range, the likelihood is that it will wind up on the X10 range. To get there, it will (nearly instantly) change to MIN, then require four seconds each to go to X3.16 and to X10. The manual override speeds this up.

Don't be surprised if it then keeps going on its own. If the Step Switch is depressed when the Auto-Man Switch is in Auto, the range change logic will respond to range change commands from both the autorange logic and the Step Switch. For instance, you might depress the Step Switch, causing one range change, then release the Step Switch and discover the instrument changes ranges again. This would happen if the manual step command caused the instrument to go off-scale or to yield a reading less than 20% of full scale. The autorange logic would sense this and cause additional range changes.

You may switch from Auto to Man (or the reverse) any time you desire. It does not affect any other operation of the instrument, nor does it affect the reading.

#6 **Blank Control.** This control is used to compensate for the residual or "blank" fluorescence, corresponding to zero concentration of the unknown.

Turning this control clockwise decreases the reading (suppresses blank); turning it counterclockwise increases the reading.

Blank Suppression. When blank is set to zero on one range, blank remains at zero at other ranges. Read the section on blank adjustment carefully (near the end of the Introduction in Section 3.3 (Calibration Instructions). Sometimes the Blank Control does not appear to be doing anything. It is, but sometimes you can't see it.

The Model 10 Rack Mount Fluorometer has a ten-turn dial graduated from 0 to 1000, with a locking mechanism. Up (counterclockwise) is free-running; down (clockwise) is locked.

On the Model 10 Field Fluorometer, a ten-turn uncalibrated dial with a locking ring is provided. A waterproof graduated dial is simply not available.

#7 **Span Control.** This control is used for fine adjustment of readings of the instrument over a range of 4:1.

Turning this control counterclockwise decreases the reading; turning it clockwise increases the reading.

The Span Control may be adjusted without affecting the setting of the Blank Control, bearing in mind that if you start with the Span Control full counterclockwise and turn it full clockwise, you have multiplied any error (in setting blank to zero) by a factor of four. It is better to have the Span Control set where you will use it, or to turn it counterclockwise after setting the Blank Control.

The dial and lock used are identical to those on the Blank Control.

#8 **On-Off Switch.** This switch turns the instrument on and off. It is not necessary to turn this off prior to unplugging the instrument.

#9 **Meter Reading.** Shows the reading of the blank, the standard, or the sample under study. These readings are not temperature corrected.

The reading is proportional to the light reaching the photomultiplier tube. Up to a concentration that is dependent on the compound and the path length (typically a concentration of about 0.1 ppm with a path length of 1 cm), the reading is proportional to the concentration of the unknown sample.

#10 **Power/Telemetry Connector.** This connector contains both the power input pins and the telemetry (recorder) output pins. The correct cable (available from Turner Designs) should be used to ensure proper connection. Severe damage to your instrument may result if the power and telemetry pins are connected improperly. (See Section 2.2.1.)

#11 **Sample Compartment.** Contains the High-Volume, Continuous-Flow Cuvette System (or cuvette holder for individual samples). Note the upper Exhaust Fitting and the lower Intake Fitting of the flow system. (Figure 1.)

NOTE: When the Cuvette Adaptor is used (for test tube sample measurement), there are no Intake or Exhaust Fittings, and a Light Shield must cover the top opening in the Sample Compartment. (See #13.)

#12 **Fuses.** The lower 1/2 amp slo-blo fuse is for DC operation and the upper 3 amp slo-blo fuse is for AC operation. Fuses can be changed by pushing in and turning counterclockwise. The instrument should be unplugged from any power source while changing a fuse.

#13 **Light Shield.** When measuring test tube samples, the Cuvette Adaptor replaces the Continuous-Flow Cuvette System, and a Light Shield must be used. The Light Shield is a black cap that fits over the top opening in the Sample Compartment and over any test tube sample. It prevents external light from falling on the light detector. It should be kept in place even when the instrument is not in operation to prevent dirt and moisture from entering the Sample Compartment.

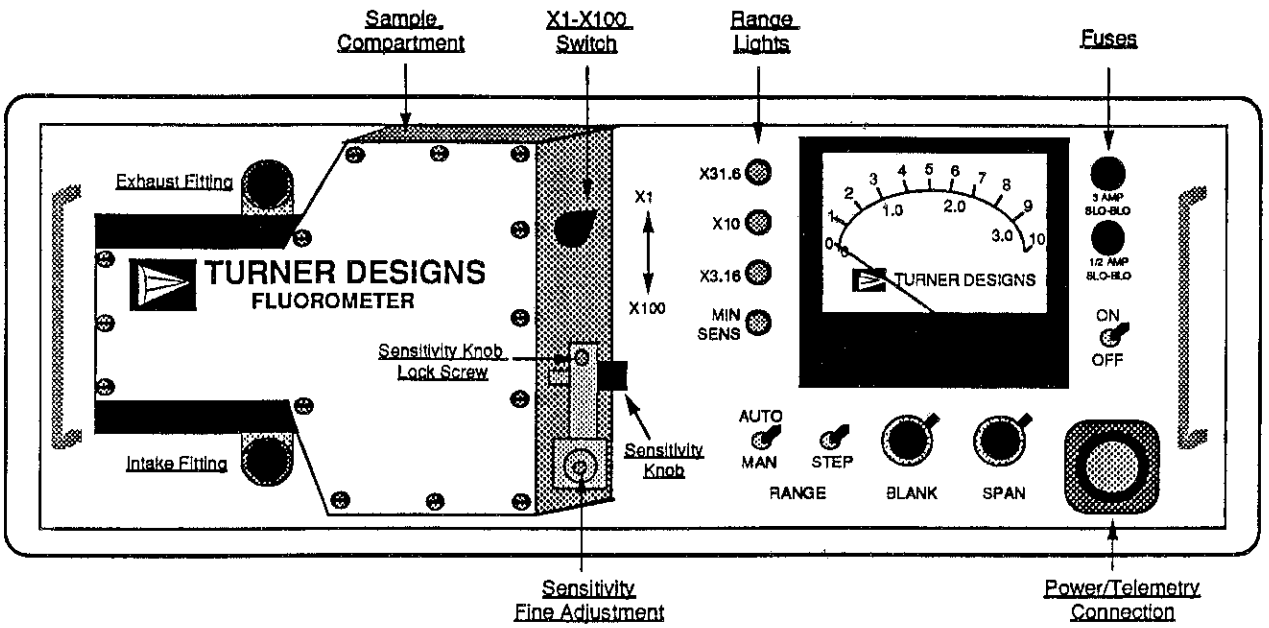

Figure 1. Model 10 Fluorometer Controls & Indicators (shown with Continuous-Flow Cuvette)

2.2 OPERATING CONDITIONS & CONSIDERATIONS

1. Power Requirements

Before operating, check your power requirements:

___ 115 volt AC (standard unless 230 V is specified). Cable with screw-in connection for attachment to fluorometer and 3-prong plug is supplied.

The operating range is 105 to 130 volts, 50 to 400 Hz. Virtually any generator, as well as normal household current, will supply power. The instrument is protected against transient spikes, and prolonged operation (several minutes) at 140 volts will cause no damage. Current drain is about .3 amperes

___ 230 volt AC

Analogous to 115 volts, the operating range is 210 to 260 volts, 50 to 400 Hz. Current drain is about .15 amperes.

___ 12 volt DC (battery). A DC power cable can be purchased from Turner Designs.

The DC operating range is 11 to 16 volts. Current drain is about 2.0 amperes, independent of voltage.

All instruments, except for certain ones manufactured for use within another system, will operate on DC as well as AC power.

To supply power, simply screw the appropriate power cable onto the power/telemetry connector at the front of the instrument (see Figure 1), and attach it to the desired power source.

The power/telemetry connector contains both power input pins and telemetry (recorder) output pins. If you intend to use the recording capabilities, ask us for an AC or DC cable wired for telemetry. (See Appendix 6.10.)

CAUTION: If you do not use a Turner Designs cable, use caution. Improper connection of power and telemetry pins may result in severe damage to your instrument.

The negative DC power line is grounded to the instrument case. Preferably, the instrument and all telemetry outputs should be isolated from the DC source. If this is not possible, ground to the negative side of the DC power source only.

To change from 115 volt AC operation to 230 volt AC operation (or vice versa) or from AC to DC, simply purchase the appropriately wired power cable from Turner Designs.

2. Telemetry Connections

(See Appendix 6.10 for details.)

The Model 10 Fluorometer is designed to allow output of information to a recording device. It is commonly used with a strip chart recorder, data logger, or computer (with an A/D converter board).

If you intend to use the recording capabilities, ask us for an AC or DC power cable wired for telemetry.

Please read the cautionary notes in Section 2.2.1 above.

3. Environmental Considerations

(See Appendix 6.5.)

The Model 10 Fluorometer is designed to operate under a wide variety of environmental conditions.

Temperature. Storage temperature is -20 degrees C to +60 degrees. Operating temperature is from the freezing point of the fluid in the cuvette (but not less than -20 degrees C ambient) to +50 degrees C ambient.

Water and Dirt. The Model 10 Rack Mount Fluorometer is NOT protected against water. All moving parts are shielded against dirt, dust, etc., by the outer case, and a tight-fitting inner box.

The Model 10 Field Fluorometer is resistant to water, including salt water, with or without the protective cover on (a flow cell must be installed

for the instrument to be watertight), and may be washed off with fresh water.

Vibration. The instrument is designed to operate satisfactorily when used in mobile equipment. It is important, however, to fasten it securely to prevent it from colliding with other hard objects.

Helium. If you are working with exotic breathing mixtures, or other systems using helium, keep them away from the fluorometer because they can cause damage to the photomultiplier.

4. **Mounting Considerations**

(See Appendix 6.6.)

Mounting Position. The average position of the instrument should be within 20 degrees of level.

Mounting Considerations. The Model 10 Rack Mount Fluorometer may be mounted directly in any standard 19" electronic rack, with four 10-32 screws, or in a 10-002 Laboratory Case for laboratory service.

Watch overall temperature rise in enclosed racks. Heat may be caused by other equipment. Temperatures should be kept low and constant.

Access Requirements. During normal operation, only access to the front panel controls is needed.

If you are taking "grab" samples with the Cuvette Adaptor Kits, be sure to allow enough room for easy cuvette insertion.

Section Three

OPERATING INSTRUCTIONS

3.1 SETTING UP YOUR FLUOROMETER

1. Relabeling For Calculations

The Model 10 has been labeled (by convention) with range multipliers, which are sensitivity multipliers. Thus, the most sensitive range (X31.6) is useful for reading low concentrations of unknown, and the least sensitive range (MIN SENS) is useful for reading high concentrations. This means that to effectively handle meter readings on more than one range, you must divide by the range multiplier. While the laboratory market did not mind the inconvenience of handling numbers less than one, it is more convenient to be able to multiply. If you agree, BEFORE PROCEEDING any further, take a labeling machine or write-on tape and relabel the front of the Model 10 as follows:

Original Sensitiviy Range Labels	New Concentration Range Labels
X31.6	X1 (MIN CONC)
X10	X3.16
X3.16	X10
MIN SENS	X31.6
Original X1-X100 Switch	**NEW X1-X100 Switch**
X1	X100
X100	X1

All further references in the Calibration and Calculation sections are to the new concentration range labels.

NOTE: If you are using a data logger, see Appendix 6.10 for information about range values for specific pins.

2. Sample System

Unless you specifically requested another configuration, the instrument, as shipped, has the High-Volume, Continuous-Flow Cuvette System installed and ready for

operation. (For information on other Continuous-Flow systems, refer to the Series 10 Ordering Information booklet, accompanying your fluorometer.)

If you wish to use one of the other cuvette adaptors, it should be installed at this time. (You will find instructions included with all accessories shipped with your order.) A Cuvette Adaptor replaces the Continuous-Flow Cuvette System when measuring test tube samples. The proper size test tubes must be used (i.e., 25 x 100 mm, etc., depending on the Cuvette Adaptor). Generally the test tube is glass; however, in some applications quartz test tubes are required. Refer to Appendix 6.8 and to the Series 10 Ordering Information booklet, accompanying your fluorometer.)

NOTE: The Light Shield must cover the opening in the top of the Sample Compartment and any test tube samples to get accurate readings.

3. Filters and Light Source

The filters and light source are installed by Turner Designs for your main application (i.e., rhodamine, chlorophyll, oil, etc.). If you change applications, you will need to change the lamp and filters, and make an adjustment for the ranges. (Refer to Section 3.5.2, and Appendices 6.7 and 6.8.)

4. Additional Items Needed

(See Appendix 6.3 for information about accessories.)

You may also need the following items, not manufactured by Turner Designs:

a. A freshly charged 12-volt car battery for field power, with connector cable. A 20 amp-hour battery will support your unit for about 10 hours. If used in connection with other equipment, this time will be reduced.
b. A small sample pump with ballast weights.
c. Opaque connecting hoses to deliver sample to the fluorometer.
d. Data acquisition equipment, your choice, from a pen to a PC. (See Appendix 6.10.)
e. A dye injection pump is needed for some kinds of dye studies.
f. The correct dyes (if a dye is to be used). (See Appendix 6.2 for information about dye studies.)
g. Appropriate standards. (See Appendix 6.2.)

5. Power Source

Connect the instrument to an appropriate power source as explained in Section 2.2.1.

3.2 PRELIMINARIES TO CALIBRATION

(See Appendix 6.2 for information about specific studies.)

1. Introduction to Standards and Blanks

Standard. Normally, your standard will be a known concentration of the material that you plan to quantitate. For flow rate measurements, absolute concentration will not, however, be needed. A known dilution of the solution of dye you will inject is all that is required. (See the monographs "Preparation of Standards in Dye Studies," "A Practical Guide to Flow Measurement," and "Flow Measurements in Sanitary Sewers by Dye Dilution," following Appendix 6.2.)

For those applications in which a known concentration is needed, the concentration should be low enough so that very little of the exciting light is absorbed by the fluorescent material. When this condition is met, the reading of the fluorometer will always be proportional to concentration (if blank is subtracted). If you wish to operate at higher concentrations, several standards should be used, so a calibration curve may be prepared.

For example, a calibration curve should be used for concentrations of rhodamine WT above 0.1 parts per million (ppm) active ingredient. (Rhodamine WT is normally supplied as a 20% aqueous solution. Thus 0.1 ppm is equal to 0.5 ppm of the 20% concentrate.)

When the concentration of fluorescent material is extremely high, readings will flatten out and then decrease as concentration goes up. This effect, known as "concentration quenching," is often noted during the early phases of circulation studies in bays and harbors. Such concentrations cannot be measured directly. If you suspect such an effect, check. Dilute a sample 1:1 or some other convenient ratio. If the reading goes up, or does not decrease as much as you diluted it, take a few "grab" samples for diluting into the linear range. (See Section 3.5.1 below.)

Blank. Before measuring sample, a blank should always be collected. A blank is a sample of the water you will work in, taken before any dye has been added or without any of the analyte of interest present. In large systems, this blank may be collected from several locations. This will be used to prepare your standard and will be used to set the instrument to zero, or at least will be measured to see if it is significant (and subtracted if it is). For in vivo chlorophyll analysis, this blank would ideally be a sub-micron filtered sample of the water you are working in.

Temperature. Most fluorescent materials have fairly high temperature coefficients. For example, the temperature coefficient of fluorescence of rhodamine WT is -2.6%/degrees C, while that of chlorophyll extracts is reported to be -.5%/degree C. Thus, the reading of any concentration of rhodamine WT decreases 2.6% for each degree C rise in temperature.

For this reason, the standard, the blank and the unknowns should all be held at the same temperature, or the raw readings corrected to what they would be at some common temperature.

Adsorption. If you are working with fluorescent dyes, see Appendix 6.3.3.

Storage. For storage of samples, we recommend polypropylene or borosilicate glass. Ordinary glass sometimes has outcroppings of soda lime. This may change the pH enough to alter readings. The pH must be between about 4 and 10.5. Readings drop off rapidly if it is lower or higher. The readings may be restored by neutralizing.

2. Calibrating With Continuous-Flow Systems

If you use the 10-020 High-Volume Continuous-Flow Cuvette System, the easiest way to calibrate it is to install the 10-027A Cuvette Adaptor System, calibrate the instrument, and switch back to the Continuous-Flow System. Both systems have the same calibration factor.

NOTE: When switching between systems, the lamp must be re-installed in its original orientation to maintain calibration! Mark it, before you remove it. See Appendix 6.7.1.

NOTE: In addition, removal and replacement of the Excitation Filter from its holder may affect calibration also. The reason for this is that some filters change color with irradiation. As a result, the excitation filter may not be uniform. In fact, with a very old one you may actually see a circle corresponding to the round hole in the plate holding the filter in place. Remove the Excitation Filter assembly as a unit, and do not change the position of the filter in its holder. See Appendix 6.7.

NOTE: See Section 3.5.2, below, for details about adjustments that must be made when switching from one application to another (i.e., from rhodamine to chlorophyll, etc.).

Accurate calibration can also be made by setting up so that the Continuous-Flow Cuvette may be poured full of the standard and blank. Several flushings are required, of course, to eliminate any trace of the preceding sample. To ensure complete filling of the cuvette without having any large bubbles trapped, it is best to fill through the lower Intake Fitting. Significant air bubbles will greatly affect the accuracy of your readings. This particular method has the disadvantage that it is difficult to know the sample temperature. Simply measuring the temperature prior to filling the cuvette is not sufficient. The cell itself may be warm and will quickly warm the sample. It is best to pump the standard through the cell and measure it while it is flowing.

Alternately, you could prepare a valving system in which standard, blank, and unknown are pumped through the fluorometer in identical fashion. Such a system could include a means of stopping flow, to conserve on standard. If you do stop flow, do it only briefly as the instrument will warm the solution.

Note that with a few exceptions, the flow rate through the Continuous-Flow Cuvette System is not important and is at the discretion of the operator. (See Appendix 6.3.2.)

3.3 CALIBRATION INSTRUCTIONS

1. Introduction

In its most general sense, calibration consists of determining the reading (above blank) of a sample of known concentration.

To calibrate, you will need a Standard of the material you wish to quantitate and a Blank solution. (See Section 3.2.1.) This standard must be at some known temperature or at the same temperature as all the samples. If the standard and samples are in a water bath, wipe each thoroughly, but quickly, with a lint-free tissue (such as Kim Wipes). Any sample read and returned to the bath should not be re-read for at least ten minutes. Even if the reading has not begun to change in the instrument (an indication that the temperature has changed), the tube will have warmed slightly and the solution temperature will increase after it is removed.

Averaging Readings. If you are working with a test tube sample, you should bear in mind that there can be slight variations from tube to tube. For this reason it is common to have three (or more) tubes of standard and to average the readings. You may also use replicate samples as well. It is for you to determine how important the reading of one sample is.

If you are working with the flow cell, multiple readings will not be required. However, unless you are pumping sample through, remember that the glass cuvette will be warm. You must flush with enough standard to cool the cuvette, or the reading may not be valid.

Suppressing Blank. There are two reasons to suppress blank:

1. Convenience. If the blank reading is a significant part of the total reading, it should be subtracted prior to doing any calculations. You can let the instrument do this, or you can do it manually.

2. Resolution of low samples. If the blank is taking up a substantial portion, or all, of the most sensitive range, then low samples will have to be read with less accuracy on a

higher range. It makes sense to suppress blank and make this portion of the readout available for sample.

As long as you are consistent, whatever you do is correct. You may have the instrument subtract all of the blank. You may have the instrument subtract none of the blank and subtract it manually. You may even have the instrument subtract part of the blank and subtract the rest manually.

At first the operation of the Blank Control seems complex, as it does not seem to do as much on some ranges as on others.

Regardless of the range, the Blank Control always subtracts the same amount of fluorescence! On the most sensitive range, the X1-X100 on X1 and the X1 range light lit (new labeling system, Section 3.1), where the multiplier is 1, with the Span Control about seven turns clockwise, it will subtract whatever is approximately full scale. In other words, a reading of about 10 can be suppressed to 0. When you change to any other range, it stays at 0. Now consider trying to initiate blank subtract on some other range. On X3.16, a full-scale reading is actually 3.16 times 10, or 31.6. You can suppress 10. Therefore you can suppress about one-third of the scale. When the X1-X100 is set to X100 and the X10 range light is lit (new labeling system), the multiplier is 1000 and full scale is 10,000. Moving the Blank Control from one end to the other will barely wiggle the needle, as its maximum motion is 0.1% of full scale.

What this all boils down to is that in order to subtract blank to zero, your blank must not exceed a reading of approximately full scale on the most sensitive range. Use the Sensitivity Knob, if necessary. (If that, alone, won't do it, some of the optical kits contain attenuator plates. See Appendix 6.8.)

2. Choosing a Calibration Method

There are three basic calibration methods you can choose, depending on your study. Detailed instructions for calibrating the fluorometer in each of these situations are set forth in part 3, below.

A. High Resolution and Blank Suppression. You wish to have the greatest possible resolution of samples of low concentrations, and background fluorescence is the limiting factor. This is another way of saying that you wish to set background to zero and you wish to expand the readings as much as possible. This will very likely be the case when you are measuring low concentrations of chlorophyll, tracking the carcinogens from an oil spill, or working with low concentrations of rhodamine WT in relatively dirty waters.

B. Highest Usable Resolution. You wish to work over the widest possible range of concentrations. You wish to set the instrument such that the "noise" is as high as you are willing to tolerate on very low concentration samples. If blank can still be subtracted, this will give you the best possible resolution on low samples, and will still generally keep samples whose concentration is in the linear range from going off-scale.

C. Interested in High Concentrations Only. You are mainly interested in high concentrations, or have a specific resolution in mind. For example if you are interested in measuring 5 parts per billion (ppb) to 1% and do not anticipate lower concentrations (or don't care if they are measured with less accuracy), there is no point in going to the trouble of adjusting the instrument to a resolution of 0.001 ppb, since it is unlikely that the blank can be set to zero. (Remember to consider the linear range for your study. See 3.5.1.)

RELAX! Some of this may look complicated. However, the worst thing that can happen to you is that the instrument may not wind up adjusted exactly the way you wish it. It will still work, and it will give you correct answers. No matter how the instrument is adjusted, if it gives you a reading (an "on-scale" reading), it is a valid answer.

3. Detailed Calibration Instructions

A. High Resolution and Blank Suppression.

REMEMBER, ALL REFERENCES ARE TO THE NEW CONCENTRATION RANGE LABELS IN SECTION 3.1.

1. Turn the instrument on and allow at least five minutes for the lamp to warm up and stabilize.

2. Unlock the Blank Control and set it fully clockwise (maximum suppression).

3. Unlock the Span Control and set it nine turns clockwise from full counterclockwise, or one turn from full clockwise.

4. Set the Auto-Man Switch to the Man position.

5. Press the Step Button until the X1 (minimum concentration or maximum sensitivity) light is on.

6. Set the X1-X100 Switch to the X1 position.

7. Unlock the Sensitivity Knob (loosen the Sensitivity Knob Lock Screw with a screwdriver; see Figure 1) and turn it full clockwise (maximum sensitivity).

   Ignore the Sensitivity Fine Adjustment.

8. Insert your blank. (Fill up the cuvette system if using the Continuous-Flow System or use a test tube if employing the Cuvette Adaptor system. Replace the Light Shield, if using test tube samples.)

9. Turn the Sensitivity Knob slowly counterclockwise (pausing until the meter stabilizes) until the meter reads just below zero. What you are doing here is reducing the reading of the blank until the blank suppression is able to handle it. You are aiming for zero, but the Sensitivity Knob is too touchy to hit this exactly.

   NOTE: With oil or chlorophyll studies, the fluorometer may be too sensitive. Even with the Sensitivity Knob full counterclockwise (minimum sensitivity), you will be unable to blank to zero. If this occurs, then install an attenuator plate onto the Excitation Filter Holder (see Appendix 6.7, #8 and the Filter Selection Guide for your study in Appendix 6.8). For oil studies, an attenuator plate is available that

reduces sensitivity approximately 75-fold. For chlorophyll, a plate reduces sensitivity 5-fold. These plates do not affect the accuracy of readings.

If you install an attenuator plate, then begin again with step 1, above.

10. Once sensitivity is adjusted, lock the Sensitivity Knob Lock Screw securely with a screwdriver.

11. Turn the Blank Control counterclockwise until the meter reads 0 (may have to average this if the needle is fluctuating).

12. Lock the Blank Control.

You have now set the instrument at the maximum sensitivity (greatest resolution) at which the instrument will subtract blank for you. You may now adjust readings, if you wish, with the Span Control, and you may change ranges -- blank will remain at zero. <u>You may not readjust the Sensitivity Knob without invalidating your zero point.</u>

13. Set the Auto-Man Switch to Auto.

14. Insert your standard. (Remember the Light Shield for test tube samples.) Check to see that you have adequate range for higher concentrations. If you do not, or if it goes off-scale, move the X1-X100 Switch to the X100 position. If the instrument is still off-scale you will need to unlock the Sensitivity Knob and turn it counterclockwise until the reading is on-scale and offers suitable range. At this point, you can accept the reading for the standard or adjust it slightly, using the Span Control. LOG THE READING (upper scale), noting the range and whether you are in the X1 or X100 position, for later ratio calculation. (See Section 3.4.2.)

NOTE: If you adjust the Sensitivity Knob, you must re-insert your blank (set the Auto-Man Switch to Man; go to Range X1, with the X1-X100 in the X1 position) and suppress the reading to zero, using the Blank Control.

(Be sure to thoroughly flush out the system before inserting the blank.) Then, re-insert your standard (set to Auto, X1-X100 in the X100 position), adjust the Span Control to an acceptable reading, and LOG THE READING for later ratio calculation.

B. Highest Usable Resolution.

REMEMBER, ALL REFERENCES ARE TO THE NEW CONCENTRATION RANGE LABELS IN SECTION 3.1.

1. Turn the instrument on and allow at least five minutes for the lamp to warm up and stabilize.

2. Unlock the Blank Control and set it fully counterclockwise. (No blank suppression.)

3. Unlock the Span Control and set it seven turns clockwise from full counterclockwise.

4. Set the Auto-Man Switch to the Man position.

5. Press the Step Button until the X1 range light is on.

6. Set the X1-X100 Switch to the X1 position.

7. Unlock the Sensitivity Knob (loosen the Sensitivity Knob Lock Screw with a screwdriver; see Figure 1), and turn it full counterclockwise (minimum sensitivity).

   Ignore the Sensitivity Fine Adjustment.

8. Insert your blank. (Fill up the cuvette system if using the Continuous-Flow System or use a test tube if employing the Cuvette Adaptor system. Replace the Light Shield, if measuring test tube samples.)

9. Slowly turn the Sensitivity Knob clockwise until the meter needle varies so much that you cannot comfortably average a reading. (If the needle goes off-scale before this happens, go directly to method A.) At this point you can back off on the Sensitivity Knob until you are comfortable with choosing an average reading.

10. Lock the Sensitivity Knob Lock Screw securely with a screwdriver.

11. Using the Blank Control, you may now suppress blank to zero, if you wish. Lock it.

12. You may increase or decrease readings with the Span Control. Increasing readings will increase the "noise," and decreasing readings will decrease it. Do not readjust the Sensitivity Knob.

    NOTE: You have now set the sensitivity at a point where variability of reading the blank (and low samples) is significant. In doing so, you have spread out readings as much as practical on low samples. If you were to increase resolution more, the value of the larger numbers obtained would be offset by increased variability.

13. You may now wish to insert the highest concentration sample you wish to read (or the highest sample in the linear range). (Remember the Light Shield, if measuring test tube samples.) Put the X1-X100 Switch in the X100 position, and Auto-Man in Auto. If the sample is "on-scale," then proceed. If it is not, you may wish to adjust the Sensitivity Knob counterclockwise until the sample is on-scale. This is up to you. You may wish to keep the high resolution and elect to read high samples by diluting them.

    NOTE: If you adjust the Sensitivity Knob, re-insert your blank, set Auto-Man to Man, X1-X100 in X1, Range X1, and adjust to zero, using the Blank Control.

14. Make sure the Auto-Man Switch is in Auto.

15. Insert your standard. You can accept the reading for the standard or adjust it slightly (i.e., to a whole number), using the Span Control. LOG THE READING (upper scale), noting the range and whether you are in X1 or X100, for later ratio calculation. Lock the Span Control. (See Section 3.4.2.)

DATA LOG

Test Name/No. _____ Date _____

Comments: _____

_____

_____

Meter Reading x X1-X100 x Range = Readout
(Based on new concentration labels, Section 3.1)

$$C_u = R_u / R_s \times C_s$$
(Where Blank is suppressed, Section 3.4.2)

BLANK

Blank	Meter Reading	X1-X100	Range	Readout	Suppressed? Yes/No	Comments

STANDARD

Standard ID	Meter Reading	X1-X100	Range	R_s (Readout)	C_s (Concentration)	Comments

UNKNOWNS

Unknown ID	Meter Reading	X1-X100	Range	R_u (Readout)	C_u (Concentration)	Comments

As in mentioned in the Averaging Readings portion of Section 3.3.1, you may wish to read several standards and average them.

C. Interested in High Concentrations Only.

REMEMBER, ALL REFERENCES ARE TO THE NEW CONCENTRATION RANGE LABELS IN SECTION 3.1.

Let's say you are interested in reading 5 ppb to 1% and do not expect (or do not care if you can read accurately) concentrations less than this. (Remember to consider the linear range. See Section 3.5.1.)

1. Turn the instrument on and allow at least five minutes for the lamp to warm up and stabilize.

2. Unlock the Blank Control and set it fully counterclockwise.

3. Unlock the Span Control and set it seven turns clockwise from full counterclockwise.

4. Set the Auto-Man Switch to the Man position.

5. Press the Step Button until the X1 light is on.

6. Set the X1-X100 Switch to the X1 position.

7. Insert your standard (for example, 5 ppb). (Fill up the cuvette system if using the Continuous-Flow System, or use a test tube if employing the Cuvette Adaptor system. Replace the Light Shield, if measuring test tube samples.)

8. Unlock the Sensitivity Knob (loosen the Sensitivity Knob Lock Screw with a screwdriver; see Figure 1) and turn it until the meter reads approximately 1 on the upper scale.

   Ignore the Sensitivity Fine Adjustment.

   If you cannot get the reading down to 1, then simply accept your lowest reading.

9. Remove the sample, thoroughly flush out the system, and insert your blank. (Remember the Light Shield for test tube samples.)

10. Suppress to zero with the Blank Control -- if desired.

11. Set the Auto-Man Switch to Auto.

12. Insert a standard with a high concentration. You can accept the reading for the standard or adjust it slightly (i.e., to a whole number) using the Span Control. LOG THE READING (upper scale), noting the range and whether you are in the X1 or X100 position, for later ratio calculation. (See Section 3.4.2.)

Now, the instrument is set up with the resolution you desire, and since there is no more resolution than needed, the instrument readings will be as stable as possible.

3.4 OPERATING THE INSTRUMENT

REMEMBER, ALL REFERENCES ARE TO THE NEW CONCENTRATION RANGE LABELS IN SECTION 3.1.

(For your convenience, a data log sheet has been provided at the end of this section for ease in recording and calculating your measurements.)

1. Operating Steps

A. Set the Auto-Man Switch.

When the instrument is used in the laboratory on individual samples or in the field on "grab" samples, the Man position will probably be best. This is because removing the Light Shield will generally cause off-scale readings. Replacing the Light Shield and absence of a sample will generally cause readings below 20% of full scale. Either of these conditions will cause the autoranging system to search out a new range with consequent delay in settling on a stable reading.

If, however, your samples vary widely in concentration, you may find it easier to leave the instrument in the Auto position, and let it find the correct range for you.

The Auto position will be particularly advantageous for unattended service where wide ranges of concentration are encountered on continuous flow samples.

B. Set the X1-X100 Switch to the X1 position.

C. Insert the unknown to be measured.

Insert the unknown. (Fill up the cuvette system if using the Continuous-Flow System, or use a test tube if employing the Cuvette Adaptor system. Replace the Light Shield, if measuring test tube samples.)

If the reading of your unknown is over-scale, select the X100 position on the X1-X100 Switch.

D. Log the reading (upper scale) for your sample.

Note 1: In addition to the meter reading, log the range and whether your sample is being read in the X1 or X100 position.

Note 2: A recording device is very useful (see Section 2.2.2) when using the Continuous-Flow Cuvette system.

2. Calculations Using the Ratio Method

After your measurements are complete, you will have to perform a few simple calculations.

First, obtain the readout (R_u) for your samples. To do this, multiply the range value times the X1-X100 position times the meter reading (upper scale). For example, if the range was X3.16, and the X1-X100 Switch was on X100 and the meter reading was 4.2, the readout for your sample would be:

3.16 x 100 x 4.2 = 1327.2

(More correctly, this would be recorded as 1.33×10^3, as 1327.2 would convey greater accuracy than exists.)

Next, obtain the readout (R_s) for your standard. To do this, multiply the range value times the X1-X100 position times the meter reading (upper scale) for the standard.

Assuming blank has been suppressed, your readings are in the linear range, and the exact concentration of your standard is known, the equation for calculating unknown sample concentration is:

$C_u = R_u/R_s \times C_s$

Concentration of unknown (C_u) = readout of the unknown (R_u) divided by the readout of the standard (R_s), times the concentration of the standard (C_s).

For example, if a 100 ppb standard has a readout of 50.00 and the unknown readout is 20.00, the concentration of the unknown is:

20.00/50.00 x 100 ppb = 40.00 ppb

If blank has not been suppressed, your readings are linear, and the exact concentration of your standard is known, the equation for calculating unknown sample concentration is:

$C_u = (R_u - R_b)/(R_s - R_b) \times C_s$

where R_b equals the readout (range setting x X1-X100 position x meter reading) of your blank.

If your readings are not in the linear range but are within the parameters for a calibration curve, plot readings of your dilutions versus known concentrations and obtain concentrations of unknowns by reading the curve.

For details about calculations to be performed when conducting flow rate measurements, where it is not required that the exact concentration of the standard be known, see the monograph entitled "A Practical Guide to Flow Measurement," in Appendix 6.2.

3.5 PRECISION OF READINGS

1. Concentration Quenching

The readout of the Model 10 Fluorometer is proportional to concentration of the sample from the smallest detectable concentration to a concentration specific to the fluorescent material, the wavelengths being used, and the path length.

Above some concentration the measurements become non-linear. (See Appendix 6.9 for approximate linear ranges.)

As the concentration of the sample is further increased, the fluorometer reading rises at a decreasing rate and eventually begins to decrease, even though the concentration is still increasing. In effect, "concentration quenching" results in non-linearity.

For example, the fluorescent dyes provide linear readings from the limit of detectability of about 10 parts per trillion to about 0.1 ppm active ingredient. (Rhodamine WT is provided as a 20% aqueous solution. Thus, 0.1 ppm corresponds to 0.5 ppm of the 20% solution.) As a rule of thumb, linearity should be checked when measuring concentrations of dye higher than 0.1 ppm, using the 10-020 High-Volume Continuous-Flow System or 25 x 100 mm cuvettes. (For other cuvettes, refer to the instructions accompanying the cuvettes.)

At dye concentrations below 0.1 ppm, a single-point calibration (one standard) may be used to calibrate the Model 10. For concentrations between 0.1 and 0.5 ppm, a multi-point calibration curve (using multiple concentrations of the standard) must be prepared, or the samples must be diluted and the reading obtained multiplied by the dilution factor. (Much above 0.3 parts per million, dilution will be more accurate.) Above 0.5 ppm, dilute the samples before taking readings.

2. Changing Applications

The Model 10 Fluorometer has internal ranges that have been preset at the factory to ±1% accuracy with the light source and reference filter for your main application. This includes switching the X1-X100 Switch. (If you ordered the combination chlorophyll and rhodamine applications, the internal ranges were adjusted for rhodamine. See Appendices 6.7 and 6.8 for more information on light sources and filters.)

If you change from one application to another, the internal ranges must be adjusted or a compensation must be made in your readings. For example, if factory-adjusted on the rhodamine light source and filters, errors as great as ±4% may occur if you switch to the chlorophyll light source and filters.

These errors are stable and may be corrected for. (See Appendix 6.7.2 for the procedure to generate a table of actual values.)

Contact Turner Designs for the procedure for adjusting the internal ranges.

Note: There will be no error if the standard and all samples are read on one range.

After the internal ranges have been adjusted or a compensation factor allowed for, calibrate the fluorometer following the steps set forth in Section 3.3.

3. Staying in One Configuration

Staying in one configuration (no range change) avoids the potential errors inherent in moving to a different analysis. Thus, the possible 1% error of a range change can be avoided by arranging for the standard and all samples to be read on one range.

This might become important in a very accurate measurement of flow using the steady-state dye-dilution method. By using the flow cell, staying on one range, and using a computer to average readings over a long period of time (and correct for temperature variations), it is possible to achieve an accuracy of about 0.25%.

Section Four

MAINTENANCE, WARRANTY, & SERVICE

4.1 MAINTENANCE

To keep your Model 10 Fluorometer in good operating condition, the following maintenance procedures should be performed on a routine basis:

1. Clean off corrosive materials, including saltwater.
2. Check the Continuous-Flow Cuvette System to make sure it is clean and that there is no evidence of leaks. (See Appendix 6.4.3.)
3. In humid areas, desiccant should be installed in the sample compartment area if the continuous-flow attachment is used. (See Appendix 6.4.5.) There is not much point in using desiccant with one of the Cuvette Adaptors (test tube sample holders). The only way to avoid condensate with these is to have the samples at a temperature above dewpoint.
4. Before storing your fluorometer, remove the sample compartment cover and make sure the sample compartment is dry and free of corrosive materials (including salt). Add fresh desiccant. If using a Cuvette Adaptor, tape the Light Shield securely over the opening in the top of the Sample Compartment. When you bring the fluorometer from storage, be sure to add fresh desiccant.

4.2 WARRANTY

Turner Designs warrants its Model 10 Series Fluorometers 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 Model 10 Series Fluorometer User's 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.

4.3 OBTAINING SERVICE

1. Warranty Service

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

a. Write or call the Turner Designs service department and describe as precisely as possible the nature of the problem.

b. Carry out minor adjustments or tests as suggested by the Service Department.

c. 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 to us, 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 our distributors, contact your distributor. If you have purchased direct, contact us. We will repair at no charge, but will not pay for shipment, documentation, etc. These charges will be billed at cost.

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

1. To ensure that the problem is not a trivial one, easily handled in your laboratory, with consequent savings to everyone.

2. To specifically determine the nature of the problem, so that repair can be rapid, with particular attention paid to the defect you have noted.

2. 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.

3. Routine Overhaul

Turner Designs offers a routine overhaul service, which may prove to be particularly advantageous to customers who use their instruments under unusually severe conditions.

The instrument will be overhauled and completely tested to the performance (but <u>not</u> appearance) specifications existent at the time the instrument was first shipped.

The charge for this overhaul will be 7% of the list price of the equipment overhauled at the time of the overhaul, plus the price of parts used for the overhaul.

The overhauled instrument will be guaranteed as if it were new, except the warranty shall be for six months only, and shall be further limited to labor and to those parts replaced at the time of the overhaul only. Original parts are <u>not</u> covered.

To obtain details, please write or phone the Turner Designs service department.

Section Five

TROUBLESHOOTING YOUR MODEL 10 FLUOROMETER

5.1 INTRODUCTION

The most common fluorometer problems relate to the lamp and to errors in calibration methods. Secondary sources of difficulties involve the filters and water or corrosion in the sample compartment. Many of these problems can be taken care of simply and easily in the field.

Whenever you address a problem, we recommend that you have available a spare lamp, desiccant packets, a screwdriver (1/4" blade), an Allen wrench (5/64"), needle-nose pliers, and a flashlight.

5.2 TROUBLESHOOTING CHECKLIST

Symptom	Possible Problem	Section to See
Erratic readings Noisy readings	1. Check to see lamp is lighting. 2. Check calibration. 3. Check for air bubbles in the sample. 4. Check Flow Cell for fouling/discoloration. 5. Check the excitation and emission filters.	1. 5.3; 6.7 2. 5.3; 3.3.3 3. 5.3; 6.2 4. 5.3; 6.4 5. 5.3; 6.7; 6.8
Unstable or drifting readings	1. Check the reference filter. 2. a. Check for moisture on Flow Cell. b. Check for leaks. c. Check for moisture on circuit boards. 3. Check the lamp.	1. 5.4; 6.7; 6.8 2. a. 5.4; 6.4 b. 5.4; 6.4; 6.5 c. 5.4 3. 5.4; 6.7
Low readings	1. Verify the Auto-Man Switch is set to Auto. 2. Select X1 on the X1-X100 Switch. 3. Check calibration. 4. Check filter selection and placement. 5. Check to see correct lamp is installed. 6. Check the flow cell for fouling or discoloration.	1. 5.5; 3.4 2. 5.5; 3.4 3. 5.5; 3.3.3 4. 5.5; 6.7; 6.8 5. 5.5; 6.7; 6.8 6. 5.5; 6.4
Off-scale readings	1. If using Cuvette Adaptor, make sure Light Shield is in place. 2. Verify the Auto-Man Switch is set to Auto. 3. Select X100 on the X1-X100 Switch. 4. Check calibration. 5. Check filter selection and placement. 6. Check to see correct lamp is installed. 7. Install an Attenuator Plate.	1. 5.6; 3.1.2; 3.3.3 2. 5.6; 3.4 3. 5.6; 3.4 4. 5.6; 3.3.3 5. 5.6; 6.7; 6.8 6. 5.6; 6.7; 6.8 7. 5.6; 3.3.3; 6.7; 6.8
No response	1. Make sure power switch is on. 2. Check to see power cable securely fastened. 3. Check power source. 4. Check fuses.	1. 5.7; 2.1 2. 5.7; 2.2.1 3. 5.7 4. 5.7; 2.1
Unable to blank to zero	1. If using Cuvette Adaptor, make sure Light Shield is in place. 2. Select X1 on the X1-X100 Switch. 3. Check filter selection and placement. 4. Check to see correct lamp is installed. 5. Install an Attenuator Plate. 6. Check calibration.	1. 5.8; 3.1.2; 3.3.3 2. 5.8; 3.4 3. 5.8; 6.7; 6.8 4. 5.8; 6.7; 6.8 5. 5.8; 3.3.3; 6.7; 6.8 6. 5.8; 3.3.3.A.

5.3 ERRATIC READINGS (sudden needle movements) NOISY READINGS (fluctuations >4% of full scale)

1. Visually check to see that the lamp is lighting. The lamp should be uniformly lit throughout its length and should not be flickering; otherwise, replace the lamp. If it is not lighting, remove it, invert it, and reinsert it. If this doesn't cure the problem, put in a new lamp. See Appendix 6.7.1. (Do not view the lamp directly when on, as it will cause a sunburn to your eyes.)

   If the instrument has been calibrated, the lamp must be reinstalled in its original position, to maintain calibration. See Appendix 6.7.1.

2. Check calibration. The Sensitivity Knob setting may be too high (too sensitive), resulting in excessive noise (needle movement). (See Section 3.3.3.)

3. On the Continuous-Flow Cuvette System, check for air bubbles in the sample. (Refer to the Section on Sampling Systems in the booklet entitled "A Practical Guide to Flow Measurements," included with Appendix 6.2.)

4. On the Continuous-Flow Cuvette System (particularly if using the 3 mm cell), check for fouling or discoloration of the cuvette. Clean the Flow Cell if necessary. See Appendix 6.4. (If using the 3 mm Flow Cell, refer to the instructions that accompanied it.)

5. Check to see that the proper Excitation and Emission filters are installed and in the correct locations. If the filters are laminated glass, check it for uniform color and density. To check, remove the filter and hold it up to a light. Slight wrinkling is acceptable. See Appendices 6.7 and 6.8.

5.4 UNSTABLE OR DRIFTING READINGS

1. Check to see that the proper Reference filter is installed in the correct location. If the Reference Filter is laminated glass, check it for uniform color and density. To check, remove the filter and hold it up to a light. Slight wrinkling is acceptable. See Appendices 6.7 and 6.8.

2. a. Moisture on the Flow Cell. In humid areas, or if there has been a leak, condensation may build up on the outside of the glass/quartz flow cell. If this occurs, wipe off the flow cell and dry inside the Sample Compartment. Add fresh desiccant. (For

instructions to access the Flow Cell, see Appendix 6.4.)

b. Leaks. In a properly sealed fluorometer, desiccant should take care of any residual moisture. It is possible for moisture to get into the sample compartment even when using the Continuous-Flow System, if the Flow Cell is improperly installed or if all 14 screws on the sample compartment cover have not been securely tightened. (Before removing the sample cover, check to see if it is loose. If it is loose, condensation may have formed inside the sample compartment.)

If there has been a leak, water may have corroded the lamp connections or other internal components. TURN OFF THE FLUOROMETER. Check inside the sample compartment by removing the 14 screws on the cover. If you find moisture or corrosion inside the sample compartment:

1. Check the lamp connections for damage.

2. Check the sensitivity polarizer. See Figure A2, Appendix 6.4, to locate it. It is a laminated piece of plastic, and the layers can separate if the compartment is flooded. To check it in place, shine a flashlight on it. Turn the Sensitivity Knob fully counterclockwise to get the best view. It should be a uniform smoky gray color, especially in the center area. If only the edges are delaminated, it will still work, although you may want to order a new one for the future. If it has been damaged in the center area, you will need to order a new one from Turner Designs.

3. If you find substantial moisture, check the reference, excitation, and emission filters for damage or separation. (See Appendices 6.7 and 6.8.)

4. Check for leaks. Check the intake and exhaust fittings, the O-rings, and the flow cell itself for leaks. See Appendices 6.4 and 6.5.

5. If there is a slow leak from the top or bottom portion of the flow cell body (inside the sample compartment), try pushing the intake and exhaust fittings more securely into the flow cell. To do this, loosen the Upper and

Lower Set Screws with an Allen wrench (see Figure A2 for location). Be careful not to let the lower intake fitting fall out of the fluorometer. Then grasp both intake and exhaust fittings and push them gently together (be firm, but do not force) into the top and bottom openings in the flow cell body until they are seated snugly. Tighten the Upper and Lower Set Screws. To check for leaks, allow solution to flow through the flow cell.

If the leak appears to be coming from the glass cuvette itself, refer to Appendix 6.4 for repair or replacement. (For the 3 mm Flow Cell, see the instructions accompanying the Flow Cell.)

6. If the problem is condensation rather than a leak, make sure the sample cover is securely tightened when you replace it. (If this does not take care of future condensation problems, contact Turner Designs.)

7. After repairing leaks or checking for condensation, thoroughly dry inside the sample compartment. (Gentle heat from a hair dryer works well.) If salt water entered the compartment, flush the affected area with distilled water. (See Appendix 6.5.) Add fresh desiccant. Two packets should be added to the left of the lamp between the Excitation Filter Holder and the sample compartment cover. (See Appendix 6.4.5, Figure A2.)

8. Replace the sample compartment cover and tighten the 14 screws snugly.

9. Recalibrate the fluorometer. (See Section 3.3.3.)

c. <u>Moisture on fluorometer circuit boards</u>. (Problem is less likely with field unit.)

In humid areas, condensation may form on the circuit boards, resulting in unstable meter readings. The instrument is protected against damage to electronic components from condensation. Burn off condensation by leaving the instrument on for about 20 minutes, and operation should return to normal.

Excessive moisture that leaks inside the fluorometer housing and onto the circuit boards may result in unstable readings and could damage the fluorometer.

To check the field unit, remove the 18 screws on the front panel of the fluorometer and pull the fluorometer from its watertight case. For the rack mount unit, remove the top panel (silver sheet metal) by removing the screws on the top sides and the rear edge. Check for moisture on the circuit boards. Drying the circuit boards with gentle heat (as with a hair dryer) may cure the problem. In any case, if you find moisture inside the fluorometer casing, locate and eliminate the source of the leak.

3. Visually check to see that the lamp is lighting. The lamp should be uniformly lit throughout its length and should not be flickering; otherwise, replace the lamp. If it is not lighting, remove it, invert it, and reinsert it. If this doesn't cure the problem, put in a new lamp. See Appendix 6.7.1. (Do not view the lamp directly when on, as it will cause a sunburn to your eyes.)

   If the instrument has been calibrated, the lamp must be reinstalled in its original position, to maintain calibration. See Appendix 6.7.1.

5.5 READINGS ARE LOW

1. Verify that Auto-Man Switch is set to "Auto."

2. Select the X1 (new concentration range labels, see Section 3.1) position on the X1-X100 Switch. (See Section 3.3.3.)

3. Check calibration. The Sensitivity Knob setting may be too low (not enough sensitivity), resulting in low readings. (See Section 3.3.3.)

4. Check filter selection and placement. (Refer to Appendices 6.7 and 6.8.) Don't forget the reference filter!

5. Make sure the correct lamp is installed. (See Appendices 6.7 and 6.8.)

6. On the Continuous-Flow Cuvette System, check for fouling or discoloration of the cuvette. Clean the Flow Cell if necessary. See Appendix 6.4. (If using the 3 mm Flow Cell, refer to the instructions that accompanied it.)

5.6 READING IS OVER-SCALE/OFF-SCALE

1. If measuring test tube samples with the Cuvette Adaptor, make sure the Light Shield is in place.

2. Verify that Auto-Man Switch is set to "Auto."

3. Select the X100 (new concentration range labels, see Section 3.1) position on the X1-X100 Switch. (See Section 3.3.3.)

4. Check calibration. The Sensitivity Knob setting may be too high (too sensitive). (See Section 3.3.3.)

5. Check filter selection and placement. (Refer to Appendices 6.7 and 6.8.) Don't forget the reference filter!

6. Make sure the correct lamp is installed. (See Appendices 6.7 and 6.8.)

7. In some applications, you will need an attenuator plate. (See Appendices 6.7 and 6.8., and Section 3.3.3.)

5.7 NO RESPONSE FROM THE INSTRUMENT

1. Make sure power switch is on.

2. Check power cable to see that it is securely tightened to Power/Telemetry connector. (See Section 2.2.1.)

3. Check power source. (Low battery level, etc.)

4. Check and replace fuses if necessary. Fuse caps can be removed by depressing and turning counterclockwise. See item #12, Section 2.1.

5.8 UNABLE TO BLANK TO ZERO

1. If measuring test tube samples with the Cuvette Adaptor, make sure the Light Shield is in place.

2. Select the X1 (new concentration range labels, see Section 3.1) position on the X1-X100 Switch. (See Section 3.3.3., method A.)

3. Check calibration. The Sensitivity Knob setting may be too high (too sensitive). (See Section 3.3.3.)

4. Check filter selection and placement. (Refer to Appendices 6.7 and 6.8.) Don't forget the reference filter!

5. Make sure the correct lamp is installed. (See Appendices 6.7 and 6.8.)

6. In some applications, you will need an attenuator plate. (See Appendices 6.7 and 6.8., and Section 3.3.3.)

Appendix 6.1
KEY OPERATING PRINCIPLES OF THE MODEL 10 FLUOROMETER

The following explanation is written for Model 10 users who are interested in some of the inner workings of the instrument but do not have a laboratory or instrument background. It is not intended to be a thorough course on fluorometry but rather an explanation that will make you feel more comfortable with the instrument as you use it.

Fluorescence

As you already know, the Model 10 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.

Light from a light source is passed through a color filter that transmits light of the chosen wavelength range (color). This is the exciting light (and excitation filter). 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. (But see Section 3.5.1 for a discussion of linearity.)

The emitted light goes out in a sphere. That which is headed for the detector (usually at right angles to the exciting beam) is passed through another optical filter (emission filter). The purpose of the emission filter is to prevent any scattered exciting light from reaching the detector (in this case a photomultiplier tube) and to pass the emitted color that is specific to the analyte of interest.

The photomultiplier tube looks something like a vacuum tube, which you may have seen in communications or laboratory equipment. Like a simple phototube or photodiode, it generates electrons (electric current) in response to photons (light). What is different about a photomultiplier tube, however, is that it contains many stages (in this case, nine), each of which multiplies the electrons coming from the previous stage. Thus the current is multiplied many times before the amplifier in the fluorometer has to take over.

The wavelength of the exciting light that falls on the sample is set by the choice of the light source and the excitation filter. This wavelength is chosen (1) for strong absorption by the material under study, and (2) for minimal absorption by any interfering fluorescent materials that may be present.

The choices of photomultiplier and emission filter are made so that (1) they respond as much as possible to the light emitted by the material under study, (2) they respond as little as possible to the emission of any interfering fluorescent materials which may be present.

Refer to Figure A1 to see the optical system of the Turner Designs Model 10 Fluorometer.

Stability

While the process just described is straightforward, it is challenging to provide an instrument that measures sample with great sensitivity and stability under harsh conditions with less than perfect power supplies. The Model 10 Fluorometer achieves stability (minimal drift) by recalibrating itself 13 times a second.

When you are in the middle of a measurement and you have difficulty with your power supply or some other environmental condition, you may wonder if this affects the accuracy of your result. In most cases, it does not, because the instrument is constantly recalibrating itself. It does this by continually looking at the light that passes through the flow cell, then looking at a reference light (that comes from the same light source), and then at total darkness. In a sense, it triangulates itself using these three readings to stay at the same electronic reference point.

Since the same light source and detector are involved in both the measurement and reference path, variations in intensity of the lamp and in sensitivity of the detector are automatically compensated for. This is no little feat when you consider that the sensitivity of a nine-stage photomultiplier tube varies with the ninth power of the voltage. This compensation scheme is so efficient that only very rapid surges in voltage will cause an error, and the instrument will compensate within seconds.

Sensitivity

The Model 10 Fluorometer is highly sensitive. It can measure samples with either very low concentrations or very high concentrations of the analyte of interest, without operator recalibration. Again, the photomultiplier tube is at the heart of this process. The following analogy will help you understand how it works.

If you were in a completely darkened room, you would be able to observe even a tiny spark of light. That same spark of light would not be noticed in bright sunlight; in fact, a

flashlight pointed at you might not be noticed in bright sunlight, because your eye adjusts as it moves from darkness to light. The instrument functions in a way that allows it to see not only that tiny spark of light in a dark room but also a larger spark of light in a brighter area. It does this by adjusting the photomultiplier tube in the instrument. The instrument, in effect, pours light into the room or darkens the room, depending on what level of sensitivity is required. You and the instrument control the "light into the room" through instrument controls that act like shutters or curtains. When you adjust the fluorometer's Sensitivity Knob (which crosses or uncrosses two polarizers) or change the X1-X100 Switch (which is a discrete light-limiting optical filter), you are in essence opening or closing devices not unlike Venetian blinds. As you let in less light, you increase the sensitivity of the photomultiplier tube and enable it to sense smaller changes in the amount of light emitted by your sample.

Once you make the coarse adjustments with the Sensitivity Knob and set the X1-X100 Switch, the instrument can make the remaining adjustments. When the Auto-Man Switch is set to Auto, it automatically adjusts the light through a process we call autoranging. The "clicking" you hear is a wheel rotating other light-limiting filters into the reference beam. Thus, if the light into the photomultiplier tube is so low that the reading registers less than a preset value, the instrument knows more sensitivity is needed. It automatically "ranges" or reduces the light (in the reference path) to the photomultiplier tube by filtering out some of the reference light. Conversely, if the reading is more than another preset value, the instrument turns up the reference light (opens the curtains) so larger amounts of light from the sample can be accurately measured.

The Model 10 is designed with range multipliers, which are, by convention, sensitivity multipliers. These ranges have the values: Min Sens, X3.16, X10, and X31.6. (See Section 3.1 for information on relabeling for concentration range mulipliers.) The range multipliers are adjusted at the factory for your main application. If you change applications, internal adjustments need to be made, or a correction factor allowed for. (See Section 3.5.2 and Appendix 6.7.2.)

In summary, we adjust the sensitivity of the instrument by adjusting the reference light, which the instrument shines on the photomultiplier tube. You make the initial adjustments using the X1-X100 Switch and the Sensitivity Knob, and the instrument makes the rest of the adjustments through the autoranging feature.

If you are interested in knowing more, consult the references below.

Why Is Fluorescence So Sensitive?

Any compound that can be measured in a fluorometer can also be measured in a colorimeter. After all, the compound has to absorb light in order to fluoresce.

Fluorescence, however, is as much as 10,000 times more sensitive.

A colorimeter (or spectrophotometer) does not measure absorbed light. It measures the transmitted light and subtracts this from the 100% (blank) transmission to get the absorbed light.

For example, you wish to measure the distance between two marks only 0.01 inch apart. The way the spectrophotometer would do it would be to measure from each of them to the wall across the room. It would then subtract these two measurements to get the desired answer. Thus, relatively small errors (on a percentage basis) would totally invalidate the answer.

The fluorometer simply uses a micrometer caliper and directly measures the distance between the marks.

Fluorometry References

1. G. K. Turner, "Measurement of Light From Chemical or Biochemical Reactions," in Bioluminescence and Chemiluminescence: Instruments and Applications, Vol. I, K. Van Dyke, Ed. (CRC Press, Boca Raton, FL, 1985), pp. 43-78.

2. J. R. Lakowicz, Principles of Fluorescence Spectroscopy (Plenum Press, New York & London, 1983).

3. I. B. Berlman, Handbook of Fluorescence Spectra of Aromatic Molecules (Academic Press, New York & London, Second Edition, 1971).

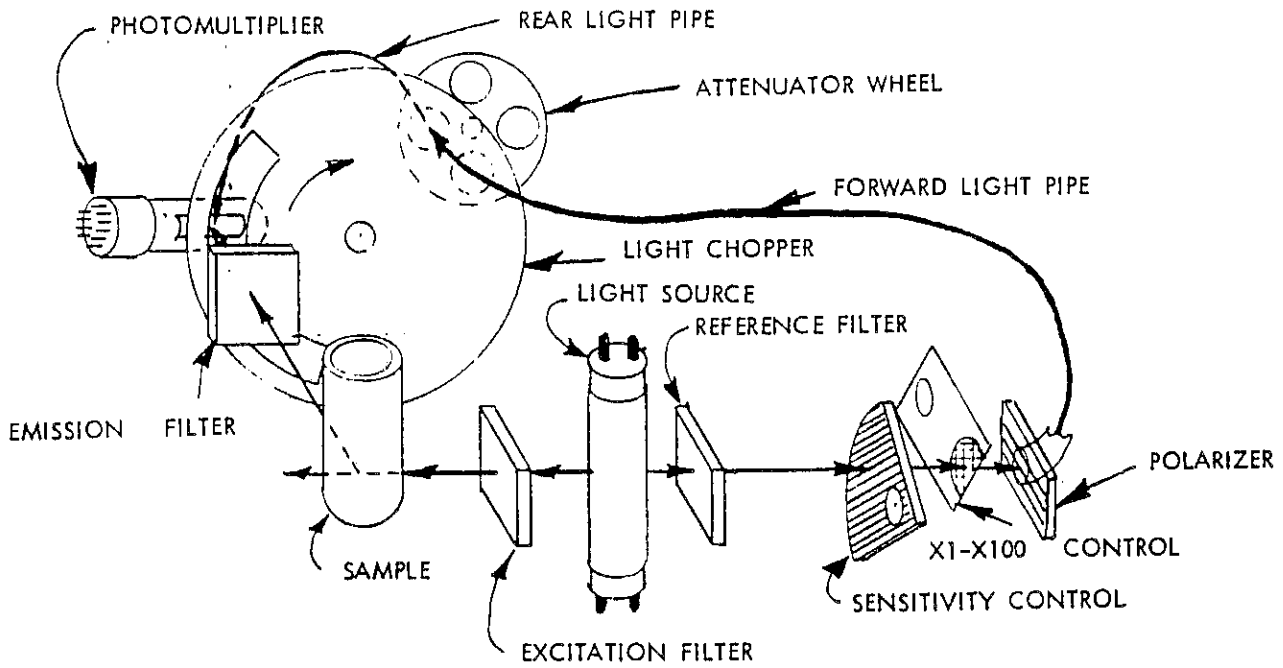

Figure A1. Optical System of the Model 10 Fluorometer

Appendix 6.2
FIELD STUDIES USING THE MODEL 10 FLUOROMETER

The Turner Designs Model 10 Fluorometers have been used for a wide variety of laboratory and field studies. The following are common, but by no means exhaustive, uses for the fluorometer.

6.2.1 Flow Measurements and Dye Tracer Studies

The fluorometer, along with fluorescent dyes, has proven to be an efficient and cost-effective means of flow measurement and pollution control. It can be used to calibrate flow meters on site; calibrate weirs and flumes in the field, correlate stream-level gauges with the flow rate; measure stream, canal, drainage ditch, and sewer flow directly; study sewer system infiltration; study time-of-travel in streams; and measure residence time in settling basins and disinfection chambers.

The following monographs, from the Turner Designs Fluorometric Facts series, can be found at the end of this Appendix for your convenient reference:

"A Practical Guide to Flow Measurement." Provides details about general principles, dyes, and equipment needed.

"Fluorescent Tracer Dyes." Provides information on dyes approved for use as tracers.

"Preparation of Standards for Dye Studies." Gives detailed procedures for preparing standards for dye studies.

The following monographs are available upon request from Turner Designs:

"Flow Measurements in Sanitary Sewers by Dye Dilution." This is basically a "how-to-do-it" manual for quantitating and localizing infiltration and calibrating any type of flow meter.

"Circulation, Dispersion, and Plume Studies." Discusses use of fluorescent dye tracers for thermal plumes, estuary flushing, underground water transport, etc.

"Fluorometry in the Water Pollution Control Plant." Shows how dye studies can be used in a water pollution control plant for everything from conceptual design to troubleshooting.

"Ground Water Tracing." An annotated bibliography comparing fluorescent tracers with other ground water tracers such as chloride, bromide, and tritium.

6.2.2 Oil Measurements

The aromatic (carcinogenic) fractions of petroleum products are fluorescent. These dissolve in the water column following a spill and move separately from the unsightly slick on the surface. Since no sample preparation is required, the fluorometer has become very popular for tracking these allowing for protective measures for downstream water supplies and shellfish beds. It is also commonly used to map a spill following the use of dispersants. Other uses include performing baseline oil studies; pinpointing oil leaks and natural seeps; and measuring oil in organisms, sediment, and air. Refer to the appended monograph, Fluorometric Facts: "Oil in the Environment."

Recently, the Model 10 has been used to monitor oil in process water and in cooling water. Refer to the appended monograph Fluorometric Facts: "Petroleum in Water."

6.2.3 Chlorophyll and Pheophytin Studies

Fluorometric techniques have many advantages for both qualitative and quantitative measurements of chlorophyll and its primary degradation product, pheophytin. These techniques are relatively simple compared with spectrophotometry, as well as faster and more sensitive. In many applications, the fluorometer can be used in vivo, on a continuous-flow basis, eliminating delays for extraction and processing. Refer to the appended monograph, Fluorometric Facts: "Chlorophyll and Pheophytin."

6.2.4 Process Control

The Model 10 can be used for on-line monitoring of industrial processes. It can be operated continuously over extended periods of time, with minimal operator supervision.

Appendix 6.3
ACCESSORIES FOR THE MODEL 10 FLUOROMETER

Turner Designs carries accessories for all aspects of the Model 10 Series Fluorometers. Refer to the Turner Designs Series 10 Ordering Information booklet at the end of this manual.

6.3.1 Batteries for DC Power

For DC operation, the Model 10 Fluorometer will function properly on any portable generator providing 105-130 volts AC at 50-400 Hz, or battery that will provide 11-16 volts DC at 2 amperes. The negative lead should be grounded.

For portable applications, a battery is more commonly used. The prime requirement is that it must deliver 2 amperes for the period of expected operation without the having the voltage drop below 11.

A 12-volt lead-acid battery is probably the best choice for most applications. One example is a battery designed for snowmobiles, with special caps to prevent any battery acid loss. The Gould SN-9L is rated at 32 amperes, weighs 21 pounds, and is 7 3/4" x 5 1/4" x 7 1/4". It has a life of about 15 hours.

A disadvantage of this battery and all automotive-type batteries is that they are not designed for complete discharge without damage unless they are recharged immediately after each discharge.

A battery designed especially for field work, permitting complete discharge without immediate recharge, is the Globe GC-1220B 20-ampere hour battery. It weighs 16 pounds, and is 7" x 6 1/2" x 5". It has a life of about 10 hours.

6.3.2 Pumps for Continuous-Flow Systems

Normally, sampling using the Model 10 High-Volume, Continuous-Flow System is done with a pump.

Flow Rate. We are frequently asked what flow rate is appropriate for continuous measurement in a flow

cell. With two exceptions (discussed below), it does not matter.

Fluorescence typically occurs about ten nanoseconds after the molecule absorbs the exciting light. For a molecule to be excited on entrance to the active zone of the continuous flow attachment, and exit prior to emission, would require a flow rate of approximately 500,000 gallons per minute. Sample velocity would be 5 million miles per hour!

The flow rate to be used, therefore, is at the discretion of the operator. The flow rate will be a compromise between the desire to minimize transit time and the pump power and size available. The maximum flow rate that will not exceed the pressure rating of the High-Volume Continuous-Flow Attachment (25 psig) is approximately 150 gallons per minute (570 liters/minute).

The two exceptions, where rate of flow could be important, are:

1. Where measurements are particularly temperature sensitive, as with rhodamine and chlorophyll. In these cases, with extremely low flow the fluorometer could raise the temperature of the sample during passage through the flow cell. Although this has not been precisely tested, we expect that a minimum flow of 50 milliliters/minute is safe.

2. In the in vivo measurement of chlorophyll, where certain species of phytoplankton exhibit an induction effect if a long opaque hose is used (i.e., the organisms partially dark-adapt). It is recommended that the flow rate not exceed 600 milliliters/minute. High flow rates may yield falsely high readings. Refer to the section on Continuous-Flow Sampling Methods in the monograph "Chlorophyll and Pheophytin," in Appendix 6.2.

Centrifugal pumps. These are the least expensive and best suited for use with the Continuous-Flow System. No matter what system is used, keep in mind that the presence of air bubbles will affect measurements; occasional bubbles are not a problem, but continuous, numerous air bubbles will invalidate the measurements.

Submersible pumps. Often used and very satisfactory. A commonly used pump for shallow sampling is a battery-operated bilge pump. Capacity is not important as long as the pump will operate against the head. Remember that once started, and returning to the surface, the head is zero. The problem is that a bilge pump will need help in getting the system primed if the fluorometer is much more than about four feet above the surface. An adequate capacity is 400 gallons per hour. Turner Designs supplies an appropriate sample pump. Refer to the Turner Designs Series 10 Ordering Information booklet for specifications.

Above-water pumps. Satisfactory, although they frequently introduce bubbles by air leakage and cavitation. Therefore, it is recommended that this type of pump be mounted on the discharge side of the fluorometer. Keep in mind, also, that the sample is under suction and there is some danger of bubble formation, unless the rate of sampling and the operating head are kept relatively low.

6.3.3 Hoses

In dye studies, adsorption of rhodamine WT is not normally a problem in nature. It is not adsorbed significantly on suspended solids. It is, however, adsorbed by soft vinyl tubing (e.g., Tygon) and by rubber and most rubber substitutes. Thus, if you use sample tubing of such material with a high concentration of dye, it may be some time before the fluorometer reading returns to a true background, even though you are sampling an area where there is no dye. In other words, it will take up some dye, then gradually bleed it out. The error will depend on the sampling rate. If the flow rate is high, the error may be minimal.

Polypropylene and high-density polyethylene do not adsorb the tracer. They are, however, stiff and somewhat difficult to work with. The most common sample hose is green garden hose. If you accidentally contaminate it with a high concentration and you must go quickly to low levels, simply be prepared to replace it.

Rubber hose is not recommended.

The hose should be completely opaque, or the portion attached to the intake and exhaust fittings of the fluorometer must be wrapped carefully with

black tape. Wrapping a distance of three or four feet from the fluorometer fittings is generally satisfactory, depending on the diameter. The object is to prevent outside light from reaching the photomultiplier tube. To check, shade the hose with the instrument on a sensitive range - direct sunlight and shade should give the same reading.

On the Model 10, both intake and exhaust fittings are 1/2" female pipe thread. For laboratory studies, where smaller intake tubing might be desired, Turner Designs makes a tubing adaptor that will accept 3/16" to 1/4" (ID) plastic tubing. Refer to the Turner Designs Series 10 Ordering Information booklet.

6.3.4 Dye Injection Pumps

For some studies using fluorescent dyes, a dye injection device is very useful. For flow rate measurements, an accurate injector is mandatory. There are three basic types of constant-rate injectors: constant displacement pumps, constant-head (gravity-feed) devices, and regulated pressure systems. Turner Designs carries a durable, battery-operated constant displacement injection pump manufactured by Fluid Metering (FMI). Refer to the Turner Designs Series 10 Ordering Information booklet for specifications.

To decide on the appropriate dye injection device for your study, refer to the section on Dye Injectors in Fluorometric Facts: "A Practical Guide to Flow Measurements," in Appendix 6.2.

6.3.5 Recorders and Data Loggers

(See Appendix 6.10 for information about telemetry pins.)

The Model 10 Series Fluorometer will operate virtually any recorder. An integrating feature may be desirable for some studies, but special features found on some more expensive recorders, such as logarithmic presentation, X-Y recording, and scale expansion are of little value.

The recorder should be a linear strip-chart recorder with an accurate time drive. A range of chart speeds is valuable, as the speed of paper drive can be adjusted to fit the experiment. A range of 0.05 inch per minute (3 inches per hour)

to 2 inches a minute (10 feet per hour) should cover most studies.

Turner Designs supplies both a galvanometer- and a servo-type recorder. Refer to the Turner Designs Series 10 Ordering Information booklet for specifications.

An IBM-compatible pocket data logger is also available from Turner Designs. Three channels will record fluorometer reading and ranges, with a fourth channel for temperature input. Refer to the Turner Designs Series 10 Ordering Information booklet for specifications.

Appendix 6.4
SAMPLE SYSTEM: REMOVAL, INSTALLATION, & MAINTENANCE

The 10-020 High-Volume Continuous-Flow Cuvette System (25 mm) is standard with your fluorometer, unless you requested otherwise. The cuvette is made of borosilicate glass. The system has a pressure rating of 25 psig, with Intake and Exhaust Fittings of 1/2" female pipe thread.

NOTE: There are other sizes of continuous flow cuvettes available, including a 3 mm flow cell, useful for measuring higher concentrations. The shorter path length increases the linear range and reduces the effects of light-absorbing materials. (See Appendices 6.1 and 6.9; consult the Series 10 Ordering Information booklet.) If you are using one of the smaller flow cells, see the installation instructions accompanying it.

6.4.1 Continuous-Flow Cuvette Removal

1. Drain the system! Remove all fittings from the Intake and Exhaust Fittings.

2. Remove the 14 #8-32 screws that retain the Sample Compartment Cover. If the gasket under this cover sticks, remember that it is glued to the cover. Inserting a thin knife gently between the gasket and the Sample Compartment Casting should be sufficient.

   See Figure A2 for locations of various parts with the Sample Compartment Cover removed. (See Figure A3 in Appendix 6.7 for a view with the filters removed.)

3. If you have just received your instrument, the Lamp should be installed.

   Note that readings on a given fluorometer are affected by the unique properties of the individual lamp installed.

   If the lamp has been installed and you want to maintain calibration, you should mark the Lamp so that it may be returned to its original position.

A dab of nail polish on the lower base of the lamp works well.

Remove the lamp (if installed), by rotating 90 degrees and pulling it out toward you.

If you are switching applications (i.e., from rhodamine to chlorophyll, etc.), please refer to Section 3.5.2 for important information about internal range adjustments.

4. Remove the Upper Stud and the Lower Stud. Remove the Excitation Filter Holder Assembly, by sliding it to your right and toward you.

5. Using the Allen wrench (5/64") stored in the Desiccant Holder, loosen the Upper Set Screw. Pull up on the Exhaust Fitting, rotating it back and forth a little, to free it. Be careful! The Cuvette may come out with the Exhaust Fitting. If it does, remove it with the Exhaust Fitting. If not, pull it up and out after you have removed the Exhaust Fitting.

6. Loosen the Lower Set Screw. Pull down on the Intake Fitting, rotating it back and forth a little, to free it.

   NOTE: The Intake and Exhaust Fittings are identical. Don't worry about getting them mixed up.

7. Wipe up any spilled liquids. If any old Desiccant bags are in place, remove and discard them.

6.4.2 Continuous-Flow Cuvette Installation

Normally, this procedure will start with the following items removed from your instrument:

1. Sample Compartment Cover, and 14 screws.
2. Lamp.
3. Upper and Lower Studs.
4. Excitation Filter Holder Assembly.

Proceed as follows:

1. Carefully inspect the two O-rings on the Exhaust Fitting and two on the Intake Fitting for nicks or tears. If there is any sign of

deterioration, replace them with new O-rings, found in your 10-021 Cuvette Replacement Kit. (This kit was supplied with your instrument.)

If you do replace the O-rings, order a 10-022 O-Ring Kit.

Be sure that the round rubber flat washers supplied on the Exhaust and Intake Fittings are in place. These flat washers are near the smaller O-rings, against the flat surface on the fittings that makes contact with the end of the cuvette.

CAUTION! These flat washers should be removed when the 10-033 Continuous-Flow Nephelometry Kit is installed.

2. Lubricate the O-rings. You'll find excess lubricant with the O-rings in your Cuvette Replacement Kit. Silicone oils will also do.

3. Work the Intake Fitting up into position. The threads can face toward you, or to the left, but not at an intermediate angle.

   Tighten the Lower Set Screw.

4. Carefully clean the cuvette. Slip it down through the hole where the Exhaust Fitting will eventually go, and press it into place on the Intake Fitting.

   The plastic handle of a screwdriver may be used to push the cuvette into place.

5. Work the Exhaust Fitting down until it almost engages the cuvette. Be sure that the cuvette is properly aligned with the Exhaust Fitting. Push the Exhaust Fitting down, to engage the Cuvette fully.

   Note that the Exhaust Fitting may also face toward you or face to the left, but may not be at an intermediate angle.

6. Loosen the Lower Set Screw on the Intake Fitting.

   Adjust the fittings up and down until the Cuvette is centered.

The ends of the Cuvette should contact the flat rubber washers described in paragraph 1, above, but should not compress them. (The liquid seal is supplied by the O-rings. The flat rubber washers protect the cuvette from direct contact with the metal Exhaust and Intake Fittings.)

7. Rotate the Fittings a little as you slowly tighten the set screws - so the set screws are centered on the flats on the fittings. Return the Allen wrench to its storage place. Wipe any fingerprints off the Cuvette.

   If you have any questions about leakage, now is the time to hook up the external connections, and make a visual check.

8. Check that the Emission Filters are in place. (See Appendices 6.7 and 6.8.) Install the Excitation Filter Holder Assembly with the Upper and Lower Studs.

9. Install the Lamp. See Appendix 6.4.1, part 3.

10. Install Desiccant, if needed. See Section 6.4.5 below.

11. Put the Sample Compartment cover in place and install all 14 screws very loosely. Progressively tighten all screws until they are snug, but not dead tight.

6.4.3 Continuous-Flow Cuvette Maintenance

As long as the cuvette appears visually clean, and there is no sign of leakage, maintenance is not required. If the fluid lines leading to the system are moved or stressed, check for leaks!

The Cuvette may be inspected by going through steps 2-4 in Section 6.4.1, above. To remove it for cleaning, drain the system, remove external connections, then continue with Section 6.4.1.

The Cuvette is borosilicate glass, and may be cleaned by normal techniques.

The O-rings are a Nitrile (Buna N) rubber. Chemical resistance is good. They must be lubricated before reassembly. See Section 6.4.2.

The Intake and Exhaust Fittings are nickel-plated brass. Do not use strong chemical cleaning agents.

6.4.4 Continuous-Flow Cuvette External Connections.

As received, your instrument has both the Intake and the Exhaust Fittings installed so that their threads face forward. If it makes your plumbing job easier, either or both of these fittings may be set so they face left. See Section 6.4.2 above. Intermediate positions are not available. Pull the fittings out before rotating them.

The following points should be considered, when making external connections:

1. The fittings accept standard 1/2" ips male pipe threads. Pipe dope or the plastic tape sealers will be required.

2. Don't over-tighten! It is possible to break the solder joint in the fittings - and also, with extreme force, you could distort the fittings to the point where the rubber-cushioned cuvette will break.

   We do not recommend rigid pipe hook-up.

3. Normally, you'll be going to a hose on both the Exhaust and Intake fitting. This hose must be opaque for the first several feet, at least. If not, light can "leak" in and upset your measurements.

   If you have any doubts about light leakage, shade the hose, and see if the reading changes. Select the most sensitive instrument range that you plan to use.

4. If you have any question about Cuvette breakage or leaks, check visually for breaks, then turn the sample system on and check for leaks. See Appendix 6.4.3.

5. Air bubbles will cause erratic readings. The packing gland of a pump is often a source of inward air leakage. This problem is often cured by putting the sample pump on the exhaust end of the system, so it sucks sample through rather than pushes it through.

6. CAUTION! Remember that the Sample System is rated at 25 psig! If, for example, you are using a pressurized system to combat dissolved gas release, etc., be sure you do not exceed this rating.

6.4.5 Desiccant Use With the Continuous-Flow Cuvette.

Condensation forming on the outside of the Cuvette can cause drifting readings as it builds up. If there is a sufficient volume of air to pull moisture from, it can also cause erratic readings as droplets break free and run down.

For this reason, the entire sample area is gasketed and sealed, and a space for desiccant is provided.

Usually, you won't have any problem, even without desiccant, since the free volume of air inside the sample area is small, and there just isn't much moisture to condense out.

If problems are encountered, proceed as follows, referring to Figure A2:

1. Remove the Sample Compartment Cover (14 screws).

2. Remove and discard any spent desiccant.

3. Remove the tape seal from the bottle of desiccant packages supplied with your instrument. Remove two packages, close and re-seal the bottle.

4. Place the packages in the position shown in Figure A2.

5. Re-install the Sample Compartment Cover with a minimum of delay. All 14 screws should be installed loosely - to allow best hold alignment. Then tighten progressively until all screws are snug, but not dead tight. (Overtightening can cause distortion of the cover and leakage.)

6. Order a 10-023 Desiccant Replacement Kit (bottle of ten), if needed.

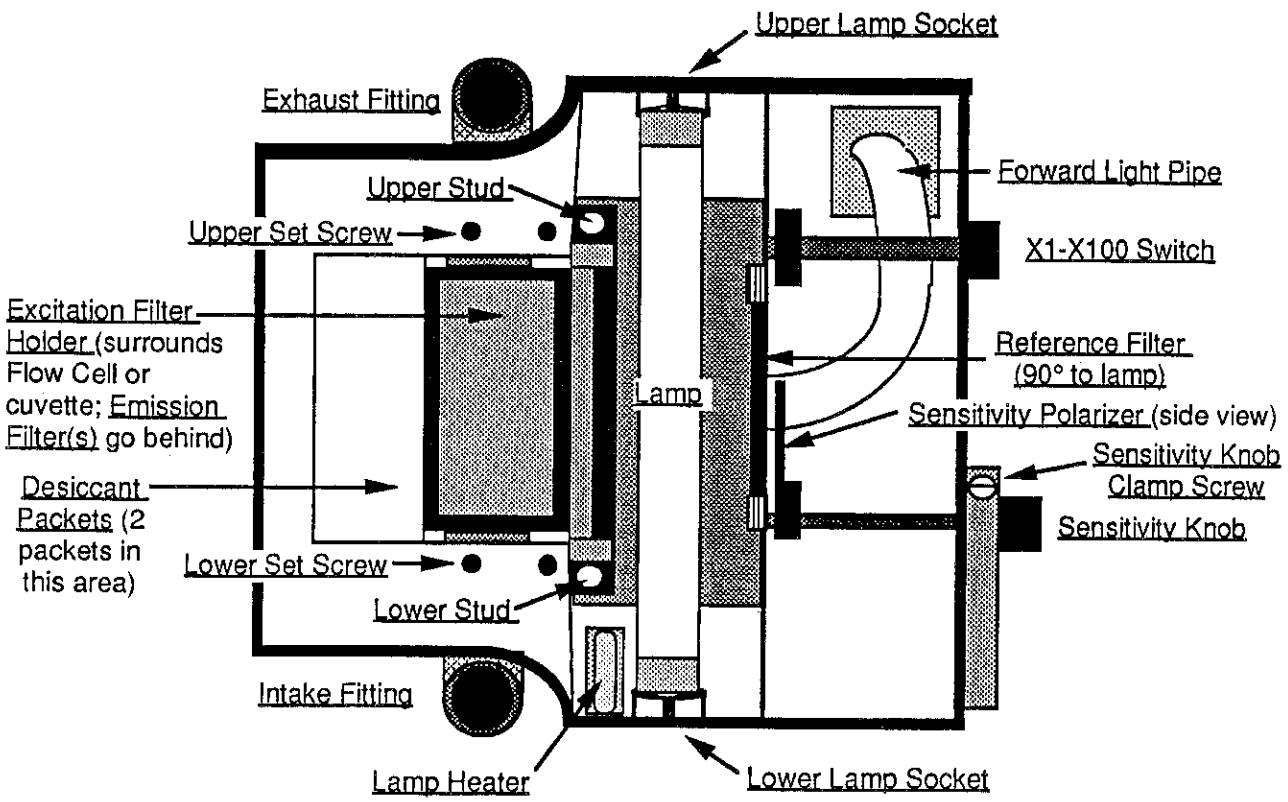

Figure A2. Sample Compartment, Cover Removed

Appendix 6.5
ENVIRONMENT

6.5.1 Temperature

Storage temperature is -20 degrees C to +60 degrees C, as long as the cuvette is empty! Operating temperature is from the freezing point of the fluid in the cuvette (but not less than -20 degrees C) to +50 degrees C, ambient. Expect slow lamp start below +4 degrees C.

6.5.2 Water and Dirt

The Model 10 Rack Mount Fluorometer is not protected against water. All moving parts are shielded against dirt, dust, etc., by the outer case and a tight-fitting inner box. The photomultiplier and the sensitive amplifier associated with it are additionally protected by a second box. Because of this, it may be used in quite dirty locations without damage or loss of performance. Corrosive atmospheres should, of course, be avoided. When the Cuvette Adaptor Kits are installed, the Light Shield should be in place as much as possible, to keep dirt from entering the cuvette well.

The Model 10 Field Fluorometer is resistant to water, when the protective cover is on (for transport) or when it is removed for operation. The design philosophy is "Drop it over-board - it will float. Wash it off with fresh water - it will work," as long as it is equipped with the Continuous-Flow Cuvette available with the instrument or available as an accessory.

When the various discrete or "grab" sample holders are installed, water can get to the optical elements and the wiring and heater associated with the light source. It cannot get to the bulk of the electronics or to any moving parts, but considerable damage can result. (See Section 5.5.)

If water does enter the Sample compartment:

1. Turn power off immediately. There is no hazard, but electricity and water together will quickly destroy the lamp wiring and lamp heater assembly.

2. Remove the Sample Compartment Cover (14 screws). Remove the light source, all filters and the Excitation Filter Holder. See Appendix 6.4.1 and Figure A2 in Appendix 6.4.

3. If the water that entered the Sample Compartment was salt water, flush the affected area with fresh water, then with deionized or distilled water.

   If only fresh water entered the Sample Compartment, flush the affected area with deionized or distilled water.

4. Dry with gentle heat. (A hair dryer works.)

5. Put it back together, and return to service.

   If the Field Fluorometer is immersed with the cover on, the cover should be removed as soon as possible, and any water in the area between panel and cover allowed to drain out.

   Saltwater should be flushed off as soon as possible.

6.5.3 Vibration

The instrument is designed to operate satisfactorily when used in an environment satisfactory for normal mobile equipment such as radio-telephones, etc. The limiting factor will be keeping "grab" samples in the cuvette.

6.5.4 Helium

Surprisingly enough, helium will ruin photomultipliers in a fairly short time. If you are working on exotic breathing mixtures, or other systems using helium, keep it away from the fluorometer.

6.6.5 Water Condensation

If the instrument has been in a cold area, then moved to a hot, humid area, it may fail to operate correctly when first turned on. Turn on the fluorometer and let it run. Internal protective circuitry will burn any condensate out in 15-20 minutes.

Appendix 6.6
MOUNTING CONSIDERATIONS

6.6.1 Mounting Position

Except for the problem of keeping "grab" samples in the cuvette, the instrument will operate in any position. However, for greatest stability, the average position of the instrument should be within 20 degrees of level, for proper convective cooling of the light source. With the continuous flow cuvette (normally supplied), this will also allow best purging of any air bubbles associated with the sample.

6.6.2 Mechanical Considerations

The Model 10 Rack Mount Fluorometer may be mounted directly in any standard 19" electronic rack, with four 10-32 screws. Panel space required is 7". The instrument extends 7.5" behind the rear surface of the front panel, and at least an extra half inch should be allowed.

For laboratory service, it may also be mounted in our 10-002 Laboratory Case. Please see the Model 10 Brochure and the Turner Designs Series 10 Ordering Information booklet for specifications.

In any boat or vehicle installation, the important thing is to tie it down! As the old story goes, "It isn't the fall that hurts, it's the sudden stop." For the Field Fluorometer, consider installing a standard automobile safety belt to hold the instrument securely in a moving vehicle.

6.6.3 Effect of Nearby Equipment

Watch overall temperature rise in enclosed racks. While your fluorometer will work fine on a scorching hot day, it will not operate as hot as some purely electronic equipment.

A second problem with temperature rise is caused by the temperature coefficient of the optical filters used in your fluorometer. See the Filter Selection Guide, Appendix 6.8, for details. Heat may be caused by other equipment; temperature should be kept low and constant.

6.6.4 Access Requirements

During normal operation, access to the front panel controls only is needed.

In situations where dirty water is being handled, you should have access to allow cleaning of the continuous flow cuvette. See Section 6.4.3 above. Refer to Figure A2, Appendix 6.4; there should be enough room to slide the Exhaust Fitting straight up 1-1/2" and to slide the Intake Fitting straight down 1-1/2" from their installed positions.

If you are taking "grab" samples with the Cuvette Adaptor Kits, be sure to allow enough room for easy cuvette insertion.

If you must install and remove the 10-030 Cuvette Holder, Temperature Controlled, and 13 x 100 mm cuvettes, allow room for a 6" long cylinder to be inserted where the Intake Fitting is shown in Figure A2, Appendix 6.4.

Appendix 6.7
FILTER AND LIGHT SOURCE REPLACEMENT

Readings on a given fluorometer are affected by the unique properties of the individual lamp and filters installed. Therefore, please note the cautionary remarks about lamps and filters when replacing them.

If you are changing from one application to another (i.e., from rhodamine to chlorophyll), the accuracy of your readings, if the instrument steps (changes range), will be affected due to factory-set internal ranges. See Section 3.5.2.

There are three different types of filters on your fluorometer (all located inside the sample compartment): the reference filter, the excitation filter (mounted in a holder that fits around cuvette), and an emission filter(s). See Figure A3 for their locations. They are made of glass and will break if dropped, so handle them carefully. See Appendix 6.8 for a description of the filters needed for your application.

Although filter problems are not common, missing, broken, or improperly mounted filters can result in erratic or unstable readings, high or low readings, or the inability to blank to zero. Many filters are laminated (a plastic filter sandwiched between two layers of glass). With age and exposure to moisture, a filter can separate; i.e., it will no longer have a uniform color and density. To check, remove the filter and hold it up to a light. Color and density should be consistent; slight wrinkling is acceptable.

6.7.1 Replacing the lamp or filters.

1. Remove the 14 #8-32 screws that retain the Sample Compartment Cover. If the gasket under the cover sticks, remember that it is glued to the cover. Inserting a thin knife gently between the gasket and the Sample Compartment Casting should be sufficient.

2. Remove the lamp (if installed), by rotating it 90 degrees and pulling it out toward you.

   NOTE: If you are changing filters within the same application and desire to maintain calibration, you should mark the Lamp so that it may be returned to its original position.

A dab of nail polish on the lower base of the lamp works well.

3. Remove the Upper Stud and the Lower Stud. Remove the Excitation Filter Holder Assembly, by sliding it toward your right and then toward you.

   NOTE: If you are changing filters within the same application and you desire to maintain calibration, do not remove the filters from their holders. Rather, remove and install the filters in their Holder Assembly, keeping an Excitation Filter Holder (part no. 10-080) for each set of filters. This is particularly important when using the clear quartz lamp, which will gradually (over a period of months or years) change the characteristics of the filter installed.

   The emission filters are not subject to such changes.

4. Locate the emission filters for your application. Refer to the Filter Selection Guide, Appendix 6.8, for details.

   Note that these filters must be in the specified order, and with the color specification marking forward.

5. The emission filters mount directly behind the cuvette. See Figure A3. Remove any filters present, and install the new ones.

6. If the Continuous-Flow Cuvette is being used, wipe off any fingerprints with a lab wipe.

7. If changing to a new application, locate the excitation filter for your application. Refer to the Filter Selection Guide, Appendix 6.8, for details.

   See Section 3.5.2 for important information about adjustments to internal ranges.

8. Where a 10-080 Excitation Filter Holder complete with excitation filters is available, go on to step 9.

   When excitation filters must be installed, remove the flat plate with the large central hole from the Excitation Filter Holder Assembly. (The plate is located on the side that is toward the light source. There are two #4-40 nuts and lock washers.)

Remove any existing excitation filter under this plate. Put the desired filter in place and clamp it to the Excitation Filter Holder Assembly with this plate.

If an Attenuator Plate is necessary, install it at this time by placing it on top of the existing flat plate, before reinstalling the nuts. (See Section 3.3.3, Appendix 6.8, and Figure A3.)

Tighten the two nuts enough to bend the plate just a little. Do not over-tighten, as the filters may crack.

9. Install the Excitation Filter Holder Assembly (fits around the cuvette) with the Upper and Lower Studs. See Figure A3.

10. Locate the Lamp for your application. (See Appendix 6.8.)

WARNING! Under no circumstances let light from the clear quartz lamp fall on your eyes! Serious eye irritation will result - with a delay of about four hours between exposure and symptoms.

11. When using the clear quartz lamp, check that there are no mercury droplets between the two pieces of black tape. If present, dislodge by gently tapping the lamp.

Install the Lamp by inserting the two end prongs into the slots on the upper and lower lamp sockets and turning 90 degrees.

If you wish to maintain calibration, the lamp should be reinstalled in the original position. See paragraph 2, above.

12. Locate the Reference Filter for your application. Refer to the Filter Selection Guide, Appendix 6.8, for details.

Install it under the two spring clips, just to the right of the lamp. See Figure A3.

13. Put the Sample Compartment Cover in place and install all 14 screws very loosely. Progressively tighten all screws until they are snug, but not dead tight.

If the Cuvette Adaptor is in place, reinstall the Light Shield.

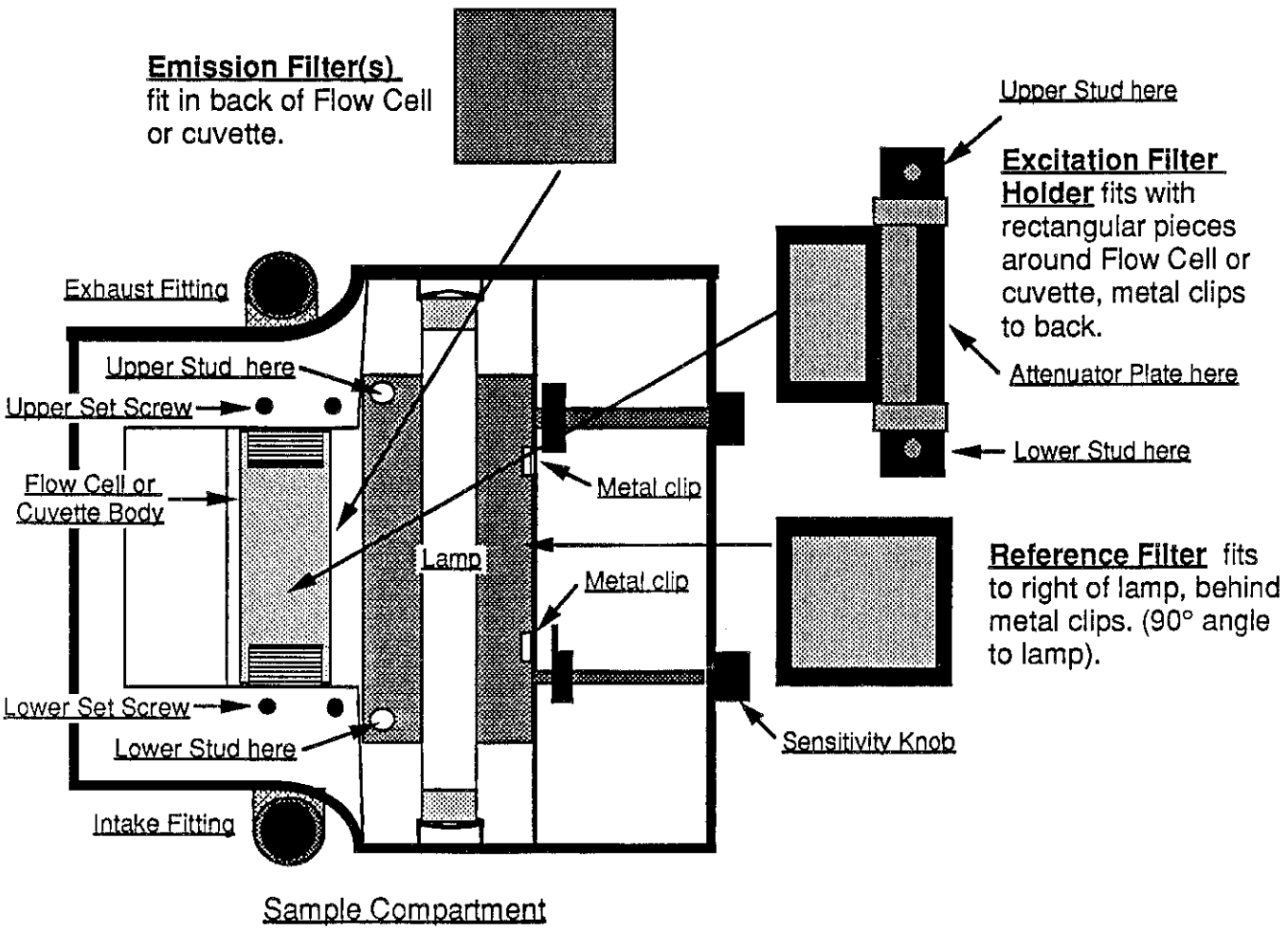

Figure A3. Filter Locations

6.7.2 Obtaining a compensation factor for internal ranges when changing applications.

Note: All references to range multipliers are in the reversed (concentration) mode where the most sensitive configuration is the X1-X100 Switch in X1 position and the range light in the 1 position. See Section 3.1.

The range multipliers on the Model 10 Fluorometer are controlled by optical filters in the reference path. There are four of these, corresponding to ranges 1, 3.16, 10, and the 1 position of the X1-X100 Switch. The 31.6 and 100 positions are empty. Of the four range lights, position 1 has a (nominal) 3.16% transmission filter. This decreases the light in the reference path 31.6-fold (compared with no filter). The instrument sees this reduced light and forces the output of a standard reference voltage. To do this, the electronics increases the photomultiplier voltage to increase the instrument sensitivity 31.6-fold.

The 3.16 position has a 10% filter; the 10 position a 31.6%; and the 1 position of the X1-X100, a 1%. These filters are what are called "neutral," meaning that their transmission is independent of wavelength. Unfortunately, this is only approximately true and of course they really are not exactly the values stated. At the factory, electronic trimming is used to correct the range multipliers to your principal use.

If you change applications, more specifically if you change the light source and/or the reference filter, the color (the wavelength distribution) of the light in the reference path will change. This will cause the internal ranges to change slightly (perhaps as much as 4%). Also, there will be a very gradual change in the ranges as the optical filters in the stepping mechanism and in the X1-X100 Switch age. If you wish to adjust the ranges internally, ask Turner Designs for a copy of "Internal Range Multiplier Adjustment for the Model 10 Fluorometer." If this is a temporary change of application, it is probably not worth it. It is easy to develop a correction factor for each range.

To obtain correction factors for the ranges, you may need two concentrations, high and low, of the analyte you will be working with. When you are no longer able to adjust with the low concentration of

dye, use the high one. It is possible that you will be able to obtain the factors with the low concentration alone.

We normally use rhodamine WT for trimming for both rhodamine and chlorophyll. It takes a higher concentration of rhodamine for the chlorophyll filters, but the solution does not have to be in the linear range. Using chlorophyll itself has the disadvantage that it is not stable.

Rhodamine. For checking the factors for rhodamine, you need a solution of about 20 ppb active ingredient (100 ppb of the 20% aqueous solution) and about 80 ppb active ingredient (400 ppb of the 20% solution). These concentrations need not be precise, as the concentration is not involved in the calculation.

Chlorophyll. For chlorophyll, you need rhodamine solutions of about 80 ppb active ingredient (400 ppb of the 20% solution) and 2 ppm active ingredient (10 ppm 20% solution).

Recorder or Meter. If you normally collect your data via recorder or computer, you should use the recorder output in generating the correction factors. At the factory, we use a digital voltmeter. You could also use a good recorder or you could use your computer. The following directions are written for the panel meter. If you use voltage, full scale corresponds to 5.00 volts. Therefore when the directions call for setting the reading to 10, or slightly under, you would aim for 5.00 volts, or slightly under.

Procedure. Turn the fluorometer on and let it stabilize for at least five minutes. During the course of the determination, the instrumental and solution temperatures will change. This is not important. It is only important that pairs of readings (which can be taken pretty rapidly) are done at the same solution temperature.

Start with the X1-X100 Switch in the X1 position. The Auto-Man Switch should be in Man. With the Step Switch, move the range light to the "X31.6" position. Put the low-concentration solution in place and use the Sensitivity Knob and/or the Span Control to make the meter read about 10. It is not important that it be exactly 10, but it should not be higher. Note exactly what the number is. Now,

switch the X1-X100 switch to X100 and step the instrument three times, by pressing the Step Switch three times. This will put you in range X1, X100. Read the meter. Your reading should be about 1/3 of the first one. Ideally it would be 1/3.16. If the first reading is 10.0 and the second reading is 2.9, then the factor when going from X1, X31.6 to X100, X1 is 10.0/2.9 or 3.45.

Now do the same thing between ranges X1 and X3.16. Remaining in range X1, adjust it to read about 10. Note the exact reading, then step three times. This should put you in range X3.16. Again read the value and determine the ratio. Then, adjust to read 10, step three times to range X10 and note the reading. Continue with range X31.6

You now will have a table of factors that will look something like the following:

Range	Factor
31.6 (X1, X31.6) - 100 (X100, X1)	3.45
100 (X100, X1) - 316 (X100, X3.16)	3.27
316 (X100, X3.16) - 1000 (X100, X10)	2.95
1000 (X100, X10) - 3160 (X100, X31.6)	3.10

The numbers are, of course, for illustration only. To obtain a complete table, fill in the factors for the ranges 1 to 3.16, 3.16 to 10, and 10 to 31.6. These factors are the same as those obtained for the last three in the above table. (I.e., the factor for X3.16, X1 is the same as for 316 -- 3.27; the factor for X10, X1 is 2.95; and the factor for X31.6, X1 is 3.10.)

To obtain the multiplier for each range, simply multiply the factors. Thus, the multiplier for range X3.16, X1 is 3.27. The one for range X10, X1 is 9.65 (3.27 x 2.95), the one for 31.6 is 29.9 (3.27 x 2.95 x 3.10), etc. Keep multiplying in the new factor.

A complete table will look like this:

Range	Factor	Corrected Multiplier
X1 (X1) - X3.16 (X3.16, X1)	3.27	3.27
X3.16 - X10 (X10, X1)	2.95	9.65
X10 - X31.6 (X31.6, X1)	3.10	29.9
X31.6 (X1) - X100 (X1, X100)	3.45	103
100 - 316 (X3.16, X100)	3.27	337
316 - 1000 (X10, X100)	2.95	995
1000 - 3160 (X31.6, X100)	3.10	3085

Now, when calculating the readout for a sample, note the range you are in (i.e., range and X1-X100 Switch position) and multiply the meter reading times the matching "Multiplier" from the above table. (See Section 3.4.)

For example, to calculate the readout for a sample, multiply the range value times the X1-X100 position times the meter reading. For example:

10 x 100 x 4.2 = 4200

To use the corrected multiplier, replace the 10 times 100 value with 995, which yields:

995 x 4.2 = 4179

Appendix 6.8
FILTER SELECTION

6.8.1 Theory of Selection

Fluorometric analysis is based on the measurement of fluorescent materials absorbing light at one wavelength and converting it into light at a longer wavelength.

Two primary considerations in selecting the proper filter, light source, and light detector are:

1. The light source and excitation filter must allow light (which the material being analyzed absorbs at certain wavelengths) to fall on the sample.

2. The light detector and emission filter system must be sensitive to the wavelength of light emitted by the material being analyzed.

The limit of sensitivity of the Model 10 series fluorometer is almost always determined by the level of extraneous background light that reaches the light detector. This background light may vary from sample to sample.

Four primary sources of such unwanted light and their impact on filter, light source, and light detector selection are:

1. Interference from other fluorescent material(s) in the sample.

   By proper choice of the wavelength of light that falls on the sample and wavelength to which the light detector is sensitive, system sensitivity to the fluorescent material being analyzed can be maximized while sensitivity to interfering fluorescent materials is minimized.

2. Scattered light in the system from either reflective surfaces or from turbidity.

   This becomes a problem when certain inherently fluorescent filters are used. These filters convert scattered light from one wavelength to another. If the final wavelength is also transmitted by the filter, it acts as an extraneous light component and will reach the light detector.

Intermediate blocking filters can reduce this interference by protecting inherently fluorescent filters from scattered light. The order of placement of these filters is important.

3. Overlap in the transmission wavelength ranges of excitation and emission filters.

   If both the excitation and emission filters transmit even a small percentage of the same wavelength of light, excitation light scattered by fixed components of the sample system and turbidity in the sample can also reach the light detector. Such "overlap" should be held to a minimum.

4. Raman fluorescence of the solvent.

   This interference, which is often significant, can be minimized by proper selection of filters that prevent the Raman fluorescence from reaching the light detector.

6.8.2 Oil Accessory Kit, Short Wavelength (#10-301)

This guide covers the proper use of the filters and light sources supplied in the 10-301 Oil Accessory Kit, Short Wavelength.

Filter and light source installation instructions are given in Appendix 6.7.

WARNING!

Under no circumstances let light from the clear quartz lamp fall on your eyes! Serious eye irritation will result, with a delay of about four hours between exposure and symptoms.

Light Source:	Use the 10-046 Clear Quartz Lamp ("Q" is scratched in one of the metal end caps).
Excitation Filter:	Use the 10-038 Color Specification 254 Interference Filter (1" square or 1" diameter, highly reflective). Comes with adaptor (black plate) and light seal (1" O-ring).
Emission Filter:	Install one of the 10-300 Soft Glass Filters ("SG" in one corner) first, nearest the photomultiplier. Install the 10-064 Color Specification 7-60 Filter (almost black square of glass with "7-60" in one corner) over it, nearest the sample.
Reference Filter:	Use one of the 10-300 Soft Glass Filters.

Attenuator Plate: In near-shore determinations, sometimes the fluorometer is too sensitive. Even with the Sensitivity Knob full-counterclockwise, you may find that you cannot set your blank to zero with the Blank Control. In this event, you can reduce the sensitivity of the instrument about 75-fold by installing a 10-327 Attenuator Plate (black plate with center hole 1/16" in diameter). Referring to Appendix 6.8, remove the Excitation Filter Holder. On the side that was toward the light source, you will see a plate with a circular hole about 1" in diameter. Remove the two nuts and lock washers retaining this plate. Install the Attenuator Plate on top of the existing plate (this way you won't lose the old one). Replace the washers and nuts, then tighten, and reassemble the fluorometer.

Cuvette: Quartz Cuvettes must be used.

6.8.3 Oil Accessory Kit, Long Wavelength (# 10-302)

This guide covers the proper use of the filters and light sources supplied in the 10-302 Oil Accessory kit, Long Wavelength.

Filter and light source installation instructions are given in Appendix 6.7.

Light Source: Use the 10-049 Near UV Lamp ("BL" scratched in one of the metal end caps).

Excitation Filter: Use the 10-069 Color Specification 7-37 Filter (almost black square of glass with "7-37" in one corner). Installs in Excitation Filter Holder.

Emission Filter: Install the 10-059 Color Specification 2A Filter (light yellow laminated, tape-bound square of glass with "2A" in one corner), first, nearest the photomultiplier. Install the 10-068 Color Specification 4-96 Filter (light blue square of glass with "4-96" in one corner) over it, nearest the sample.

Reference Filter: Use the 10-300 Soft Glass Filter (clear tape-bound square of glass with "SG" in one corner).

Attenuator Plate: In near-shore determinations, sometimes the fluorometer is too sensitive. Even with the Sensitivity Knob full-counterclockwise, you may find that you cannot set your blank to zero with the Blank Control. In this event, you can reduce the sensitivity of the instrument about 5-fold by installing a 10-318 Attenuator Plate (black plate with 1/4" diameter center hole). Referring to Appendix 6.7, remove the Excitation Filter Holder. On the side that was toward the light source, you will see a plate with a circular hole about 1-1/4" in diameter. Remove the two nuts and lock washers retaining this plate. Install the Attenuator Plate on top of the existing plate (this way you won't lose the old one). Replace the washers and nuts, then tighten, and reassemble the fluorometer.

6.8.4 Chlorophyll Accessory Kit (#10-040)

This guide covers the proper use of the filters and light sources supplied in the 10-040 Chlorophyll Accessory Kit.

Filter and light source installation instructions are given in Appendix 6.7.

Light Source: Use the 10-045 Daylight White Lamp ("D" scratched in one of the metal end caps).

Excitation Filter: Use the 10-050 Color Specification 5-60 Filter (deep blue square of glass with "5.60" in one corner). Mounts in holder.

Reference Filter: Use the 10-052 Color Specification 3-66 Filter (orange square of glass with "3-66" in one corner).

Emission Filter: See the following chart for uses.

10-051 Color Specification 2-64 Filter (deep red square of glass with "2-64" in one corner).

10-053 Color Specification 16 Filter (yellow square of laminated glass with "16" in one corner).

10-054 Color Specification 29 Filter (red tape-bound square of laminated glass with "29" in one corner).

10-055 Color Specification 70 Filter (deep red tape-bound square of laminated glass with "70" in one corner.)

Attenuator Plate: In in vivo determinations, sometimes the fluorometer is too sensitive. Even with the Sensitivity Knob full-counterclockwise, you may find that you cannot set your blank to zero with the Blank Control. in this event, you can reduce the sensitivity of the instrument about 5-fold by installing a 10-318 Attenuator Plate (black plate with 1/4" center hole). Referring to Appendix 6.7, remove the Excitation Filter Holder. On the side that was toward the light source, you will see a plate with a circular hole about 1-1/4" in diameter. Remove the two nuts and lock washers retaining this plate. Install the Attenuator Plate on top of the existing plate (this way you won't lose the old one). Replace the washers and nuts, then tighten, and reassemble the fluorometer.

Emission Filter Selection Chart for Chlorophyll Accessory Kit:

Type of Measurement	**Measuring**	**Install Filter (in order of installation):**
In Vivo	Chlorophyll	10-051 Color Specification 2-64
**Extractive	Chlorophyll A	10-055, Color Specification 70 (nearest photomultiplier) 10-053, Color Specification 16 (nearest the sample)

If a high blank is experienced while using the above filter combination (10-055/10-053), the 10-051 Color Specification 2-64 Filter should be used. In using this filter, expect a significant temperature coefficient, requiring that the instrument be warmed up for the best precision of measurements.

Extractive	Chlorophyll A, B, & C	10-054, Color Specification 29 (nearest photomultiplier) 10-053, Color Specification 16 (nearest the sample)

** These filter combinations will greatly reduce fluorescence due to Chlorophyll B and C.

6.8.5 10-041 Rhodamine Accessory Kit (#10-041)

This guide covers the proper use of the filters and light sources supplied in the 10-041 Rhodamine Accessory Kit.

Filter and light source installation instructions are given in Appendix 6.7.

WARNING!

Under no circumstances let light from the clear quartz lamp fall on your eyes! Serious eye irritation will result, with a delay of about four hours between exposure and symptoms.

The following recommendations apply equally well for Rhodamine B, Rhodamine WT, Sulpho Rhodamine B extra, and Pontacyl Brilliant Pink B.

Light Source:	Use the 10-046 Clear Quartz Lamp ("Q" scratched in one of the metal end caps).
Excitation Filter:	Use the 10-056 Color Specification 546 Filter (thick green tape-bound square of laminated glass with "546" in one corner).
Reference Filter:	Use the 10-053 Color Specification 16 Filter (yellow square of laminated glass with "16" in one corner).
Emission Filter:	See the following chart for uses.
	10-052 Color Specification 3-66 Filter (orange square of glass with "3-66" in one corner).
	10-057 Color Specification 23A Filter (deep reddish-orange, tape-bound square of laminated glass with "23A" in one corner).
	10-058 Color Specification 4-97 Filter (light blue square of glass with "4-97" in one corner).

Emission Filter Selection Chart for Rhodamine Accessory Kit:

Instrument	Effect of Ambient Temperature Variation	Algae Level	Filter (order of installation)
10-000/005	Slight	Low	10-057, CS 23A (nearest photomultiplier) 10-052, CS 3-66 (nearest the sample)
10-000/005	Slight	*High	10-052, CS 3-66 (nearest photomultiplier) 10-058, CS 4-97 (nearest the sample)
10-000/005	Significant	High	10-057, CS 23A (nearest photomultiplier) 10-058, CS 4-97 (nearest the sample)
10-000R/005R	Slight	*All	10-052, CS 3-66 (nearest photomultiplier) 10-058, CS 4-97 (nearest sample)
10-000R/005R	Significant	All	10-057,CS 23A (nearest photomultiplier) 10-058,CS 4-97 (nearest sample)

* Expect a significant temperature coefficient, requiring that the instrument be warmed up for the best precision of measurements.

6.8.6 Combination Chlorophyll and Rhodamine Accessory Kit (#10-042)

A combination kit is available that contains filters and light sources for both chlorophyll and rhodamine studies. To see the correct filters and light sources, refer to Section 6.8.4 (chlorophyll) and Section 6.8.5 (rhodamine).

6.8.7 Fluorescein Accessory Kit (#10-086)

This guide covers the proper use of the filters and light sources supplied in the 10-086 Fluorescein Accessory Kit.

Filter and light source installation instructions are given in Appendix 6.7.

Light Source: Use the 10-045 Daylight White Lamp ("D" scratched in one of the metal end caps).

Excitation Filters: Use the 10-062 Color Specification 3 Filter (yellow tape-bound square of laminated glass with "3" in one corner), and the 10-061 Color Specification 47B Filter (purple tape-bound square of laminated glass with "47B" in one corner) combined.

Reference Filter: Use the 10-063 Color Specification 8 Filter (dark yellow tape-bound square of laminated glass with "8" in one corner).

Emission Filters: 10-060, Color Specification 12 Filter (gold tape-bound square of laminated glass with "12" in one corner), nearest Photomultiplier;

10-059, Color Specification 2A Filter (very light yellow tape-bound square of laminated glass with "2A" in one corner), next to 10-060, Color Specification 12 Filter;

10-058, Color Specification 4-97 Filter (light blue square of glass), nearest the sample.

Attenuator Plate: We have found that background fluorescence can be very high in natural systems. Even with the Sensitivity Knob full-counterclockwise, you may find that you cannot set your blank to zero with the Blank Control. In this event, you can reduce the sensitivity of the instrument about 5-fold by installing a 10-318 Attenuator Plate (black plate with 1/4" center hole). Referring to Appendix 6.7, remove the Excitation Filter Holder. On the side that was toward the light source, you will see a plate with a circular hole about 1-1/4" in diameter. Remove the two nuts and lock washers retaining this plate. Install the Attenuator Plate on top of the existing plate (this way you won't lose the old one). Replace the washers and nuts, then tighten, and reassemble the fluorometer.

Appendix 6.9
MODEL 10 FLUOROMETER SENSITIVITY

6.9.1 Oil Measurements.

Where high concentrations of crude or very heavy oils are to be studied, long wavelength excitation may be used. The limit of detectability is about 0.1 parts per million (ppm); the linear range extends to about 50 ppm; and the useful range using a calibration curve extends to about 200 ppm.

Where #2 fuel or lighter oils are to be measured, short wavelength excitation is required. The limit of detectability is about 2 parts per billion (ppb); the linear range extends to about 2 ppm; and the useful range using a calibration curve is about 10 ppm.

Refer to the monograph "Oil in the Environment," in Appendix 6.2.

A 3 mm Continuous-Flow Cuvette has been developed that allows measurements at higher concentrations. The shorter path length increases the linear range and reduces the effects of light-absorbing materials. (Contact Turner Designs for details.)

6.9.2 Fluorescent Dye Studies.

The limit of detectability in pure water of the most commonly used fluorescent dyes (rhodamine WT, rhodamine B, and fluorescein) is about 10 parts per trillion. Pontacyl brilliant pink is less detectable by a factor of three. Factors such as fluorescent background affect the detectability. Nonetheless, measurements can be made to 0.1 ppb of rhodamine WT (<u>active</u> ingredient) in raw sewage. The linear range extends to about 0.1 ppm; and the useful range using a calibration curve is 0.5 ppm. Refer to the monograph "A Practical Guide to Flow Measurement," in Appendix 6.2.

A 3 mm Continuous-Flow Cuvette has been developed that allows measurements at higher concentrations. The shorter path length increases the linear range and reduces the effects of light-absorbing materials. (Contact Turner Designs for details.)

6.9.3 Chlorophyll and Pheophytin.

All chlorophyll-containing organisms are fluorescent. Where the organisms are small, such as phytoplankton, fluorescence may be measured directly without extraction or chemical treatment. Sensitivity of the fluorometer varies, depending upon such factors as the amount of organic substance associated with a given quantity of plant pigment, the presence of humic materials, and the fluorescence efficiency of the particular species. However, the fluorometer is at least 20 times more sensitive than spectrophotometric techniques. In vivo measurements are commonly used at open-ocean levels. In one field demonstration, chlorophyll was successfully measured in vivo in Crater Lake, Oregon. The Secchi disk reading was 39 meters!

When used with extractive techniques, the limit of detectability is about 5 parts per trillion in the final extract. For information about linearity and factors affecting measurements, refer to the monograph "Chlorophyll and Pheophytin," in Appendix 6.2.

A 3 mm Continuous-Flow Cuvette has been developed that allows measurements at higher concentrations. The shorter path length increases the linear range and reduces the effects of light-absorbing materials. (Contact Turner Designs for details.)

Appendix 6.10
TELEMETRY OUTPUTS

The Model 10 Fluorometer is equipped with recorder output capability through the power/telemetry connector. This connector contains both power input and telemetry output pins.

It can be used with a strip-chart recorder, data logger, or a computer (with an A/D converter board). (See Appendix 6.3 for a discussion of recording devices.)

If you wish to use the recording capabilities, purchase an AC or DC power cord, wired for telemetry, from Turner Designs.

CAUTION: If you do not use a Turner Designs cable, use caution. Improper connection of power and telemetry pins may result in severe damage to your instrument.

The negative DC power line is grounded to the instrument case. Preferably, the instrument and all telemetry outputs should be isolated from the DC source. If this is not possible, ground to the negative side of the DC power source only.

The Model 10 is designed with range multipliers, which are, by convention, sensitivity multipliers. The ranges have the values: Min Sens, X3.16, X10, and X31.6. The range multipliers are adjusted at the factory for your main application. If you change applications, internal adjustments need to be made, or a correction factor allowed for. (See Section 3.5.2 and Appendix 6.7.2.)

The power/telemetry pins and their functions are:

Pin A: External chassis ground.

Pin B: AC voltage input, neutral wire.

Pin C: AC voltage input, hot wire.

Pin D: Serves both as negative DC power input and as the ground return for all TTL-compatible signals.

Pin E: Positive 12 Volt DC power.

Pin F: A TTL-compatible digital output. A "1" means MIN SENS; a "0" means any other range. (NOTE: if you are using the new concentration range labels discussed in Section 3.1, a "1" means X31.6; a "0" means any other range.) This

output is only valid if Pin N - the analog range output is not used.

Pin G: Like Pin F, except a "1" means X3.16 and a "0" means any other range. (NOTE: if you are using the new concentration range labels discussed in Section 3.1, a "1" means X10; a "0" means any other range.)

Pin H: Like Pin F, except a "1" means X10 and a "0" means any other range. (NOTE: if you are using the new concentration range labels discussed in Section 3.1, a "1" means X3.16; a "0" means any other range.)

Pin J: Like Pin F, except a "1" means X31.6 and a "0" means any other range. (NOTE: if you are using the new concentration range labels discussed in Section 3.1, a "1" means X1 or MIN CONC; a "0" means any other range.)

Pin K: A TTL-compatible digital output. A "0" means on-scale; a "1" means off-scale.

Pin L: Precision ground. This is the ground return for analog outputs. Be careful not to ground this pin externally, as "ground loops" can cause significant errors in the analog output. For best results, a recorder or A/D converter used with the Model 10 series Fluorometer should have a floating (ungrounded) input amplifier.

Pin M: Analog signal output. 0 to +5 volts DC represents zero to full scale. Internal impedance is 3900 ohms.

To use with various common millivolt recorders, connect a resistor with the following values from Pin M to Pin L, and connect the recorder across the resistor:

0-1 volt	- use 1000 ohms
0-100 millivolts	- use 82 ohms
0-10 millivolts	- use 8.2 ohms
0-1 millivolts	- use 0.82 ohms

If your recorder does not have a span control, it will be necessary to adjust these resistance values, so that the recorder and the front panel meter "track."

Most current recorders do not have span controls. Therefore, it is recommended that a potentiometer be used for this purpose. Its CCW lead is hooked to Pin M. The slider goes to a fixed resistor. The fixed resistor goes to the positive recorder terminal. The negative recorder terminal goes to Pin L as follows:

0-1 milliampere -	a 2500-ohm potentiometer only, slider to recorder.
0-100 microamperes -	25,000-ohm potentiometer and a 33,000-ohm resistor.

Pin N: This pin supplies an analog signal to indicate what range the instrument is on. When it is used, the digital range signals at pins F, G, H, and J must not be used.

An external load resistor must be used. A 0-1 volt recorder sensitivity is the maximum full-scale range recommended.

If a 0-1 volt recorder channel is available, connect a 1000 ohm resistor from Pin N to Pin L, and connect the recorder across the resistor. If you are using the original sensitivity range labels discussed in Section 3.1, expect the following output:

MIN SENS	0V
X3.16	0.4V
X10	0.7V
X31.6	1V

If you are using the new concentration range labels discussed in Section 3.1, expect the following output:

X31.6	0V
X10	0.4V
X3.16	0.7V
X1	1V

For lower-range recorders, connect a resistor from Pin N to Pin L, and connect the recorder across the resistor, as follows:

0-100 millivolts	- use 75 ohms
0-10 millivolts	- use 7.5 ohms
0-1 millivolts	- use 0.75 ohms

A current recorder may be connected up precisely as described for Pin M above - except start at Pin N instead of Pin M, of course.

This pin may also be used for remote range selection. An external momentary switch that applies +11 to +16 volts to Pin N will operate in the same manner as the Step Switch. See Section 2.4, #4. Current drain is about ten milliamperes.

A single-pole double-throw switch should be used, with the arm going to pin N, the normally open contact to +11 to +16 volts, and the normally closed contact to the range recorder channel, so that power does not get applied to the recorder.

Pin P: Not used.

Pin R: Not used, but internally grounded.

Pin S: A TTL-compatible digital output. If you are using the original sensitivity range labels discussed in Section 3.1, a "1" means the X1-X100 knob is in the X1 position. A "0" means it is in the X100 position.

(NOTE: If you are using the new concentration range labels discussed in Section 3.1, a "1" means the X1-X100 is in the X100 position; a "0" means it is in the X1 position.)

Pin T: Positive 5 volts. This regulated voltage is reserved for accessories. Maximum available current is 100 milliamperes. Return should be made to pin D, not to pin L, unless specified on the accessory.

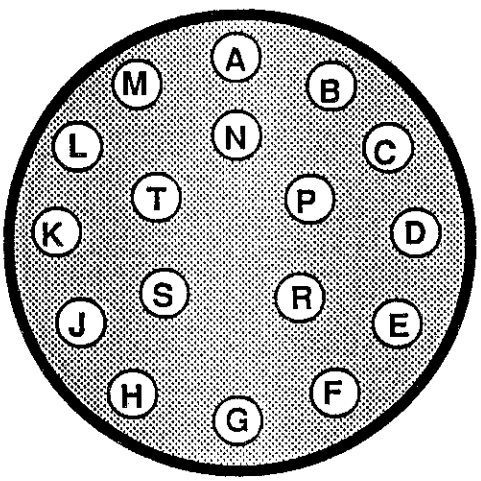

Figure A4. Power/Telemetry Connector Pins