Extracts From Manual

Turner Quantech Fluorometer

General Description

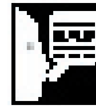
Components

Display:
2 x 24 character LCD display to indicate software menus and fluorescence values.

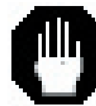
Power Switch:
Rocker switch located on back panel.

Sample Chamber and Cuvette Holder:
The large sample chamber will accommodate nonstandard size cuvettes and up to four flow tubes. The standard cuvette holder supplied with the units accepts 12 x 75 mm round cuvettes and 12.5 x 12.5 x 45 mm square cuvettes.
To insert the cuvette in the holder, open the chamber cover and carefully insert the cuvette by positioning it between the spring clip and the angular sides of the holder. Two flat, clear faces of a square cuvette should oppose the circular openings that form the light paths.

Take care not to touch the curved mirror which is located next to the spring clip.



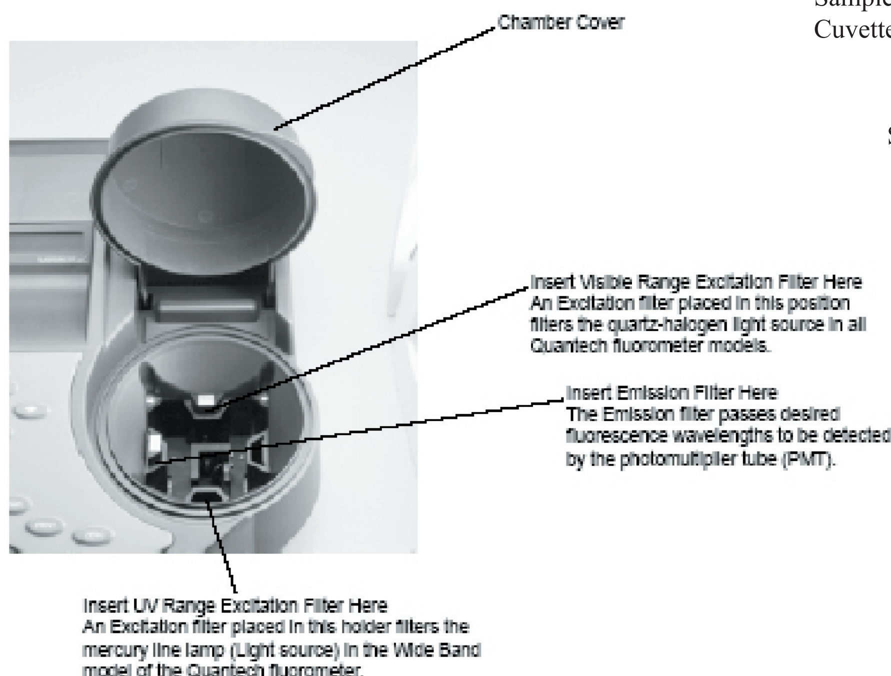
Note
The Spring clip in the cuvette holder is adjustable and can be pushed inward to accommodate 10 mm diameter tubes or outward to accommodate 16 mm diameter tubes. (Maximum tube height is 130 mm 13 cm).



Caution
Do not touch the curved mirror located in the cuvette holder.

Filter Block and Filter Slides:
The filter block lines the perimeter of the sample and serves as a holder for the Excitation and Emission filter slides. The Visible Range Excitation filter slide is placed in the trapezoidal-shaped holder located on the back side of the sample chamber (refer to Figure 1&2). The Emission filter slide is placed in the trapezoidal-shaped holder located on the left side of the sample chamber (refer to Figure 1&2).

To Change the Filter Slide:
Open the chamber cover and grip the top of the filter slide with your thumb and index finger so you can lift the slide up and out of the filter slide holder. When replacing a filter slide be sure that the circular filter is properly aligned with the light path opening in the bottom of the filter holder.



Sample chamber/
Cuvette holder

Spring clip

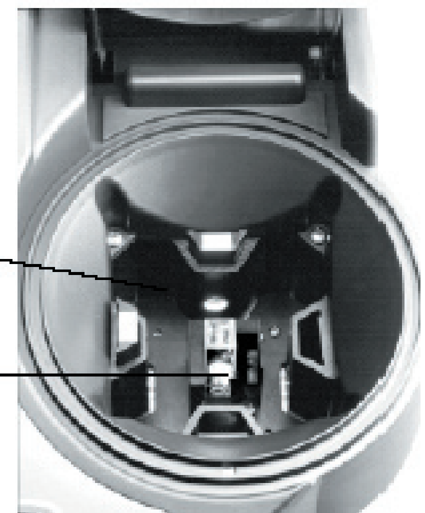


Figure 1&2

Theory of Fluorescence

When a molecule absorbs radiation, an electron is promoted from one energy level in the molecule to a higher energy level within the molecule, absorbing the energy. The molecule may release the absorbed energy and return the electron to its ground state by converting this energy to vibrational energy or releasing the energy in the form of light emitted by the molecule.

If part of this energy is converted to vibrational energy the reminder (if radiated within 10-8 seconds), is emitted as light of lower energy (longer wavelength) than the absorbed energy. This property is called fluorescence.

Atoms or molecules which fluoresce have well-defined excitation and emission spectra which allow both qualitative and quantitative analysis of the material. The shape of the excitation spectrum is that of the absorbance curve of the molecule and is independent of the wavelength at which fluorescence is measured.

Each molecule also has a characteristic number called the quantum efficiency, which is the ratio of the total number of emitted photons to the total number of absorbed photons.
A non-fluorescent molecule is one whose quantum efficiency is zero or so close to zero that the fluorescence is not measurable.

Both the quantum efficiency and the shape of the emission spectrum are independent of the wavelength of the exciting light. If the exciting light used is of a wavelength which is different from that of the absorption peak, a smallest portion of the light will be absorbed and proportionately less light will be emitted, illustrating the constancy of the quantum efficiency. However, the shape and location of the emission spectrum will not change.

Fluorescence/Concentration Relationships

Fluorescence is related to and dependent on concentration according to the following equation:

F=KIøCl

Where;
F = Fluorescence reading observed on the instrument.
K = A constant which accounts for instrumental factors, pH, T, electronics.
I = The intensity of the exciting light at a wavelength.
C = The concentration of the fluorescing molecule.
l = The path length of the solution or solid being measured. ø = The quantum efficiency of the molecule. An examination of this equation shows that for a given molecule, at a constant concentration, the amount of fluorescence observed can be affected by:

- I, the intensity of the exciting wavelength.
- D, the path length of the molecules.

As a practical matter, this means that the only limits on the sensitivity of fluorescence measurements are the electrical noise in the instrument, competing radiations, physical limitations, i.e. sample volume available, maximum energy available and maximum acceptable cell size.

The accuracy of measurement is excellent because the technique is a direct measure of the radiant energy being made.

Considerations

Blank Fluorescence

One of the most common limits on sensitivity is set by blank fluorescence. Blank fluorescence arises from the reagents or other sources separate from the compound of interest. Ideally, the fluorescence of the "blank" should be 000, relative to the fluorescence of the material being analyzed.

Reagents

Usually, little difficulty will be encountered with commercially available reagent-grade chemicals. This is particularly true in laboratories utilizing procedures where the majority of assays are done with visible light activating the fluorescence of the sample. If the samples are under ultraviolet excitation, or at a high instrument sensitivity, then high "reagent blank" fluorescence may be encountered. Even spectro-quality solvents sometimes contain traces of impurities which may fluoresce under ultraviolet light. There are several companies marketing fluorescent-grade reagents for those who must work at very low concentration. A frequent source of contaminants are the plasticizers in some plastics and rubber. For dispensing distilled water, an all polyethylene or glass system is recommended. Water standing in rubber tubing may develop high fluorescence from moieties contained in the tubing during manufacture.

Standards

Standards should always be stored as concentrated solutions, out of the light and at low temperature. Standard solutions of low concentrations may degrade with time. Dilutions to working concentrations should always be prepared as required using appropriate diluent since fluorescence is usually pH dependent.

Glassware

Borosilicate glassware is recommended for general storage of reagents. Test tube or flasks may be capped with parafilm for mixing and storage, however, care should be taken when using this material. For work at very low levels of fluorescence, it may be necessary to rinse all glassware with an appropriate solvent. See the Operation section.

Temperature

Fluorescence is usually more temperature sensitive than absorption. Although not all fluorescent material exhibit marked sensitivity, there are instances where as much as a 2% reduction in fluorescence is observed for every degree centigrade temperature rise. Standards, reagent blanks and sample should be at the same temperature prior to measurement of fluorescence. Sometimes drifting of fluorometer readings may be observed due to a change in the temperature of the sample while in the sample chamber.

pH

The pH of the solution may affect the fluorescent species being tested. Protonation changes the resonance structures of the molecule, the equilibrium of these species and consequently, the concentration of the fluorescent molecule. Check your applications carefully for the possibility of this problem. The pH of standard solutions may change over time due to the acidic nature of storage container materials such as glass. Similarly, the fluorescent compound may decompose under a variety of pH ranges.

Miscellaneous

Some materials are photosensitive; that is, they decompose when exposed to wavelengths they absorb. This property can be determined by inserting a sample solution into the instrument and observing its change in fluorescence over a period of time.

Operation

Unit Power-Up

1. Turn main power switch located on back of unit ON.

2. Display reads, "Turner Quantech Fluorometer Manufactured by Barnstead/Thermolyne Fluorometer, Software Version 1.00.03."

3. Display proceeds to the Language selection option. The default language is "English." To select another language (Espanol, French, Deutsch), press the LEFT or RIGHT ARROW key.

4. Press ENTER to continue operation of the fluorometer.

5. Display reads, "Unit Initializing, Please Wait;" "Auto Calibrating (Please Wait);" "Unit Initializing, "15 Minutes Remain."

6. The unit will perform a 15 minute warmup countdown to ensure the stability of the fluorometer electronics. The countdown timer on the display allows you to monitor the time remaining before the warm-up period is complete.

Keypad and Function Keys (See Figure 3.)

UP ARROW (ON/YES) key:
Allows operator to scroll through "Method" parameters in an incremental manner and select an option or select the "ON/YES" option in response to a question posed by the fluorometer software user interface.

DOWN ARROW (OFF/NO) key:
Allows operator to scroll through "Method" parameters in descending order and select an option or select the "OFF/NO" option in response to a question posed by the fluorometer software user interface.

LEFT ARROW key:
Allows operator to scroll between "Menu," "Method" and "Function" options and select an option, or scroll across the display.

RIGHT ARROW key:
Allows operator to scroll between "Menu," "Method" and "Function" options and select an option, or scroll across the display.

ENTER key:
Allows operator to activate or proceed with a selected option visible on the display screen.

MENU key: Serves as a home key to allow operator to immediately return to the "Main Menu."

BACK key:
Allows operator to go back one step within a "Method," "Menu" or "Function."

PRINT key:
Initiates the signal to send standard curve data, sample concentration data and method settings to a printer or computer.

ZERO KEY:
Sets fluorescence value to zero when blank solution is in the cuvette holder.

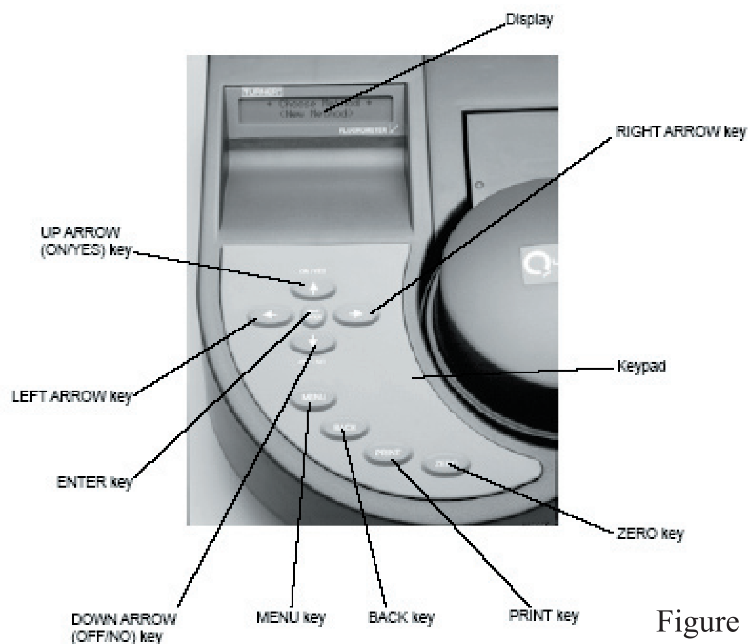


Figure 3

Choosing a Method of Analysis

1. Starting from the "Main Menu," press the ENTER key until display reads, "Choose Method."

2. Press the LEFT or RIGHT ARROW key to choose from the following methods:

- Aflatoxin
- DNA
- Histamine
- Rhodamine
- New Program
- Raw Fluorescence

3. Select the desired method and press ENTER. Follow the proceeding steps according to the method selected.

Aflatoxin, DNA, Rhodamine and Histamine Methods

After choosing either the Aflatoxin, DNA, Rhodamine or Histamine method, proceed with the following steps:

1. Display reads, "Change Name? (Y/N?)."
a. Press ENTER to select "No." (System will automatically default to "No.") Proceed to step 2.

b. If you wish to change the "Method" name, press the UP ARROW key to select the "Yes" option and press ENTER. See the New Method section for instructions on how to change the method name.

2. Display reads, "Proper Filters in Unit?" "Yes." (System defaults to "Yes.")
a. If you are satisfied with the filters that you have in the unit for your method of choice, press the ENTER to select
"Yes" and proceed to step 3.

b.If you don't have the right filter, change filter according to manual and then press "Yes". See filter selection guide if you need some guidance on which filters to use.

3. Display reads, "Std Curve from Memory (Y/N?); No." (System will automatically default to "No.")
a. If no, press ENTER and proceed to step 4.

b. If yes, press the UP ARROW to select "Yes" and press ENTER. Proceed to step 8.

4. Display reads, "Enter Number of Points;" "2." The default setting for the number of points in a standard curve is 2. Enter a value for the number of standards of known concentration that you will be working with by pressing the UP and DOWN ARROW keys to scroll between numbers in positive and negative increments, respectively.
Press ENTER.

5. Display reads, "Enter Concentration;" "0000.000 <FIU>." Press LEFT or RIGHT ARROW key to scroll horizontally across the display and select a character. Each "0" represents a numerical character field that can be changed. The <FIU> field can also be changed when the ">" is highlighted. In order to select a concentration value and unit of measure, press the UP and DOWN ARROW keys to scroll between numbers and concentration units (ppm, ppb, ppt, µg/ml, µg/dl, ng/ml, pg/ml or ng/µl). When the display reflects the correct numerical value and units for the first standard (known sample), Press ENTER.

6. Display reads, "Insert Known Sample #1." Open the chamber cover, insert sample and close cover. Press ENTER. Display then reads, "Auto Gain Setting;" Please Wait" and runs through consecutive gain settings before showing, "Taking Reading." Next the display reads, "Enter Concentration;" "0000.00 <FIU>." Select a new concentration value and press ENTER. Display reads, "Insert Known Sample 2." Open the chamber cover, insert known sample #2 and close the cover. Press ENTER. Display will read, "Taking Reading." Repeat these steps for each known sample in the standard curve.

Note
If you are using a standard curve from the memory of the Quantech unit, the display will first indicate the age of the standard curve data and the coefficient of determination for the curve. The display will then read, "Insert Unknown Sample."

7. Display reads, "Insert Blank." Open chamber cover and insert cuvette with blank solution in the cuvette holder. Close chamber cover and press ZERO. The display will read, "Taking Reading" and then show the "Coefficient of Determination;" "1.00" (example value). Proceed to step 8.
If an unacceptable coefficient (<0.75) is obtained, the unit will display the value and indicate "Curve Unacceptable;" Press Enter." After you press ENTER, the display will indicate "Enter Number of Points;" "2." Please check the concentrations of your standard samples before repeating the steps of the standard curve procedure.

8. If an acceptable "Coefficient of Determination" is obtained, the display will read "Insert Unknown Sample." After inserting the cuvette containing the unknown sample into the cuvette holder and closing the chamber cover press ENTER. The display will read, "Taking Reading" and the concentration value of the unknown sample is displayed. The standard curve data for your method is now stored in memory. A concentration reading of the first unknown sample is required to store the standard curve data.

To continue fluorescence analysis of unknown samples, remove the previous sample for which the concentration value was determined and insert the next unknown sample. After closing the chamber cover, press ENTER to obtain the concentration value of the sample. Repeat these steps for all unknown samples. If the concentration of an unknown sample does not fall within the range of your standard curve, the unit will display an "errant value" or indicate "range error." A new standard curve will need to be set up in order to correctly analyze an over or under range sample. Alternatively, the unknown sample can be diluted by a given factor and the dilute sample analyzed.

Note
The standard curve data that you have just obtained can be utilized for future analyses. However, please note that it is good laboratory practice to perform new standard curves at least once a week. The Quantech fluorometer will keep track of the age of your standard curve data. After 5 days the unit will indicate that the curve should be considered marginal by prompting you on whether or not (YES or NO) to use the data before proceeding with analysis.

Note
The blank for a stored standard curve may be reset to zero. When resetting the blank to zero using the ZERO key, you are replacing the last blank setting with the new blank setting. This reset procedure will not compensate for errors in sample readings due to instrument drift. For maximum accuracy it is best to redo your curve.

New Method

1. Display reads, “New Method (Y/N?); No.” (System will automatically default to “No.”) and provides an alphabetical/character listing. Alternatively, the display will read “New Program” and provide the same options as “New Method.”

2. To change the method name, press the LEFT and RIGHT ARROW keys to select a letter or character from the alphabetical/character listing on the display. Each time an ARROW key is manually depressed, the display cursor will advance or step back one character. After selecting a character (cursor highlights the character), press the UP ARROW key. The letter you have selected will appear at the top of the display window. Continue selecting letters in this manner until you have entered the desired program name, then press ENTER.

3. Display reads, “Proper Filters in Unit?”; “Yes” (System defaults to “Yes.”)
a. If you are satisfied with the filters that you have in the unit for your method of choice, press ENTER to select “Yes” and proceed to step 3.

b. If you don’t have the right filter, change filter according to manual and then press “Yes”. See filter selection guide if you need some guidance on which filters to use.

4. Display reads, “Std Curve from Memory (Y/N?); No.” (System will automatically default to “No.”)
a. If no, press ENTER and proceed to step 5.

b. If yes, press the UP ARROW to select “Yes” and press ENTER. Proceed to step 9.

5. Display reads, “Enter Number of Points;” “2.” The default setting for the number of points in a standard curve is 2. Enter a value for the number of standards of known concentration that you will be working with by pressing the UP and DOWN ARROW keys to scroll between numbers in positive and negative increments, respectively. Press ENTER.

6. Display reads, “Enter Concentration;” “0000.000 <FIU>.” Press the LEFT or RIGHT ARROW key to scroll horizontally across the display and select a character that is to be changed. Each “0” represents a numerical character field that can be changed. The <FIU> field can also be changed when the “>” is highlighted. In order to select a concentration value and unit of measure, press the UP and DOWN ARROW key to scroll between numbers and concentration units (ppm, ppb, ppt, µg/dl, µg/ml, ng/ml, pg/ml or ng/µl). When the display reflects the correct numerical value and units for the first standard (known sample), Press ENTER.

7. Display reads, “Insert Known Sample #1.” Open the chamber cover, insert sample and close cover. Press ENTER. Display then reads, “Auto Gain Setting;” “Please Wait” and runs through consecutive gain settings before showing “Taking Reading.” Next the display reads, “Enter Concentration;” “0000.00 <FIU>.” Select a new concentration value and press ENTER. Display reads, “Insert Known Sample 2.” Open the chamber cover, insert known sample #2, and close the cover. Press ENTER. Display will read, “Taking Reading.” Repeat these steps for each known sample in the standard curve.

8. Display reads, “Insert Blank.” Open chamber cover and insert cuvette with blank solution in the cuvette holder. Close chamber cover and press ZERO. The display will indicate “Taking Reading” and then show the “Coefficient of Determination;” “1.00” (example value).
Proceed to step 9.

If an unacceptable coefficient (<0.75) is obtained, the unit will display the value and indicate “Curve Unacceptable;” Press Enter.” After you press ENTER, the display will indicate “Enter Number of Points;” “2.” Please check the concentrations of your standard samples before repeating the steps of the standard curve procedure.

9. If an acceptable “Coefficient of Determination” is obtained, the display will read “Insert Unknown Sample.” After inserting the cuvette containing the unknown sample into the cuvette holder and closing the cover press ENTER.
The display will read, “Taking Reading” and the concentration value of the unknown sample will be displayed. The standard curve data for your method is now stored in memory. A concentration reading of the first unknown sample is required to store the standard curve data. To continue fluorescence analysis of unknown samples, remove the previous sample for which the concentration value was determined and insert the next unknown sample.
After closing the chamber cover, press ENTER to obtain the concentration value of the sample. Repeat these steps for all unknown samples.

If the concentration of an unknown sample does not fall within the range of your standard curve, the unit will display an “errant value” or indicate “range error.” A new standard curve will need to be set up in order to correctly analyze an over or under range sample. Alternatively, the unknown sample can be diluted by a given factor and the diluted sample analyzed.

Note
If you are using a standard curve from the memory of the Quantech unit, the display will indicate the age of the standard curve data and the coefficient of determination for the curve. The display will then read, “Insert Unknown Sample.”

Note
The standard curve data that you have just obtained can be utilized for future analyses. However, please note that it is good laboratory practice to perform new standard curves at least once a week.

Note
The blank for a stored standard curve may be reset to zero. When resetting the blank to zero using the ZERO key, you are replacing the last blank setting with the new blank setting. This reset procedure will not compensate for errors in sample readings due to instrument drift. For maximum accuracy it is best to redo your curve.

Raw Fluorescence (Mode I)

1. Display reads, “Enter Concentration;” “000.000 <FIU>.” Press the LEFT or Right ARROW key to scroll horizontally across the display and select a character field that is to be changed. Each “0” represents a numerical character field that can be changed. The <FIU> field can also be changed when the “>” is highlighted. In order to select a concentration value and unit of measure for your reference sample, press the UP or DOWN ARROW key to scroll between numbers and concentration units (ppm, ppb, ppt, µg/ml, µg/dl, ng/ml, pg/ml, or ng/µl). When the display reflects the correct value and units for the reference sample, press ENTER.

2. Display reads, “Insert Known Sample;” Open the chamber cover, insert reference sample and close the cover. Press ENTER. Display then reads, “Auto Gain Setting;” Please Wait” and runs through consecutive gain settings before showing “Taking Reading.” 3. Display reads, “Insert Blank;” “No” System defaults to “No.” If you choose to use a blank sample in “Raw Fluorescence,” press the UP ARROW key to select “Yes.” Display reads, “Insert Blank Sample.” Open the chamber cover, insert blank sample and close the cover. Press ZERO. Display then reads, “Taking Reading.” Proceed to step 4.

If you do not want to use a blank sample, press the ENTER key to select “No” and proceed to step 4.

4. Display reads “Insert Unknown Sample.” Open the chamber cover, insert unknown sample, close cover and press ENTER. The display will show the “Raw Concentration” value of the sample per the reference sample unit of measure.

5 To continue determination of “Raw Concentration” values for subsequent unknown samples, open the chamber cover, insert next unknown sample and close cover. The display will show the “Raw Concentration” of the unknown sample within approximately two seconds.

6. If the unknown sample is significantly more fluorescent than the reference sample, the display will indicate an overrange error. The unknown sample can be diluted by a given factor and read again or the unknown can serve as the new reference sample in a raw fluorescence analysis.

Note

<FIU> indicates a generic unit of measure or Fluorescence Intensity Units.

Note

Raw Fluorescence (Mode I) is designed for applications that do not require 2 - 9 point standard curves. This mode essentially provides a 1 point standard curve in reference to zero concentration of a compound. This 1 point is only stored in memory for the duration of your raw fluorescence analysis.

Note

A reference sample is generally of the highest concentration you would expect to analyze for the compound of interest. An arbitrary numerical value is usually assigned as the concentration of this reference sample (e.g. 1000.000 <FIU> or 1000.000 ppb.

Raw Fluorescence (Mode II)

1. Display reads, “Enter Concentration;” “0000.000 <FIU>.” Press ENTER.

2. Display reads, “Insert Known Sample.” Open the chamber cover, insert reference sample and close the cover. Press ENTER. Display then reads, “Auto Gain Setting;” Please Wait” and runs through consecutive gain settings before showing “Taking Reading.”

3. Display reads, “Insert Blank;” “No.” (System defaults to “No.”) If you choose to use a blank sample in “Raw Fluorescence,” press the UP ARROW key to select “Yes.” Display reads, “Insert Blank Sample.” Open the chamber cover, insert reference sample and close the cover. Press ZERO. Display then reads, “Taking Reading.” Proceed to step 4.

If you do not want to use a blank sample, press the ENTER key to select “No” and proceed to step 4.

4. Display reads “Insert Unknown Sample.” Open the chamber cover, insert unknown sample, close cover and press ENTER. The display will indicate the “Raw Concentration” value of the sample per the reference sample unit of measure.

5 To continue determination of “Raw Concentration” values for subsequent unknown samples, open the chamber cover, insert next unknown sample and close cover. The display will show the “Raw Concentration” of the unknown sample within approximately two seconds.

6. If the unknown sample is significantly more fluorescent than the reference sample, the display will indicate an overrange error. The unknown sample can be diluted by a given factor and read again or the unknown can serve as the new reference sample in a raw fluorescence analysis.

Note

Raw Fluorescence (Mode II) is designed for basic research applications in which a reference standard of “known” concentration is not available.

The Turner Quantech fluorometer assigns a value to a reference sample based upon the auto gain function response. In general, the reference sample would be chosen such that it represents the maximum fluorescence response expected in the range of unknown samples that are to be analyzed.

The “Raw Fluorescence” of the unknown samples is determined relative to the reference sample. The fluorescence response for the reference sample is only stored in memory for the duration of your “Raw Fluorescence” analysis.

Filter Selection Guide

Filters

The filters in the Fluorometer are used to perform two functions. The first is to allow only light of a specific wavelength to pass into the sample cell and excite a specific molecule. The excitation filters are always Narrow Band (NB) filters and are specific for the compound of interest. The emission filters are generally Sharp-Cut (SC) filters that allow only emitted light above a specific wavelength to pass into the photomultiplier tube. Since the photomultiplier tube is sensitive to a wide range of wavelengths, a filter allows the user to choose the wavelength range detected by the photomultiplier tube to reduce background noise and increase sensitivity. Selection For nearly all fluorescent determinations, the required filters are recommended by B/T. In the event that a new procedure is being developed, the following considerations should be applied in selecting the proper filters:

1. Some compounds have the ability to discharge some of the energy obtained when they absorb light by emitting light of a longer wavelength. These compounds are fluorescent. The efficiency of this process may be anywhere from a fraction of a percent to almost 100 percent. A portion of the absorbed energy is released in heat.

2. The intensity of the light emitted is proportional to the amount of exciting light absorbed. At low concentrations, the emitted light may be considered proportional to the concentration. In practice, the meter reading of the fluorometer is linear with concentration of the fluorescent molecule involved. The range between the lowest detectable sample and the point where significant nonlinearity occurs is normally a factor of 104 to 105 in concentration of the fluorescent molecule.

3. If a compound is fluorescent, a wide range of energy which it absorbs causes it to fluoresce. The spectrum of the emitted light is normally quite broad and its shape and peak are independent of the wavelength of the exciting light. The only variation is in intensity; where less light (energy) is absorbed, less light will be emitted. Proportionality of fluorescence and concentration are maintained even when the exciting light and measured fluorescence are far removed from the peaks.

Narrow Bandpass Filter (NB)

This filter is used when the excitation and emission wavelengths are close together. Typical bandwidths are in the 8 to 10 nm range. They require precision manufacturing techniques and cost more than the other types of filters. The filter consists of a miniature interferometer in conjunction with blocking glasses. For the filter designated NB390 the range of wavelength passed by the filter is 385-395 nm. These filters are constructed with a thin coating of material and are susceptible to scratching. Extreme care should be taken when handling these filters. See the Preventive Maintenance section for maintenance.

Some Narrow Bandpass Filters are comprised of special optical glasses or a combination of glass or gelatin materials, and have bandwidths of 50 to 100 nm. Examples of these excitation filters are the NB360 (bandpass 40 nm) and the NB430 (bandpass 90 nm). These filters are particularly useful where high sensitivity is required, and lower resolution is not a factor in distinguishing between different fluorescent moieties present in the solution. By allowing more light to pass to the sample, a greater number of the fluorescence molecules are excited and more emission light is produced per sample volume. Narrow Band Filters can also be used in the emission side filter holder when specific emitted wavelengths need to be distinguished among many emitting fluorescence present in solution.

Sharp-Cut Filters (SC)

This type of filter passes all light of a longer wavelength than a given value and blocks all light of shorter wavelengths. The transition from zero to full transmittance (approximately 80-90 percent) usually takes place over a region of about 40 nm. The characteristic wavelength of a Sharp-Cut Filter is defined as the wavelength at which the filter has a transmittance of 37 %. With most filter manufacturers, there is about a 10 nm tolerance in the location of the longest wavelengths of emitted light which can be measured

Compatibility of Filters

Two filters are compatible and may be used as excitation and emission filters if the wavelengths of the light they transmit do not overlap significantly. If such an overlap is present, scattered light from an even slightly turbid sample or from an optical defect in the cuvette will reach the photomultiplier and be registered as fluorescence. The steps involved in selecting the best combination of excitation and emission filters for a given compound are as follows:

1. Ideally the fluorescence spectra of the compound will be available. Look for the excitation and emission filters required in Tables I and II. If the name of the compound is known, the user may consult the manufacturer of the fluorescent compound to obtain the absorbance maxima for the compound and the fluorescent spectrum maxima for the compound. Direct the inquiries to the technical service department of the chemical manufacturer.

2. If the fluorescence spectra are not available, then proceed as follows:
Refer to the transmittance curves for this discussion.
a. Determine the maximum absorption wavelength of the compound with a
spectrometer. Choose a filter which is closest to the peak from Table I and use this for excitation.
b. Having chosen a tentative excitation filter, it requires but a few minutes to insert a series of Sharp-Cut (SC) emission filters compatible with the excitation filter, comparing the ratio of sample reading to reagent blank. If the data obtained indicates a fairly sharp-peaked emission curve, the possibility of using a Narrow Pass Emission filter may be considered. Usually there is little advantage to a Narrow-Pass Emission filter, though they are occasionally of the value where the emission peak falls close to the excitation bandpass.

Filter Selection Chart

(See Table I, Table II and Filter Transmittance Curves)

All filters are mounted in filter holders specifically designed for simple wavelength selection in the Quantech Digital Filter Fluorometer. With the exception of the NB360(40 nm) and the NB430 (90 nm), all narrow band filters designated by the center wavelength in nanometers have a 10nm nominal bandpass curve.

Sharp-Cut Filters (SC) are long pass filters. The number following the SC is wavelength, in nanometers, at which they exhibit a transmission of 37%. They typically have a transmission of less than 1% at a wavelength 10 nm less than the rated wavelength. The NB excitation filters may be used as emission filters where wavelengths are close together but sensitivity will be reduced.

Table I - Quantech Excitation and Emission Filters

B/T PART # DESCRIPTION
Narrow Band (NB) Filters May Be Used As Excitation or Emission Filters.Sharp Cut (SC) Filters Are Only Used As Emission Filters

B/T PART #	DESCRIPTION	B/T PART #	DESCRIPTION
LE1095X1	NB254 Filter	LE1095X18	SC415 Filter
LE1095X3	NB297 Filter	LE1095X19	SC430 Filter
LE1095X4	NB313 Filter	LE1095X20	SC450 Filter
LE1095X30	NB360 Filter	LE1095X21	SC475 Filter
LE1095X6	NB365 Filter	LE1095X22	SC500 Filter
LE1095X7	NB390 Filter	LE1095X23	SC515 Filter
LE1095X8	NB405 Filter	LE1095X24	SC535 Filter
LE1095X9	NB420 Filter	LE1095X25	SC550 Filter
LE1095X31	NB430 Filter	LE1095X26	SC585 Filter
LE1095X10	NB440 Filter	LE1095X27	SC605 Filter
LE1095X11	NB450 Filter	LE1095X28	SC635 Filter
LE1095X12	NB460 Filter	LE1095X29	SC665 Filter
LE1095X13	NB490 Filter		
LE1095X14	NB520 Filter		
LE1095X15	NB540 Filter		
LE1095X16	NB590 Filter		
LE1095X17	NB680 Filter		

Available NBTC NB filters
Available NBTC SC filters

Table II - Filter Requirements for Common Applications

Application	Method	Primary Excitation	Secondary Emission
Fluorescein		NB490	SC515
Chlorophyll		NB440	SC665
Quinine Sulfate		NB360	SC415
Thiamine		NB360	SC430
Riboflavin		NB440	SC535
Tocopherols		NB360	SC515
Catecholamines		NB405	SC515
Rhodamine		NB540	SC585
Protoporphyrin		NB405	SC585
Niacin	condensation	NB360	NB440
Niacin	cyanogen bromide	NB440	SC515
Vitamin A		NB360	NB490
Vitamin B1		NB360	SC430
Vitamin B2		NB440	SC535
Vitamin B6	condensation	NB360	NB440
Vitamin B6	CN:pyridoxal	NB360	NB440
Vitamin B12		NB440	SC515
Ascorbic Acid(Vitamin C)	Deutsch	NB360	SC440
Vitamin E	Kofler	NB360	SC515
Vitamin K	Kofler	NB360	SC430
Vitamin K	Jansson	NB360	SC415
DNA	DABA	NB405	SC515
DNA	thymine	NB360	NB440
DNA	ethidium bromide	NB360	SC585
DNA	Hoechst 33258	NB360	SC430 or SC415
DNA	Thiazole orange	NB490	SC515
DNA	Oxazole yellow	NB460	SC500
DNA	PicoGreen	NB490	SC515
Protein	NanoOrange	NB490	SC585
Aflatoxin	Methanol Extraction	NB360	SC430
Beta-Galactosidase		NB360	NB460
Histamine	OPT	NB360	SC450 or NB450
RNA	RiboGreen	NB490	SC515
ssDNA/Oligos	OliGreen	NB490	SC515

PicoGreen, NanoOrange, RiboGreen, and OliGreen are registered trademarks of Molecular Probes, Inc.

NB versus SC Filter Use

The previous discussions detail which filters to use for an individual application, however there are times when the results of the fluorescent measurements are not linear, or are affected by undesired fluorescent moieties. In these instances using a Narrow Bandpass Filter (NB) in the emission side of the filter holder may give more accurate results. In some instances more than one compound is present which can absorb the excitation light. If the undesired molecule subsequently does not fluoresce, then the undesired molecule will have no effect on the reading of the fluorometer. If however the undesired molecule does fluoresce then the proper choice of emission filter is important. The SC filter allows light of a specific wavelength and longer to be passed through to the photomultiplier tube. In the case when this light is the result of emissions from two or more different compounds in the sample, then a choice of using a Narrow Bandpass versus Sharp-Cut filter must be made. This is where fluorometry has a distinct advantage over absorption spectroscopy. If the fluorescence maximum of the desired compound is greater than the undesired moiety, choose a Sharp-Cut (SC) filter with a longer wavelength cutoff, above the fluorescence maximum of the moiety. In the case where the undesired moiety fluoresces at a wavelength longer than the compound of interest, choose a Narrow Bandpass (NB) filter closest to the fluorescence maximum of the compound of interest.

If the fluorescent wavelengths of two compounds overlap, then careful choice of the excitation bandpass filter can decrease the contribution to the fluorescent light emitted by the undesired moiety. Choose the excitation range for the desired compound using an NB filter at wavelength where there is significant absorption of light by the desired compound while there is little or no absorbance of light by the undesired compound.

Compromises Between NB and SC Filters

Use of an NB filter in the Excitation side of the filter holder allows light of a specific wavelength to excite a molecule. The intensity of the light passed through to the sample depends on the light source and the bandpass filter. Choosing an NB filter with a larger bandpass, like for the NB360 and NB430 filters, will allow for a larger spectral distribution of light to pass to the sample and excite more molecules.
This may increase sensitivity of the rest. Care should be taken when using these filters. Use of an NB filter on the emission side of the filter holder passes less of the fluorescent light produced by the sample to the photomultiplier tube. While being more specific for a wavelength range, the sensitivity of the test may be reduced.

Advanced Functions

To access the “Advanced Functions” parameter from the “Main Menu,” press the LEFT or RIGHT ARROW key until the display reads, “Advanced Functions,” then press ENTER. Press the LEFT or RIGHT ARROW key to step through the following options:

• Set Date and Time: User can define month, day, year, hour, minutes and seconds.

• View Stored Data: User can view standard curve data for defined methods.

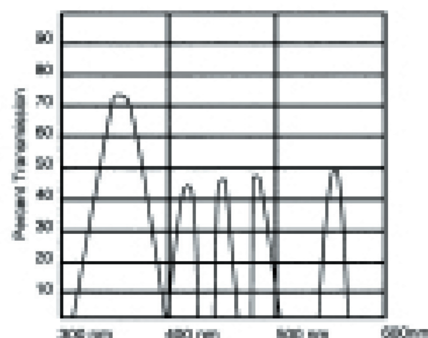
• Reset System to Default: User can reset the system to default settings in the rare event that the RAM is corrupted.

• View Diagnostic Information: User can perform a status check on fluorometer component functions to aid in troubleshooting.

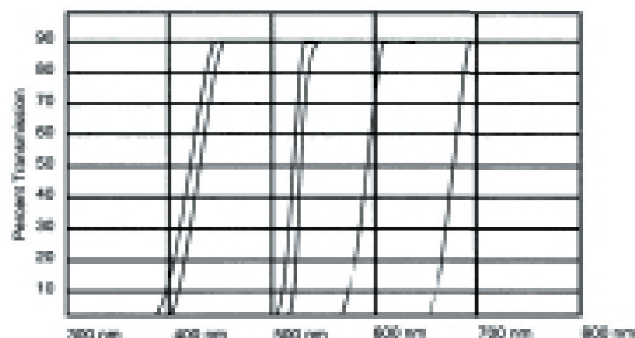
• Set Printer and Print Options: User can set data output configurations for optional printer or computer communication.
• UV Lamp Options: User can activate the UV lamp (mercury line lamp) on the Wide Band model of the Quantech.
• Manually Set Gain and PMT Voltages:
User can turn off the auto gain function and manually select gain and PMT Voltage.
• Injector Pump Options: User can activate optional injector pump for kinetics studies.

Please refer to the flowchart in the Full Manual Appendix for additional navigational information.

Note
Resetting the system will result in loss of all standard curves.



Bandpass Filter Transmittance Curves



Sharp Cut Filter Transmittance Curves