FL-45A

February, 1997

MF-9066 INSTRUCTION MANUAL

Fluorescence Detector

Bioanalytical
Systems, Inc

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Indiana 47906

MANUFACTURER'S NOTE

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This instrument, either wholly or in part, is manufactured for research purposes only. Use for medical diagnosis is not intended, implied or recommended by the manufacturer. Use for this purpose and accountability for the same rests entirely with the user.

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Safety Information

INSTRUMENT CERTIFICATION

In accordance with our long standing commitment to customers, the FL-45A fluorescence detector and its accompanying documentation have satisfied the requirements necessary to receive the FCC (Class A) certification.

IDENTIFYING SAFETY INFORMATION

This manual contains warnings and precautionary statements that can prevent personal injury, instrument damage, and loss of data if properly followed. All statements of this nature are called to your attention through the use of bold type.

SPECIFIC HAZARDS

Every instrument has specific hazards, so be sure to read and comply with the following precautions. They will help ensure the instrument's safe, long term use.

1. Before plugging your detector in and turning the power on, always make sure that the voltage and fuses are set appropriately for your local power supply. And never run the instruments at more than 10% below the nominal line voltage!

2. The supplied power cord must be inserted into a power outlet with a protective earth contact (ground). When using an extension cord, make sure that it's also grounded.

3. Do not change the external or internal grounding connections. Tampering with or disconnecting these connections could endanger you and/or damage the detector.

NOTE: The instruments are properly grounded when shipped. To ensure safe operation, do not alter the electrical connections or the instrument's chassis.

4. Never run the instruments without the top cover on. Permanent damage can occur.

5. Don't turn the instrument on if you suspect that it's incurred any kind of electrical damage. Instead, disconnect the power cords and/or power supplies and contact a BAS Representative for a product evaluation. Do not attempt to use the instrument until it's been evaluated (electrical damage may have occurred if the detector shows visible signs of damage, or has been handled roughly in transport).

6. Damage can also result if the instruments are stored for prolonged periods under unfavorable conditions (e.g., subjected to heat, water, etc.).

7. Always disconnect from power supply before attempting any type of maintenance.

8. Capacitors inside the instrument may still be charged even if the instrument is turned off.

9. Never try to repair or replace any instrument component that's not described in this manual without the assistance of BAS.

GOOD LABORATORY PRACTICES

Always follow good laboratory practices whenever you operate any high performance liquid chromatograph.

Keep Good Records

We recommend that you keep good records of all system conditions (e.g., %RDSs on retention times and peak area, peak shape and resolution, column pressure and detector sensitivity). At a minimum, keep a chromatogram of a standard mixture, well documented with system conditions. Careful comparison of retention times, peak shapes, column pressure, peak sensitivity, and baseline noise can provide valuable clues to identifying and solving problems.

Chemical Toxicity

Although the large volume of toxic and flammable solvents used and stored in laboratories can be quite dangerous, don't ignore the potential hazards posed by your samples. Take special care to read and follow all precautions that ensure proper ventilation, storage, handling, and disposal of both solvents and samples. Become familiar with the toxicity data and potential hazards associated with all chemicals by referring to the manufacturers' Material Safety Data Sheets (MSDS).

Sample Preparation

Always consider the solubility of your sample in the mobile phase. Sample precipitation can plug the system by obstructing the flow through the injector and/or the column. This obstruction may result in irreparable damage to parts of the system. Particulate matter can be avoided by filtering the samples through 0.45- or 0.2- micron (or less) filters.

Solvent Requirements

Many chemical manufacturers provide a line of high purity or spectro-quality reagents that are free of chemical impurities. Routine filtration of all solvents through a 0.45- or 0.2-micron (or less) fluorocarbon filter before analysis will significantly prolong the life and effectiveness of the pump's inlet filters, check valves and seals, injector and column.

Choose a mobile phase that's compatible with the sample and column you've selected for your separation. Remember that some solvents are corrosive to stainless steel.

Degas the Solvents

Degas you solvents by vacuum degassing or sparging with inert gas. Complete information for using either of these techniques is found in separate documentation provided with degas accessories.

Solvent Disposal

Make sure you have a solvent waste container or other kind of drain system available at or below the benchtop level. Most solvents have special disposal requirements and shouldn't be disposed of directly down a drain. Follow all governmental regulations when disposing of any chemical.

High Pressure Systems and Leaks

LC systems operate at high pressures, but since liquids aren't highly compressible, they don't store much energy. Thus, little immediate danger arises from the high pressure in an LC system. However, if a leak occurs, it should be corrected as soon as possible. Finally, we recommend that you always wear eye and skin protection when working on an LC system and that you always shut down the system and return it to atmospheric pressure before attempting any maintenance.

Section 1. Getting Started

1.1 INTRODUCTION

This section provides you with the three basic rules you'll need for using your Model FL-45A fluorescence detector. It also introduces you to the instrument's command center and describes the conventions we'll use in this manual.

Before you start this section, be sure to read the Safety Information located at the beginning of this manual and to install your detector as described in Section 6.

Throughout our explanations, we encourage you to explore the general architecture of the instrument's menus and screens. Use the Menu Tree in Section 7 as your guide if you wish.

1.2 LEARNING YOUR WAY AROUND

It's easy to learn to use your Model FL-45A detector. Just remember these three rules:

1. The arrow keys ([∧], [∨], [<], [>]) move the cursor in the direction printed on the key.

   HINT: Press [MENU] to jump quickly to the top of the menu structure.

2. The shape of the cursor determines how you make a selection:

   a. If a triangular cursor appears, press [ENTER].

   b. If a blinking square cursor (❑) appears, press the [+] or [–] keys to change values. Depending on the field, you'll scroll up or down through preset choices, or change alphanumeric entries one letter or digit at a time.

3. There are four ways to accept (and automatically save) an entry. Just move the cursor out of the field by any of the following methods:

   a. Pressing [ENTER]

   b. Using the arrow keys

   c. Pressing [menu]

   d. Pressing [STATUS]

   NOTE: You won't be able to leave a menu if errors are present or if you haven't filled in all the necessary entries.

Visual Clues

The following conventions are used on the detector's display:

1. Top-level menu choices are displayed in all-capital letters.

2. A field's square cursor (❑) changes to an underscore cursor (_) when you're scrolling through preset choices or entering numerical values and characters.

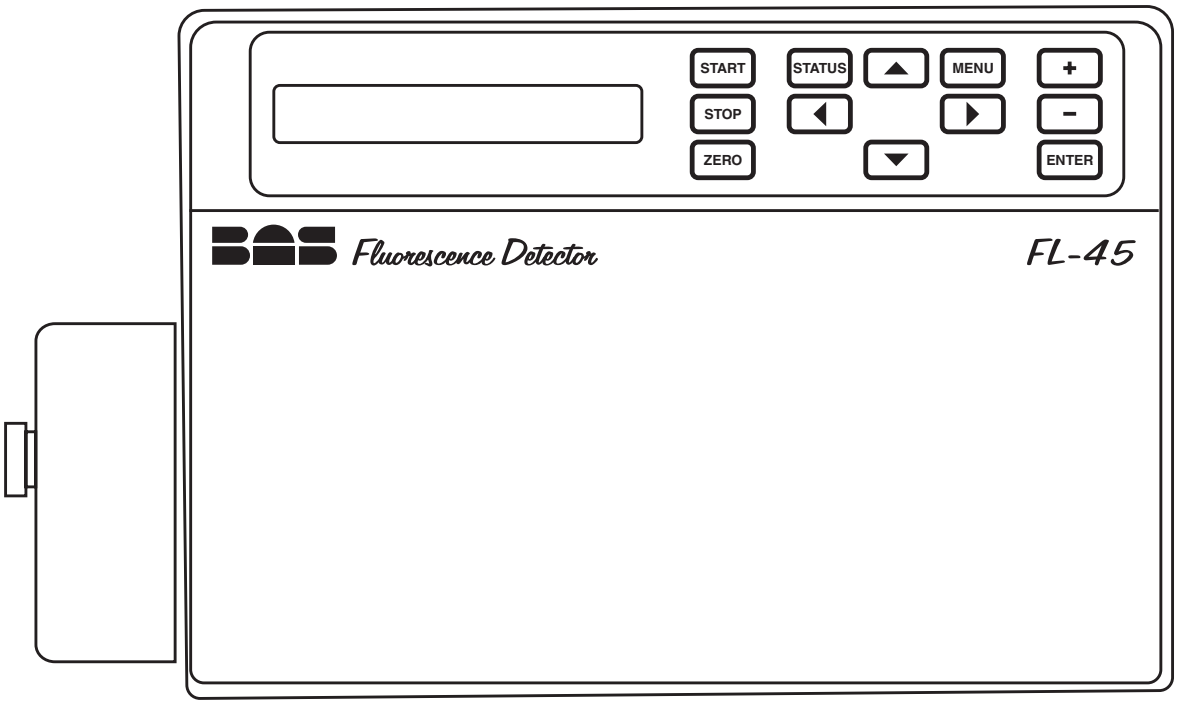
3. A solid down-arrow (▼) on the right side of some displays indicates that the current menu continues on additional screens. To access additional menu lines, press the down-arrow key, [∨].

4. The last line of a longer menu is frequently a blank display line without a solid down-arrow (▼).

1.3 INSTRUMENT CONTROL

Take a look at the keypad and two-line display located on the front panel (Fig. 1.1). This is the command center from which you'll access menus and control the instrument's operations. A brief explanation of the keys (Fig. 1.2) and the main menus and screens follows.

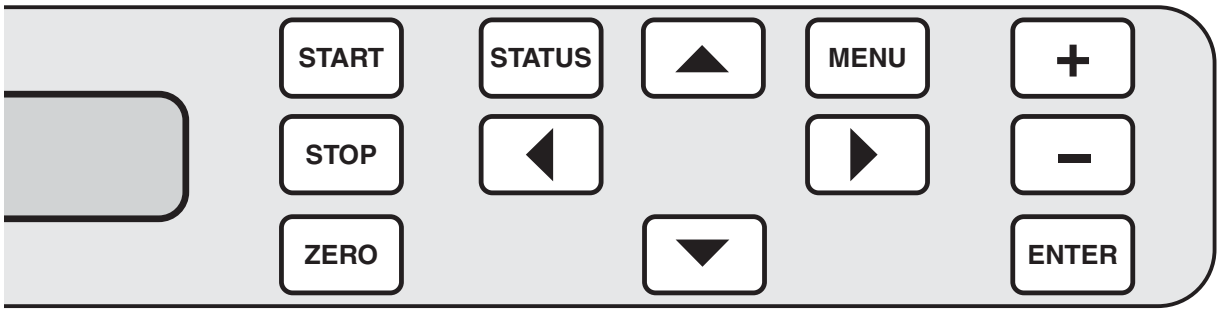
Figure 1.1 The detector's command center



The Keypad

The keypad of each Model FL-45A detector consists of twelve keys (Fig. 1.2). Four keys directly control the instrument's operation: [START], [STOP], [STATUS], and [ZERO]. The remaining keys either access commands ([MENU] and [ENTER]), or are used to set parameters and move around the display ([∧], [∨], [<], [>], [+], [–]). The function of each is explained below.

Figure 1.2 The detector's keypad



[START]
Pressing [START] begins a run. The detector must be in the READY state (or QREADY if a queue is loaded), indicating that the detector is stabilized and waiting to begin a run.

[STOP]
Pressing [STOP] halts a run, stops the internal clock, and returns the detector to a READY state. If a wavelength program is operating, pressing [STOP] halts the program and returns the detector to its initial conditions.

[STATUS]
Pressing [STATUS] displays the Status Screen (Fig. 1.4). From the Status Screen you can monitor the run in progress. You can also access the Status Menu.

[ZERO]
Pressing [ZERO] resets the detector output to zero volts, plus or minus any offset.

[MENU]
Pressing [MENU] displays the Main Menu (Fig. 1.3).

[ENTER]
Pressing [ENTER] accepts a selected choice or menu entry. The [ENTER] key also advances the cursor to a new field, either on the same line of the display or in the line below.

[∧], [∨], [<], and [>]
Pressing any arrow key (up, down, left, or right) moves the cursor in the direction indicated on the key. The up- and down-arrow keys also move the cursor between menus and displays.

[+] and [–]
Pressing the [+] and [–] keys scrolls you through a field's available choices or changes the value of alphanumeric entries. Holding down either key will continuously scroll the list of choices forward or backward until you release the key.

In fields that require numerical entries, the value of each digit is increased or decreased by one unit each time you press the [+] or [–] key. In fields that accept either numeric or character entries, such as the File Name field, the [+] and [–] keys scroll through the alphabet from A to Z, then through the numbers 0 to 9, and finally to a slash, hyphen, and blank space.

In other fields, the [+] key advances you through a preset list of choices while the [–] key takes you backward through the list.

Menus and Screens

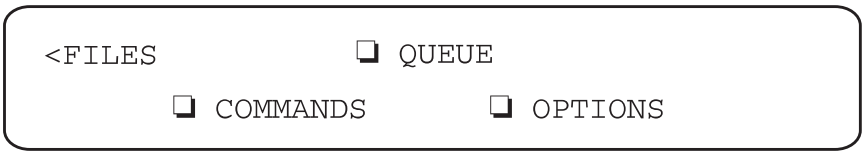
Your detector's display can show you three kinds of information: menus, screens, and messages. Menus require you to make selections or enter specific values. Screens display

information that cannot be edited. Messages confirm actions and point out errors. The Menu Tree in Section 7 outlines the structure and content of the detector's menus and screens, three of which are discussed here.

MAIN MENU

The Main Menu (Fig. 1.3) is the top level of the menu structure. It gives you access to five menus: FILES, QUEUE, TESTS, COMMANDS, and OPTIONS. To see the Main Menu, press the [MENU] key at any time.

Figure 1.3 The Model FL-45A's Main Menu

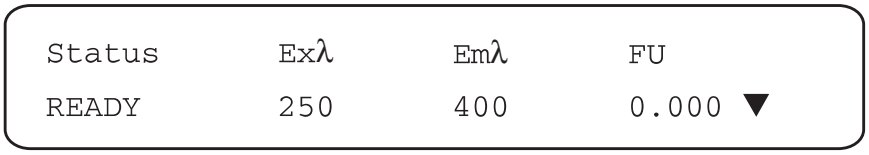


From the Files Menu you can edit, load, copy, or delete files. The Commands Menu lets you initiate spectral scanning, replay spectra, insert an event mark onto your chromatogram, short outputs, or shut down the detector. The Tests Menu lets you run built-in instrument tests and diagnostics. In the Options Menu, you can set up or change your instrument's configuration. From the Queue Menu you can edit or change the order of files in the sample queue. Refer to Sections 3, 4, 5, and Section 7 for more information on any of the instrument's menus.

STATUS SCREEN

The Status Screen (Fig 1.4) displays the detector status, excitation and emission wavelength settings, and the fluorescence intensity reading. It appears automatically whenever the instrument is powered on or the [STATUS] key is pressed. No entries are made on the Status Screen.

Figure 1.4 The Status Screen



STATUS MENU

Just below the Status Screen is the Status Menu. To access the Status Menu, press [∨] from the Status Screen. The Status Menu lets you review and edit run parameters during a run. Section 3 discusses the Status Menu in more detail.

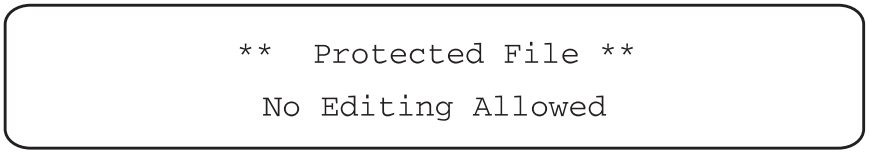
Messages

There are three kinds of messages that can appear on your detector's display: user messages, confirmation messages, and error messages.

USER MESSAGES

User messages, indicated on the display by two sets of double asterisks, tell you about an existing instrument condition or ask for further actions. Some of these messages will only appear on the display for three seconds. An example of a message requiring further action is shown in Figure 1.5.

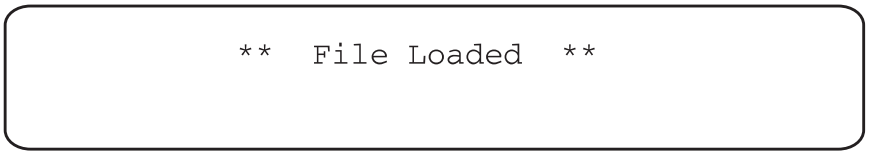
Figure 1.5 An example of a user message



CONFIRMATION MESSAGES

Confirmation messages (Fig. 1.6), also indicated on the display by two sets of double asterisks, appear for one second after an operation has been carried out successfully.

Figure 1.6 An example of a confirmation message



ERROR MESSAGES

Error messages (Fig. 1.7) are indicated on the display with capital letters and two sets of double exclamation points. They're shown whenever an undesirable condition exists that prevents the instrument from carrying out an operation. Error messages remain on the display until you press a key.

Figure 1.7 An example of an error message

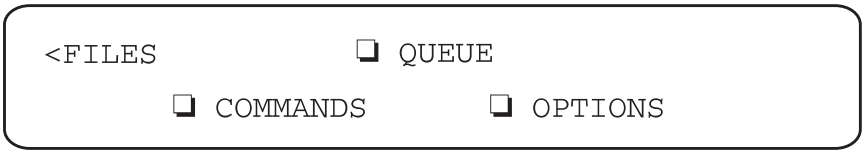
1.4 MANUAL CONVENTIONS

This manual uses several conventions. Among them are menu displays, text conventions (brackets, slashes, etc.), standard words, and several different notice words.

Displays

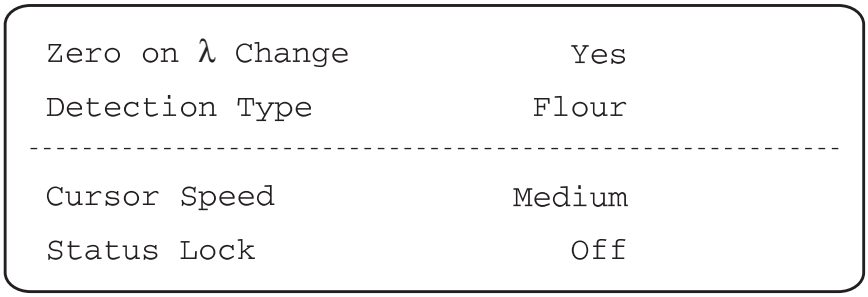
Figure 1.8 shows how we depict the two-line display. Note that, in menu illustrations, the triangular cursor location is indicated by a caret.

Figure 1.8 A two-line menu display



Frequently the two lines shown on the display are only part of a longer menu. In this manual, menus having more than two lines are represented as in Figure 1.9.

Figure 1.9 A menu longer than two lines



Text

Three typographic conventions are used to differentiate between keys, menus, and fields.

Brackets
Brackets, [ ], indicate instrument keys. For example: Press [MENU].

SLASHES
Slashes, / /, are used around menu choices. For example: From the Main Menu, select /FILES/.

CAPITALIZATION
Capitalization is used to make field and menu names appear just as they do on the display. Generally the first letters of field names are capitalized. For example: Select /FILES/, /Copy/, Copy File #.

Standard Words

We have also standardized the meanings of two words: “select” and “enter.”

SELECT
The word “select” is used when you need to choose from among available options. For example, to “select” a particular menu choice, you would move the cursor to the appropriate choice and press [ENTER]. To “select” a field entry, move the cursor to the appropriate field and use the [+] and [–] keys to scroll to the desired preset value.

ENTER
The word “enter” is used when you need to specify individual alphanumeric digits. To “enter” a particular value, move the cursor to the desired field and use the [+] and [–] keys to increment or decrement each digit in the field until the desired value or letter appears.

Notice Words

The following notice words are found within the text of this manual and will alert you to the following situations.

WARNING! Warnings alert you to situations that could result in personal injury. They also tell you how to avoid them. In addition to general warnings, this word is used to call out chemical hazard warnings. All warnings appear in bold type.

WARNING – Chemical Hazard! Chemical hazard warnings alert you to the potential dangers of handling chemicals. The warnings also tell you how to avoid chemical hazards. All chemical hazard warnings appear in bold type.

WARNING – High Voltage! This warning alerts you to the presence of high voltage and to the potential injury that could occur from electrical shock were you to come in contact with a specific instrument area or component. It also tells you how to avoid contact with the high-voltage areas in your instrument. All high-voltage warnings appear in bold type.

CAUTION! Cautions alert you to the correct operating or maintenance procedures needed to prevent equipment or data damage. All cautions appear in bold type.

HINT: Hints call out general rules or shortcuts. They specify ways to obtain the best performance and results from your instrument. All hints appear in italics.

NOTE: Notes alert you to important exceptions, side effects, or unexpected occurrences that may result from certain action(s). All notes appear in italics

1.5 WHAT’S NEXT?

Now you’re ready to try the practice example in Section 2, A Quick Example.

Section 2. A Quick Example

2.1 INTRODUCTION

In Section 1, you read about the three easy rules for using your detector's command center and some of its menus and screens. In this section, you'll find an example of a procedure that shows you how the rules and keys work as you move through the various menus.

This quick example uses only a fraction of the features available on your detector and is included only as a first step in becoming familiar with your new instrument.

After experimenting with this example, you'll want to turn to Sections 3 and 4, which cover the detector's basic and more advanced operations. It's in those sections that you'll learn about the full capabilities of your detector. First though, to give you a general understanding of the detector's capabilities and design, we'll briefly describe the features and benefits of the Model FL-45A here.

The Model FL-45A is a full-featured, time-programmable, fluorescence detector. The instrument employs an optical system design that provides high sensitivity and low baseline noise. With the advanced features of spectral scanning, multiple file storage, and file linking (with the Queue feature), the Model FL-45A provides increased versatility for your chromatography laboratory.

Before You Begin

Before you begin this quick example, the detector should be fully installed in your chromatographic system according to the procedures described in Section 6.

2.2 AN EXAMPLE

In this example, we'll show you how to prepare a file and how to load the file into the detector's operating parameters. After a practice run, we'll add a stop time.

HINT: You may wish to keep the Menu Tree in Section 7 on hand as you work through this example. If you lose your place at any time, you can:

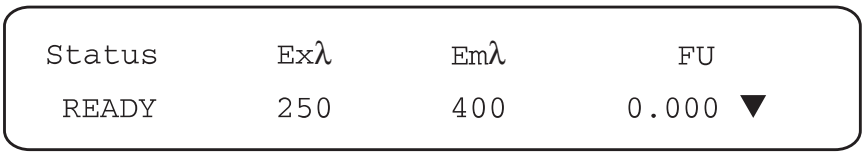
1. Press the [∧] key to move back to a previous screen.

2. Or, press [STATUS] to return to the Status Screen and retrace your steps.

Startup

Set the power switch located on the detector's rear panel to On. After a series of power-up tests, the Status Screen (Fig. 2.1) appears on the display. We will discuss the Status Screen after you've set up your operating parameters.

Figure 2.1 The Model FL-45A's Status Screen

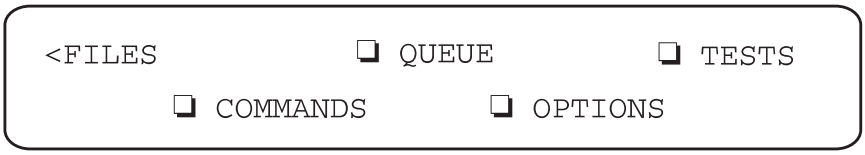


Setting Parameters

To set your parameters, you need to prepare an edit file. The following steps will show you how to access the Edit Menu and prepare the file:

1. Press the [MENU] key. The detector's Main Menu appears on the screen (Fig. 2.2).

Figure 2.2 The Model FL-45A's Main Menu

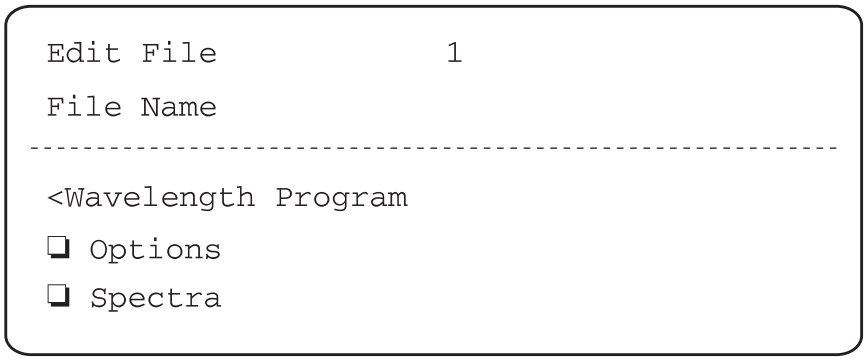


2. Now select /FILES/ to display the Files Menu (Fig. 2.3).

Figure 2.3 The Model FL-45A's Files Menu.

3. Select /Edit/ to display the Edit Menu (Fig. 2.4).

Figure 2.4 The Model FL-45A's Edit Menu



For this example, we'll use a file designation of 1 and leave the File Name field blank.

WAVELENGTH

The excitation and emission wavelengths are examples of fields that require a numeric entry. To set each wavelength:

1. From the Edit Menu (Fig. 2.4), select /Wavelength Program/ to display the Wavelength Program (Fig. 2.5). The cursor will automatically be in the excitation wavelength field.

Figure 2.5 The Model FL-45A's wavelength program

```
Time          Exλ        Emλ
0.00          250        400
```

2. Using the [+] and [–] keys, set each wavelength field to the desired setting for your analysis. Remember that each digit must be edited individually.

3. Press [ENTER] to accept the new wavelength settings.

LAMP STATUS

Lamp Status is an example of a field that gives you a preset list of choices. To set the lamp status:

1. Select /Options/ from the Edit Menu (Fig. 2.4) to display the Options Menu (Fig. 2.6).

Figure 2.6 The Model FL-45A's Options Menu

```
Range 1                    10
Range 2                    10
------------------------------------------------------------
Rise Time                   2
Autozero Time            0.00
Lamp Flash Rate           100
Lamp Status               run
PMT Voltage               600
```

2. Use the [∨] key to scroll to the Lamp Status Line.

3. Using the [+] or [–] key, select On from the list of choices.

4. Press [ENTER] to turn the lamp on.

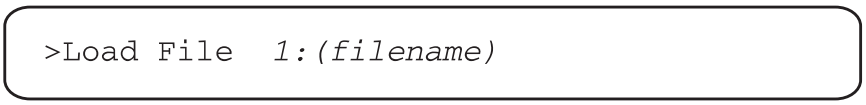
For this example, we'll use the default settings for the remaining parameters. You will learn more about setting these parameters in Section 3.

LOADING THE FILE
You're now ready to load the settings from File 1 into the detector's operating parameters. To load the file:

1. Return to the Files Menu (Fig. 2.3) using the [∧] key.

2. Select /Load/. The screen in Figure 2.7 appears.

Figure 2.7 The Load file command



3. Press [ENTER] to execute. The confirmation message shown in Figure 2.8 appears for one second.

Figure 2.8 The file-loaded message

You're returned automatically to the Status Screen and are ready to run your detector.

A Practice Run

Now you're ready for a practice run! Note that the Status Screen (Fig. 2.1) now displays your excitation and emission wavelength settings, the detector's status, and the fluorescence intensity. If the Status reads READY, the detector is stabilized and ready to run.

When the detector is stabilized:

1. Press the [ZERO] key to zero the detector's analog output signal.

2. Inject your sample.

During setup, you may have noticed that there was no stop time entered in the detector's parameters. In this case, the detector stays in the READY state and continually monitors the column eluant. You don't need to manually start or stop a run with this set-up.

Adding A Stop-time

To add a stop-time, you need to use the following steps to modify the detector's operating parameters. You will then start and stop a run, using the new setting.

1. From the Status Screen, press the [∨] key to move down to the Status Menu (Fig. 2.9), which is the programming area below the Status Screen.

Figure 2.9 The Model FL-45A's Status Menu

```
File  :1
-----------------------------------------------------------

Time          Emλ           Emλ
0.00          250           400

Range 1                      10
Range 2                      10
Rise Time                     2
Autozero Time                 0
Lamp Flash Rate             100
Lamp Status                 run
PMT Voltage                 600
```

2. Using the [∨] key, move the cursor to the blank line below the 0.00 time line and press [+]. This adds a second line, with a time of 1.00 and the same wavelength settings as the first. Change 1.00 to the desired stop-time for the run, and leave the wavelengths unchanged.

3. To save your edits, scroll down to the words “Save File” (which now appear below PMT Voltage), and press [ENTER]. The message shown in Figure 2.10 appears and you're returned automatically to the Status Screen.

Figure 2.10 The file-saved message

```
**  File Saved  **
```

Running With A Stop-time

Now that you've entered a stop time, you'll need to start the run with each injection. To do this:

1. Zero the detector's analog output signal by pressing the [ZERO] key.

2. When the detector is stabilized, inject your sample and press [START].

Notice that Status now shows the run time. If you wish to stop your run before the set stop-time, simply press [STOP].

2.3 WHAT'S NEXT?

Once you've completed this example and are comfortable with the keypad and display, proceed to Section 3, *Basic Operations*, to learn more about your detector.

Section 3. Basic Operations

3.1 INTRODUCTION

This section provides you with step-by-step instructions for the most frequently used detector operations, including setup and run procedures for single (emission and excitation) wavelength mode, detector file management and protection, and analog output operations. You may wish to keep the Menu Tree and Menu Listing from Section 7 on hand as you work through this section.

NOTE: Your display's values may differ from those presented in this manual, especially if the detector has been programmed previously.

3.2 BEFORE YOU BEGIN

Before you begin this section, your detector should be installed in a chromatographic system (see Section 6). We also recommend that you review Section 1, *Getting Started*, which gives general instructions for using the detector keypad and the conventions used throughout this manual.

3.3 SINGLE-WAVELENGTH OPERATION

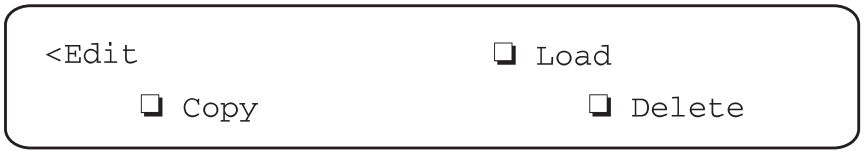
This section will show you how to perform single-wavelength operation. You'll learn how to identify and edit a file, how to load that file into the detector's current operating parameters, and how to start and stop a run. This section will also show you how to modify the detector's operating parameters.

Setting Parameters

Before you start setting any detector parameters, you need to identify the file you wish to edit. To do this, access the Files Menu.

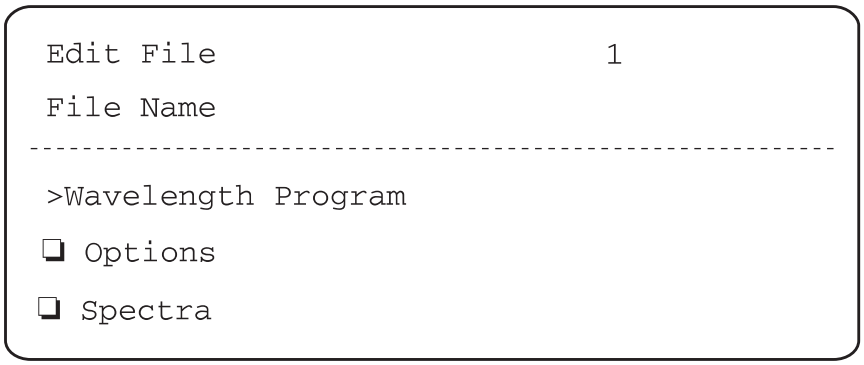
To access the Files Menu, first press [MENU]. The Main Menu appears on the screen. From the Main Menu, select /FILES/. The menu shown in Figure 3.1 will appear.

Figure 3.1 The Model FL-45A's Files Menu



Select /Edit/ from the Files Menu to display the Edit Menu (Fig. 3.2).

Figure 3.2 The Model FL-45A's Edit Menu



FILE IDENTIFICATION

Enter the file number you wish to edit in the Edit File field. The Model FL-45A can store up to four files in memory, so file numbers from 1 to 4 are allowed. You may also enter a name of up to eight characters in the File Name field.

WAVELENGTH PROGRAM

From the Edit Menu, select /Wavelength Program/. The Wavelength Program (Fig. 3.3) consists of a table showing time and the excitation and emission wavelengths.

Figure 3.3 The Model FL-45A's Wavelength Program

You can operate with either a one-line or a two-line wavelength program. Using a one-line program, the detector is always in the READY state and you can monitor the chromatographic eluant continually. Using a two-line program, you can add a stop-line and you can start and stop the detector during a chromatographic run. (Stop-lines are useful, for example, in a series of automated runs where you want to autozero the detector's baseline after each injection.)

For a one-line program, enter the excitation and emission wavelengths for your analysis (in the Exλ and Emλ fields, respectively), that correspond to the start time of 0.00.

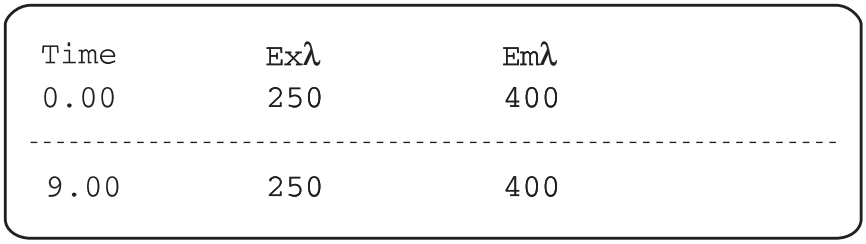
HINT: Always set the emission wavelength greater than the excitation wavelength by more than 1.5 to 2 times the monochromator's slit widths to minimize light-scattering effects. Also, avoid setting the excitation wavelength to exactly one-half the value of the emission wavelength. This condition creates second-order (Rayleigh) scattering that will require an optical filter in conjunction with the emission slit.

HINT: Switch to the optional Extended Range PMT if your emission wavelength setting is in the 600 to 800 nm range.

For a two-line program, add an additional line (the stop-line) by scrolling down to the blank line below and pressing [+]. The second line automatically will have a time setting of 1.00 and the same wavelength setting as the first. (You will learn more about time lines in Section 4.) Change 1.00 to the desired stop time for the run, and leave the wavelength values unchanged.

An example of a two-line wavelength program for a nine-minute run at an excitation wavelength of 250 nm and an emission wavelength of 400 nm is shown in Figure 3.4.

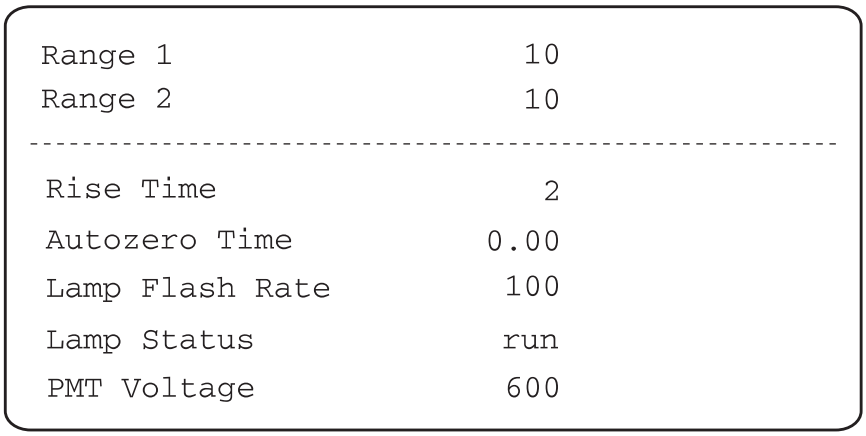
Figure 3.4 An example of a wavelength program with a stop time



OPTIONS

Select /Options/ from the Edit Menu to display the Options Menu (Fig. 3.5). Use this menu to set the detector's ranges, rise time, autozero time, lamp flash rate, lamp status, and PMT voltage.

Figure 3.5 The Model FL-45A's Options Menu (as selected from /Files/, /Edit/)



Range 1 and Range 2

Set each range value to an appropriate full-scale fluorescence intensity for your sample. For example, a peak of five fluorescence units will appear full-scale on a recorder at a range setting of 5 FUFS (fluorescence units full-scale). Note that Range 1 and 2 correspond

to Analog Outputs 1 and 2 (labeled CH1 and CH2) on the rear panel of your detector. For more information on the use of ranges and analog outputs, see pages 25 and 68.

Rise Time
This field affects the detector's response time. Rise time is inversely proportional to the amount of baseline noise. For example, the longer the rise time, the less noise detected. The two-second default value is appropriate for most applications.

HINT: To minimize baseline noise while retaining maximum resolution, select a rise time that's at least one-tenth of the peak width at the base of the narrowest peak of interest.

Autozero Time
This parameter tells the detector when to perform an automatic zero of the baseline. If you don't want to set an automatic autozero and you're using a stop-line in your wavelength program, simply set the autozero time to a value greater than your stop-time.

HINT: It's good practice to zero the detector automatically at the start of each run. This will keep the detector output in range throughout an automated series of runs.

Lamp Flash Rate
Set the rate at which the xenon lamp should pulse on and off. The 20 Hz setting prolongs lamp life, while the 100 Hz setting results in greater sensitivity at the expense of reduced lamp life. Most likely you'll use the 100 Hz setting for most applications.

Lamp Status
Set the lamp mode you wish to use. You can choose from the following selections:

a. *On and Off*. These selections turn the lamp on or off, as soon as you accept the setting.

b. *Run*. Choosing “run” automatically turns the lamp on at the beginning of each run and off at the end of each run.

c. *Off@End*. This selection turns the lamp off at the end of a queue. (You will learn about the queue feature in Section 4.)

HINT: Lamp life is increased significantly by turning the lamp off whenever the detector isn't in use.

PMT Voltage
Set the voltage to be applied to the photomultiplier tube (PMT). The PMT's sensitivity is proportional to the applied voltage, but higher voltages also cause a shortening of the PMT's service life.

HINT: The default value of 600 satisfies most applications' sensitivity needs, while providing an acceptable service life.

LOADING A FILE

When you're ready to load a file, select /Load/ from the Files Menu. The screen will display the words “Load File 1:(filename).” Enter the desired file number and press [ENTER]. The message shown in Figure 3.6 will appear for one second. You're then returned to the Status Screen.

Figure 3.6 The file-loaded message

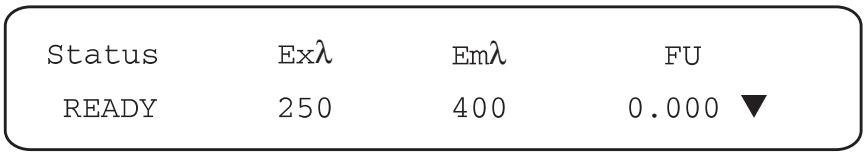
RUNNING YOUR DETECTOR

Once you've set your detector parameters in the designated file and have loaded the file into the detector's operating parameters, you're ready to run your analysis. First check the detector's status by viewing the Status Screen. If you're using a stop-line in your wavelength program, you'll start and stop the run with each injection.

STATUS SCREEN

You can check the detector's status, wavelength settings, and fluorescence reading from the Status Screen (Fig. 3.7). To access the Status Screen, press [STATUS].

Figure 3.7 The Model FL-45A's Status Screen



If the Status Screen reads READY, the detector is stabilized and ready to run. The Exλ and Emλ fields display the current excitation and emission wavelength settings. The FU field is the current fluorescence intensity reading.

INJECT YOUR SAMPLE

When the detector is stabilized and you're ready to inject your sample, manually zero the detector by pressing the [ZERO] key.

If you're not using a stop-line in the wavelength program, the detector remains in the READY state throughout your chromatographic runs. If you're using a stop-line, you must start and stop the run with each injection, following the procedures below.

STARTING A RUN

If you're using a stop-line in your wavelength program, you need to start the run with each injection. There are two ways to start a run using the Model FL-45A:

1. *Manually*, by pressing [START] each time you make an injection.

2. Automatically, by interfacing the detector with a remote run-signal from the injector (see Section 6 for details). In this scenario, a signal that's equivalent to pressing [START] is sent from the injector to the detector automatically with each injection.

During the run, you can monitor the run time from the Status Screen.

STOPPING A RUN
There are two ways to stop a run:

1. *Manually*, by pressing [STOP] before the programmed stop time.

2. *Automatically*, by allowing the run to finish at the preset time.

In either case, the detector returns to READY.

Changing Run Parameters

There are two ways to change the detector's run parameters:

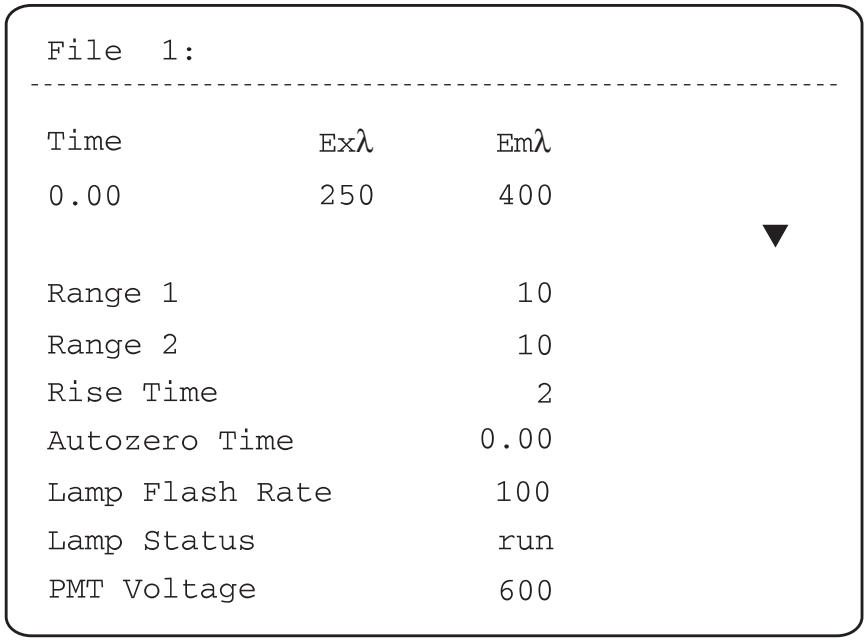
1. You can use the Files Menu and follow the procedures outlined under “Setting Parameters” on page 15.

2. You can use the Status Menu, which is the programming area below the Status Screen.

Each has a distinct advantage. Programming in the Status Menu allows you to change the detector's current operating parameters, even while the detector is running. Programming in the Files Menu allows you to prepare a file containing the changes without altering the current detector settings. The file may then be loaded at a later time.

STATUS MENU
From the Status Screen, scroll down to the Status Menu (Fig. 3.8), which contains the loaded file identification (its number and name), Wavelength Program, Ranges, Rise Time, Autozero Time, Lamp Flash Rate, Lamp Status, and PMT Voltage.

Figure 3.8 The Model FL-45A's Status Menu



The detector's parameters are set following the same instructions previously given under "Wavelength Program" and "Options Menu," starting on page 16. However, you cannot modify the file identification while in the Status Menu.

NOTE: When you modify a file's parameters from the Status Menu, you don't change the contents of the same file number stored in the detector's memory. Only the copy of the active file is modified.

SAVING THE FILE

When you modify a loaded file from the Status Menu, each change is effective as soon as you leave the field. You're reminded of the file's changed status in two ways: the file name shown on the first line of the Status Menu (Fig. 3.8) now reads "-changed" and a Save File command appears at the very end of the Status Menu (below PMT Voltage). Press [ENTER] at the Save File command to save the new values. The message shown in Fig. 3.9 appears.

Figure 3.9 The message that's displayed when a file is saved.

If you wish to keep the original file without saving the changes, don't press [ENTER]. Instead, reload the unaltered file by using the Files Menu as follows:

1. Press [MENU].

2. Select /FILES/.

3. Select /Load/.

4. The words “Load File N: (filename)” will appear on the screen. Enter the desired file number and press [ENTER].

The confirmation message shown in Figure 3.6 will appear for one second. You're then returned to the Status Screen, where all settings will contain their original values.

3.4 MORE ABOUT FILES

On page 15 you learned how to edit and load files from the Files Menu, but the Files Menu also allows you to copy and delete files in a few easy steps. This section will show you how. It will also show you how to protect files from being edited, copied to, or deleted.

Copying Files

To copy a file:

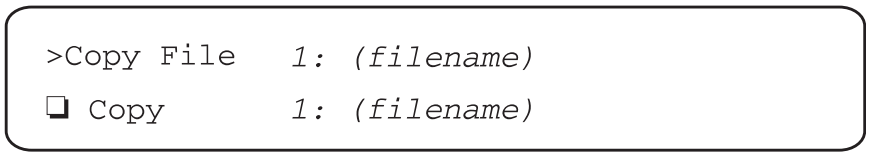
1. Press [MENU].

2. Select /FILES/ to display the Files Menu selections (Fig. 3.10).

Figure 3.10 The Model FL-45A's Files Menu

3. Select /Copy/. The Copy Menu will appear on the screen (Fig. 3.11).

Figure 3.11 The Model FL-45A's Copy Menu



4. Enter the identification number for the file you wish to *copy* in the Copy File field.

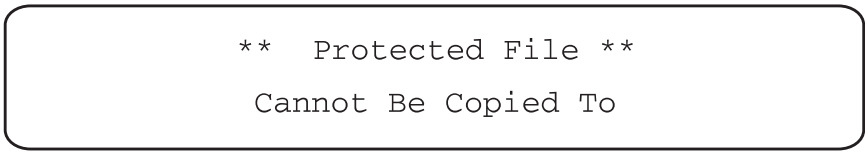
5. Enter the number of the file to which you wish to copy *to* in the To File field.

6. Press [ENTER]. The message shown in Figure 3.12 appears briefly, and you're returned to the Files Menu.

Figure 3.12 The file-copied message

If you attempt to copy to a protected file (see the section below, titled “Protecting Files”), you'll get the message shown in Figure 3.13. If a file isn't protected, make sure it's empty or unwanted before you copy to it, as it will be overwritten.

Figure 3.13 The message that's displayed when you attempt to copy to a protected file.



Deleting Files

To delete a file:

1. Press [MENU].

2. Select /FILES/ to display the Files Menu (Fig. 3.10).

3. Select /Delete/. The words “Delete File N:(filename)” will appear on the screen.

4. Enter the identification number of the file you wish to delete. When you press [ENTER], the message shown in Figure 3.14 appears briefly, and the display returns to the Files Menu. (The parameters in the file you've just deleted return to their default values.)

Figure 3.14 The file-deleted message

If you attempt to delete a protected file (see the next section, “Protecting Files”), you'll get the message shown in Figure 3.15.

Figure 3.15 The message that's displayed when you try to delete a protected file

```
**  Protected File **
   Cannot Be Deleted
```

Protecting Files

The Model FL-45A allows you to protect files from being edited, copied to, or deleted. To access the file protection operation, follow these steps:

1. Press [MENU].

2. Select /OPTIONS/. The Options Menu appears in Figure 3.16

Figure 3.16 The Model FL-45A's Options Menu

```
>Analog Outputs
❑ More
```

3. Select /More/ to display the More Menu (Fig. 3.17).

Figure 3.17 The Model FL-45A's More Menu

```
Zero on λ Change              Yes
Detection Type              Fluor
-----------------------------------------------------
Cursor Speed               Medium
Status Lock                   Off
File Name                 Protect
1:                            Off
2:                            Off
3:                            Off
4:                            Off
```

4. Scroll down to the table containing the fields File Name and Protect. To protect a file from being edited, copied to, or deleted, select On in the Protect field that corresponds to the appropriate file number. To remove the file protection, select Off.

3.5 ANALOG OUTPUT OPERATIONS

The Model FL-45A has two outputs, Analog Output 1 and Analog Output 2. Labeled CH1 and CH2 on the detector's rear panel, these outputs are useful for monitoring analyses at two different sensitivity settings simultaneously. For example, analog outputs allow you optimally to detect very small peaks and very large peaks in the same sample run.

For information on how to make rear-panel connections for analog outputs, see page 68.

Analog Offsets

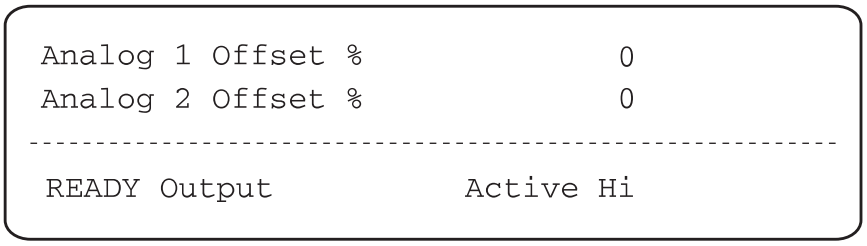
Analog offsets may be used when there's a high background fluorescence reading, or when there's considerable baseline drift from your chromatographic system and you're unable to keep your integrator's (recorder's) signal on-scale.

Because integrators have a very limited capacity for handling negative signals, you may wish to set a small positive offset (1%) when using an integrator.

Use negative offsets with recorders, where you may wish to set the pen at either side of the strip chart.

The offset options are selectable from the Analog Outputs Menu shown in Figure 3.18.

Figure 3.18 The Model FL-45A's Analog Outputs Menu



To access the Analog Outputs Menu:

1. Press [MENU].
2. Select /OPTIONS/.
3. Select /Analog Outputs/.

HINT: We recommend a 1% offset setting for use with your integrator.

Section 4. Advanced Operations

4.1 INTRODUCTION

In this section, you'll learn to use the Model FL-45A's more advanced capabilities, such as wavelength programming, automatic zeroing, scanning, and queues. You should be familiar with the instructions presented in Section 3, *Basic Operations*, before you begin.

4.2 WAVELENGTH PROGRAMMING

Your detector can change excitation and/or emission wavelength as a function of time, a feature we call Wavelength Programming. This feature gives you maximum detection sensitivity for each component in a mixture without making multiple injections of the sample.

Building The Program

In wavelength programming, you enter time lines into a "Wavelength Program." Each time line specifies the time at which you want a wavelength change to occur.

You can build a wavelength program in either the Status Menu or the Files Menu using the procedure outlined in this section.

Figure 4.1 The Model FL-45A's Wavelength Program

The initial time entry is 0.00. Move the cursor to the Exλ and Emλ fields, and enter the initial excitation and emission wavelengths for your analysis.

ENTERING A SECOND TIME LINE

To add a second time line, scroll down to the first blank line and press [+]. The second line automatically will have a time setting of 1.00 and the same wavelength settings as the first. Change the Time and corresponding Exλ and Emλ fields to the desired values.

ADDING SUBSEQUENT LINES

A wavelength program may contain as many as ten lines for a single run. If you enter time lines out of sequence, the detector will automatically sort the lines and place them in chronological order.

THE STOP-LINE

The last line of the program (the stop-line) lists the time at which the detector automatically will end the run and return to initial conditions. Since wavelengths aren't important in the stop-line, they can be set to any value(s).

NOTE: Remember, the last line of the program is always the detector's signal to end a run; it's not a programmed wavelength change!

DELETING A LINE
To delete an entire time line, place the cursor in the Time field and press [–] repeatedly until the value goes blank. When you leave the line, it will be deleted.

Figure 4.2 shows a completed wavelength program.

Figure 4.2 An example of a completed wavelength program

Time	Exλ	Emλ
0.00	250	400
2.50	280	375
6.00	280	375

In Figure 4.2., the initial excitation and emission wavelengths are 250 and 400 nm, respectively. At 2.50 minutes into the run, the wavelengths change to 280 and 375 nm. The run ends at 6.00 minutes, and the detector returns to its initial wavelengths of 250 and 400 nm and to its READY state.

Running The Program

After you set the rest of your parameters, the detector is ready to run. It's good practice to zero the detector at the beginning of every run and at each wavelength change. See the next section, titled “Programmed Autozero,” for details.

Once you start the run, you may edit any timed event (wavelength change, autozero, or stop-time) that has not yet taken place. These changes can be made only from the Status Menu however! Each edit is entered immediately into the detector's operating wavelength program.

For example, for the program displayed in Figure 4.2, the stop time is 6.0 minutes. If, at 5.00 minutes into the run, you determine that the run should be 9.00 minutes long, you can edit the last line of the program and the current run will stop at 9.00 minutes.

4.3 PROGRAMMED AUTOZERO

The Model FL-45A can be programmed to perform an automatic zero with each wavelength change during a run using the Zero on λ Change field. To access this feature:

1. Press [MENU] and select /OPTIONS/ to access the Options Menu (Fig. 4.3).

Figure 4.3 The Model FL-45A's Options Menu

2. Select /More/ to display the More Menu (Figure 3.17, p. 24).

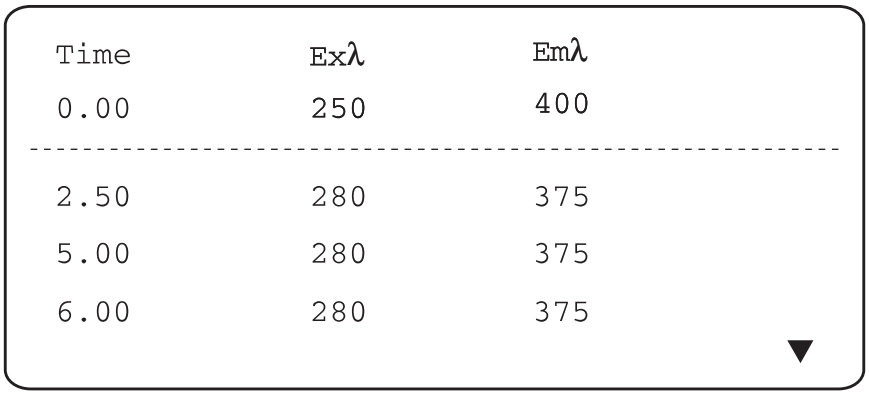
3. Place the cursor on the Zero on λ Change field. This field appears on the first line of the More Menu.

4. Select Yes, to zero the detector response automatically with each wavelength change during a run, or No, to turn this feature off.

You can also use this automatic zero feature to add autozeros into your wavelength program *without* changing the detector's wavelength settings. To do this, simply add additional time lines. Adding autozeros in this way is convenient in cases such as solvent programming, where the detector's baseline may drift due to changes in solvent background.

An example program is shown in Figure 4.4.

Figure 4.4 Example of wavelength program with automatic autozeros



With the Zero on λ Change field set to Yes, the detector will autozero at 2.50 and 5.00 minutes into the run, even though the wavelength will only change once (at 2.50 minutes into the run).

4.4 SCANNING

The Model FL-45A is uniquely capable of performing an excitation, emission, or synchronous (delta) spectral scan on eluting peaks without stopping the eluant flow. This ability greatly simplifies the determination of wavelength maxima for individual compounds in your sample during method development work.

How It Works

When a scan is initiated, either manually or automatically, the monochromator moves from the run-wavelength to the scan's start-wavelength. The detector scans by stepping through the defined spectral range at specified wavelength increments and at a rate of 100 Hz. Individual fluorescence intensities are read at each increment until the monochromator reaches the last wavelength. The scan is then repeated in reverse, from the last wavelength to the first. Up to 32 scans can be averaged to minimize any effects from changing peak concentrations.

The number of data points in each scan determines the number of spectra that the Model FL-45A can collect and store for a single chromatographic run. Use the following equations to calculate the number of data points and the number of spectra you'll be able to collect.

Equation 1. To calculate the number of data points for any scan between λ1 (the lower wavelength), and λ2 (the higher wavelength):

$$\#\ of\ data\ points < \frac{\lambda 2 - \lambda 1}{step\ size} + 1$$

Equation 2. To calculate the number of spectra you can collect:

$$\#\ of\ spectra < \frac{12{,}800}{(\#\ of\ data\ points \times 4) + 14}$$

For example, if you want to scan from 200 to 400 nm in 2-nm steps, there would be 101 data points and the Model FL-45A would be able to store up to 30 spectra:

$$\#\ of\ spectra < \frac{12{,}800}{(101 \times 4) + 14} = \frac{12{,}800}{418} = 30.62$$

Each spectrum is corrected for baseline fluorescence before being played back as individual data points or as a continuous curve.

Preparing A File

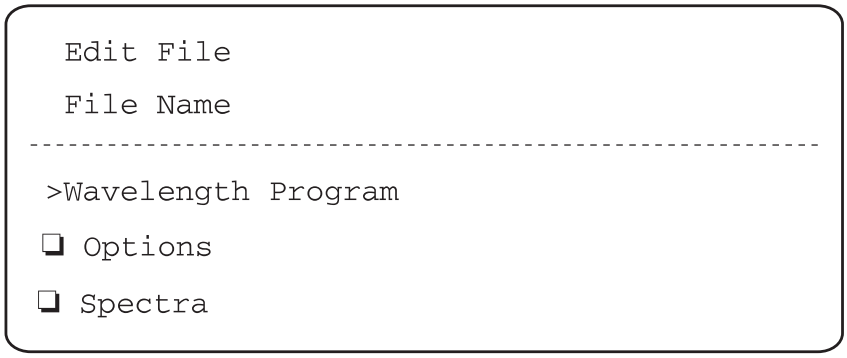
To prepare a scan file, you need to set up both the detector's run parameters and its scan parameters.

SETTING THE RUN PARAMETERS
Use the following steps to set the detector's run parameters:

1. Press [MENU]. Select /FILES/.

2. Select /Edit/ to display the Edit Menu (Fig. 4.5).

Figure 4.5 The Model FL-45A's Edit Menu



3. Enter whatever file identification you wish to use in the Edit File and File Name fields.

4. Set the detector's run parameters in the Wavelength Program and Options Menus.

NOTE: To perform an excitation scan, the Model FL-45A uses the emission wavelength programmed in the file through the Wavelength Program (not the Spectra Menu). To perform an emission scan, the detector uses the excitation wavelength programmed in the file. For a delta scan, the monochromators move to the start wavelengths programmed in the Spectra Menu.

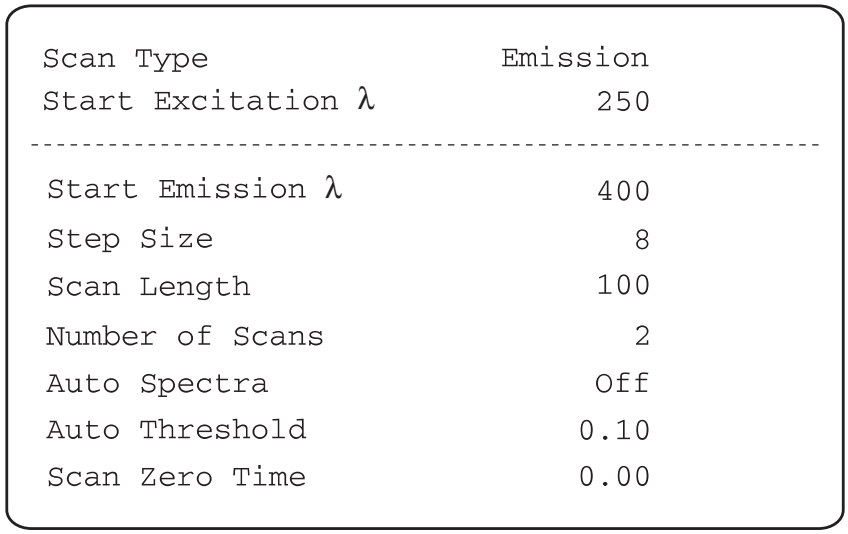
5. Select /Spectra/. The Spectra Menu, described in the next section, is where you'll set up your spectral scanning parameters.

SETTING THE SCAN PARAMETERS

Use the Spectra Menu as follows to set the detector's scan parameters:

1. From the Edit Menu, select /Spectra/. The Spectra Menu shown in Figure 4.6 appears.

Figure 4.6 The Model FL-45A's Spectra Menu



2. In the Scan Type field, select the scan mode you wish to run. For an excitation scan, the emission wavelength is held constant while the excitation monochromator performs the scan. For an emission scan, it's the opposite. In a delta scan, both monochromators move simultaneously, keeping the same wavelength span (delta) between them.

3. In the Start Excitation λ field, enter the excitation wavelength at which each scan should begin. When you're performing emission scans, this parameter is ignored.

4. In the Start Emission λ field, enter the emission wavelength at which each scan should begin. When you're performing excitation scans, this parameter is ignored.

5. In Step Size, enter the wavelength increment at which the detector will scan.

NOTE: If you chose starting and ending wavelengths that were not an exact multiple of your step size, the ending spike (event mark) on your chromatogram would be placed at the last multiple of the step size that falls within the scanning range. For example, with a starting wavelength of 200 nm, an ending wavelength of 365 nm, and a step size of ten, the end spike on your chromatogram would be at 360 nm, the last full step size within the range.

6. In Scan Length, enter the spectral range for each scan.

7. In Number of Scans, enter the number of times the monochromator should perform each scan (for averaging).

8. In Auto Spectra, select On, if you want the detector to scan automatically, or Off, if you choose to scan manually.

9. In Auto Threshold, specify the minimum peak fluorescence intensity that will signal the detector to perform an automatic scan. If you're scanning manually, disregard this parameter.

10. In Scan Zero Time, enter the run time at which the detector should perform an automatic baseline scan.

When you're finished with setup, return to the Files Menu. Load your scan file and you're ready to run.

While The Scan File Is Running

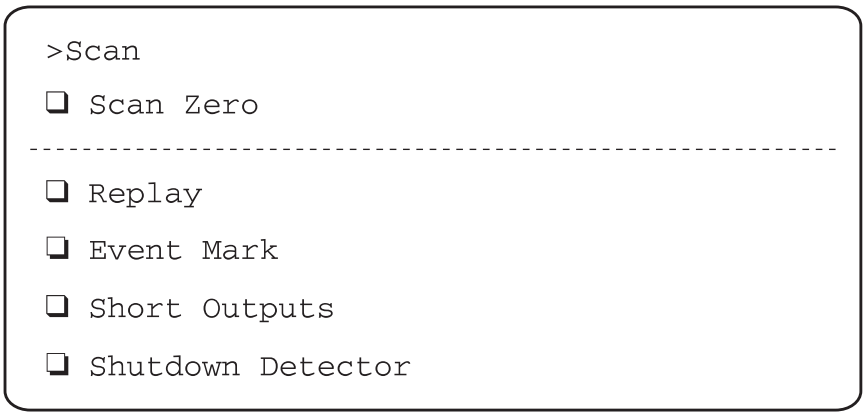
As was noted in the introduction, you can perform scans manually or automatically during a run. In either case, you'll see a “wiggle” in the chromatographic trace each time a scan is taken. For this reason, quantitative analysis should never be performed when scanning.

MANUAL SCANNING
To perform manual scanning, access Scan and Scan Zero in the Commands Menu as follows:

1. Press [MENU].

2. Select /COMMANDS/. The menu shown in Figure 4.7 appears.

Figure 4.7 The Model FL-45A's Commands Menu



3. Select /Scan/ or /Scan Zero/.

SCAN
Select Scan whenever you want to perform sample scans of the chromatographic peaks. Press [ENTER] each time you wish to perform a sample scan.

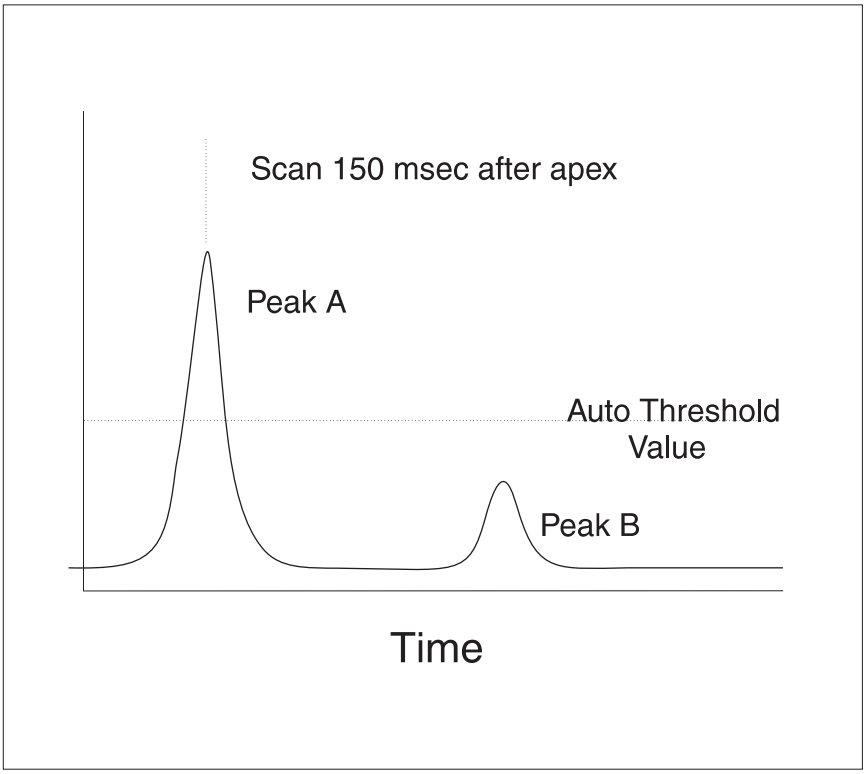
SCAN ZERO
Use Scan Zero to perform baseline scans of the solvent background fluorescence. With the detector baseline stabilized and the cursor on the Scan Zero command, press [ENTER]. The Model FL-45A will perform and store a baseline scan using the parameters in your scan file. Each subsequent sample scan is corrected automatically for any background absorbance.

Baseline scans may be taken at any time during the run, as long as no peak is eluting at that time. Subsequent sample scans are corrected using the last baseline scan taken.

For example, let's say you perform a baseline scan before you initiate a run, and then again at 5.00 minutes into the run. You also perform sample scans of your eluting peaks at 2.4 and 5.6 minutes into the run. The sample scan taken at 2.4 minutes will be corrected using the baseline scan taken before the run began. The sample scan taken at 5.6 minutes will be corrected using the baseline scan taken at 5.0 minutes.

AUTOMATIC SCANNING
If you've set the Auto Spectra field in the Spectra Menu to On (see the section, “Setting The Scan Parameters” on page 31), your detector will perform an automatic scan at 150 msec after a peak apex whenever the fluorescence intensity exceeds the value set in the Auto Threshold field. In our example chromatogram (Fig. 4.8), a scan would occur automatically for Peak A, since it exceeds the value set in Auto Threshold.

Figure 4.8 An example of how automatic scanning works



An automatic baseline scan will occur at the time specified in the Spectra Menu's Scan Zero Time field. Make sure that no peaks are eluting at the specified time.

Replaying Your Spectra

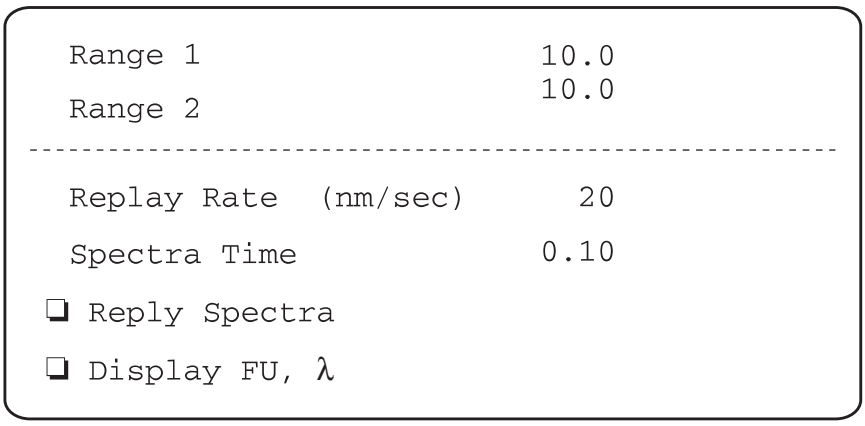
Once you've completed your run (either by pressing [STOP] or by the file having completed each time line), you can retrieve your stored sample spectra by selecting /Replay/ in the Commands Menu (Fig. 4.7). If no spectra are stored in memory when you select Replay, the message shown in Figure 4.9 will appear on the display.

Figure 4.9 The message that's displayed when you try to replay spectra and none are in memory

```
**  No Spectra Available  **
```

The Replay Menu appears in Figure 4.10.

Figure 4.10 The Model FL-45A's Replay Menu



SETTING REPLAY PARAMETERS

From the Replay Menu (Fig. 4.10), set the parameters for replaying your spectra as follows:

1. Set Range 1 and Range 2 for Analog Output 1 and Analog Output 2, respectively. If you're using only one output, disregard the appropriate range.

2. Enter the Replay Rate (nm/sec). This is the rate at which the detector will read out the spectral data to your integrator chart. You will use this value and an appropriate chart speed to calculate wavelength increments on your printed sample spectrum.

   For example, if your sample scan was taken from 250 to 350 nm (a span of 100 nm), a replay rate of 5 nm/sec would print the spectrum in 20 seconds. A chart speed of 30 cm/min would give you a scan of 10 cm in increments of 10 nm/cm.

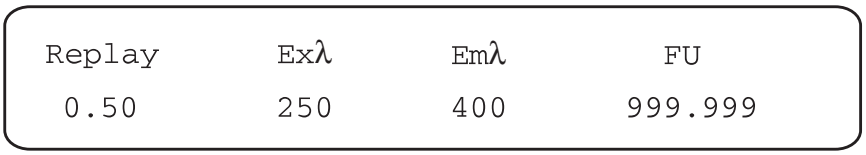
3. Select the spectrum you want to replay by displaying its start time in the Spectra Time field. Each spectrum taken during the run is individually identified by the run time at which it was initiated.

When you've finished setting your replay parameters, you're ready to send the spectral data to your chart by selecting the Replay Spectra command in the Replay Menu (Fig. 4.10).

RUNNING REPLAY

To replay your stored spectrum, initiate the Replay Spectra command in the Replay Menu (Fig. 4.10) by moving the cursor to /Replay Spectra/ and pressing [ENTER]. While the replay is occurring, the screen in Figure 4.11 appears on the display.

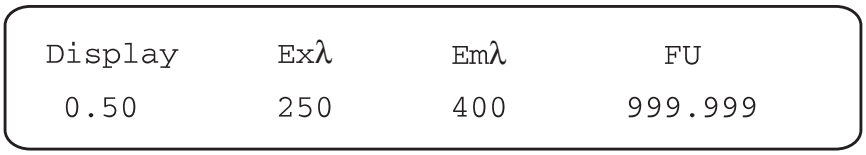
Figure 4.11 The display as it appears while running replay



The screen's Replay field displays the start time of the spectra being played. The Exλ, Emλ, and FU fields display the individual data points being plotted. When the replay is finished, the display returns to the Replay Menu. You may stop a replay at any time by pressing [STOP].

DISPLAY FU, λ
You can display the individual data points of a stored spectrum by selecting /Display FU, λ/ in the Replay Menu (Fig. 4.10). The screen shown in Figure 4.12 appears on the display.

Figure 4.12 The Display FU, I screen



The screen's Display field shows the time the spectrum was taken, the excitation and emission wavelengths (Exλ and Emλ), and the corresponding fluorescence intensity (FU). To scroll through the data, use the [+] and [–] keys. To return to the Replay Menu, press [∧].

Spectral Data Storage

Spectral data are stored in the Model FL-45A's memory until the detector is turned off, or until you press [START] (after loading a new file or queue).

4.5 SAMPLE QUEUE

Sometimes it's convenient to run groups of samples under different detector conditions in an automated run. For these occasions, the Model FL-45A offers a queuing feature. Using a queue, you can program the detector to load and run one file for your first group of samples, then automatically load a second file to run your next group of samples. The queue feature allows you to run as many as ten files in a single queue.

To access the Queue Menu, follow these steps:

1. Press [MENU].

2. Select /QUEUE/.

When no queue is loaded, the Queue Menu appears as shown in Figure 4.13. On page 39, we'll see how the menu appears when a queue is loaded.

Figure 4.13 Queue Menu with no queue loaded

Setting Up A Queue

To set up a queue, select /Edit/ from the Queue Menu. If the queue is empty, the display will look like Figure 4.14.

Figure 4.14 An empty queue

ENTERING A LINE
A “1" is placed automatically in the Order field of the first file to be run. You can't change that, so the cursor appears under the first editable field, "File:Name." Scroll through the available files and press [ENTER] when your choice appears.

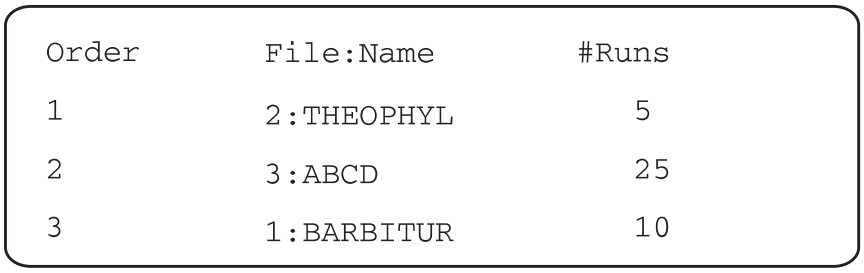
Enter the number of injections to be made in the #Runs field and press [ENTER]. You can have as many as 999 injections per file.

ADDING SUBSEQUENT LINES
After completing the first line, a second line appears automatically. The Order field reads 2, and the rest of the line is blank. Select the proper file and the number of injections to be made for that file. You can have as many as ten groups for a single queue.

DELETING A LINE
To delete a line, use the [–] key in the File:Name field until it goes blank. When you leave the line, it's deleted and the queue is resorted automatically.

An example of a queue appears in Figure 4.15.

Figure 4.15 An example of a queue



In Figure 4.15., we have programmed the detector to run File 2 for the first five injections, File 3 for the next 25 injections, and File 1 for the last ten injections.

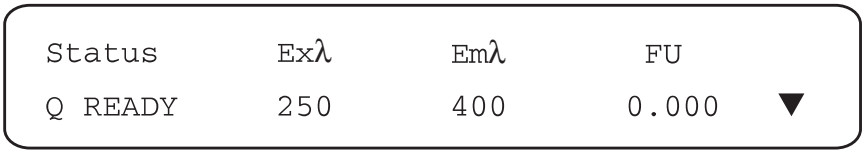
Loading A Queue

To load a queue, select /Load/ in the Queue Menu (Fig. 4.13). When the words “Load Queue” appear, press [ENTER]. The confirmation message shown in Figure 4.16 appears for one second.

Figure 4.16 The confirmation message when a queue is loaded

When a queue is loaded, the letter “Q” appears at the extreme left of the Status Screen (Fig. 4.17).

Figure 4.17 The Status Screen when a queue is loaded



If you attempt to load a queue when no queue exists, the message shown in Figure 4.18 will appear on the display.

Figure 4.18 The message that's displayed when no queue is available

Running A Queue

When the detector receives its first start signal, it loads and runs the file designated in Order 1. It will continue to run this file each time it receives a start signal until the file has run the number of times specified in the #Runs field. The detector will then load and run the file designated in Order 2 and run it the number of times specified in that line, and so on, until the entire queue has run.

Viewing its Progress

To view the progress of a queue that's running:

1. Press [MENU].

2. Select /QUEUE/. Note that when a queue is loaded, the Queue Menu (Fig. 4.19) looks different. The Load field has been replaced by “Pause,” which we'll discuss on page 40.

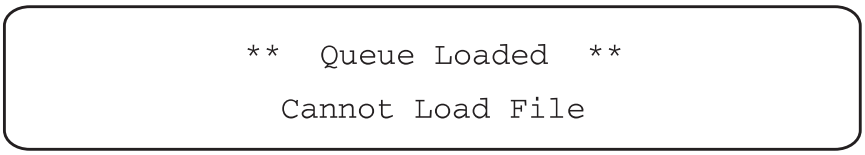
Figure 4.19 The Queue Menu with a queue loaded

3. Select /Edit/ to display the queue. (Refer to Figure 4.15 for an example queue.)

While the queue is running, the #Runs field decreases automatically by one with each injection. When a particular file's last injection is made, the queue is resorted automatically. In other words, the information for Order 2 is now moved up to Order 1, the information for Order 3 is moved up to Order 2, and so forth. This process continues until the queue becomes empty, is paused, or is deleted.

LOADING OTHER FILES
When a queue is loaded or running, you may not load any other file from the Files Menu without first pausing or deleting the queue. If you try to load a different file without pausing or deleting the queue, you'll get the message shown in Figure 4.20. You're then returned to the Files Menu.

Figure 4.20 The message that appears when you attempt to load a file while a queue is loaded or running



Editing A Queue

To edit an existing queue, follow the procedures outlined in “Setting Up a Queue” on page 37. You're allowed to edit the queue while it's running, but if you want to edit anything in Order 1, you'll have to pause the queue first.

Pausing A Queue

The following steps tell you how to pause a queue:

1. Select /Pause/ from the Queue Menu (Fig. 4.19).

2. When the words “Pause Queue” appear, press [ENTER]. If a file is running, the run continues until it's completed, at which point the detector returns to its READY state. The letter Q no longer appears in the Status Menu.

To continue, you must reload the queue. When the detector receives a start signal, the queue will resume operation at the point where it left off.

Deleting/Stopping A Queue

Use the following steps to delete an existing queue or to stop a running queue:

1. Display the Queue Menu (Fig. 4.19).

2. Select /Delete/.

3. When the words “Delete Queue” appear, press [ENTER]. If a file is running, the run continues until it's completed. The message shown in Figure 4.21 appears for one second and you're returned to the Queue Menu.

Figure 4.21 The queue-deleted message

```
**  Queue Deleted  **
```

You may delete or stop a queue at any time, but remember that the queue will be subsequently erased from the detector's memory. It's good practice to delete an existing queue prior to designing a new one.

4.6 PHOSPHORESCENCE

Throughout this manual we have been talking about fluorescence, the *virtually instantaneous* and *temperature-independent* emission of light by a sample that has been excited by incident light energy.

But the Model FL-45A can also detect phosphorescence. Phosphorescence is defined as the *delayed* and *temperature-dependent* emission of light by a sample when that sample is excited by incident light energy. In both fluorescence and phosphorescence, the wavelength of light that excites the sample is typically shorter than the wavelength of light that the sample emits (i.e., the sample emission is a Stokes emission).

To select the type of detection:

1. Press [MENU].

2. Select /OPTIONS/.

3. Select /More/.

4. Scroll down to Detection Type. Select Fluor for fluorescence or Phos for phosphorescence. [Fluor sets the PMT's (photomultiplier tube) voltage integration for immediately after the lamp flash; Phos sets it for approximately 20 msec after each lamp flash.]

4.7 ZERO ORDER

You can enter a value of zero for the excitation wavelength, the emission wavelength, or both. This sets the respective monochromator to what is known as the “zero order” position. In the zero-order mode, the monochromator doesn't diffract light into its spectral components. Rather, it functions like a mirror, simply reflecting all wavelengths of incident light.

The zero-order mode is useful when you don't know the proper wavelength to monitor for your sample. To find the excitation wavelength, set the emission wavelength to zero and scan the excitation wavelengths for activity. Then use the excitation wavelength that you find to scan for the emission wavelength.

When the excitation monochromator is set to zero order, all wavelengths of light emitted by the xenon lamp are reflected toward the flowcell. When the emission monochromator is set to zero order, you may get a “PMT Overloaded” message, since all wavelengths of light emitted by the fluorescing compound are reflected toward the PMT.

Setting either or both monochromators to zero order may increase sensitivity, but selectivity will decrease and baseline noise can increase significantly.

If you plan to use zero-order settings on your detector's monochromators, try experimenting with various excitation and emission slit-width settings (see page 57) to determine the best conditions for your application.

4.8 OTHER FEATURES

Additional features offered by the Model FL-45A include the abilities to lock the Status Screen, to short the detector outputs, to place an event mark on the chromatogram, and to send a ready signal to external devices. You can also control the display's contrast and cursor speed, and quickly shut down the detector's lamps and motors.

Status Lock

You can lock the detector's display by using the Status Lock field. This feature lets you prevent accidental changes to a file that's currently being run. With the lock on, only the Status Screen appears. You will not be able to move the cursor below the Status Menu's File Name field. You will still be able to access the Main Menu and the rest of the menu structure using the [MENU] key however.

To access Status Lock:

1. Press [MENU].
2. Select /OPTIONS/.
3. Select /More/.
4. Scroll down in the More Menu to Status Lock. Select On or Off to turn the lock on or off, respectively.
5. Press [STATUS].

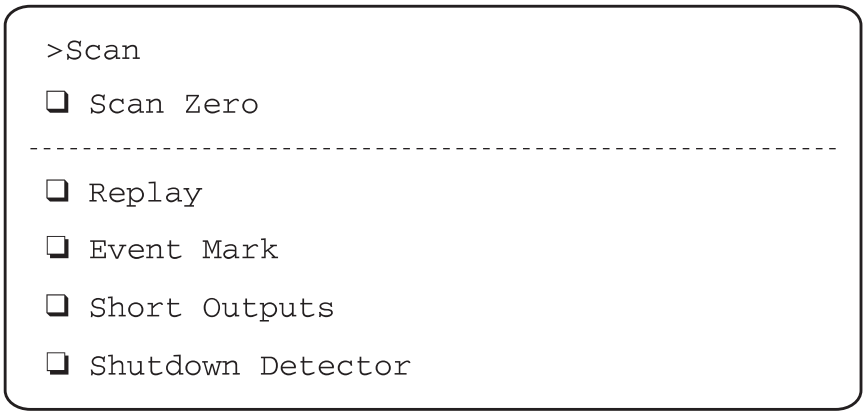
Short Outputs

When zeroing a readout device such as an integrator or recorder, it's convenient to be able to short the detector outputs. You can do this by using the detector's Short Outputs feature.

To access Short Outputs:

1. Press [MENU].
2. Select /COMMANDS/. The Commands Menu (Fig. 4.22) appears.

Figure 4.22 The Model FL-45A's Commands Menu



When you select Short Outputs, the detector analog outputs are shorted together (zero volts) and the field changes to "Unshort Outputs." To remove the short and return the outputs to their normal operating state, select Unshort Outputs, and the field changes back to "Short Outputs." (When you leave this screen, the field returns automatically to Short Outputs.)

Event Mark

You can place an event mark on your chromatogram to note the occurrence of certain events, such as the turning of a sampling valve. The event mark is a spike (15% of full-scale for one second) on both of the detector's outputs.

To set an Event Mark:

1. Press [MENU].

2. Select /COMMANDS/.

3. Place the cursor on Event Mark (Fig. 4.22) and press [ENTER] each time you wish to place an event mark on your chromatogram.

CAUTION! You may not want to use event marks if your data will be analyzed by an integrator. Integrators can misinterpret event marks as peaks!

Ready Output

Using the Accessory Relay terminals on its back panel, the detector can send a signal to other devices each time it goes to its READY state. This feature is frequently used with autosamplers to signal that the detector is ready for the next injection.

To access the READY Output field:

1. Press [MENU].

2. Select /OPTIONS/.

3. Select /Analog Outputs/.

4. Scroll down to the READY Output field. Select Active Hi or Active Lo depending on which signal you wish to send.

HINT: The Model FL-45A detector is set to receive high signals, so select Active Hi *if you're hooking up to this type of chromatograph. For any other type of instrument, refer to the appropriate reference manual.*

For details on interfacing your detector with other devices, see page 69.

Display Contrast

You can vary the display's contrast to make it easier to read.

To change the display's contrast, first press [STATUS] to access the Status Screen. Then simultaneously press the [>] key and the [+] key to *increase* the contrast, or the [>] key and the [–] key to *reduce* the contrast.

Cursor Speed

The detector lets you control the cursor speed to make it easier to use.

To access Cursor Speed:

1. Press [MENU].

2. Select /OPTIONS/.

3. Select /More/.

4. Scroll down to the Cursor Speed field and select Fast, Medium, or Slow.

Shutdown Detector

This shutdown feature offers a quick shutdown and subsequent startup of the detector's lamp and motors. The electronics stay on to maintain the detector's memory.

To shut down the detector:

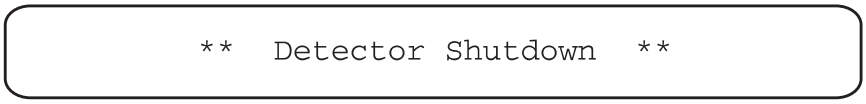
1. Press [MENU].

2. Select /COMMANDS/.

3. Scroll down to the Shutdown Detector field (Fig.4.22).

Press [ENTER]. The confirmation message shown in Figure 4.23 appears on the display.

Figure 4.23 The shutdown confirmation message



To start the detector up again, press any key on the keypad. The detector will come up under the same conditions present when the Shutdown field was activated.

Section 5. Maintenance

5.1 INTRODUCTION

The Model FL-45A detector is a finely tuned scientific instrument that we at BAS are proud to stand behind. Even so, routine maintenance is necessary to ensure peak performance, so we can only guarantee our detectors' performance if you follow proper care and maintenance procedures.

This section describes the routine maintenance procedures for your detector, including flowcell cleaning, and replacement of the xenon lamp and photomultiplier tube.

CAUTION! Failure to complete the necessary maintenance of your detector may void your warranty.

If you have any questions regarding proper maintenance, please contact your local BAS representative.

5.2 THE FLOWCELL

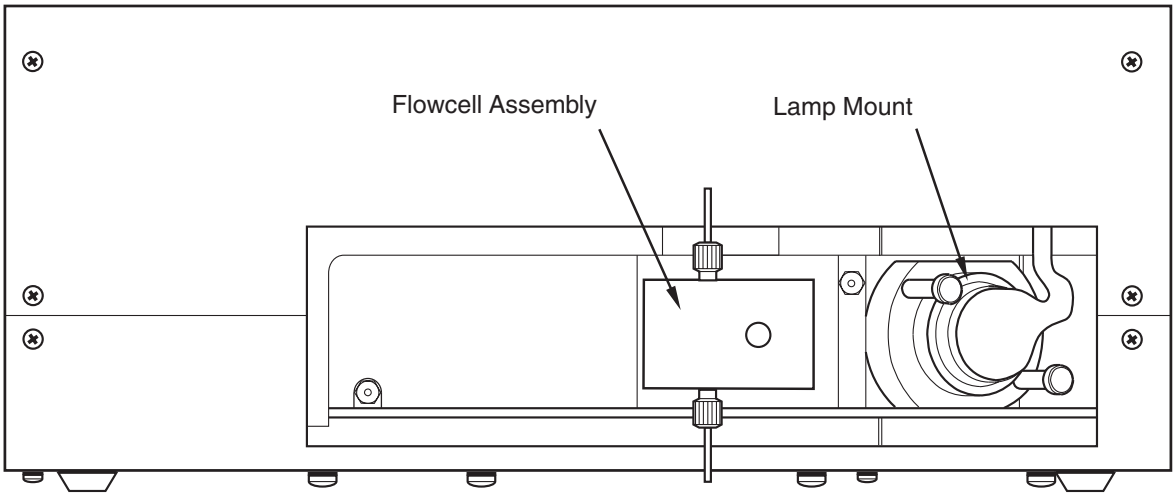
This section describes the changing and general cleaning of your detector's flowcell. For other flowcell problems, such as cracks or leaks that occur in locations other than at the inlet/outlet fittings, contact your BAS representative.

Changing The Flowcell

You will need to remove the detector's flowcell whenever you replace a broken cell or clean either the cell's exterior or interior with nitric acid.

To access the flowcell, remove the enclosure on the left side of the detector. The flowcell is premounted in a holder assembly that's easily identified by the inlet and outlet lines shown in Figure 5.1. The holder assembly makes it easy to install and align the flowcell.

Figure 5.1 Flowcell assembly and xenon-lamp mount locations



Removing the Flowcell Assembly

Use the following steps to remove the flowcell assembly.

CAUTION! Avoid touching the flowcell's quartz tube, the lenses, the lamp's surface, or the photomultiplier tube, all of which are exposed during these procedures. The oils on your skin can be permanently etched into the quartz surface when exposed to UV light. If necessary, use an eye-dropper or Pasteur pipette filled with spectroscopic-grade methanol to clean the contaminated surface, taking care not to drip methanol on the interior surfaces of the detector.

1. Disconnect the power cord from the detector's rear panel and turn the power switch off.

2. Unscrew and remove the small thumbscrew on the face of the flowcell assembly. Gently rock the entire flowcell assembly outward and pull it away from the detector (Fig. 5.2).

3. Carefully unscrew and remove the inlet and outlet fittings and their associated tubing from the flowcell assembly, taking care not to lose the ferrules.

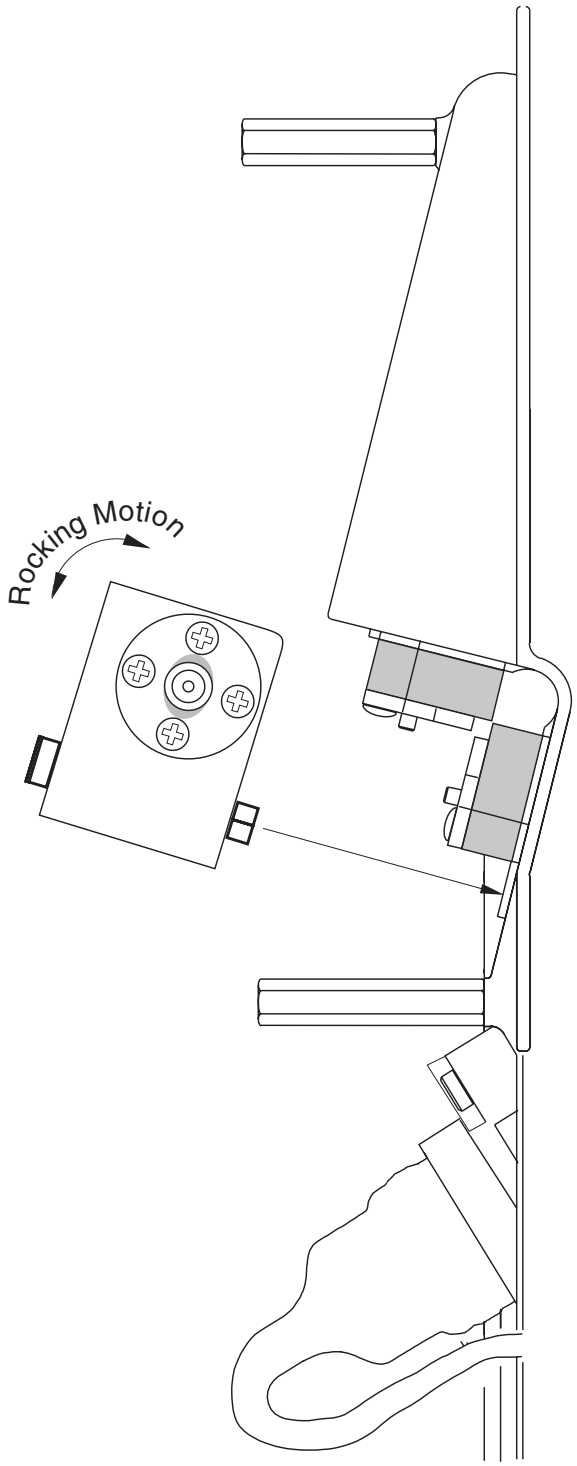
REPLACING THE FLOWCELL ASSEMBLY
To replace the flowcell assembly, follow these steps:

1. Slide the flowcell assembly onto the alignment pin, gently rocking it inward, until it's seated securely. Fasten it in place with the small thumbscrew (Fig. 5.2).

2. Replace the inlet and outlet tubing into their fittings, making sure that the tubing is fully inserted. Then reattach the detector housing.

3. Connect the power cord to the detector's rear panel.

Figure 5.2 Top view of the left side of the Model FL-45A with the side enclosure removed to show the location of the flowcell assembly and the rocking motion recommended for flowcell removal



Cleaning The Flowcell

The exterior and interior surfaces of the flowcell can become contaminated, depending on the quality of your mobile-phase solvents and the cleanliness of your samples. Signs of a contaminated flowcell include increased baseline noise, spikes in the chromatogram, an erratic or drifting baseline, and increased back-pressure.

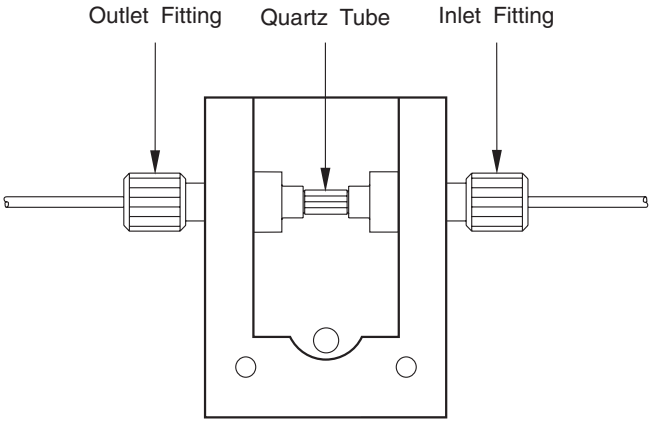
As a first step toward resolving contamination problems, use the external cleaning procedure described below. If the problem persists, continue with the two internal cleaning procedures that begin on page 51.

CAUTION! Do not store or handle optics-cleaning solvents in plastic labware. Fluorescent compounds present in and/or on the plastic can contaminate the solvents, making them worthless for optical cleaning procedures. Store and handle cleaning solvents in glass containers only!

CLEANING THE EXTERIOR

Inspect the exterior surface of the quartz tube (Fig. 5.3) carefully (with magnification if necessary) for evidence of fingerprints, dust, or other contaminants. If you see any contaminants, remove the entire assembly from the detector (using the procedure described on page 47). Without taking the flowcell assembly apart, rinse the outside surfaces of the quartz tube carefully with a clean eye-dropper or Pasteur pipette filled with spectroscopic-grade methanol.

Figure 5.3 The flowcell assembly, inside view



NOTE: Don't clean the flowcell's quartz tube with methanol-dampened wipes or lens paper. Oils from your skin will be dissolved in the solvent and disbursed over the surfaces you're attempting to clean.

CLEANING THE INTERIOR

There are two ways to clean the interior of a flowcell. Try using organic solvents (see below) before resorting to the use of a nitric acid solution.

CLEANING WITH ORGANIC SOLVENTS

If you suspect that your flowcell needs to be cleaned, start with the following procedure using organic solvents.

1. Make certain that the cleaning solvent(s) you plan to use is/are miscible with the solvent already present in the flowcell and pump. Isopropanol is a good choice for most applications.

HINT: If the last solvent in the pump was an aqueous buffer solution, pump 25–40 mL of HPLC-grade water (or equivalent) through the system to remove any salts before flushing with the cleaning solvent(s).

2. Flush the flowcell with 40–50 milliliters of solvent (HPLC-grade water, methanol, or isopropanol). You can either pump the solvent through the flowcell with the chromatographic pump, or you can *draw* the solvent through the flowcell using a large-volume syringe.

If you use an LC pump to flush the flowcell, first remove the column from your chromatographic system to avoid column degradation. Replace the column with an appropriate length of tubing, ensuring that all connections are snug and leak-free. If you use a syringe, always *draw* the solution through the flowcell.

WARNING! Never use a syringe to force solvent through a flowcell. Pressurizing the syringe could cause a leak or rupture that would result in an extremely dangerous, uncontrolled spraying of solvent.

CLEANING WITH NITRIC ACID

Methanol or isopropanol is generally sufficient for cleaning a flowcell. However, if the flowcell is still contaminated after flushing with organic solvents, follow this nitric acid procedure.

WARNING – Chemical Hazard! Nitric acid is extremely corrosive and can react explosively with alcohols (especially methanol). Be sure to adhere to your company's safety procedures for handling and disposal of corrosive acids. Flush the flowcell with water to remove all traces of alcohol prior to flushing with nitric acid!

1. Remove the flowcell assembly from the detector (following the procedure on page 47) before cleaning with a nitric acid solution. This will prevent possible leaks from harming the mechanical or electronic components of the detector.

2. Flush the flowcell with water before proceeding. This step is very important!

3. Prepare a 20% (v/v) solution of nitric acid in HPLC-grade water.

4. Pump the nitric acid solution through the flowcell with the chromatographic pump or draw it through with a large-volume syringe.

If you use an LC pump, replace your column with tubing and make sure water was the last solvent in the pump and solvent reservoir. If you use a syringe, always draw the solution *through* the flowcell.

WARNING! Never use a syringe to force nitric acid through a flowcell. Pressurizing the syringe could cause a leak or rupture that would result in an extremely dangerous, uncontrolled spraying of acid.

5. After you've finished the cleaning procedure and before returning to the buffer solution, pump another 25–40 mL of water through the flowcell to remove all traces of nitric acid before returning to your chromatographic solvents. Reinstall the flowcell assembly.

CAUTION! Flush the pump with water *immediately* after the nitric acid flush. Leaving nitric acid solution in the pump for prolonged periods can damage pump seals.

5.3 CHANGING THE XENON LAMP

The Model FL-45A has a pulsed xenon flashlamp that requires no warm-up time. This lamp provides exceptional performance and reliability across the entire UV/Visible spectrum. The lamp is aligned and permanently seated inside a slotted mount for easy installation and alignment.

As lamps age, there's a reduction in light output, resulting in decreased peak height. If you notice a reduction in peak height, to where sensitivity is no longer acceptable, you may need to change the lamp.

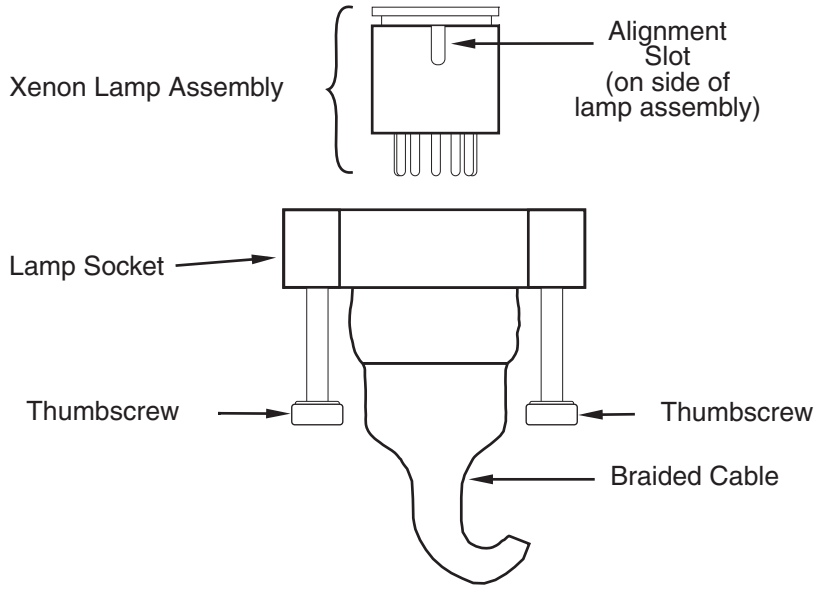
REMOVING THE LAMP ASSEMBLY
To remove the lamp assembly from the detector:

1. Turn off the power switch and unplug the power cord from the detector's back panel.

2. Remove the detector's side enclosure to expose the lamp (Fig. 5.1).

3. Loosen the two thumbscrews that secure the lamp assembly and socket to the detector (Fig. 5.4).

Figure 5.4 Xenon lamp assembly and socket



4. Pull the socket (with the lamp assembly still attached) straight out of the detector.

5. Grasp the lamp assembly in one hand, and the braided cable (that attaches the socket to the detector) in the other. Gently pull the lamp assembly out of the socket.

REPLACING THE LAMP ASSEMBLY

Before replacing the lamp assembly, note that the pins on the base of the lamp are arranged in a C-shaped pattern that exactly matches the holes in the lamp socket.

NOTE: The detector must be recalibrated any time the lamp is replaced. A recalibration procedure is provided on page 59.

1. Orient the pins and socket holes. Slide the pins into the socket holes until the lamp assembly is fully seated in its socket.

NOTE: The face of the lamp must be clean of all fingerprints and debris at the end of the installation procedure. Don't clean the optical surfaces with methanol-dampened wipes or lens paper. Oils from your skin will be dissolved in the solvent and disbursed over the surfaces you're attempting to clean. If necessary, use an eye-dropper or Pasteur pipette filled with spectroscopic-grade methanol to flush fingerprints or debris from the newly mounted lamp.

2. Grasp the socket (with the lamp assembly seated firmly inside it) and insert it in the lamp recess. Make sure that the keyed slot in the lamp assembly aligns with the silver guide pin in the lamp recess.

HINT: Position the lamp assembly such that the slot in its side is towards the right-hand side of the detector as you're facing it. If the slot is perpendicular to the flat, right-hand side of the socket, it will slide easily into place in the lamp recess.

3. Align the thumbscrews on the socket with the screw holes on the detector, and tighten the thumbscrews. (The part of the assembly that contains the thumbscrews can be moved independently of the socket to make it easier to align the thumbscrews with the screw holes.)

4. Replace the external cover, tighten the thumbscrews, and reconnect the power cord.

TRACKING LAMP LIFE

The Lamp Count display found in the Tests Menu (page 89) can be used to track the age of the xenon lamp. You should set the lamp count display value to zero when a new lamp is installed.

5.4 CHANGING THE PMT

The photomultiplier tube (PMT) is a device that generates a current whenever photons of light strike it. The digital electronics of the Model FL-45A integrate and process this current to produce a voltage signal that drives your recorder/integrator.

If you still have baseline noise problems after cleaning the flowcell and replacing the lamp, you may need to change the PMT. However, bear in mind that under normal operating conditions the PMT is designed to perform to specifications for several years. If the instrument is less than three years old, it's more likely that the tube needs cleaning than replacement.

CAUTION! Never touch the PMT's surface with your fingers. Contamination of the PMT's quartz bulb can cause increased baseline noise and diminished sensitivity. Use a lint-free tissue or clean cotton gloves to protect the PMT from contact with your skin during these procedures.

REMOVING THE PMT

The PMT is accessed from the underside of the detector. To remove it, use the following steps:

1. Turn off the power switch, disconnect the power cord from the detector's rear panel, and lay the detector on its side.

2. Loosen the screw that holds the rectangular panel located on the detector's underside. Remove the panel.

3. Locate the 1/8-inch, Allen-head screw that secures the PMT slit-wheel holder in position. Remove the Allen-head screw and then pull the holder straight out toward you to expose the PMT.

4. Put on a pair of vinyl laboratory gloves to provide grip and to prevent your fingerprints from getting on the PMT in Step 5.

5. Grasp the PMT and gently pull it straight out from its socket with minimum force.

If the instrument is relatively new, you should first clean the PMT using an eye-dropper or Pasteur pipette filled with spectroscopic-grade methanol to rinse the PMT's outer surfaces. Reinsert the PMT following the next procedure, and run an analysis. If problems still exist, install a new PMT.

NOTE: Don't clean the flowcell's optical surfaces with methanol-dampened wipes or lens paper. Oils from your skin will be dissolved in the solvent and redisbursed over the surfaces you're attempting to clean.

REPLACING THE PMT
To replace the PMT:

NOTE: The detector must be recalibrated any time the PMT is replaced. A recalibration procedure is provided beginning on page 59.

1. Grasp the glass portion of the PMT and insert the PMT fully into the socket.

HINT: Note the guide-pins on the PMT before you install it. They make the PMT much easier to align in the socket.

2. Replace the slit-wheel holder and secure it in place with the 1/8-inch Allen-head screw.

3. Reconnect the power cord.

5.5 CHANGING SLIT-WHEELS

The Model FL-45A's signal-to-noise ratio is directly proportional to the square root of the available emitted light. Thus, if the amount of light coming from the monochromators increases by a factor of four, the signal-to-noise ratio, and therefore the sensitivity, of the detector increases by a factor of two.

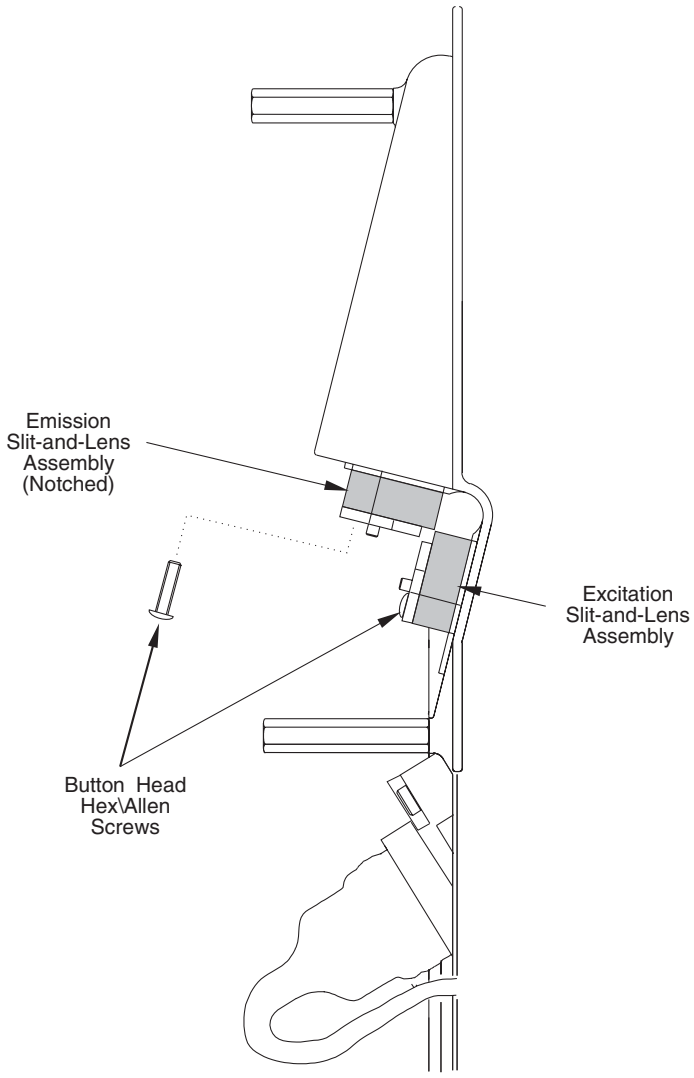
In some cases you can improve the detector's sensitivity by increasing the monochromators' bandwidths, thus allowing more light to pass through to the PMT.

Bandwidth is controlled by three slit-wheels: the excitation, emission, and PMT slit-wheels. Each slit-wheel has three manually-selectable slit settings: 8, 20, and 30 nm. Your detector is shipped with each slit set to 20 nm. The excitation and emission slit-wheels are accessible when the flowcell is removed. The PMT slit-wheel is accessed from the underside of the detector.

To change the emission monochromator's bandwidth, set both the emission slit and the PMT slit to the same setting. To set the excitation monochromator's bandwidth, you need only change the excitation slit.

HINT: The Model FL-45A is designed to provide optimized performance for most applications when all three slit-wheels are set to 20 nm. Larger slit-widths increase sensitivity, but also decrease specificity, and you may start to see interfering compounds. Conversely, smaller slit sizes increase specificity but decrease sensitivity.

Figure 5.5 Top view of removing the excitation and emission slit-and-lens assemblies



CHANGING THE EXCITATION AND EMISSION SLIT-WIDTHS

Use the following steps to change either the excitation or emission slit-widths.

1. Disconnect the power cord from the detector's rear panel, turn the power switch off, and remove the detector's side housing.

2. Unscrew and remove the small thumbscrew on the face of the flowcell assembly and pull the entire assembly away from the detector.

CAUTION! Never touch either lens surface with your fingers. Contamination of the lenses can cause increased baseline noise and diminished sensitivity. If necessary, use an eye-dropper or Pasteur pipette filled with spectroscopic-grade methanol to rinse the lenses, taking care not to drip methanol on the detector's interior surfaces.

3. You can now see the assemblies that hold the slits and lenses. Remove the screw (Fig. 5.5) that secures the slit-and-lens assembly to the monochromator.

4. Remove the lens-holder from the slit-wheel holder.

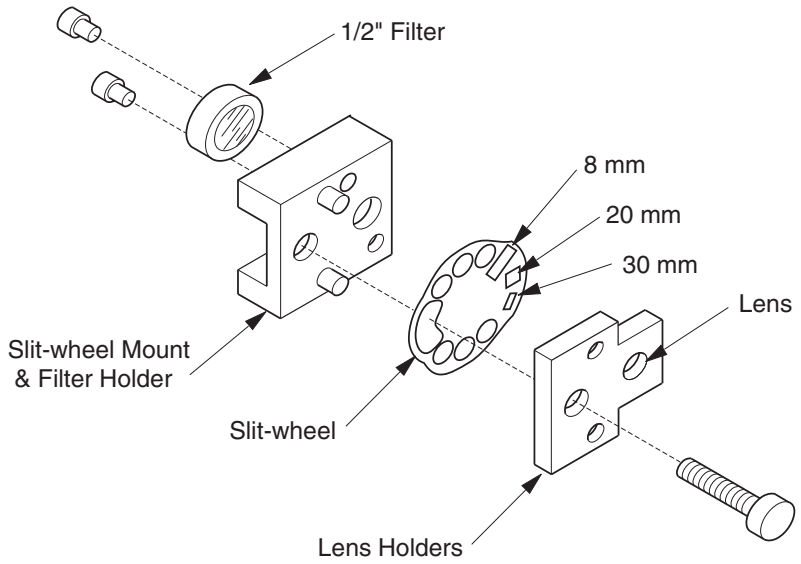
5 Lift the slit-wheel clear of the two guide pins.

6. Rotate the wheel (Fig. 5.6) until the desired slit is directly over the opening into the monochromator.

7 Replace the slit-wheel and lens-holder on the slit-wheel holder, and tighten the Allen-head screw.

8. Replace the flowcell following the procedure on page 48.

Figure 5.6 An exploded view of the excitation slit-wheel assembly. Emission assembly is similar.



CHANGING THE PMT SLIT-WIDTH

Use the following procedure to change the PMT slit-width:

1. Turn off the power switch, disconnect the power cord from the detector's rear panel, and lay the detector on its side.

2. Loosen the screw that holds the rectangular panel located on the detector's underside. Remove the panel.

3. Locate and remove the Allen-head screw that secures the PMT slit-wheel holder in position. Pull the holder straight out toward you to expose the PMT.

4. Next, remove the Allen-head screw that holds the slit-wheel to the holder and lift the slit-wheel from the two guide pins (Fig. 5.7).

5. Rotate the wheel until the desired slit setting is directly over the center opening on the slit-wheel holder.

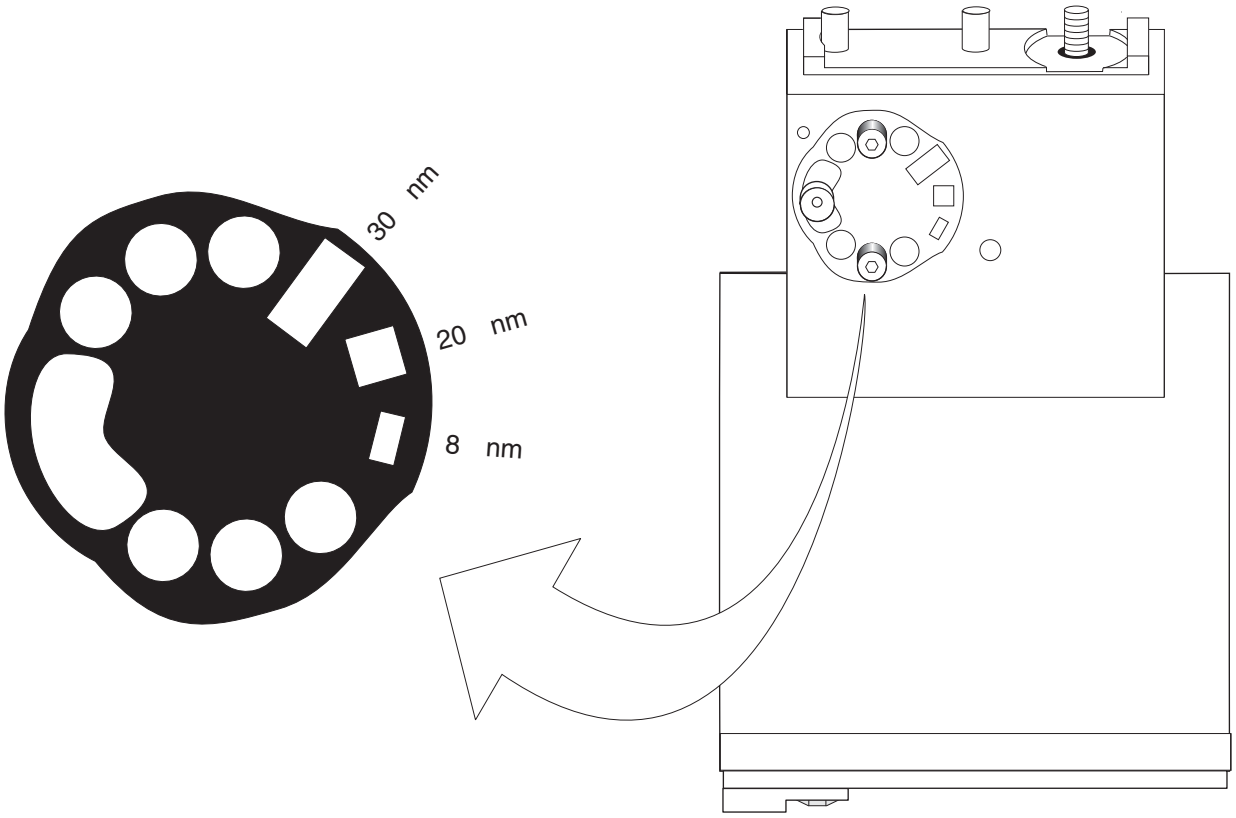
6. Replace the slit-wheel and the Allen-head screw.

7. Replace the slit-wheel holder and secure it in place with the Allen-head screw.

8. Reattach the rectangular panel and secure it in place.

9. Reconnect the power cord.

Figure 5-7 The PMT slit-wheel and slit-wheel holder



5.6 RECALIBRATING THE DETECTOR

You should recalibrate the detector each time you replace the lamp and/or PMT. This process normalizes the difference in light output or PMT response that is certain to exist between the old component and its replacement. Although the procedure that follows uses anthracene, you can calibrate your detector with the standard of your choice.

NOTE: Recalibration also may be required if a user has inadvertently activated the fluorescence-response function (described on page 91).

CAUTION! If you don't perform a calibration following a lamp or PMT change, the results of subsequent analyses—while comparable to each other—cannot be compared to data that you generated prior to the lamp/PMT replacement.

The standard anthracene calibration procedure requires the following equipment, tools, and materials:

a. Precision micro-balance (accurate to ± 0.1 mg)

b. LC gradient pump

c. 1/8-inch Allen wrench

d. HPLC-grade methanol and water

e. Reagent-grade anthracene

Use the following procedures to prepare and use a standard anthracene solution to recalibrate the detector.

PREPARING A STANDARD ANTHRACENE SOLUTION

This calibration procedure requires a 4.1 μg/L solution of anthracene in methanol. The solution should be created using scrupulously clean, particle-free laboratory glassware. Use these steps to prepare the standard anthracene solution:

1. Weigh out 0.0041 gm of reagent-grade anthracene and transfer it quantitatively to a clean, one-liter, volumetric flask.

2. Fill the volumetric flask to the mark with HPLC-grade methanol.

CAUTION! You must use methanol. If you use a solvent other than methanol, the emission and excitation maxima will shift.

3. Add a clean magnetic stir bar and place the flask on a stir plate. Allow the solution to mix at a moderate speed for 1 hour.

4. Pipette 1.0 mL of the resulting anthracene solution into a second, clean, one-liter, volumetric flask and dilute to the mark with HPLC-grade methanol to produce the working solution.

5. Transfer the solution just created to a clean, one-liter, screw-top bottle.

HINT: The anthracene/methanol calibration solution has a shelf-life of approximately one week under normal laboratory conditions. To extend the shelf-life, store the solution in the dark and refrigerate it.

PERFORMING THE CALIBRATION

Once you've prepared the anthracene solution, use it and the following procedure to perform the calibration:

NOTE: If using a different calibration standard, make the appropriate changes to the items that are marked with asterisks in the following procedure. Since these changes of solvent, standard, and wavelength settings are specific to your calibration standard, we can't document them here.

1. Fill a clean, one-liter, screw-top bottle with HPLC-grade methanol*. Place that bottle and the one containing the standard anthracene* solution near the gradient pump's inlet tubing.

2. Ensure that all three sets of optical slits (excitation, emission, and PMT) are set for 20 nm. Instructions for setting the slit-widths are provided beginning on page 55.

3. Verify that the excitation and emission wavelengths are set for 250nm* and 400nm,* respectively.

4. Place a flow restrictor on the outlet side of the detector to ensure adequate back-pressure.

5. Flush the detector's flowcell by running several milliliters of HPLC-grade water or methanol through it (your choice of solvent should be the same as the last fluid that was run through the flowcell). If your initial flushing solvent was water (due to analyzing salt samples), finish the flushing process by running several milliliters of HPLC-grade methanol* through the flowcell. Once flushing is completed, press [ZERO] and then end the run.

6. Begin a run using the standard solution. While the calibration standard is flowing, press [MENU] to select the Main Menu.

7. Press [>] until the cursor is in the /Test/ field, then press [ENTER]. Press [v] to move the cursor to the /Fluorescence Response/ field, then press [ENTER].

8. Observe your display. If it looks like Figure 5.8, go on to Step 9A. If it looks like Figure 5.9, go to Step 9B.

Figure 5.8 Fluorescence-response display, up to v. 3.01

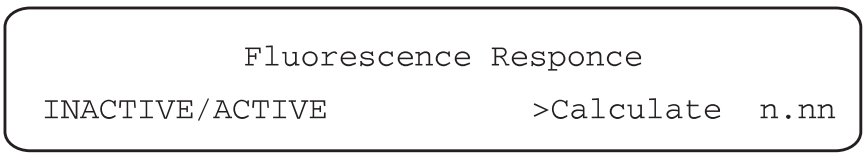


Figure 5.9 Fluorescence-response display, v. 3.06 and newer1

9A. The blinking cursor should appear in the /Active-Inactive/ field. Press [+] until the word "Active" appears on the display. Press [ENTER] twice to invoke the response factor. Press [STATUS] and observe the emission level that appears on the display. The level

should be 20 ± 1 FU. If the displayed value is unacceptable, repeat Steps 5 through 9A. If repeated calibrations fail to produce an acceptable fluorescence-response value, contact your nearest BAS service center. Now, skip on to Step 10 to complete this procedure.

9B. Press [>] to move the blinking cursor to the /Active/Inactive/ field, then press [+] until the word "Active" appears on the display. Next, press [∨] to move the cursor to the /Factor/ field. You can press [<] or [>] to select the digit(s) you want to change and then press [+]/[–] to change the value of a selected digit. As you change the digit(s) you'll see the FU reading changing in real-time response to your modifications of the value displayed in the /Factor/ field. When the desired FU reading appears, press [ENTER] and go on to Step 10.

NOTE: Although the fluorescence-response factor set in Step 9 is stored in non-volatile RAM (NOVRAM) and is always available at power up, you must select "Active" on the Fluorescence Response screen for the factor to take effect.

10. Following completion of Step 9, dispose of all waste solvent in accordance with local regulations.

HINT: It's good practice to maintain a record of the response factor in the back of your reference manual.

NOTE: If you desire, you can have your detector upgraded to allow you to use the calibration routine specified in Step 9B, rather than the one in 9A. This change allows you to set the FU reading to the value of your choice, rather than having the detector's internal programming set it to a value of 20 FU automatically. If you desire to have this change, contact your local BAS Service Representative and request an upgrade of your detector's E-PROM to version 3.06 or newer.

Section 6. Installation, Specifications, and Warranty

6.1 INTRODUCTION

This section covers the initial installation of your fluorescence detector, including hookup to other chromatographic instrumentation. After installation, verify that the detector is working properly by running the diagnostic tests described on page 89.

Also included in this section is a list of your detector's specifications and a copy of BAS' warranty.

6.2 INSTALLATION

Unpacking

Carefully remove the detector from the shipping container and inspect both the detector and packing for any signs of damage. If you find any damage, contact the shipping company immediately.

The shipping container should contain the detector, an accessory kit, any options you ordered for your detector, and this manual. The accessory kit should contain the following items:

a. 8-pin connector

b. 12-pin connector

c. 7/64" Allen wrench

d. Filter: UV cutoff 340 nm

e. Finger-tight nuts (2)

f. Finger-tight ferrules (2)

g. Teflon® tubing

h. Other hardware as appropriate

Check carefully to make sure you received all the items listed on the packing list. If any items are missing, contact your BAS representative immediately.

You will need the following tools for installation:

a. a narrow-tip screwdriver (2 mm wide)

b. a #2 Phillips screwdriver

Place the detector on the benchtop as close as possible to the chromatographic column outlet, thus minimizing the length of tubing necessary for connection to the flowcell inlet. Allow at least five inches (13 cm) of clear space between the detector's rear panel and any wall or obstruction. This provides both access to the rear-panel connectors and a free flow of cooling air.

Power Checkout

The detector is shipped with the voltage and fuses preset for 120 Vac. Verify the setting by looking through the cut-out window on the voltage selector cover (Fig. 6.1). The cover is located at the bottom left of the detector's rear panel. If the voltage setting satisfies your local site requirements, skip to "Fuses" on page 65. If not, proceed to the next section, "Voltage Selection."

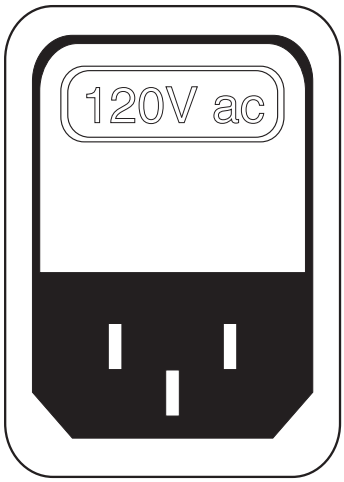
WARNING! Do not plug in the instrument without first verifying that the voltage is properly set for your location. And *never* run the detector at more than 10% below the nominal line voltage!

VOLTAGE SELECTION

If the preset voltage does *not* satisfy your local site requirements, select the correct voltage by following these steps:

1. Insert a small flat-blade screwdriver into the slot at the top of the voltage selector cover (Fig. 6.1).

Figure 6.1 Opening the voltage selector cover (note current voltage setting through the cut-out window)



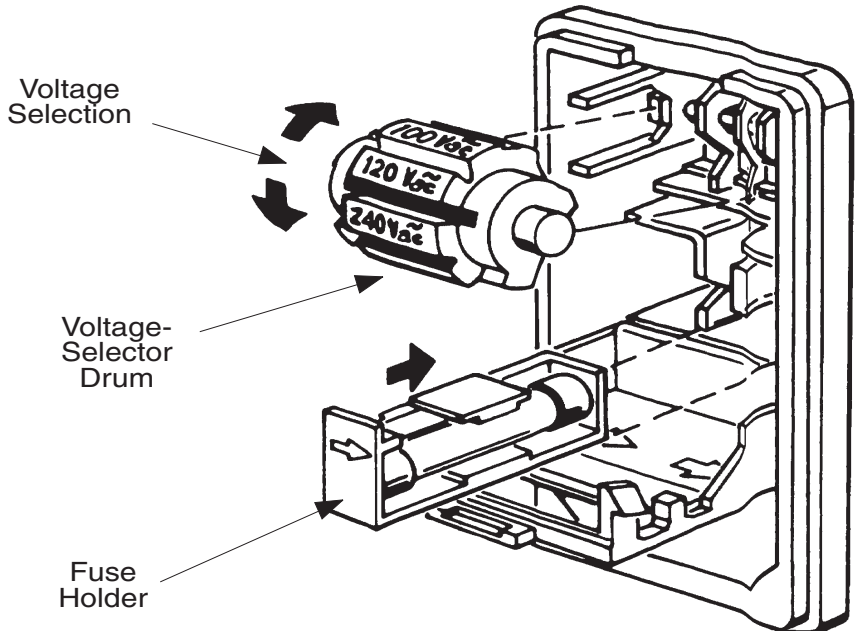
2. Gently pry open the cover. Once unlatched, the cover will swing downward to reveal the voltage selector barrel and the two fuses.

3. Remove the voltage selector barrel from the detector. The selector resembles a drum imprinted with four settings: 100, 120, 220, and 240 V (Fig. 6.2).

4. Rotate the barrel such that the desired voltage setting will be visible through the cut-out in the cover when it's replaced.

5. Replace the barrel in the detector. Before closing the cover, check the fuses according to the procedure below.

Figure 6.2 Voltage selector barrel and fuse holders



FUSES

To verify that your detector is fitted with the correct fuses, follow these steps. (If you haven't already done so, first open the voltage selector cover according to Step 1 in the "Voltage Selection" procedure provided above.)

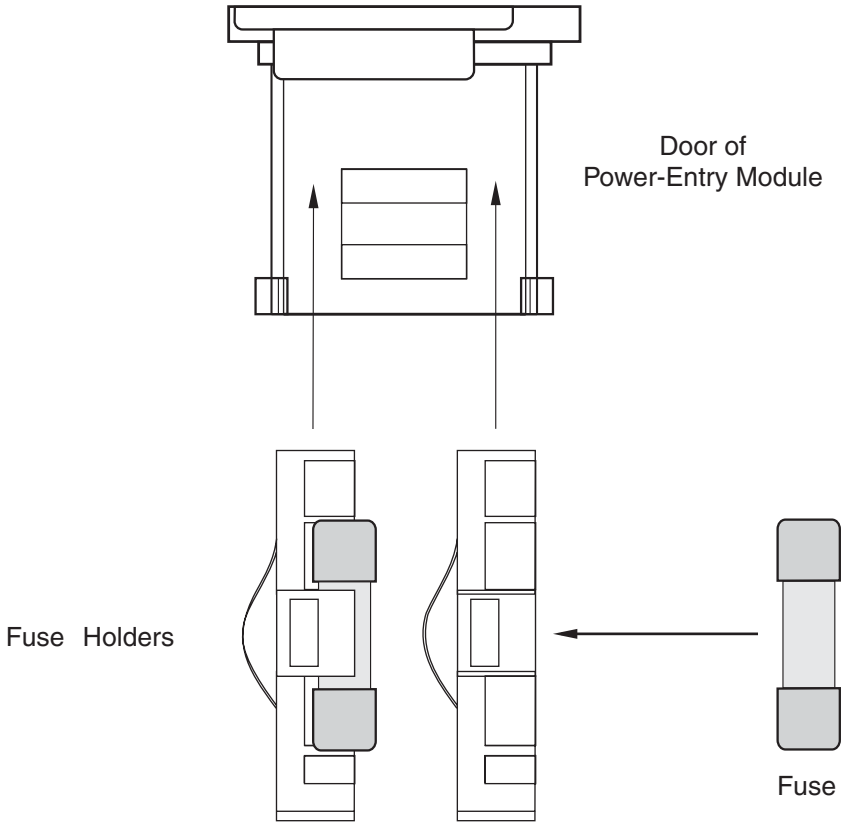
1. Pull each fuse holder straight toward you. The fuse holders are the black squares with arrows located directly beneath the voltage selector (Fig. 6.2).

2. Remove each fuse from its holder. Check the fuse's amperage, voltage, and type according to the following description. You should have either:

    a. two 2-amp, sloblow fuses (for 100 or 120 V), or

    b. two 1-amp, sloblow fuses (for 220 or 240 V)

Figure 6.3 Fuses



3. Assuming that you have the proper fuses on hand, reinsert the fuses and fuse holders, making sure that the arrows on the holders are oriented in the same direction as the arrow inside the cover panel (Fig. 6.3).

4. Close the cover panel by swinging it upward and pressing it in until it snaps shut. The correct voltage should appear in the cut-out opening.

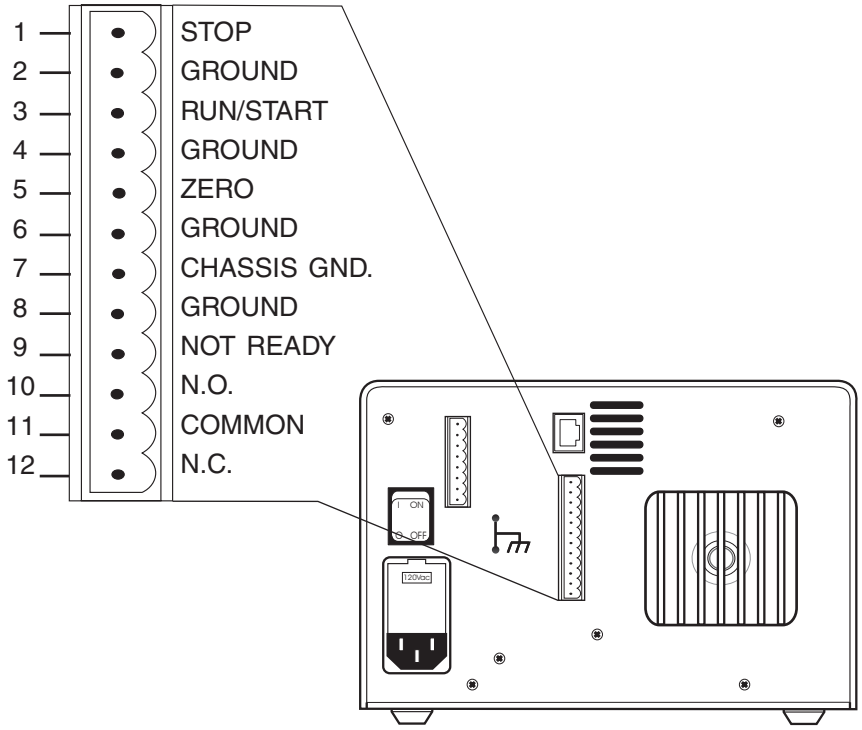
CAUTION! To avoid damaging the instrument, verify that the new voltage setting (displayed in the cut-out window) is correct before you turn it on!

POWER CORD
Attach the power cord at the lower left of the detector's rear panel.

Rear Panel Connections

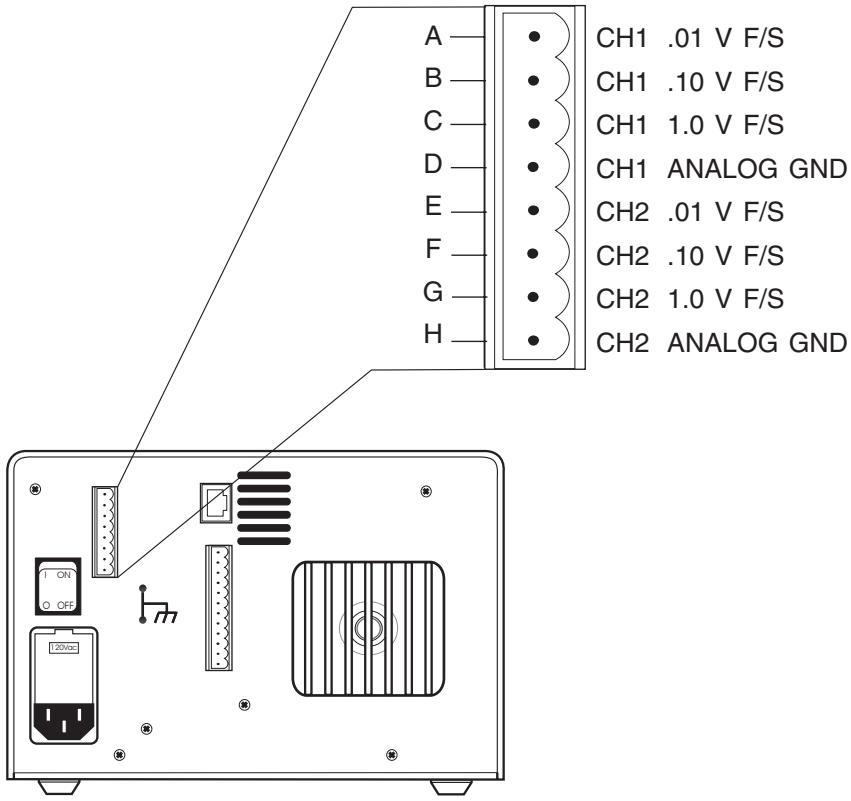
Locate the two in-line connectors (8-pin and 12-pin) in your accessory kit and insert them in the appropriate sockets on the detector's rear panel (Fig. 6.4 or Fig. 6.5). Note that the connectors are both keyed to their sockets, making it impossible to insert them incorrectly.

Figure 6.4 The rear panel of the Model FL-45A, showing analog-input connections



The 12-pin connector (Fig. 6.4) is for analog inputs. It allows the detector to communicate with other devices in your liquid chromatographic system. The 8-pin connector (Fig. 6.5) delivers the detector's two analog outputs (CH1 and CH2). There's also an RS-232 communications port, labeled Serial Port.

Figure 6.5 The rear panel of the Model FL-45A, showing analog-output connections



ANALOG OUTPUT CONNECTIONS

The terminals on the Model FL-45A's analog output connector are labeled CH1 and CH2 (Fig. 6.5). Each output channel has four terminals. These terminals are labeled:

a. 0.01 V F/S (full-scale)

b. 0.10 V F/S

c. 1.0 V F/S

d. ANALOG GND

CAUTION! Analog outputs are driven to twice their range. In other words, their maximum output is twice the selected range. To avoid clipping the voltage, be sure to connect integrators and data systems to the 1.0 V terminal and to use caution when connecting recorders to the 0.01 or 0.10 V terminal.

Connecting To An Integrator Or Workstation

Connect your integrator/workstation to the 1.0 V F/S and corresponding ANALOG GND terminals.

NOTE: The 0.01 and 0.10 V F/S terminals are provided for recorders and special applications. We recommend that you use only the 1.0 V F/S terminal for an integrator or workstation.

Connecting to a Recorder

Connect the positive input from your recorder to the full-scale voltage (0.01, 0.10, or 1.0 V) appropriate for your recorder. Connect the recorder's floating-ground (negative) input to the corresponding ANALOG GND terminal.

NOTE: Do not connect the detector's ANALOG GND to any earth ground on your recorder. This would lead to creation of a ground loop resulting in an increased noise level and a decrease in sensitivity.

REMOTE COMMUNICATIONS CONNECTIONS

The Model FL-45A can accept inputs from, as well as send inputs to, remote devices through the Analog Input connector (Fig. 6.5). For example, if your chromatographic system has programmable timed events (contact closures or TTL), you can use one to zero the detector signal automatically during a run.

The terminals available on the Model FL-45A's remote communications connector are labeled STOP, RUN/START, and ZERO (each with an associated ground terminal), and Accessory Relay.

Stop

You can use a timed event from your chromatographic system to take the detector out of run by connecting the system's event to the detector's STOP and ANALOG GND terminals.

Run/Start

You can use the remote-start event on your injector or autosampler to put the detector into run automatically whenever an injection occurs by connecting the event to the detector's RUN/START and ANALOG GND terminals.

Zero

You can zero the detector signal automatically by connecting a timed event on your chromatograph to the detector's ZERO and ANALOG GND terminals.

Accessory Relay

The detector is capable of driving one TTL load through the Accessory Relay terminals each time it goes to its READY state. This ability to signal other instruments is particularly useful with autosamplers, where the detector can signal that it's ready for the next injection in an automated series of runs. To hook up the Accessory Relay terminals, connect the one input lead from the other instrument to the detector's COM (common) terminal and the other lead to either the N.O. (normally-open) or N.C. (normally-closed) terminal.

Flowcell Connections

Use the following steps to connect the detector's flowcell to the rest of your LC system:

1. Remove the side enclosure of the detector.

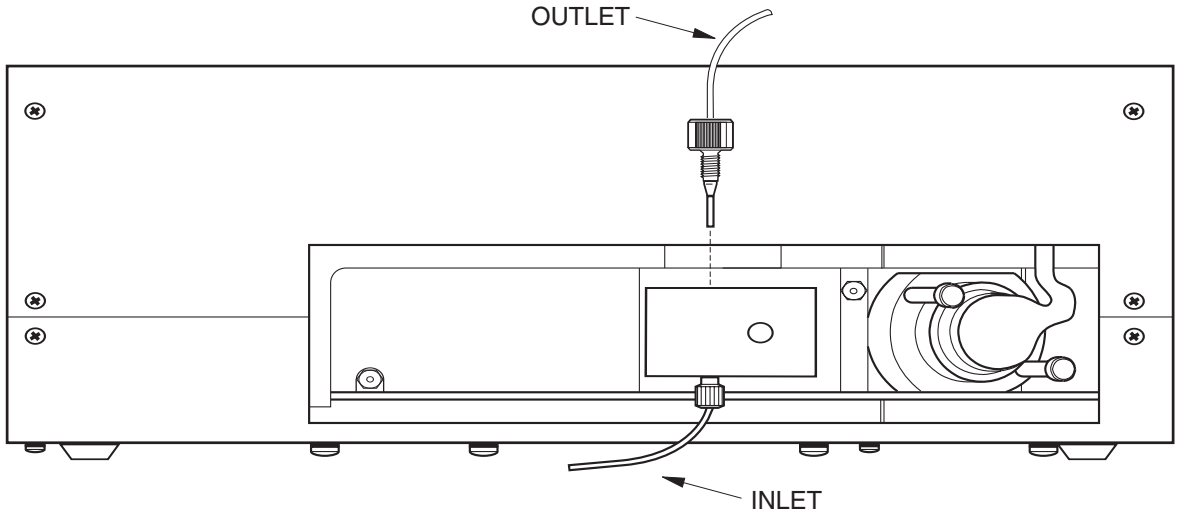
2. Using the finger-tight nut and ferrule sets included with the installation kit, connect the column outlet directly to the detector's fluid inlet on the left side of the flowcell (Fig. 6.6).

3. Connect the detector's fluid outlet to waste tubing.

HINT: If you have several detectors hooked up in series, place the Model FL-45A last to avoid back-pressure problems that could damage its flowcell.

4. Replace the side enclosure of the detector, making sure that the tubing passes through the slots without being pinched.

Figure 6.6 The Model FL-45A with the side enclosure removed



6.3 SPECIFICATIONS

DETECTOR: PMT, 200–650 nm

WAVELENGTH RANGE:
200–650 nm zero order excitation;
200–650 nm emission;
200–800 nm emission with optional
extended range (red-sensitive) PMT

WAVELENGTH ACCURACY: ± 2 nm @ 248 nm ex., 398 nm em.

WAVELENGTH PRECISION: < 0.5 nm

SPECTRAL BANDWIDTH: 8, 20, or 30 nm, selectable

SENSITIVITY: S/N > 3000 for 4.1 µg/L anthracene in MeOH, 248 nm ex., 398 nm em.

LAMP: Pulsed xenon lamp

LAMP FREQUENCY: Selectable 20 or 100 Hz

FLOWCELL:
High-purity quartz,
8 microliter illuminated volume
maximum pressure 200 PSI (14 bar)

RANGE SELECTIONS:
500, 200, 100, 50, 20, 10, 5, 2, 1, 0.5,
0.2, 0.1, 0.05, 0.02, 0.01 FUFS

ANALOG OUTPUTS:
OUTPUTS 1 AND 2: Range-selectable Over Entire Fluorescence Range

COMMUNICATIONS:
REMOTE INPUTS: Run/Start, Stop, and Zero
REMOTE OUTPUTS: Ready

DISPLAY: 2 x 24 character, high-contrast LCD

DIMENSIONS: 17 cm x 30 cm x 40 cm (H x W x D)

WEIGHT: 11 kg

POWER REQUIREMENTS: 100/120/220/240 VAC nominal; 50/60 Hz; 2-amps max.

6.4 WARRANTY

Bioanalytical Systems, Inc. (BAS) guarantees all components of the FL-45A, excluding lamp and flowcell, to be free from defects of material and workmanship for a period of one year after Initial purchase. BAS will repair or replace, at its discretion, all defective components during the aforementioned warranty period.

BAS warrants all flowcells to be free of defects of material and workmanship upon arrival. BAS will repair or replace, at its discretion, all flowcells which prove to be defective due to workmanship or materials.

BAS guarantees all Xenon lamps to be free of defects of materials or workmanship upon arrival and for a period of 90 days. BAS reserves the right to waive all warranties in the case of problems due to improper handling and use of said lamps. All Xenon lamp replacements will be warranted for the unused portion of the original 90 days.

For warranty service or repair, all BAS products must be returned to BAS. The Buyer shall prepay shipping charges to BAS, and BAS shall pay shipping charges to return the product to the buyer. However, the buyer shall pay all shipping charges, duties, and taxes for products returned from another country.

BAS warrants that its software and firmware designated for use with a BAS product will execute its programming instructions when properly installed on that product. BAS does not warrant that the operation of the instrument, software, or firmware will be uninterrupted or error-free.

The foregoing warranty shall not apply to defects resulting from improper or inadequate maintenance by the Buyer, Buyer-supplied software or interfacing, unauthorized modification or misuse, operation outside of the environmental specifications for the product, or improper site preparation or maintenance.

For any product expressly covered under this warranty, Bioanalytical Systems is liable only to the extent of replacement of defective items. Bioanalytical Systems, Inc. shall not be liable for any personal injury, property damage, or consequential damages of any kind whatsoever. The foregoing warranty is in lieu of all other warranties of merchantability and fitness for a particular purpose.

6.5 DAMAGED SHIPMENTS

Breakage of any part of this instrument during shipping should be reported immediately to BAS Customer Service. You must retain the original packing box and contents for inspection by the freight handler. BAS will replace any new instrument damaged in shipping with an identical product as soon as possible after the claim filing date. Claims not filed within 30 days after the shipping date will be invalid.

Do not return damaged goods to Bioanalytical Systems without first contacting Customer Service for a Return Authorization Number (RA#). When a defective part is returned to BAS, the RA# immediately identifies you as the sender, and describes the item being returned. Bioanalytical Systems refuses all unauthorized return shipments.

6.6 SERVICE

Bioanalytical Systems provides a skilled service staff available to solve your technical problems if an equipment-oriented problem should arise. For further details, call customer service personnel (765/463-4527) who may choose to route your problem to the correct individual. Following discussion of your specific difficulties, an appropriate course of action will be described and the problem resolved accordingly. Do not return any products for service until a RETURN AUTHORIZATION NUMBER (RA#) has been obtained. The RA# indentifies you as the sender and describes to us the problem you are having in full detail. Turnaround time on service can be quoted to you at the time your RA# is issued, although we can not determine the actual amount of service required until we have received your unit and diagnosed the problem. All correspondence and shipments should be sent to:

RA # ,Service Department
Bioanalytical Systems, Inc.
2701 Kent Avenue
West Lafayette, IN 47906

Section 7. Menu Reference

7.1 INTRODUCTION

This section provides you with an alphabetical description of all the instrument's display fields and a Menu Tree. Fortunately, it's not necessary to read this Section in order to learn how to use your detector. It's included in the manual simply as a quick reference and aid to using your instrument.

The Menu Reference is an alphabetical listing of each menu field and command. Included in each listing is the field's definition and, where appropriate, all allowable and default values for the field.

The Menu Tree is a representation of the detector's overall menu structure. It shows the location and interrelation of all the instrument's menus. This is a good reference to keep on hand while you work through the operating instructions in Sections 3 and 4. It will also help if you become "lost" while moving through the detector's menus.

7.2 MENU REFERENCE

For quick reference, we have included this alphabetical list of all possible menu fields. The listing includes a short definition as well as the field's allowable and default values. For a more detailed explanation of the functions of your detector, you should refer to Section 3, *Basic Operations*, and Section 4, *Advanced Operations*.

Analog 1 Offset %
This field offsets the Analog 1 output signal (CH1 on the back panel) by a positive or negative 50, 20, 10, 5, 2, 1, or 0 percent of the full-scale range. Default is 0%.

Analog 2 Offset %
This field offsets the Analog 2 output signal (CH2 on the back panel) by a positive or negative 50, 20, 10, 5, 2, 1, or 0 percent of the full-scale range. Default is 0%.

Analog Outputs
This menu allows you to offset the analog output terminals located on the back panel of the instrument, and access the READY Output field.

Auto Spectra
This field allows you to tell the detector to automatically scan a peak whenever the output signal exceeds the Auto Threshold value. Selections are Off and On. Default is Off.

Auto Threshold
This field allows you to set the minimum signal at which the detector will automatically scan a peak. Allowable values range from 0 to 99.999 FUs. Default is 0.010.

Autozero Time
This field tells the detector when to perform an automatic zero. Allowable values are 0.00 to 99.99 minutes. Default is 0.00 minutes.

COMMANDS
The Commands Menu lets you perform a sample or baseline scan manually, replay spectra, put an event mark into your chromatogram, short detector outputs, and shut down the Model FL-45A.

Copy
This menu choice accesses the Copy File field.

Copy File
This field, along with the To File field, allows you to copy *from* the specified file to another file designation.

Cursor Speed
This field may be set to Slow, Medium, or Fast according to your need. Default is Medium.

Delete
Under the top-level menu FILES, this field accesses the Delete File command.

Under the top-level menu QUEUE, this field accesses the Delete Queue command.

Delete File
This field deletes the designated file, setting all fields to their default values. After pressing [ENTER], the message **File Deleted** appears for one second.

Delete Queue
This field deletes the designated queue. After pressing [ENTER], the message ** Queue Deleted ** appears for one second.

Detection Type
This field allows you to put the detector into the fluorescence (Fluor) or phosphorescence (Phos) mode. Default is Fluor.

Display
This field shows the time at which the displayed scan was taken.

Display FU, λ
This field displays a screen that shows the actual incremental wavelength versus fluorescence intensity data for the selected spectral scan.

Edit
Under the top-level FILES Menu, the Edit Menu allows you to set up or edit files. The edits don't change the current settings of the detector until the file is loaded.

Under the top-level QUEUE Menu, the Edit Menu allows you to set up or edit a queue. Edits may not be made to Order 1 while a queue is loaded or running unless you pause the queue first.

Edit File
This field allows you to identify the file you wish to edit. Allowable designations are 1 to 4. Default is 1.

Emλ
This field shows the detector's emission wavelength.

Event Mark
The Event Mark field applies a 15% of full-scale spike on the detector's output signals.

Exλ
This field shows the detector's excitation wavelength.

FILES
The Files Menu allows you to edit, load, copy, or delete files.

File Name
This field allows you to enter a file name for a designated file (numbered 1 to 4). The name can contain up to eight characters from the following list: A to Z, 0 to 9, /, -, and blank. Default is blank.

FU
This field gives the intensity of fluorescence in fluorescence units (FU). It's a six-digit number, ranging from 0.001 to 999.999 FUFS.

Lamp Count
This field allows you to track the total number of lamp flashes. The field must be reset when a new lamp is installed.

Lamp Flash Rate
This field allows you to set the pulse frequency applied to the xenon lamp. Allowable values are 100 and 20 Hz. Default is 100 Hz.

Lamp Status
This field allows you: to *manually* turn the lamp on (On) or off (Off) at any time; or to *automatically* turn the lamp on and off at the beginning and end of a *run* (Run); or to automatically turn the lamp off at the end of a *queue* (Off@End). Default is Run.

Load
Under the top-level menu FILES, the Load selection accesses the Load File command.

Under the top-level menu QUEUE, the Load selection accesses the Load Queue command.

Load File
The Load File command loads the designated file settings into the active run file. After pressing [ENTER], the message **File Loaded** appears for one second.

Load Queue
The Load Queue command loads the designated queue. After pressing [ENTER], the message **Queue Loaded** appears for one second.

More
The More Menu allows you to access the Zero on λ Change, Detection Type, Cursor Speed, and Status Lock fields, and the file protection feature.

Number of Scans
This field designates the number of times the detector should scan for each spectrum. In scanning, the detector scans from the low to the high wavelength, and then from high to low. The total number of scans are subsequently averaged to produce the final spectrum. Allowable values are 2 to 32, in multiples of two. Default is 2.

OPTIONS
Found in the Main Menu, the Options Menu allows you to access the Analog Outputs and More menus.

Options
The Options selection in the Edit Menu of FILES allows you to edit Range, Rise Time, Autozero Time, Lamp Flash Rate, Lamp Status, and PMT Voltage.

Order
This field designates the order in which the detector is to run the selected files in a queue.

Pause
This field accesses the Pause Queue command.

Pause Queue
This command pauses an active queue. If a file is running, the file continues until it's completed, and the detector returns to a READY state.

PMT Voltage
This field designates the voltage to be applied to the photomultiplier. Allowable values are 0 (to turn it off), 500, 600, 700, 800, 900, and 1000 volts. Default is 600.

Protect
This field, in conjunction with the File Name field, protects a specified file from being edited, copied to (overwritten), or deleted. The field toggles between On, allowing no changes to the file, and Off, where changes may be made. Default is Off.

QUEUE
The Queue Menu allows you to edit, load, delete, or pause a queue. A queue is a series of files which are run in a specific order, and is typically used for automated runs.

Range 1
This field controls the full-scale output range for the Analog Output 1 terminal. Allowable full-scale ranges are 500, 200, 100, 50, 20, 10, 5, 2, 1, 0.5, 0.2, 0.1, 0.05, 0.02, and 0.01 FUFS. Default is 10 FUFS.

Range 2
This field controls the full-scale output range for the Analog Output 2 terminal. Allowable full-scale ranges are 500, 200, 100, 50, 20, 10, 5, 2, 1, 0.5, 0.2, 0.1, 0.05, 0.02, and 0.01 FUFS. Default is 10 FUFS.

READY Output
This field is used to communicate with other devices through the READY(Output) terminal. This TTL terminal switches the transistor between high and low states whenever the detector starts a run. Select “Active Hi” or “Active Lo,” for the high or low state, respectively. Default is Active Hi.

Replay
Under the top-level menu COMMANDS, this field accesses the Replay Menu where you can set the parameters for replaying stored spectra.

During a replay, this field shows the time at which the scan was taken.

Replay Spectra
This command initiates replay of the designated spectrum.

Replay Rate
This field designates the rate at which you wish to replay a stored spectrum. Allowable values are 2, 5, 10, 20, and 40 nm/sec. Default is 20 nm/sec.

Rise Time
This field controls the detector’s response time. Rise time is inversely proportional to the amount of baseline noise. For example, the longer the rise time, the less noise detected. Allowable values are 0, 0.1, 0.2, 0.5, 1, 2, 5, and 10 seconds. The default value of 2 seconds is appropriate for most applications.

#Runs
This field displays the number of runs to be made for each file in a queue.

Scan Length
This field designates the wavelength span for each scan. Allowable values are 0 to 600 nm. Default is 50 nm.

Scan
This command initiates a sample spectral scan.

Scan Type
This field designates the type of scan to be performed. The choices are Emission, Excitation, and Delta. In a delta scan, the detector scans both the excitation and emission spectra, keeping a constant wavelength differential between the two monochromators. Default is Excitation.

Scan Zero
This command initiates a background scan.

Scan Zero Time
This field allows you to set the time at which the detector should perform a baseline scan automatically. Allowable values are 0.00 to 99.99 minutes. Default is 0.00 minutes.

Self-Tests
This command tells the detector to run through its internal diagnostic tests.

Short Outputs
This field is used to short the detector analog outputs together (zero volts). When the outputs are shorted, the field changes to Unshort Outputs. To remove the short and return the outputs to their normal operating state, select Unshort Outputs, and the field changes back to Short Outputs. When you leave this screen, the field returns automatically to Short Outputs.

Shutdown Detector
This field shuts down the detector's lamp, photomultiplier, and motors, leaving the electronics on to preserve memory. Press any key to return the detector to the same settings as when this field was activated.

Software Version
This field displays the E-PROM version of your detector's software.

Spectra
The Spectra Menu allows you to set up the parameters for scanning.

Spectra Time
This field contains a list of the scans that are currently stored in memory. Each scan is identified by the runtime at which it was initiated.

Start Emission λ
This field defines the wavelength at which the detector should begin an emission scan. Allowable values are 0 and 200 to 800 nm. Default is 400 nm.

Start Excitation λ
This field defines the wavelength at which the detector should begin an excitation scan. Allowable values are 0 and 200 to 650 nm. Default is 250 nm.

Status
This field in the Status Screen gives the current condition of the detector. The possible conditions are: READY (the detector is stabilized and waiting for initiation of a run), and NRDY (Not Ready; the detector isn't stabilized, is performing internal tests, or has a possible internal problem). The run time is displayed when the running file has a programmed stop-time. The letter Q appears at the beginning of this field when a queue is loaded.

Status Lock
The Status Lock field limits accessibility to the Status Menu (the programming area below the Status Screen). When set to On, only the Status Screen is shown on the display and the down-arrow icon is not seen. Default is Off.

Step Size
This field defines the wavelength interval at which the detector should perform scanning. Allowable values are 2, 4, 8, and 16 nm. Default is 8 nm.

TESTS
The Tests Menu allows you to access the detector's software version, lamp-count, data acquisition, and fluorescence response screens, as well as its internal diagnostic tests.

Time, Emλ, Exλ
The Wavelength Program is a table containing the Time, Emλ, and Exλ fields. It allows you to program changes in the detector's emission and excitation wavelengths as a function of time.

Time refers to the amount of time into the run, in minutes, when a specified wavelength change is to occur. Allowable values range from 0.00 to 999.999 minutes. Default is 0.00 minutes.

Emλ and Exλ show the emission and excitation wavelength, respectively, to be set at a specified time. You can program up to nine different wavelengths for a single run. Allowable values are 0 and 200 to 800 nm for emission wavelengths, and 0 and 200 to 650 nm for excitation wavelengths. Defaults are 400 and 250 nm, respectively.

To File
This field, along with the Copy File field, allows you to copy a file *to* the specified file number.

Wavelength Program

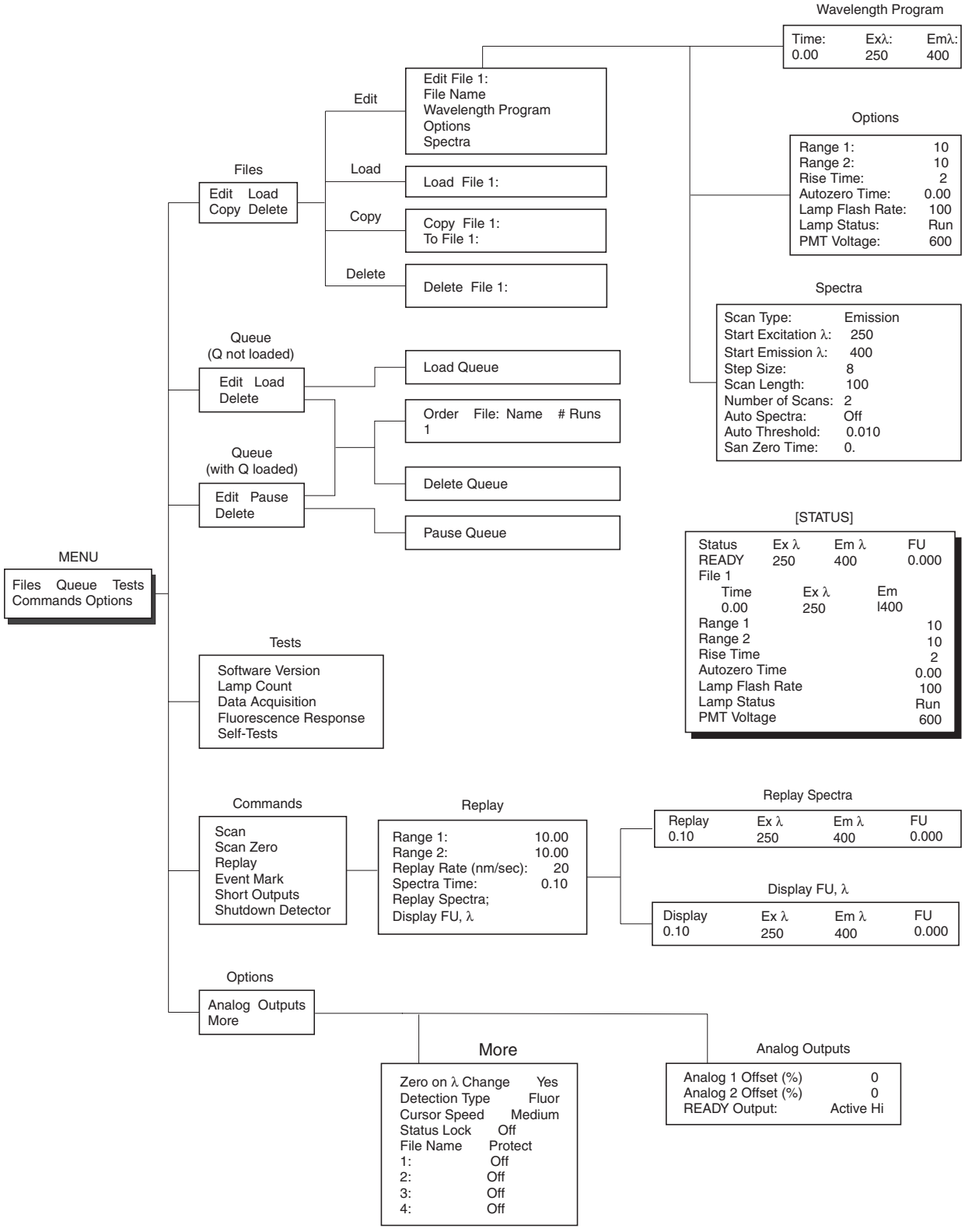
This command allows you to access the Wavelength Program. See the “Time, Emλ, Exλ” description above for details.

Zero on λ

This field toggles between Yes, where the detector baseline automatically zeroes each time the wavelength changes during a programmed run, and No. Default is Yes.

7.3 MENU TREE

The Menu Tree is useful for learning your way around the detector. You may wish to keep it handy while you learn where each display is located in the overall menu structure.



Section 8. Troubleshooting

8.1 INTRODUCTION

This Section provides you with helpful information for troubleshooting possible detector and chromatographic system problems. We have divided it into four sections:

a. a brief theory of operation

b. a troubleshooting guide that lists symptoms, possible problems, remedies

c. possible error messages

d. a description of the detector's diagnostic tests

8.2 THEORY OF OPERATION

The Model FL-45A detector consists of a pulsed xenon lamp, an excitation monochromator, an emission monochromator, a flowcell, a photomultiplier tube (PMT), and an optical system, all coordinated by supporting software and electronics.

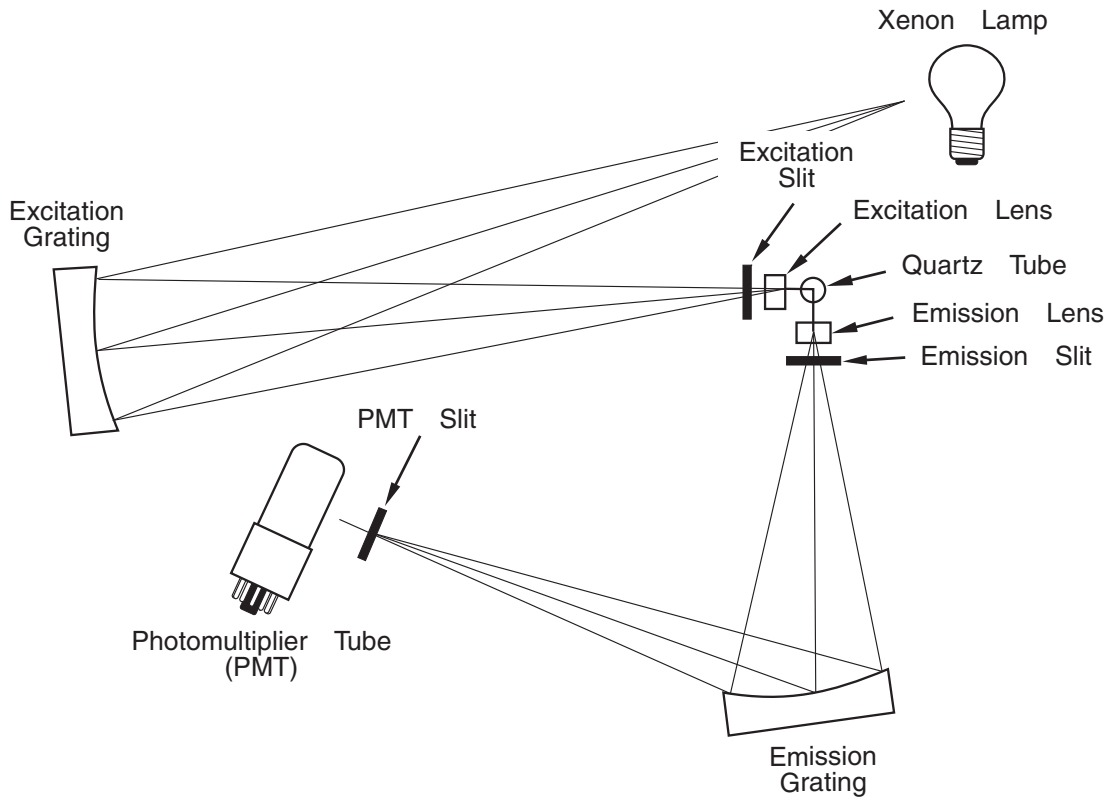
As shown in Figure 8.1, a beam of light from the xenon lamp is directed through the excitation monochromator (diffraction grating). From there, a bandwidth of light passes through the excitation slit and lens into the quartz flowcell, illuminating the sample as it passes through. If the sample is fluorescent or phosphorescent, it absorbs energy from the excitation light beam and subsequently emits light of a different wavelength.

The emitted light passes from the flowcell, through the emission lens and slit, to the emission monochromator. User-selected wavelengths of the emitted light are reflected through the PMT slit to the PMT. The PMT and its supporting circuitry convert the transmitted light into a current and ultimately into a voltage signal that's proportional to the intensity of the light received. The voltage signal is read out to an integrator/recorder.

The emission side of the detector's optics is positioned at right-angles (90°) relative to the excitation side to minimize the amount of scattered excitation light reaching the PMT tube. As a result, the PMT receives virtually no incident light unless a fluorescent material is passing through the flowcell.

A PMT generates some current (called dark current), even when no light is present. Dark current can contribute to detector background noise, reducing the signal-to-noise ratio, and ultimately reducing sensitivity. The Model FL-45A only integrates the current from the PMT during a lamp flash, virtually eliminating the contribution of dark current to detector noise.

Figure 8.1. Model FL-45A's optical system



8.3 TROUBLESHOOTING

This section contains a table of symptoms, possible causes, and remedies for some common problems you may observe in detector response. Many of the problems attributed to the detector may actually be due to other components in the chromatographic system, so we have included references to these types of problems and solutions as well.

SYMPTOM	CAUSE	REMEDY
No peaks, or peaks much smaller than expected.	Incorrect excitation and/or emission wavelength. Incorrect slit width(s).	Check excitation and emission wavelength settings. Ensure that the correct file is loaded. Check all slit widths (excitation, emission, and PMT).
	Lamp not lighted.	Check Status Menu to verify that the lamp is on. Run Self-Tests (Lamp) and replace lamp if necessary.
	Lamp power supply or connector defective.	Contact your BAS representative for assistance.

SYMPTOM	CAUSE	REMEDY
	Integrator/recorder input voltage mismatched with detector output voltage.	Reconnect positive lead of the integrator's or recorder's connecting-cable to correct terminal on detector. Verify correct integrator attenuation.
	Dissolved oxygen in mobile phase quenching fluorescence response.	Degas mobile phase.
	Defective PMT or PMT power supply.	Replace PMT and/or contact your BAS representative for assistance.
	Insufficient sample reaching the detector.	Check entire chromatographic system for leaks. Verify sample injection volume.
Spikes on recorder baseline.	Bubbles in the flowcell.	Flush flowcell with solvent. Check fittings for leaks.
	Electrical interference.	Check electrical connections for good continuity. Check for RFI (Radio Frequency Interference) from nearby sources such as computers, monitors, printers, etc. Verify that detector's GND input isn't connected to the recorder's earth-ground terminal, creating a ground loop.
	Large AC-line voltage fluctuations.	Connect detector to a power outlet not shared with heavy-current-draw devices (refrigerators, large electric motors, etc.).
Random noise on integrator/recorder baseline.	Flowcell contamination.	Clean flowcell with solvents (see Section 5.)
	Leaking sample-inlet line.	Check all fittings from the column outlet and to the flowcell inlet for leaks. Tighten or replace fittings as necessary.
	Bubble trapped in flowcell.	Increase flowrate to dislodge bubble. Supply back-pressure device to flowcell (max. 500 psi).
	Flowcell leaking.	Replace flowcell.

SYMPTOM	CAUSE	REMEDY
	Ground-loop between detector and integrator/ recorder.	Verify that detector's GND input isn't connected to the recorder's earth-ground terminal, creating a ground loop. Ensure that both devices are connected to the same AC outlet.
	Dirty optics (flowcell, lamp, PMT, or lenses).	Clean appropriate system optics (see Section 5).
	Integrator/recorder input voltage mismatched with detector output voltage.	Reconnect positive lead of integrator's or recorder's connecting-cable to correct terminal on detector. Verify correct integrator attenuation.
	Incorrect rise-time setting.	Determine and enter appropriate rise-time.
	Mobile phase contaminated with fluorescent material.	Replace with fresh mobile phase made with high-purity solvents.
	Excitation wavelength too close to emission wavelength. Scattered light interferes with detection.	Adjust wavelengths and/or slit settings.
Excessive drift in recorder baseline.	Contaminated flowcell.	Clean flowcell with solvent (see Section 5). Check fittings for leaks.
	Contaminated mobile phase.	Replace with fresh mobile phase.
	Contaminated column.	Clean or replace column.
	Oxygen diffusing into degassed mobile phase.	Apply a continuous degassing technique (e.g., sparge with inert gas).
	Leaking flowcell.	Replace flowcell.
	Leaks in system.	Leak-test all fittings.
Detector won't power up.	Tripped circuit breaker at AC wall outlet.	Resolve problem, reset circuit breaker.
	Blown detector fuse.	Resolve problem, replace fuse.
	Incorrect voltage selected.	Reset detector for correct incoming line-voltage (see Section A).
	Power cord not connected.	Connect power cord.

8.4 ERROR MESSAGES

Three types of errors may appear on your detector's display:

a. System

b. Real-time

c. User-input

Each type is explained below in further detail.

System Errors

System errors are indicated on the display by exclamation points (!! !!), and occur whenever an undesirable condition exists that prevents the detector from operating. If one of these messages appears, first try turning the detector's power switch off and on. If the message reoccurs, contact your BAS representative.

a. SYSTEM RESET

b. RAM ERROR

c. ADDRESS ERROR

d. BUS ERROR

e. DIVIDE BY ZERO

f. LOW L0 ERROR

g. LOW L1 ERROR

h. DISTANT QUEUE ERROR

i. PARAM QUEUE ERROR

Real-time Errors

The following real-time error messages may appear on the display of your detector.

PMT OVERLOADED
The PMT is saturated from too much incident light. This is usually caused by operating the instrument with a dry flowcell, leaving out the PMT slit-wheel, or operating in the zero-order mode with the wrong PMT slit-size selected and/or no optical filter installed.

CASE OPEN
The detector case is open, allowing stray light to enter. You must either close the PMT slit-mount access door or replace the side enclosure.

User-input Errors

The following error messages indicate improper use of the detector's menu system.

A File Is Already Running

You cannot start a file while a different file is already running.

Invalid Parameters, Spectrum Not Allowed

Invalid scanning setup parameters have been entered, so the detector cannot perform a spectral scan.

No More Available Memory

All available system memory is full.

No Queue Available

You cannot load a queue if none has been set up first.

No Spectra Available

You cannot run the replay command when no spectra are available in memory.

Protected File, Cannot Be Copied To

You cannot copy to a protected file.

Protected File, Cannot Be Deleted

You cannot delete a protected file.

Protected File, No Editing Allowed

You cannot modify a protected file.

Queue Loaded, Cannot Load File

When a queue is loaded, you cannot load any other file.

Run In Progress, No Testing Allowed

You cannot run the detector's built-in diagnostics while a run is in progress.

Run Not In Progress, Spectrum Not Allowed

A spectral scan can only be performed when a run is in progress.

8.5 DIAGNOSTIC TESTS

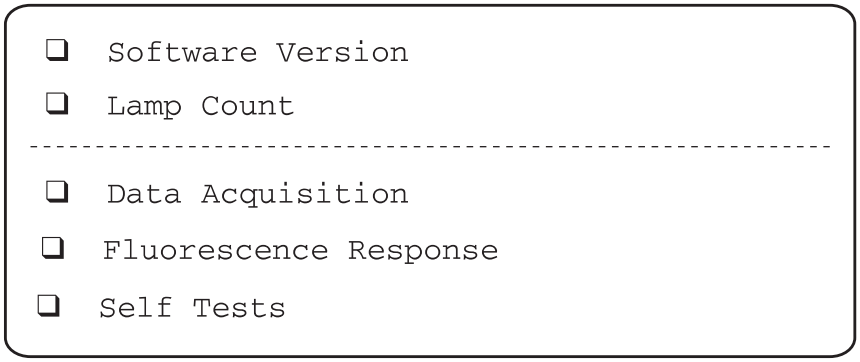
This section describes diagnostic tests you can use if you suspect that your detector isn't working properly. All are built into your detector.

To access the detector's internal diagnostic tests, follow these steps:

1. Press [MENU].
2. Select /TESTS/.

The Tests Menu appears in Figure 8.2.

Figure 8.2. The Tests Menu



Software Version

Select this field to display the E-PROM version of your detector's software (Fig. 8.3).

Figure 8.3. The Model FL-45A's software version

Lamp Count

The lamp count display (Fig. 8.4) shows the number of hours your detector's xenon lamp has operated. Access it by pressing [MENU], and selecting /Tests/, /Lamp Count/.

Figure 8.4. Lamp count display

```
Flashes = 1 * 360000
```

The detector's xenon lamp has an expected service life of approximately two billion flashes (about 28,000 hours at the 20 Hz strobe rate or 5,600 hours at the 100 Hz rate). The Lamp Count test shows the number of hours your detector's xenon lamp has operated (based on the 100 Hz strobe rate).

To calculate the number of times the lamp has flashed during its service life, multiply the number of hours of operation (the number to the immediate left of the asterisk in Figure 8.4) by 360,000.

Be sure to reset the lamp count to zero each time a new lamp is installed. To zero the lamp-hour count, move the cursor to the leftmost digit and hold down the [–] key.

HINT: Before changing the lamp-hour count, write down the current value.

For example, if the display reads 2800, you'd place the underline beneath the 2, press the [–] key, and hold it down. The underlined digit would rapidly and automatically decrease until it reached zero. The cursor would then automatically move one digit to the right and decrement it to zero. When you've reached the last digit to the right, decrement it manually by repeatedly pressing the [–] key until only an underlined zero (0) is displayed.

Data Acquisition

The Model FL-45A's Data Acquisition test is the third test in the Tests Menu. Access it by pressing [MENU], and selecting /Tests/, /Data Acquisition/. The display shown in Figure 8.5 appears.

NOTE: The words "(signal)" and "(offset)," shown in the center of Figure 8.5, don't appear on the detector's display. We show them here only to help you differentiate the values.

Figure 8.5. Data acquisition display

```
PMT:      nnnnnnn      nnnnn      Hz
          (signal)     (offset)
PHO:      nnnnnnn      nnnnn      Hz
```

The values shown in the data acquisition screen represent the analog-to-digital (A/D) conversion frequencies of the photomultiplier tube (PMT) and the photodiode (PHO). These frequencies can vary between instruments and with different applications. They assume a

dry flowcell with an excitation wavelength of 250 nm and an emission wavelength of 400 nm.

HINT: Be sure to run the data acquisition test at least once a month and keep a written record of the values!

Fluorescence Response

The fourth test in the Tests Menu is Fluorescence Response. You can use this selection to activate/inactivate the response factor and to recalibrate the detector using the standard solution of your choice. To access this function, press [MENU], and then use the arrow and [ENTER] keys to select /Tests/ and /Fluorescence Response/.

Your detector has one of two possible screen displays. Detectors having E-PROMs up to and including Version 3.01 show the display illustrated in Figure 8.6; detectors having E-PROM versions 3.06 and higher show the display in Figure 8.7. Each type of display is detailed separately below.

VERSIONS UP TO 3.01
The Fluorescence Response display that appears for E-PROM versions up to and including 3.01 is shown in Figure 8.6. If you move the blinking cursor to the /Active-Inactive/ field and press [+], you can enable or disable the response factor. If you move it to the /Calculate/ field and press [ENTER] twice while a standard calibration fluid is flowing, you can recalibrate your detector. Using this display, the calibrant peak is automatically set for a value of 20 ± 1 FU. The mathematical factor used to make the calculation appears onscreen on the right side of /Calculate/. Complete details of the recommended calibration process are provided on page 59.

Figure 8.6. Display for E-PROMs up to and including Version 3.01

```
          Fluorescence Responce
INACTIVE/ACTIVE          >Calculate   n.nn
```

VERSION 3.06 AND HIGHER
The Fluorescence Response display that appears with E-PROM Versions 3.06 and higher is shown in Figure 8.7. If you move the blinking cursor to the /Active-Inactive/ field and press [+], you can enable or disable the response factor. If you move the cursor to the /Factor/ field while analyzing your standard calibration fluid, you can recalibrate your detector.

Using this display, the calibrant peak is set for the FU value of your choice. You do this by changing the value of the /Factor/ field, one digit at a time, beginning with the “tens” digit and moving toward the “hundredths” digit. You select the digit by pressing [<] or [>] and then you change its value by pressing [+] or [–]. As you change the value of the digit(s)

you can watch the real-time effect that your changes have by viewing the FU value that's displayed to the right side of the /FU:/ field.

Complete details of the recommended calibration process are provided on page 59.

Figure 8.7. Display for E-PROM versions 3.06 and higher

Self-tests

The detector automatically runs five internal diagnostic tests every time the power is turned on. To initiate the tests at any other time, simply select /Self-Tests/.

If any test (other than the lamp test) fails, you'll see a message to that effect on the display. Clear the message and run the remainder of the self-tests by pressing [ENTER]. Repeat this process as many times as necessary until all self-tests are completed and the Status Screen appears. If any test has failed, the Status Screen will read "NRDY" (Not Ready).

Although you can frequently get back to the ready state on your own (e.g., you can load a new file or queue after the failure of particular self-tests), the detector may not function properly and your results may be affected.

For troubleshooting purposes, the most likely solution to any failed self-test other than the lamp test, is to replace the appropriate motor or PCB. Motor replacements must be done at a BAS Service Center due to special calibration equipment needs. PCBs, however, may be replaced in the field.

The five self-tests are:

1. RAM. This test checks both non-volatile and volatile RAM with a read/write test. The "RAM" message only appears during self-initiated testing. On power-up, the test occurs without any special message. Instead, you'll see words like "Version No." on the screen.

2. Excitation Motor. This test verifies proper operation of the excitation-monochromator's motor and of the motor's optical encoders.

3. Emission Motor. This test verifies proper operation of the emission-grating's monochromator motor and of the motor's optical encoder.

NOTE: You may be able to hear the motors operating during both motor tests.

4. Internal Voltages. The internal-voltages test checks the circuitry-supply voltages that supply the detector's electronics. Voltages tested include:

   a. Lamp off
   b. Lamp on
   c. PMT
   d. ± 12 Vdc
   e. Analog-output
   f. Motor voltage

   NOTE: You may hear "clicking" during the Internal Voltages test.

5. Lamp. The lamp test verifies that the xenon lamp is operating (flashing) properly. The lamp test does not check lamp intensity. If the lamp test does not pass, you'll get a "Fail" message. In that case, simply replace the lamp and try the test again.

   If, during the power-up test sequence, the lamp test passes, a new screen (Fig. 8.8) appears.

Figure 8.8. The calculating reference table message

```
** Calculating **
** Reference Table **
```

Although you'll never see the reference table that's being calculated, the message lets you know that a table is being generated that will correct (offset) any changes in excitation energy across the operating spectrum.

Section 9. Glossary

INTRODUCTION — We have included a glossary to define certain technical terms used throughout the manual's text. These terms should be consistent with standard definitions used throughout the analytical industry, and are added here as a quick reference only.

A/D — Analog-to-digital. Converts a detector's analog signal to a digital (binary-coded) signal.

analog offset — Voltage applied to the output signal in order to keep the signal "on-scale" throughout a run.

background scan — The reference spectrum of the mobile phase. It's subtracted from the sample spectral scans to correct for baseline absorbances.

bandwidth — The width of a band, measured at its base. Also called peak width.

baseline — The reference line at the bottom of a chromatogram from which measurements are made. A baseline represents the chromatogram that would be drawn if only the mobile phase (with no sample) were run through the column.

defaults — The values or choices built into a system. If no specific choice is made, the detector will run using the default settings.

degassing — The practice of removing air from the mobile phase, usually by sparging with inert gas or applying a vacuum.

delta scan — A synchronous scan of both the excitation and emission spectra that keeps a constant wavelength differential between the two monochromators.

diagnostics — Ways of detecting and isolating instrument or software problems.

display — The two-line liquid-crystal screen.

emission wavelength	The wavelength of the light emitted from a fluorescing/phosphorescing compound.
error message	A displayed message that notifies you of a problem.
excitation wavelength	The wavelength of the light used to excite a fluorescing/phosphorescing compound.
FUFS	Fluorescence units, full-scale; a measure of sensitivity.
fields	Areas in a display, screen, or menu where an entry is required or a choice must be made.
file	A list of detector parameters that contains the desired settings for an analysis.
fluorescence	The instantaneous and temperature-independent emission of light by a sample that has been excited by incident light energy; the excitation wavelength is always shorter than the emission wavelength.
gradient elution	A liquid chromatographic technique where the mobile phase composition changes over time; changes may be continuous or in steps. Also called solvent programming.
ground terminal	A terminal used to connect the ground or earth lead of a signal or contact closure cable.
keypad	All of the keys which you use to communicate with your instrument or computer.
menu	A list of choices.
NOVRAM	This abbreviation stands for Non-volatile RAM. Non-volatile RAM consists of Random-Access Memory (RAM) chips that retain data even if the instrument's power source fails. NOVRAM is used to store critical setup data in the Model FL-45A detector.

parameter A value or set of values used to define the characteristics of behavior of an instrument or system.

peak broadening The dilution of a peak as it moves through the chromatographic system.

phosphorescence The delayed and temperature-dependent emission of light by a sample that has been excited by incident light energy; the excitation wavelength is always shorter than the emission wavelength.

queue A set of items (i.e., samples, files) in a prearranged order.

RAM Random Access Memory.

range A detector parameter that controls the full-scale range for the output signal.

replay Retrieving a stored spectrum, which can be played back as either individual data points or a smoothed spectrum.

rise time A detector parameter that controls the detector's response time. Rise time is inversely proportional to the amount of baseline noise.

run time The duration of a sample run from injection to detection.

signal-to-noise A measurement of the sensitivity of a detector, the ability to measure a very small sample response over the baseline noise.

spectral scan A sample spectrum.

status The current condition.

timed event	An instrument action triggered to occur at a specific, preset time during a run (e.g., autozero, wavelength change, stop-time).
troubleshooting	Refers to locating the cause of problems with equipment or procedures, and solving these problems.
wavelength programming	Programming the detector to change the monitoring wavelength as a function of time during a run.
zero order	In fluorescence, the monochromator is set to act as a mirror, reflecting all wavelengths of incident light.

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