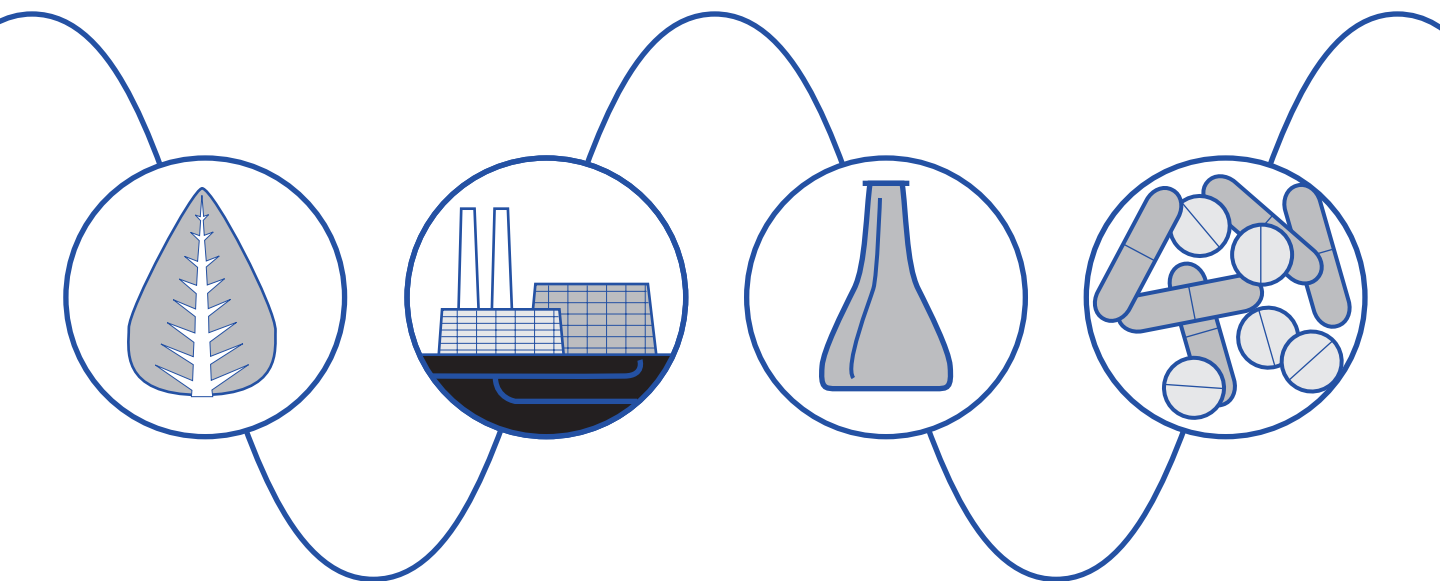


Waters 474 Scanning Fluorescence Detector

Operator's Guide



Waters

34 Maple Street
Milford, MA 01757

WAT047467, Revision 3

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Attention: When you use the instrument, follow generally accepted procedures for quality control and methods development.

If you observe a change in the retention of a particular compound, in the resolution between two compounds, or in peak shape, immediately take steps to determine the reason for the changes. Until you determine the cause of a change, do not rely upon the results of the separations.











Caution: For continued protection against fire hazard, replace fuses with those of the same type and rating.



Attention: Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

Note: The Installation Category (Overvoltage Category) for this instrument is Level II. The Level II category pertains to equipment that receives its electrical power from a local level, such as an electrical wall outlet.

Symbols Used on 474 Detector

	Direct current
	Alternating current
	Protective conductor terminal
	Frame or chassis terminal
	Caution, risk of electric shock
	Caution or refer to manual
	Ultraviolet light
	Hot surfaces

474 Scanning Fluorescence Detector Information

Intended Use

The Waters 474 Scanning Fluorescence Detector can be used for in-vitro diagnostic testing to analyze many compounds, including diagnostic indicators and therapeutically monitored compounds. When developing methods, follow the “Protocol for the Adoption of Analytical Methods in the Clinical Chemistry Laboratory,” American Journal of Medical Technology, 44, 1, pages 30 – 37 (1978). This protocol covers good operating procedures and techniques necessary to validate system and method performance.

Biological Hazard

When analyzing physiological fluids, take all necessary precautions and treat all specimens as potentially infectious. Precautions are outlined in “CDC Guidelines on Specimen Handling,” CDC – NIH Manual, 1984.

Calibration

Follow acceptable methods of calibration with pure standards to calibrate methods. Use a minimum of five standards to generate the standard curve. Concentration range should cover the entire range of quality control samples, typical specimens, and atypical specimens.

Quality Control

Routinely run three quality-control samples. Quality-control samples should represent subnormal, normal, and above-normal levels of a compound. Ensure that quality-control sample results are within an acceptable range, and evaluate precision from day to day and run to run. Data collected when quality control samples are out of range may not be valid. Do not report this data before ensuring that chromatographic system performance is acceptable.

Table of Contents

How to Use This Guide.....	19
Chapter 1	
Introduction and Theory of Operation	22
1.1 Introduction	22
1.2 Waters 474 Detector Features	23
1.3 Fluorescence Theory of Operation	24
1.3.1 Excitation Sources	24
1.3.2 Filtering	25
1.3.3 Exciting the Sample	26
1.3.4 Measuring Fluorescence	26
1.3.5 Scanning	27
1.3.6 References	27
1.4 Principle of Operation	28
1.4.1 Optics Bench	28
1.4.2 Control System	30
Chapter 2	
Installing the 474 Detector	32
2.1 Site Selection and Power Requirements.....	32
2.2 Unpacking and Inspection.....	33
2.3 Voltage Selection and Fuse Installation	35
2.4 Making Fluid Connections.....	36
2.5 Making Electrical Power Connections.....	39

2.5.1 Making AC Power Connections	39
2.6 Making Connections to Other Devices	40
2.6.1 Analog Connector Overview	40
2.6.2 Connecting the 474 to the Millennium Data System	43
2.6.3 Connecting the 474 to the Waters 2690 Separations Module	48
2.6.4 Connecting the 474 to the Waters 600 Series Controller	53
2.6.5 Connecting the 474 to a Waters 745/745B/746 Data Module	58
2.6.6 Connecting the 474 to a Chart Recorder	59
2.6.7 Connecting the 474 to the Waters 717plus	60
2.6.8 Connecting the 474 to the Carbamate System	62
2.6.9 Connecting the 474 to the AccQ•Tag System	65
2.6.10 Connecting a Voltmeter to the Excitation Out Terminals	67

Chapter 3

Using the 474 Detector	68
3.1 Powering On	68
3.2 Using the Front Panel	69
3.3 Key Descriptions	71
3.4 Operating Parameters	77
3.4.1 Displaying and Changing Parameter Settings	78
3.4.2 Excitation Wavelength	79
3.4.3 Emission Wavelength	79

3.4.4 Gain	79
3.4.5 Attenuation.....	80
3.4.6 Response.....	80
3.5 Special Parameters.....	81
3.5.1 Program Number	82
3.5.2 Diagnostics	82
3.5.3 Energy Output.....	83
3.5.4 Lamp Off Timer.....	83
3.5.5 Signal Filter.....	84
3.5.6 Auto Zero Enable	87
3.5.7 Offset	89
3.5.8 Polarity	90
3.5.9 Lamp Hours	91
3.5.10 Bandwidth.....	92
3.5.11 Wavelength Range	94
3.5.12 Temperature Correction	95
3.6 Using External Functions	95
3.6.1 Excitation Energy Out	96
3.6.2 Mark Out	96
3.6.3 Cell Leak.....	96
3.6.4 Mark In.....	97
3.6.5 Auto Zero In	97
3.6.6 Program Reset.....	97
3.7 Calibration Check.....	98
3.7.1 Excitation Wavelength Accuracy (Raman Band of Water)Test	98

3.7.2 Signal-to-Noise Test.....	102
3.8 Powering Off	103

Chapter 4

Programming the 474 Detector 105

4.1 Using the Program Mode	105
4.2 Writing a Multi-Step Time Program.....	107
4.2.1 Setting the Initial Conditions of a Time Program	109
4.2.2 Setting Steps in a Time Program.....	111
4.2.3 Saving the Time Program	113
4.2.4 Example Time Program	114
4.3 Running a Time Program	119
4.3.1 Manual Time Program Start.....	119
4.3.2 External Time Program Start	120
4.4 Modifying a Time Program.....	121
4.4.1 Accessing the Program Number.....	121
4.4.2 Modifying a Program Step	122
4.4.3 Adding a Program Step	123
4.4.4 Deleting a Program Step	124
4.4.5 Deleting a Group of Steps	124
4.5 Performing a Wavelength Scan in a Time Program	125
4.5.1 Storing a Spectrum.....	126
4.5.2 Entering the Spectrum Parameters	128

Chapter 5	
Using the Scan Mode	129
5.1 Scan Parameters	130
5.2 Performing Spectral Scans	132
5.2.1 Manual Emission Spectrum Scans	133
5.2.2 Manual Excitation Spectrum Scans	134
5.3 Spectrum Output.....	136
5.3.1 Outputting Emission Spectra	137
5.3.2 Outputting Excitation Spectra	139
5.4 Difference Spectra	142
5.4.1 Emission Difference Spectrum	143
5.4.2 Excitation Difference Spectra.....	146
 Chapter 6	
Maintenance Procedures	149
6.1 Routine Maintenance	151
6.2 Cleaning the Ventilation Filters.....	151
6.3 Cleaning the Flow Cell	152
6.4 Replacing the Flow Cell	153
6.4.1 Removing the Flow Cell Cassette.....	153
6.4.2 Disassembling the Flow Cell Cassette.....	154
6.4.3 Reassembling the Flow Cell	157
6.4.4 Reassembling the Flow Cell Cassette	158
6.4.5 Reinstalling the Flow Cell Cassette	159
6.5 Changing the Lamp.....	159
6.5.1 Removing the Lamp Housing	160

6.5.2 Replacing the Lamp Housing.....	164
6.5.3 Adjusting the Lamp.....	164
6.6 Replacing AC Fuses	166
Chapter 7	
Error Messages and Diagnostics	167
7.1 Error Messages.....	167
7.1.1 Power-On Diagnostic Tests.....	167
7.1.2 Operating Errors	168
7.2 Diagnostics	170
Chapter 8	
Troubleshooting	171
8.1 General Troubleshooting Hints.....	172
8.2 Hardware Troubleshooting	173
Appendix A	
Specifications	175
Appendix B	
Spare Parts and Accessories	178
B.1 Using the Optional Cuvette Cell.....	179
B.2 Using the Optional Optical Filters	181
2.3 Optional Photomultiplier Tubes	185

Appendix C
Warranty Information 187
 C.1 Limited Express Warranty..... 187
 C.2 Shipments, Damages, Claims, Returns..... 191

Index 192

List of Figures

1-1	Waters 474 Scanning Fluorescence Detector	22
1-2	The 474 Detector Optics Bench.....	29
1-3	Block Diagram of the 474 Detector.....	31
2-1	Voltage Selection Switch and Fuse Holders	35
2-2	Fluid Connections	37
2-3	Ferrule and Compression Screw Assembly.....	38
2-4	Rear Panel Electrical Connectors.....	39
2-5	Analog Connector	41
2-6	Bus SAT/IN Module (Front Panel).....	44
2-7	Bus SAT/IN to Bus LAC/E Connections.....	45
2-8	Bus SAT/IN Module Connections.....	46
2-9	Waters 2690 Separations Module Auto Zero Connections.....	49
2-10	Waters 2690 Separations Module Chart Mark Connections.....	50
2-11	Waters 2690 Separations Module Method Start Connections.....	51
2-12	Waters 2690 Separations Module Cell Leak Connections.....	52
2-13	Waters 600 Series Controller Auto Zero Connections	54
2-14	Waters 600 Series Controller Chart Mark Connections.....	55
2-15	Waters 600 Series Controller Connections.....	56
2-16	Waters 600 Series Controller Cell Leak Connections.....	57
2-17	Waters 745/745B/746 Connections	58
2-18	Chart Recorder Connections	60
2-19	Waters 717plus Auto Zero Connections	61
2-20	Waters Carbamate System Chart Mark Connections.....	63
2-21	Waters Carbamate System Inject Start Connections	64
2-22	AccQ•Tag System Chart Mark Connections	66
2-23	Waters AccQ•Tag System Connections.....	67

3-1	474 Detector Front Panel	70
3-2	Typical Display in Normal Mode	71
3-3	Keypad Layout	73
3-4	Typical Display in the Normal Mode.....	78
3-5	Typical Energy Output Display	83
3-6	Lamp Off Timer Selection Screen.....	84
3-7	Display Screen After Lamp Timer Has Elapsed	84
3-8	Filter Selection Screen.....	85
3-9	Effect of Different Settings in the RC Filter Mode	86
3-10	Effect of Different Settings in the Digital Filter Mode	87
3-11	Auto Zero Selection Screen	89
3-12	Offset Selection Screen	90
3-13	Effect of Changing the Polarity Parameter	91
3-14	Polarity Selection Screen.....	91
3-15	Lamp Hours Display Screen	92
3-16	Bandwidth Selection Screen.....	93
3-17	Wavelength Range Selection Screen	94
3-18	Temperature Correction Display Screen	95
3-19	Error Message for a Cell Leak	97
3-20	Entering the Emission Spectrum Memory Location	99
3-21	Entering the Excitation Wavelength for an Emission Spectrum	99
3-22	Prompt to Begin an Emission Spectrum Scan.....	99
3-23	Starting an Emission Spectrum Output	100
3-24	Entering the Emission Spectrum Memory Location	100
3-25	Entering the Start and End Wavelengths in the Emission Spectrum	100
3-26	Prompt to Start Output of a Spectrum	101
3-27	Typical Raman Band Test Results	102
3-28	Measuring the Maximum Pen Deflection	103
4-1	Typical Time Screen in the Program Mode	106
4-2	Typical Fluorescence Intensity Screen in the Program Mode	106

4-3	Flowchart for Writing a Time Program	108
4-4	Typical Initial Parameter Settings	109
4-5	First Step of a New Time Program.....	111
4-6	Typical First Step of an Existing Time Program	111
4-7	Typical Empty Program Step.....	112
4-8	First Line of the Program Menu	112
4-9	Initial Conditions of the Example Time Program.....	115
4-10	Step 1 of the Example Time Program.....	116
4-11	Step 2 of the Example Time Program.....	117
4-12	Step 3 of the Example Time Program.....	118
4-13	Step 4 of the Example Time Program.....	119
4-14	Fluorescence Intensity Screen at the End of a Program Run.....	120
4-15	Editing the Parameters in a Time Program.....	122
4-16	Typical Spectrum Output by the 474 Detector	125
4-17	Conditions for Storing a Spectrum.....	126
4-18	Typical Values for Storing an Emission Spectrum.....	127
5-1	Initial Display in the Scan Mode.....	129
5-2	Selecting the Scan Parameters	131
5-3	Entering the Scan Parameters.....	131
5-4	Entering the Emission Spectrum Memory Location	133
5-5	Entering the Excitation Wavelength for an Emission Spectrum ...	134
5-6	Prompt to Begin an Emission Spectrum Scan.....	134
5-7	Starting an Excitation Spectrum Scan	135
5-8	Entering the Excitation Spectrum Memory Location	135
5-9	Entering the Emission Wavelength for an Excitation Spectrum ...	135
5-10	Prompt to Begin an Excitation Spectrum Scan.....	136
5-11	Starting an Emission Spectrum Output	137
5-12	Entering the Emission Spectrum Memory Location	138
5-13	Entering the Start and End Wavelengths in the Emission Spectrum	138
5-14	Prompt to Start Output of a Spectrum	139

5-15	Typical Display During Output of a Spectrum	139
5-16	Starting an Excitation Spectrum Output	140
5-17	Entering the Excitation Spectrum Memory Location	140
5-18	Entering the Start and End Wavelengths in the Excitation Spectrum	140
5-19	Prompt to Start Output of a Spectrum	141
5-20	Typical Display During Output of a Spectrum	141
5-21	Starting an Emission Difference Spectrum Output	143
5-22	Entering the Memory Locations for an Emission Difference Spectrum	143
5-23	Incorrect Wavelengths for a Difference Spectrum	144
5-24	Entering the Start and End Wavelengths for an Emission Difference Spectrum	144
5-25	Prompt to Begin an Emission Difference Spectrum.....	145
5-26	Typical Display During Output of an Emission Difference Spectrum	145
5-27	Starting an Excitation Difference Spectrum Output	146
5-28	Entering the Memory Locations for an Excitation Difference Spectrum	146
5-29	Incorrect Wavelengths for a Difference Spectrum	147
5-30	Entering the Start and End Wavelengths for an Excitation Difference Spectrum	147
5-31	Prompt to Begin an Excitation Difference Spectrum.....	148
5-32	Typical Display During Output of an Excitation Difference Spectrum	148
6-1	Removing the Flow Cell Cassette.....	154
6-2	Flow Cell Cassette.....	155
6-3	Exploded View of Flow Cell Parts.....	156
6-4	Position of Cell Mask	158
6-5	Accessing the Lamp Housing	161
6-6	Moving the Printed Circuit Board Panel.....	162
6-7	Removing the Screws That Secure the Fan Access Door.....	162

6-8	Removing the Securing Screws on the Lamp Housing	163
6-9	Horizontal Adjustment of the Lamp.....	165
7-1	Error Message for Fluid Leak	169
7-2	Auto Zero Error Message	169
B-1	Installing the Optional Cuvette Cell Cassette	180
B-2	Characteristics of Excitation Filters.....	182
B-3	Characteristics of Emission Filters.....	183
B-4	Installing Optical Filters on the Flow Cell.....	184
B-5	Sensitivity of Optional Photomultiplier Tubes.....	186

List of Tables

2-1	Installation Site Requirements	32
2-2	Voltage and Fuse Selection.....	33
2-3	Analog Connector Terminal Descriptions.....	42
3-1	Display Parameters	71
3-2	Key Descriptions	74
3-3	Parameters, Ranges, and Keys	78
3-4	Filter Modes and Response Settings	80
3-5	Special Parameter Menu	81
3-6	Effects of Manual and Automatic Auto Zero Settings	88
4-1	Program Menu	113
4-2	Example Program	114
5-1	Scan Menu	129
5-2	Scan Parameters	130
6-1	AC Fuses	166
7-1	Power-On Diagnostic Tests	168
7-2	Possible Causes of the Auto Zero Error Message.....	170
8-1	General System Troubleshooting	173
A-1	Operational Specifications	175
B-1	Spare Parts and Accessories	178
B-2	Optional Optical Filter Characteristics	181
B-3	Photomultiplier Tube Type Numbers	185
C-1	Waters 474 Scanning Fluorescence Detector Warranty Periods	190

How to Use This Guide

Purpose of This Guide

The *Waters 474 Scanning Fluorescence Detector Operator's Guide* details the procedures for unpacking, installing, operating, maintaining, and troubleshooting the 474. It also includes appendixes describing specifications, spare parts and accessories, and warranty information.

Audience

This guide is intended for use by personnel who need to install, operate, maintain, or troubleshoot the 474 detector.

This guide assumes that you understand the principles of chromatography.

Structure of This Guide

The *Waters 474 Scanning Fluorescence Detector Operator's Guide* is divided into eight chapters. Each chapter page is marked with a chapter-specific tab and a footer to help you quickly find information. Each appendix page is identified in a similar manner.

The following table describes the material covered in each chapter.

Chapter/Appendix	Content
Chapter 1, Introduction and Theory of Operation	Presents a description of the 474 Scanning Fluorescence Detector, including the theory of operation and its features.
Chapter 2, Installing the 474 Detector	Describes how to install the 474 detector, including making all electrical and fluidic connections.
Chapter 3, Using the 474 Detector	Provides the power-on and power-off procedures, and describes how to set and change operating parameters.
Chapter 4, Programming the 474 Detector	Describes how to write a time program.
Chapter 5, Using the Scan Mode	Describes how to make spectral scans and perform spectral manipulation.

Chapter/Appendix	Content
Chapter 6. Maintenance Procedures	Covers routine maintenance procedures.
Chapter 7. Error Messages and Diagnostics	Explains 474 detector error messages and start-up diagnostics.
Chapter 8. Troubleshooting	Describes troubleshooting procedures for the 474 detector, including troubleshooting decision trees and symptom-cause-solution tables.
Appendix A. Specifications	Describes the specifications of the 474 detector.
Appendix B. Spare Parts and Accessories	Lists recommended and optional spare parts and procedures for installing options.
Appendix C. Warranty Information	Provides warranty and service information.

Related Adobe™ Acrobat Reader Documentation

For detailed information about using the Adobe Acrobat Reader, refer to the *Adobe Acrobat Reader Online Guide*. This Online Guide covers procedures such as viewing, navigating and printing electronic documentation from Adobe Acrobat Reader.

Printing From This Electronic Document

Adobe Acrobat Reader lets you easily print pages, pages ranges, or the entire electronic document by selecting **Print** from the File menu. For optimum print quantity, Waters recommends that you specify a Postscript printer driver for your printer. Ideally, use a printer that supports 600 dpi print resolution.

Conventions Used in This Guide

This guide uses the following conventions to make text easier to understand.

- **Purple Text** indicates user action. For example:
Press **0**, then press **Enter** for the remaining fields.
- *Italic* text denotes new or important words, and is also used for emphasis. For example:
An instrument method tells the software how to acquire data.

- [Underlined, Blue Color](#) text indicates hypertext cross-references to a specific chapter, section, subsection, or sidehead. Clicking this topic using the hand symbol automatically brings you to this topic within the electronic document. Right-clicking and selecting **Go Back** from the popup context menu brings you back to the originating topic. For example:

Remove the standard flow cell cassette as described in [Section 6.4.1. Removing the Flow Cell Cassette](#).

Notes, Attentions, and Cautions

- Notes call out information that is important to the operator. For example:

Note: *Record your results before you proceed to the next step.*

- Attentions provide information about preventing possible damage to the system or equipment. For example:



Attention: *To avoid damaging the detector flow cell, do not touch the flow cell window.*

- Cautions provide information essential to the safety of the operator. For example:



Caution: *To avoid chemical or electrical hazards, always observe safe laboratory practices when operating the system.*



Caution: *To avoid the possibility of electrical shock and possible injury, always turn off the detector and unplug the power cord before performing maintenance procedures.*



Caution: *To avoid the possibility of burns, turn off the lamp at least 30 minutes before removing it for replacement or adjustment.*

1 Introduction and Theory of Operation

This chapter introduces you to the Waters® 474 Scanning Fluorescence Detector. It provides a summary of the features and a brief description of the theory and principle of operation.

1.1 Introduction

The Waters 474 Scanning Fluorescence Detector ([Figure 1-1](#)) is a high-performance unit that provides a high level of sensitivity for spectrofluorometric analysis.

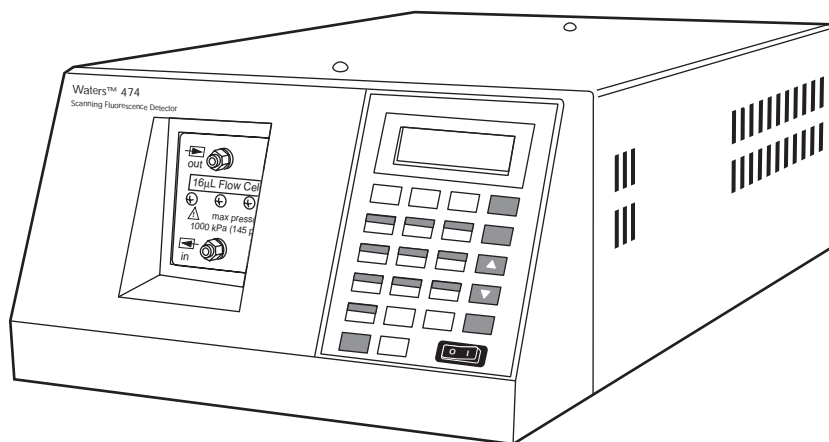


Figure 1-1 Waters 474 Scanning Fluorescence Detector

1.2 Waters 474 Detector Features

The Waters 474 Scanning Fluorescence Detector includes:

- An optical design that maximizes sensitivity
- Microprocessor control for greater versatility and reliability
- An emission wavelength sweep function so you can obtain an emission spectrum
- An excitation wavelength sweep function so you can obtain an excitation spectrum
- An automatic second-order filter to enhance applications in the visible wavelength range
- Optional optical filters that can be installed on the flow cell for specialized measurements
- Quartz flow cell or cuvette cell, permitting measurement of ultraviolet fluorescence
- Nine-stage photomultiplier tube for amplifying the emission radiation
- Photodetector to compensate for excitation fluctuations throughout a scan
- An RS-232 port to allow connectivity to the Millennium³² data system

There are three critical optical design features in the 474 detector:

- A highly energy-efficient optics system that does not use lenses, minimizing reflection loss
- An optics system that uses holographic concave diffraction gratings and aspherical mirrors to provide highly efficient monochromators in a compact package
- A 150 W Xenon lamp that provides greater light energy for excitation

There are five critical microprocessor-based features:

- Operating parameters that are set using the keypad
- A multi-line liquid crystal display (LCD) that provides information on the status of the 474 detector
- Self-diagnostic messages that are displayed on the LCD
- Digital processing that controls the entire signal processing system, assuring higher reliability and operational stability
- An auto-zero feature that provides a wide range of offset adjustment for the analog output

1.3 Fluorescence Theory of Operation

This section describes the general theory of operation of fluorescence detection. The process of fluorescence detection involves several components and processes:

- An excitation source
- Filtering the source light
- Exciting the sample with filtered light
- Collecting and filtering the emitted fluorescence
- Measuring the emitted fluorescence
- Amplifying the emitted signal

Overview

When certain molecules or atoms are exposed to an energy source such as high-intensity light, the molecules or atoms absorb the light energy and enter into an excited state. As a molecule or atom moves from this excited state back to its normal state, some of the absorbed energy is released in the form of a photon. This process is called fluorescence.

Fluorescence is a type of luminescence. The atoms and molecules in different elements and compounds emit different levels of fluorescence when exposed to the same energy levels.

The scanning fluorescence detector illuminates a sample with a narrow band of high-intensity light from a carefully controlled light source. The detector then measures the low levels of fluorescence emitted from the sample. The emitted light is filtered, amplified, and converted to electrical signals that can be recorded and analyzed.

Applications

Fluorescence detectors are widely used for detecting:

- Trace metals
- Traces of organic material
- Trace elements in biological systems

1.3.1 Excitation Sources

The typical energy source used for fluorescence detection is a lamp that provides an intense, stable spectrum of light in the UV and visible range. The fluorescence intensity is directly related to the intensity of the excitation spectrum, so high-sensitivity detectors use the most intense excitation source available.

Types of Light Sources

Common excitation light sources include the following:

- Vapor lamps: Mercury, Cadmium, or Zinc
- Arc lamps: Deuterium or Xenon

Of these light sources, the vapor lamps provide high-intensity, narrow-band outputs in the UV and visible ranges. The arc lamps provide a wider, lower intensity spectrum.

Among the arc lamps, the Xenon lamp provides the widest spectrum, making it the excitation source of choice for general-purpose fluorescence detectors.

1.3.2 Filtering

You select the desired excitation wavelength using some form of filtering. Two common methods of filtering the source light are typically used in modern detectors:

- Optical filters
- Monochromators

Optical Filters

Optical filters are generally low in cost and simple to use. They transmit a wide range of wavelengths, and, therefore, do not provide the degree of selectivity that a monochromator does.

Short-wavelength cut-off filters transmit light at wavelengths shorter than their rated value. Long-wavelength cut-off filters transmit wavelengths longer than their rated value. The rated value of a filter is defined as the wavelength at which 50 percent of the peak transmission occurs. This value is fixed; to change the excitation wavelength when you are using filters, the filter must be changed.

Monochromators

A monochromator is a device that can be adjusted to select wavelengths over a wide range of the spectrum. A grating monochromator uses a diffraction grating that at any one setting, passes only a small range, or bandwidth of wavelengths. By moving the grating, you can select wavelengths within the desired range of wavelengths. Relative to filters, the grating provides the following benefits:

- A wider range of wavelengths can be selected
- A smaller bandpass of wavelengths (greater selectivity)
- Scans of the spectrum

A grating monochromator also passes fractions, or orders, of the selected wavelength. For example, if the monochromator is set to pass light energy at 600 nm, it also passes energy at the second-order wavelength of 300 nm. A long-pass filter can be used to absorb the higher-order energy produced by a monochromator.

Just as the excitation, or source energy, is selected by a monochromator, the emission, or radiated energy, can be selected. This permits precise selection of emission wavelengths. Detectors with excitation and emission monochromators can perform scans by holding one monochromator at a constant setting while varying the setting on the other. This type of operation is necessary when you are evaluating mixtures or analyzing the chemical structure of a substance.

1.3.3 Exciting the Sample

The broad band of high-intensity light from the lamp passes through a filter or monochromator, which selects a narrow band of wavelengths. This narrow band of light is directed onto the flow cell (or the optional cuvette cell) where it excites the analytes as they pass through the cell.

Flow Cell

The flow cell is a glass or quartz cell designed to minimize the amount of stray light that could affect the measurement, and to maximize the fluorescence signal. The sample compartment is arranged so that the fluorescence energy is collected at an angle perpendicular to the excitation (lamp) beam.

1.3.4 Measuring Fluorescence

To measure the fluorescence from the flow cell, the detector must balance the need for high selectivity (to distinguish narrow fluorescence peaks) with the need for high sensitivity (to measure low fluorescence intensities).

As you increase the selectivity, you decrease the bandpass, or range of fluorescence wavelengths that pass through the filter. The narrower the filter band, the less light of fluorescence passes through it, and therefore, the less sensitive the measurement. If higher sensitivity is needed, more fluorescence must pass through the filter, that is, the bandpass must increase. This correspondingly decreases the ability of the filter to detect narrow fluorescence peaks.

Emission Monochromator

An emission monochromator provides the best means of balancing between sensitivity and selectivity. If greater selectivity is desired and the fluorescence level is sufficiently high, some detectors allow you to select a narrower slit. This can be useful when you need to eliminate or reduce the effects of coeluting compounds or impurities.

Photomultiplier Tube (PMT)

The fluorescence intensity is typically very low, especially if you are dealing with low levels of analyte. A sensitive photomultiplier tube (PMT) converts the fluorescence energy (in the UV, visible, or IR ranges) to an electric current. As its name implies, the PMT amplifies the low-level fluorescence signal.

1.3.5 Scanning

Detectors equipped with excitation and emission monochromators can easily scan a range of excitation or emission wavelengths. Changing the wavelength involves changing the monochromator setting. During a scan, the setting on one monochromator is held constant while the other monochromator scans a range of wavelengths.

As the excitation wavelength changes in a scan, the intensity from the source lamp tends to fluctuate. To compensate for these fluctuations, a beam splitter diverts a portion of the excitation energy to a photodetector. The photodetector monitors the fluctuations caused by changes in the excitation wavelength and provides a reference signal to the PMT. The 474 detector compensates for the changes in the fluorescence intensity caused by the fluctuations in excitation energy by using the ratio of the fluorescence and reference signals.

1.3.6 References

Refer to the following texts for additional information on fluorescence detection:

N. Ichinose, G. Schwedt, F.M. Schnepel, and K. Adachi, *Fluorometric Analysis in Biomedical Chemistry*, Chapter 5, Wiley-Interscience: New York, 1991.

Detectors for Liquid Chromatography, E.S. Yeung, ed., Chapter 5, Wiley: New York, 1986.

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S.G. Schulman, *Fluorescence and Phosphorescence Spectroscopy: Physicochemical Principles and Practice*, Pergamon Press: New York, 1977.

J.D. Winefordner, S.G. Schulman, and T.C. O'Haver, *Luminescence Spectroscopy in Analytical Chemistry*, Wiley-Interscience: New York, 1972.

1.4 Principle of Operation

The Waters 474 Scanning Fluorescence Detector consist of two basic internal systems:

- An optical system, often called the *optics bench*
- A control system

1.4.1 Optics Bench

The optical bench consists of the following components:

- Light source
- Excitation monochromator
- Beam splitter
- Flow cell
- Emission collector optics
- Emission monochromator
- Photomultiplier tube

[Figure 1-2](#) shows these components and the light path through the 474 detector.

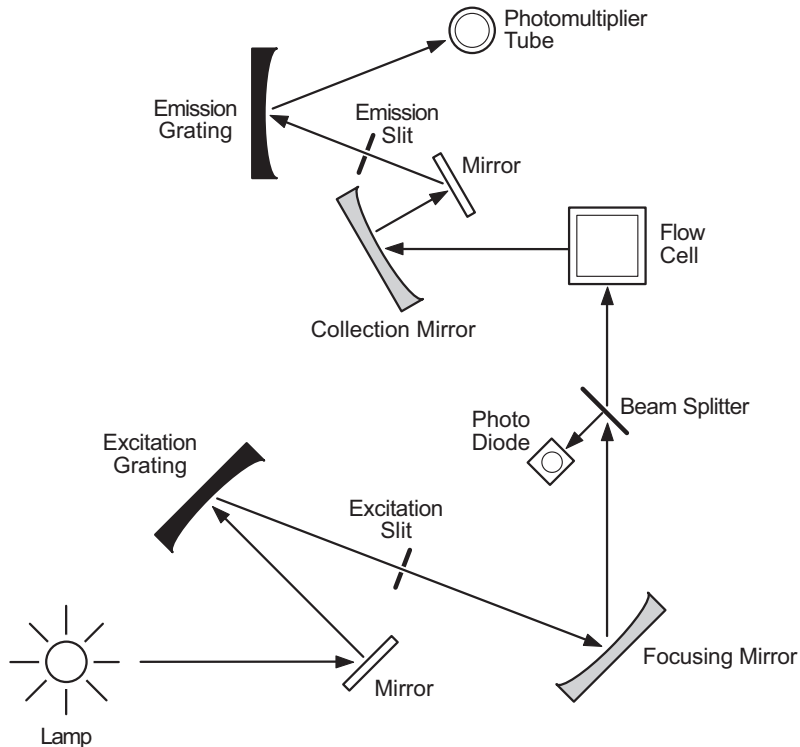


Figure 1-2 The 474 Detector Optics Bench

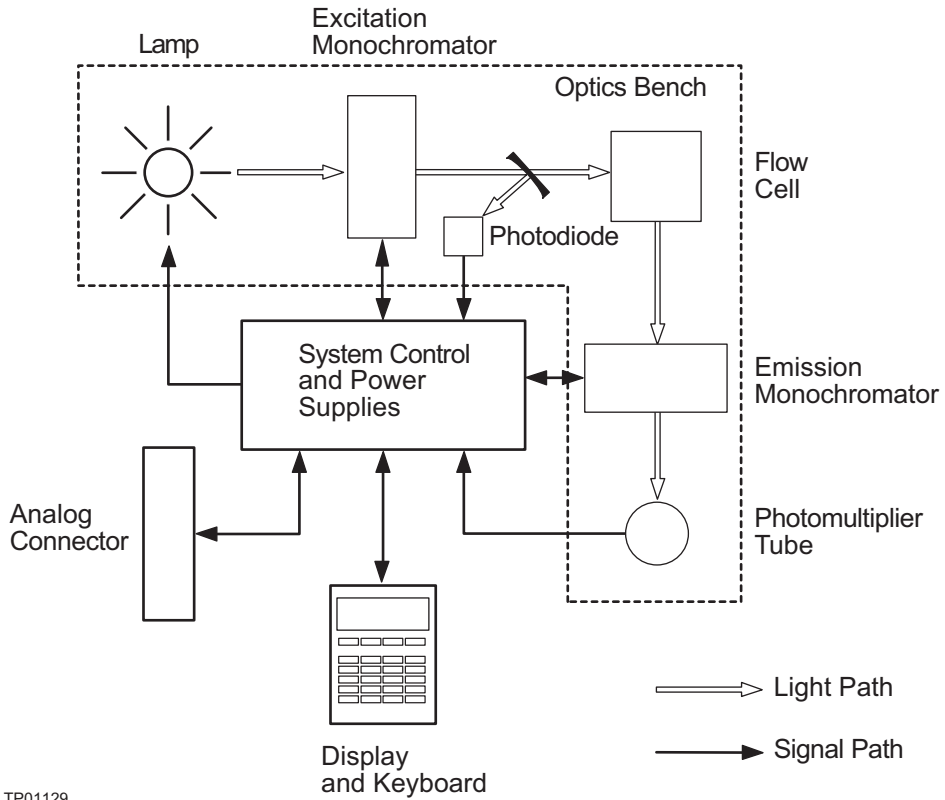
The optics bench operates as follows:

1. Energy from the light source is concentrated and directed toward the entrance slit of the excitation monochromator by an elliptical mirror.
2. The excitation monochromator diffracts the broadband source light and projects it onto a second slit to obtain the excitation spectrum. This spectrum is directed to the flow cell by the illumination optics.
3. A beam splitter diverts part of the excitation beam toward a photodiode, which converts the light to an electrical signal. This signal is used as a reference to compensate for intensity fluctuations during operation.
4. Light emitted by the sample in the flow cell is collected by the emission collector optics and is focused on the entrance slit of the emission monochromator. This diffracted emission spectrum is directed toward the photomultiplier tube, where it is amplified and converted into an electrical signal.

1.4.2 Control System

The control system consists of a microprocessor and drivers for controlling the components of the detector ([Figure 1-3](#)). The control system operates as follows:

1. Optical signals from the excitation and emission monochromators are converted into electrical signals by a photodiode and the photomultiplier tube, respectively. The electrical signals are then amplified by a preamplifier and converted to digital signals by an analog-to-digital (A/D) converter.
2. The microprocessor calculates the ratio of the two electrical signals to compensate for fluctuations in the intensity of the light source. The microprocessor then sends the resultant corrected signals to a digital-to-analog (D/A) converter for output on the analog connector.
3. The microprocessor receives and executes commands entered from the keypad or from the time program to control the operational parameters of the 474 detector, including the positions of the excitation and emission monochromators.



TP01129

Figure 1-3 Block Diagram of the 474 Detector

2

Installing the 474 Detector

The Waters 474 Scanning Fluorescence Detector operates in any standard laboratory environment. The detector requires connection to electrical power and to sample and waste fluid lines.

This chapter describes how to install the 474 and connect it to the electrical and fluid supplies and to other equipment.



2.1 Site Selection and Power Requirements

Install the 474 at a site that meets the requirements listed in [Table 2-1](#).

Table 2-1 Installation Site Requirements

Parameter	Requirement
Ambient temperature	10 to 32 °C (50 to 90 °F)
Relative humidity	20 to 80%, noncondensing
Bench space	5 inches (12.7 cm) clearance at rear 2 inches (5 cm) clearance on the sides
Vibration	Negligible
Static electricity	Negligible

Note: Be sure to provide at least 2 inches (5 cm) clearance for the vent on the side panel.

To prevent instability, make sure that the four rubber feet are secure on the bench top.

Power Requirements

The Waters 474 requires:

- One properly grounded AC outlet
- No nearby source of electronic noise
- Correct amperage fuse for your AC voltage

[Table 2-2](#) lists the fuse requirements for the two operating voltages.

Table 2-2 Voltage and Fuse Selection

Nominal Voltage (VAC)	Voltage Range (VAC)	Fuse Required
115	90 to 132	5 A, fast-blow
230	187 to 264	2.5 A, fast-blow



Caution: To avoid electric shock, power down the 474 and unplug the power cord before you replace fuses.



Warning: To reduce the risk of fire hazard, always replace fuses with the same type and rating.

2.2 Unpacking and Inspection

The 474 is packed and shipped in one carton that contains the following items:

- Waters 474 Scanning Fluorescence Detector
- Start-up kit
- Validation certificate
- Operator's guide
- Packing list

Unpacking

To unpack the 474:

1. Locate the packing list.
2. Unpack the contents of the shipping carton. As you unpack the carton, check the contents against the list to make sure you received all items.
3. Check the contents of the start-up kit against the list enclosed with the kit.
4. Save the shipping carton for future transport or shipment.

Inspection

If you see any damage or discrepancy when you inspect the contents of the carton, immediately contact the shipping agent and Waters Technical Service at (800) 252-4752, U.S. and Canadian customers only. Other customers, call your local Waters subsidiary or your local Waters Technical Service Representative, or call Waters corporate headquarters for assistance at (508) 478-2000 (U.S.).

Note: *Make sure the serial number on the detector nameplate located on the side panel matches the number on the validation certificate.*

For more information about the instrument warranty and serial number, refer to [Appendix C, Warranty Information](#).

2.3 Voltage Selection and Fuse Installation

The voltage selection switch and fuse holders are located on the rear panel of the detector ([Figure 2-1](#)).

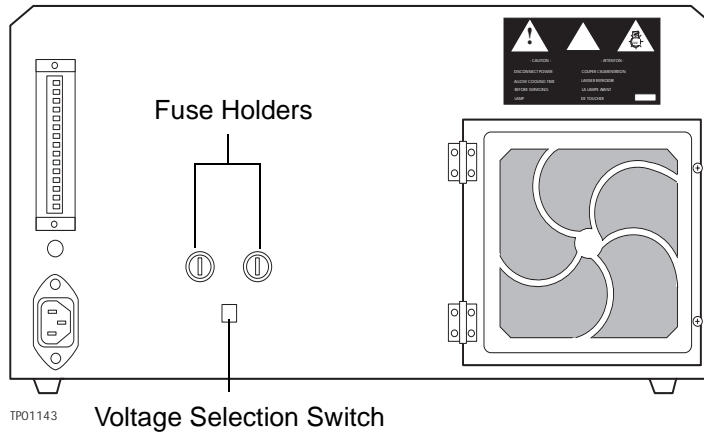


Figure 2-1 Voltage Selection Switch and Fuse Holders

Checking the Voltage Selector

Make sure that the voltage selection switch is properly set for your requirements.



Caution: To avoid electric shock, turn off the Power switch on the front panel, and disconnect the power cord before you change the voltage selector switch position or change a fuse.

With the rear panel of the detector facing you, check that the voltage selection switch is:

- In the up (115 V) position for 100 to 120 VAC power
- In the down (230 V) position for 220 to 240 VAC power

If the switch is in the wrong position, use a small, flat-blade screwdriver to move it to the correct position.

Installing Fuses

To install the fuses:

1. Use a small, flat-blade screwdriver to press in on the fuse holder and turn the fuse holder counterclockwise one-quarter turn. The spring-loaded fuse holder pops out when you withdraw the screwdriver.
2. Insert the fuse into the fuse holder. Make sure that the fuse is properly rated for your requirements. Refer to [Table 2-2](#) to determine your fuse requirements.
3. Insert the fuse holder into the receptacle. Press in with the screwdriver and turn the holder until it is securely in place.
4. Repeat steps 1, 2, and 3 for the second fuse holder.

2.4 Making Fluid Connections

Before the initial start-up you must complete:

- The fluid connections described in this section.
- The electrical connections described in [Section 2.5, Making Electrical Power Connections](#).

If you are using more than one detector in series in your HPLC system, Waters strongly recommends that you install the 474 as the last detector in the series. This avoids the higher pressures that can occur on the upstream side of the fluid pathway.



Attention: *The pressure rating of the 474 flow cell is approximately 145 psi for the 16 μ L cell (500 psi for the 5 μ L cell). If this pressure is exceeded, the flow cell could be damaged. Do not connect any tubing or device that might cause backpressure to build.*

The fluid connections to the 474 are located in the center of the front panel ([Figure 2-2](#)). To make the inlet and outlet tubing connections, you need to:

- Cut the tubing
- Attach the inlet and outlet compression fittings
- Connect the tubing to the detector

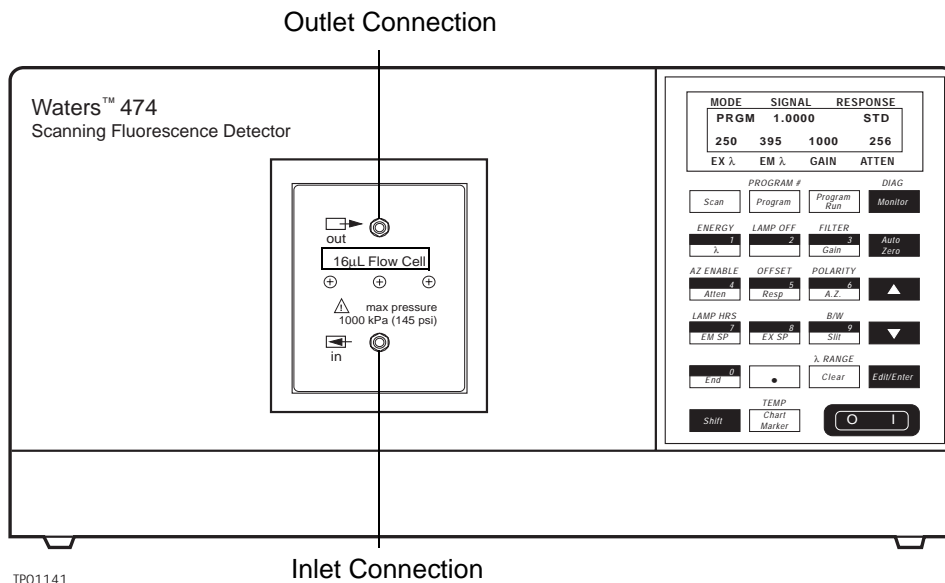


Figure 2-2 Fluid Connections

Cutting Tubing

You need the following tools to cut the tubing:

- File with cutting edge
- Two cloth- or plastic-covered pliers

To cut the tubing:

1. Measure the length of tubing (1/16-inch O.D. stainless steel) you need to make the following connections:
 - Column to the detector inlet
 - Detector outlet to a suitable waste container
2. Use a file with a cutting edge to scribe the circumference of the tubing at the desired length.
3. Grasp the tubing on both sides of the scribe mark with cloth-covered pliers. Gently work the tubing back and forth until it separates.
4. File the ends smooth.

Assembling Fittings

To assemble each fitting:

1. Slide the compression screw over the tubing end, followed by the ferrule ([Figure 2-3](#)).
2. Mount the ferrule with its taper end facing the end of the tubing ([Figure 2-3](#)).

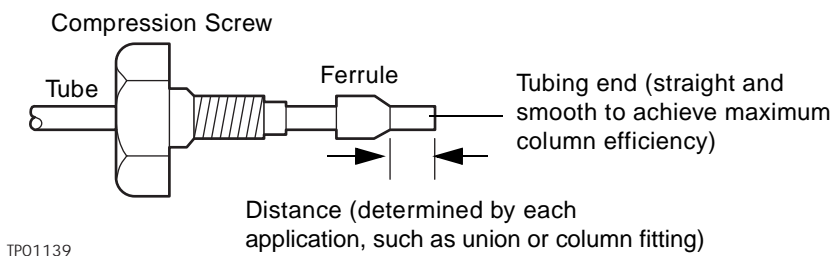


Figure 2-3 Ferrule and Compression Screw Assembly

Making Connections

Make connections at the column outlet and detector inlet, and at the detector outlet by performing the following steps:

1. Seat each tubing end in the appropriate fitting.
2. Seat each ferrule by tightening the compression screw 3/4 turn past finger-tight.

Flushing the System

Since the Waters 474 is shipped dry, you must pump solvent through the unit before initial startup.

Flush the lines with filtered HPLC-grade methanol. Then pump your mobile phase, provided that there are no miscibility problems.

2.5 Making Electrical Power Connections

The two electrical connectors on the rear panel of the 474 are:

- The AC input connector that provides the unit with the operating voltage
- The analog connector that provides status information and data to auxiliary equipment

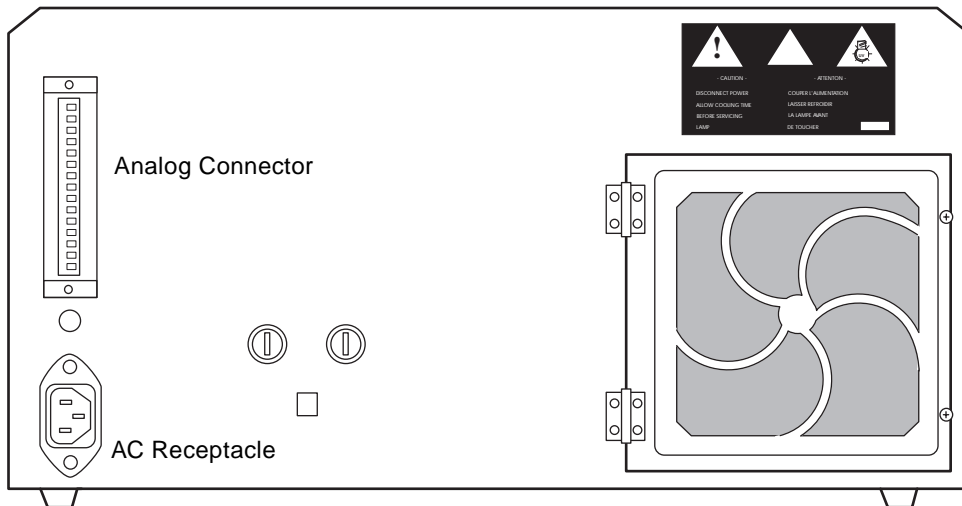
2.5.1 Making AC Power Connections



Attention: Be sure that the voltage selection switch on the rear panel is set correctly and the correct fuses are installed before applying AC power. Refer to [Section 2.3. Voltage Selection and Fuse Installation](#), for details.

To connect the 474 to the AC power supply:

1. Plug the receptacle end of the power cord into the AC input connector on the rear panel of the detector ([Figure 2-4](#)).
2. Plug the other end of the power cord into a properly grounded AC power source.



TPO1143

Figure 2-4 Rear Panel Electrical Connectors

2.6 Making Connections to Other Devices

You can connect the 474 to a wide range of HPLC system devices. This section describes how to connect the 474 to a:

- Millennium Data System
- Waters 2690 Separations Module
- Waters 600 Series Controller
- Waters 745/745B/746 Data Module
- Chart Recorder
- Waters 717plus Autosampler
- Waters Carbamate System
- Waters AccQ•Tag System
- Voltmeter

Required Materials

When connecting cables to the analog connector terminals, you need the following tools:

- Small flat-blade screwdriver
- Electrical insulation stripping tool

Cable Connecting Procedure

To connect cables from other HPLC system devices to the analog connector on the rear panel:

1. Strip 1/4 inch (5 mm) of insulation from the end of each wire.
2. Insert the bare wire into the appropriate terminal and secure it by tightening the screw.
3. Test the connection by gently pulling on the wire. The connection is secure if you cannot pull the wire out with a gentle tug.

2.6.1 Analog Connector Overview

The 474 is usually installed as an integral part of a data collection system. Although the 474 can be controlled manually via the keypad on the front panel, several functions can be controlled externally through connections to the analog connector on the rear panel ([Figure 2-4](#)). These functions are described in [Section 3.6, Using External Functions](#).



Attention: To meet the regulatory requirements of immunity from external electrical disturbances that may affect the performance of this instrument, do not use cables longer than 9.8 feet (3 meters) when you make connections to the analog connector. Waters recommends a maximum cable length of 6.5 feet (2 meters). In addition, ensure that you always connect the shield of the cable to chassis ground at one instrument only.

The analog connector is a 15-pin terminal strip on the rear panel ([Figure 2-5](#)). This connector is used for analog data and signal output to other instruments, and analog signal input from other instruments. [Figure 2-5](#) shows the pinout of this connector, and [Table 2-3](#) describes the function of each terminal. Refer to [Section 2.6. Making Connections to Other Devices](#), for information on connecting other instruments to the 474.

2

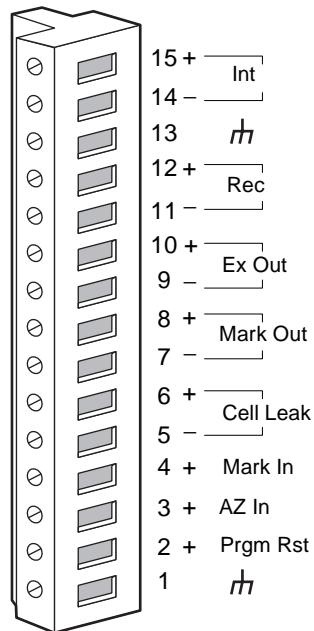


Figure 2-5 Analog Connector

[Table 2-3](#) describes the terminals on the analog connector.

Table 2-3 Analog Connector Terminal Descriptions

Terminal	Function	Reference
Int (+ and –)	Integrator output terminals. A 1 V full-scale unattenuated analog output signal appears on these terminals. This signal is unaffected by the setting in the Attenuation parameter.	“Using Gain and Attenuation” in Section 3.4.5. Attenuation
Rec (+ and –)	Recorder output terminals. A 10 mV full-scale analog output signal appears on these terminals. The signal range is determined by the settings in the Gain and Attenuation parameters.	“Using Gain and Attenuation” in Section 3.4.5. Attenuation
Ex Out (+ and –)	Excitation out output terminals. A 1 V full-scale analog signal that corresponds to the photodiode voltage. Use this signal to monitor the excitation energy during scans.	Section 3.6.1. Excitation Energy Out
Mark Out (+ and –)	Marker output terminals. A momentary 1 to 3 mV marker signal appears on these terminals when either of the following occurs: <ul style="list-style-type: none"> • The Chart Mark key on the keypad is pressed • A contact closure signal occurs between the Mark In terminal and the (–) terminal 	Section 3.6.2. Mark Out
Cell Leak (+ and –)	Output terminals to signal a fluid leak. When these contacts close, a leak has occurred inside the flow cell of the detector.	Section 3.6.3. Cell Leak
Mark In (+)	Mark input terminal. A chart mark is added to the recorder output when a contact closure signal appears between this terminal and the (–) terminal.	Section 3.6.4. Mark In
AZ In (+)	Auto Zero input terminal. The voltages on the Rec (+ and –) and Int (+ and –) terminals are set to zero when a contact closure signal appears between this terminal and the (–) terminal.	Section 3.6.5. Auto Zero In

Table 2-3 Analog Connector Terminal Descriptions (Continued)

Terminal	Function	Reference
Prgm Rst (+)	Time program input terminal. When a contact closure signal appears between this terminal and the (–) terminal, the currently selected program starts immediately. If a time program is running, the program stops, the initial conditions are reset, and the program starts again	Section 3.6.6. Program Reset

2

2.6.2 Connecting the 474 to the Millennium Data System

The Millennium® Data System is a single or multi-system computer that performs data acquisition, processing, and management of chromatographic information.

The Millennium Data System includes a menu-driven software program specifically designed by Waters for chromatographers. Use the Millennium Data System to:

- Acquire data
- Process data
- Generate and print reports
- Store information (or data) in a central area and share this information with users who have proper security access

The connections between the 474 detector and the Millennium Data System vary depending on your version of the Millennium Data System.

2.6.2.1 Millennium Data System Version 3.05.01 or Lower

Millennium software version 3.05.01 requires that the detector analog signal be converted to a digital representation. Data acquisition and control with the Millennium Data System version 3.05.01 and lower requires connections between the Waters Bus SAT/IN module and the Laboratory Acquisition and Control/Environment (LAC/E) card.

Bus SAT/IN Module

The Waters Bus SAT/IN module ([Figure 2-6](#)) converts the analog signals from devices such as the 474 into digital form. It then transmits these digital signals to the Bus LAC/E card inside the Millennium Data System computer.

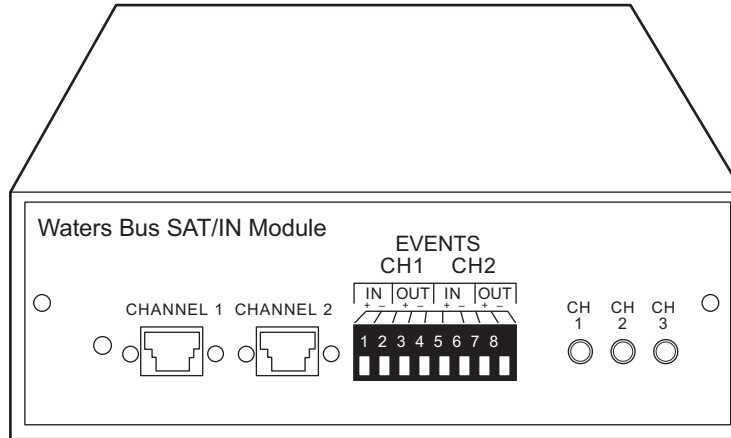


Figure 2-6 Bus SAT/IN Module (Front Panel)

To connect the 474 to the Millennium Data System:

1. Connect the Bus SAT/IN module to the Bus LAC/E card in the Millennium Data System computer.
2. Connect the 474 to the Bus SAT/IN Module.



Attention: Do not power up the Bus SAT/IN module until you perform all procedures described in the Waters Bus SAT/IN Module Installation Guide. Improper powerup can damage the unit and void the warranty.



Attention: To avoid damaging the Bus SAT/IN module, always disconnect the power cord at either the wall outlet or the power supply before attaching or removing the power connection to the Bus SAT/IN module. The Bus SAT/IN module does not have a power switch.

Connecting the Bus SAT/IN Module to the Bus LAC/E Card

The Bus SAT/IN module connects to the Bus LAC/E card through an I/O Distribution Box ([Figure 2-7](#)).

To connect the Bus SAT/IN module to the Bus LAC/E card:

1. Connect the I/O Distribution Box to the 9-pin I/O distribution port on the Bus LAC/E card using the I/O distribution cable.
2. Connect the data terminal on the back of the Bus SAT/IN to Port 2 of the I/O Distribution Box using a serial cable.

3. Configure the serial port for the Bus SAT/IN module as described in the *Millennium 2010 Chromatography Manager Software User's Guide*, Chapter 4, Configuring a Chromatographic System.

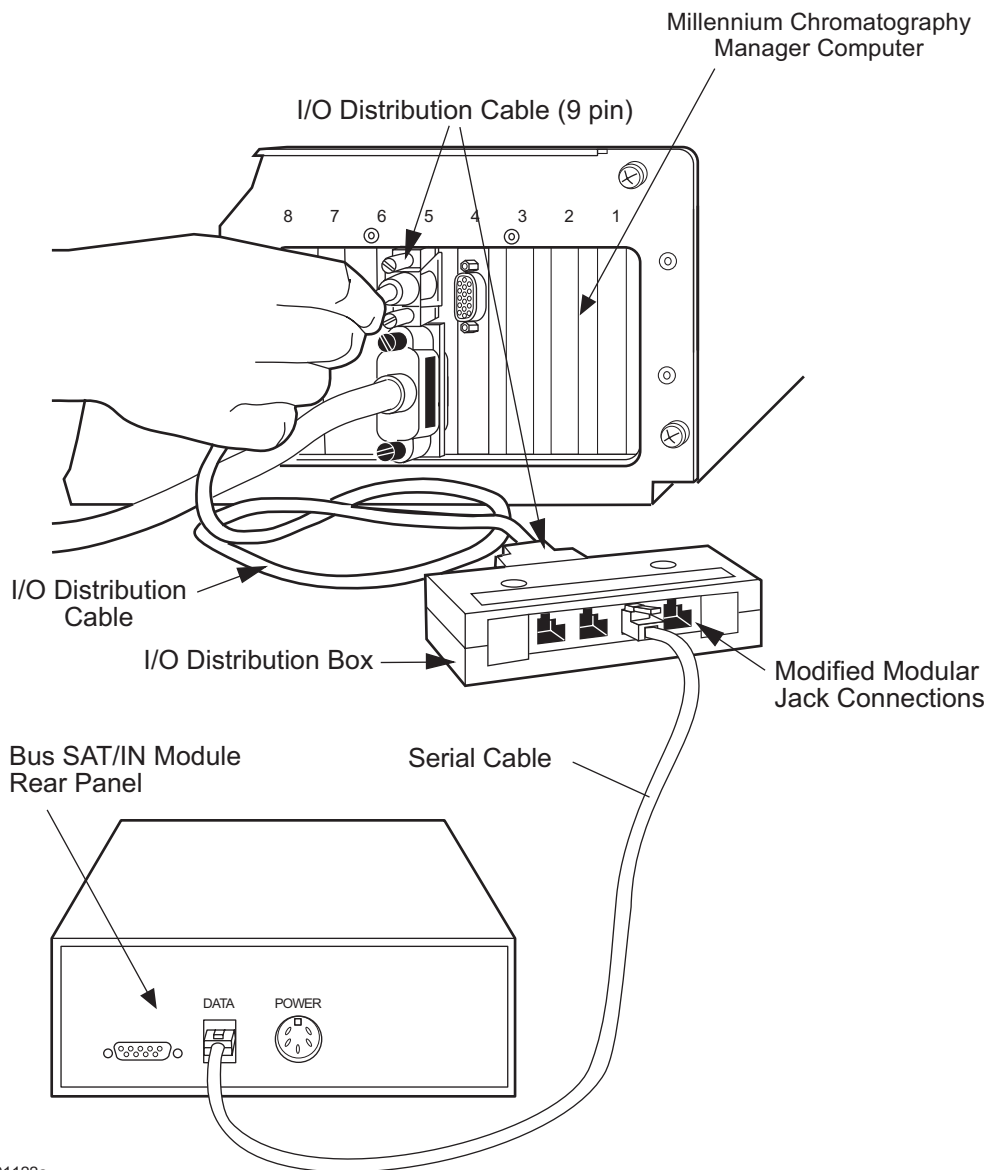


Figure 2-7 Bus SAT/IN to Bus LAC/E Connections

Connecting the 474 to the Bus SAT/IN Module

The 474 connects to the Bus SAT/IN module as shown in [Figure 2-8](#).

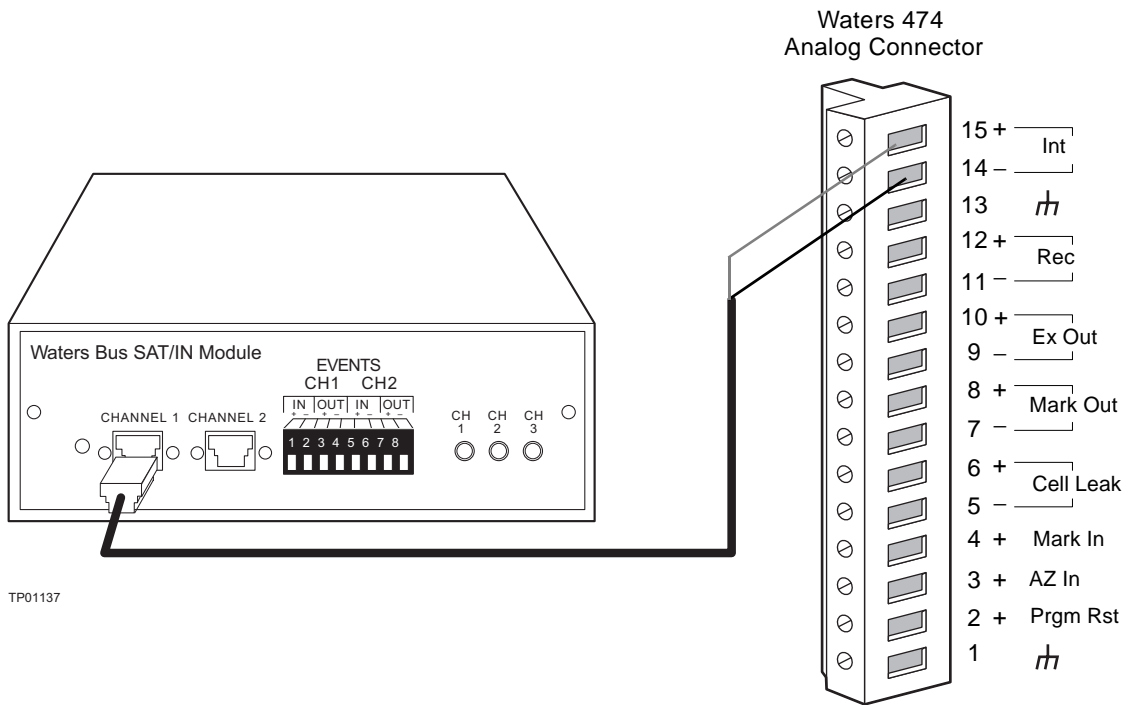


Figure 2-8 Bus SAT/IN Module Connections

To connect the 474 to the Bus SAT/IN module:

1. Connect the analog cable (included with the Bus SAT/IN module) to the Int (+ and -) terminals on the rear panel of the 474.
2. Connect the other end of the cable to either the Channel 1 or Channel 2 connector on the front panel of the Bus SAT/IN module.

The 474-to-Bus SAT/IN connections are summarized in the following table.

474 Analog Connector Terminal	Bus SAT/IN Connector
Int (+)	+ Lead from Channel 1 or 2
Int (-)	- Lead from Channel 1 or 2

2.6.2.2 Millennium³² Data System Version 3.2 or Higher

Before you connect the 474 to the Millennium³² Data System version 3.2 or higher, ensure that:

- The Windows® NT operating system is installed in the Millennium³² computer
- The 474 detector is equipped with the RS-232 Control Option Kit (Part Number 176000204)

For detectors with a serial number:

- B00474712M or higher, the RS-232 Control Option kit is factory installed.
- B00474711M or lower, the RS-232 Control Option kit is installed on site by a Waters Field Service Representative.

See the *Waters 474 Scanning Fluorescence Detector RS-232 Control Option Guide* for information about installing the RS-232 Control Option Kit.

To connect the 474 to the Millennium³² Data System:

1. Connect the 25-pin end of the null modem cable (included in the RS-232 Control Option Kit) to the RS-232 port on the rear panel of the 474.
2. Connect the other end of the cable to one of the COM ports on the rear panel of the Millennium³² computer.

Note: *If your Millennium³² computer does not have an available COM port, install the optional eight-port serial card in the Millennium³² computer. Use one of the cables provided with the card along with a 25-pin adapter (part number 340000103) to connect the 474 to one of the serial ports. For more information about using the eight-port serial card, see the Millennium³² Installation and Configuration Guide, section 2.3, Connecting Chromatographic Devices.*

To configure the 474 detector address on the eight-port serial card, use the Acquisition Server Properties window in the Millennium³² Configuration Manager. For more information about configuring the serial ports, see the Configuring Serial Ports topic in the Millennium³² Online Help.

3. Use a signal cable to make the connections between the 474 and a Waters 2690 Separations Module or a Waters 600 Series Controller that are indicated in the following table.

474 Analog Connector Terminal	Waters 2690 Connector B Terminal or Waters 600 Series Controller Terminal
Mark In (+)	Inject Start
\overline{m}	Inject Start

2.6.3 Connecting the 474 to the Waters 2690 Separations Module

To connect a standalone Waters 2690 Separations Module to the 474, you must make the following connections:

- Auto Zero
- Chart Mark
- Method Start
- Cell Leak

If your 2690 Separations Module is part of a Carbamate Analysis System or an AccQ•Tag system, see [Section 2.6.8, Connecting the 474 to the Carbamate System](#) or [Section 2.6.9, Connecting the 474 to the AccQ•Tag System](#).

Auto Zero

A contact closure signal between the AZ In (+) and the (–) terminals of the 474 starts the auto zero function. This function sets the analog voltage to zero on the Rec (+ and –) and Int (+ and –) terminals of the 474.

To provide an auto zero signal to the 474, use a signal cable to make the connections between the 474 and a Waters 2690 Separations Module as summarized in the following table, and as illustrated in [Figure 2-9](#).

474 Analog Connector Terminal	Waters 2690 Connector B Terminal
AZ In (+)	Inject Start
\overline{m}	Inject Start

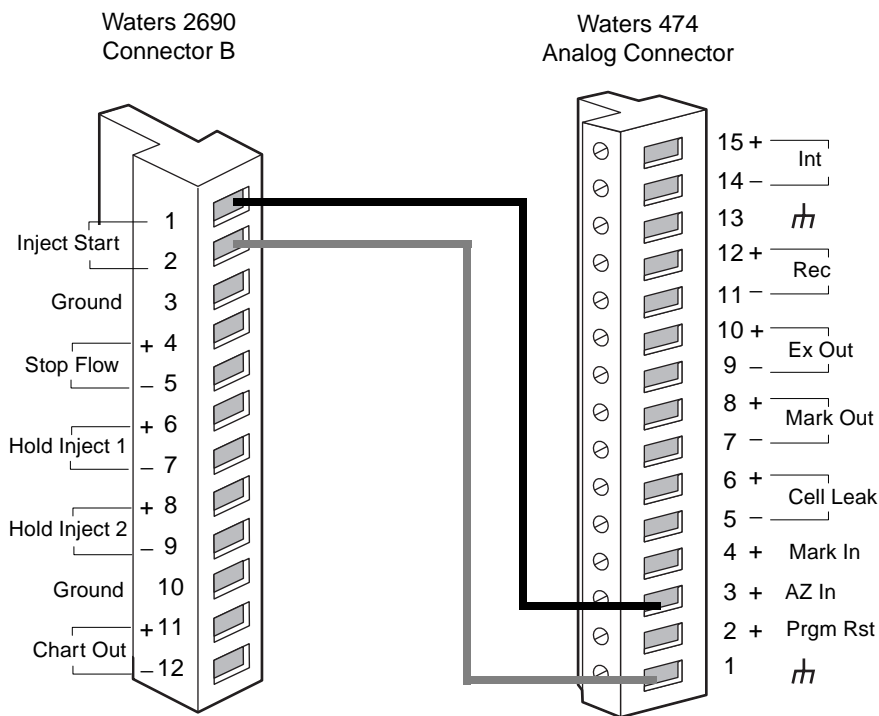


Figure 2-9 Waters 2690 Separations Module Auto Zero Connections

Chart Mark

A contact closure signal between the Mark In (+) and the – terminals of the 474 creates a chart mark signal, which is a momentary 1- to 3-mV increase in the analog voltage on the Rec (+ and –) and Mark Out (+ and –) terminals. The chart mark can signal events such as the start of an injection.

To provide a chart mark signal from a Waters 2690 Separations Module to the 474:

1. Use a signal cable to make the connections between the 474 and a Waters 2690 Separations Module as summarized in the following table, and as illustrated in [Figure 2-10](#).

474 Analog Connector Terminal	Waters 2690 Connector A Terminal
Mark In (+)	Switch 1, 2, 3, or 4 (+)
\overline{m}	Switch 1, 2, 3, or 4 (-)

- Program the 2690 Separations Module to provide a pulse output on the switch at the beginning of each run. Refer to the *Waters 2690 Separations Module Operator's Guide*, Section 2.7.1, I/O Signal Connections, for more details.

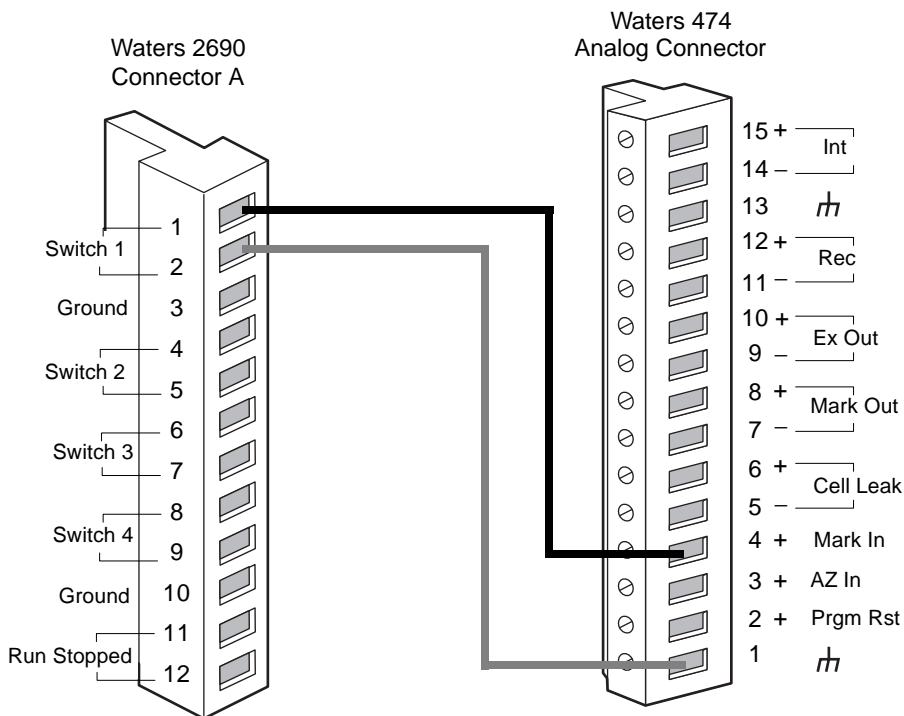


Figure 2-10 Waters 2690 Separations Module Chart Mark Connections

Refer to [Section 3.6.4, Mark In](#), for a complete description of the Mark In terminal.

Method Start

To provide a method start signal to the 474, use a signal cable to make the connections between the 474 and a Waters 2690 Separations Module as summarized in the following table, and as illustrated in [Figure 2-11](#).

474 Analog Connector Terminal	Waters 2690 Connector B Terminal
Prgm Rst (+)	Inject Start (+)
\overline{m}	Inject Start (-)

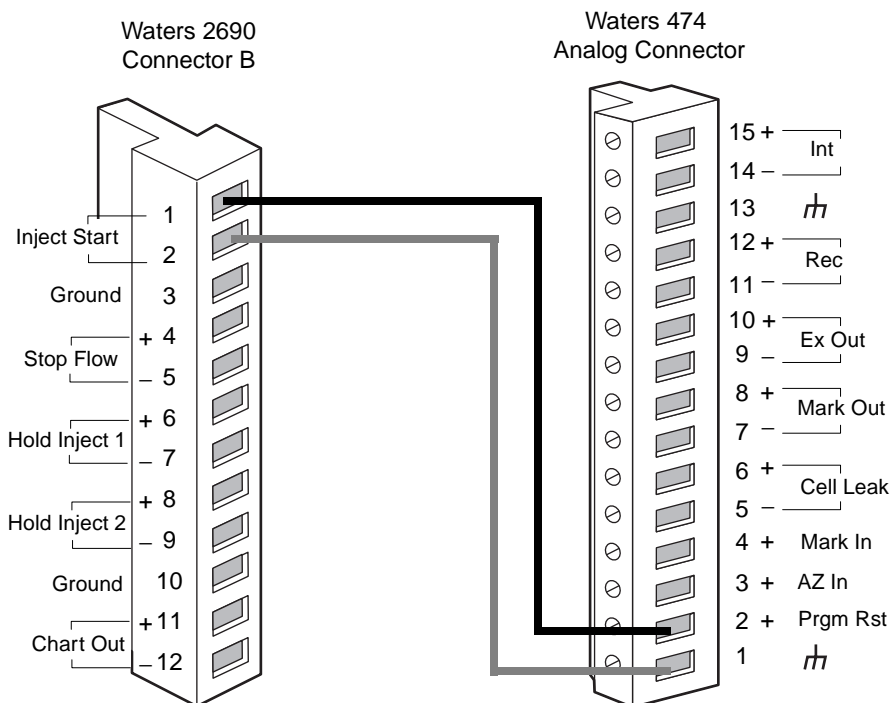


Figure 2-11 Waters 2690 Separations Module Method Start Connections

Cell Leak

The 474 generates a contact closure signal when a leak occurs in the flow cell cassette. You can use this signal as an alarm signal to the 2690 Separations Module.

To provide a signal for an external warning when a leak occurs in the flow cell, make the connections summarized in the following table, and as illustrated in [Figure 2-12](#).

474 Analog Connector Terminal	Waters 2690 Connector B Terminal
Cell Leak (+)	Stop Flow (+)
Cell Leak (-)	Stop Flow (-)

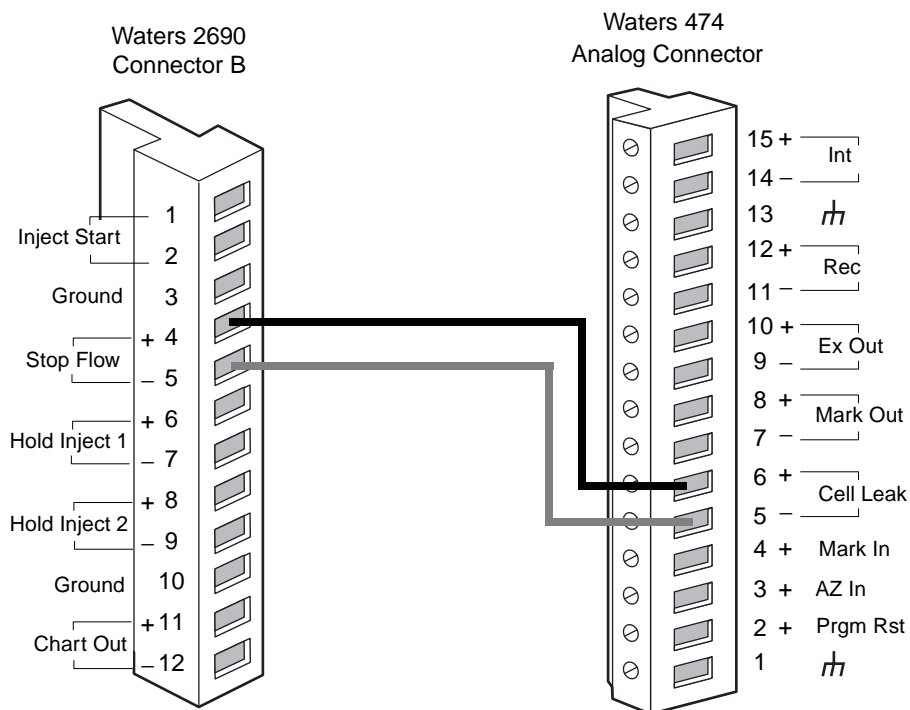


Figure 2-12 Waters 2690 Separations Module Cell Leak Connections

2.6.4 Connecting the 474 to the Waters 600 Series Controller

To connect a standalone Waters 600 series controller to the 474, you must make the following connections:

- Auto Zero
- Chart Mark
- Method Start
- Cell Leak

Auto Zero

A contact closure signal between the AZ In (+) and the (–) terminals of the 474 starts the auto zero function. This function sets the analog voltage to zero on the Rec (+ and –) and Int (+ and –) terminals of the 474.

To provide an auto zero signal to the 474, use a signal cable to make the connections between the 474 and a Waters 600 series controller as summarized in the following table, and as illustrated in [Figure 2-13](#).

474 Analog Connector Terminal	Waters 600 Series Controller Terminal
AZ In (+)	Inject
\overline{m}	\overline{m}

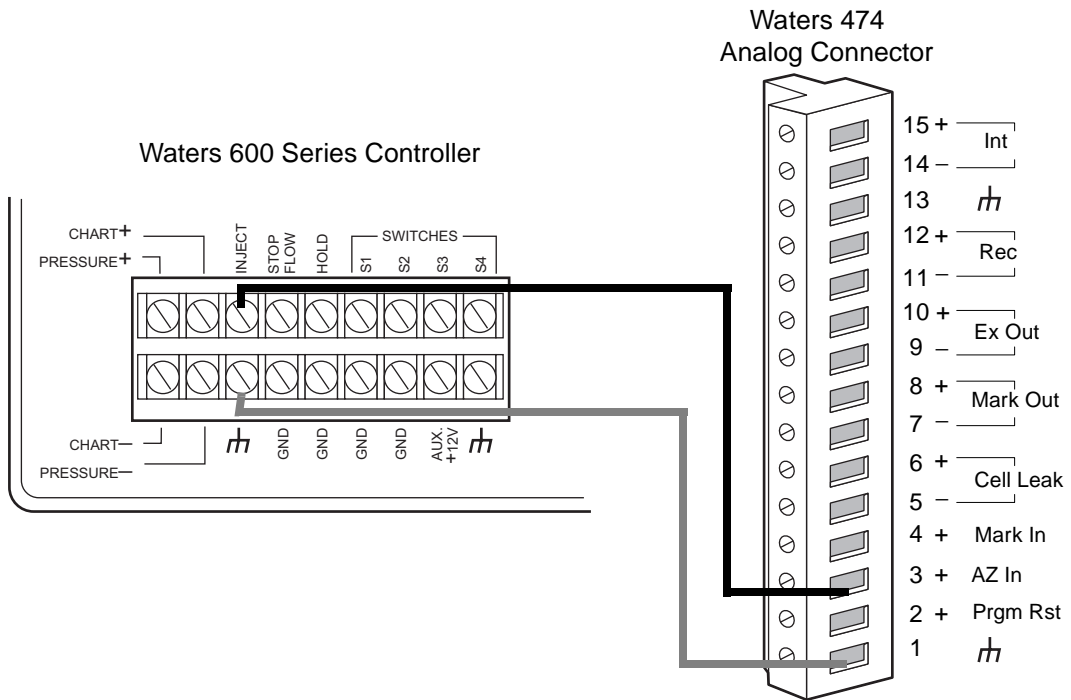


Figure 2-13 Waters 600 Series Controller Auto Zero Connections

Chart Mark

A contact closure signal between the Mark In (+) and the – terminals of the 474 creates a chart mark signal, which is a momentary 1- to 3-mV increase in the analog voltage on the Rec (+ and –) and Mark Out (+ and –) terminals. The chart mark can signal events such as the start of an injection.

To provide a chart mark signal from a Waters 600 series controller to the 474:

1. Use a signal cable to make the connections between the 474 and a Waters 600 series controller as summarized in the following table, and as illustrated in [Figure 2-14](#).

474 Analog Connector Terminal	Waters 600 Series Controller Terminal
Mark In (+)	S1, S2, S3, or S4
\overline{m}	GND

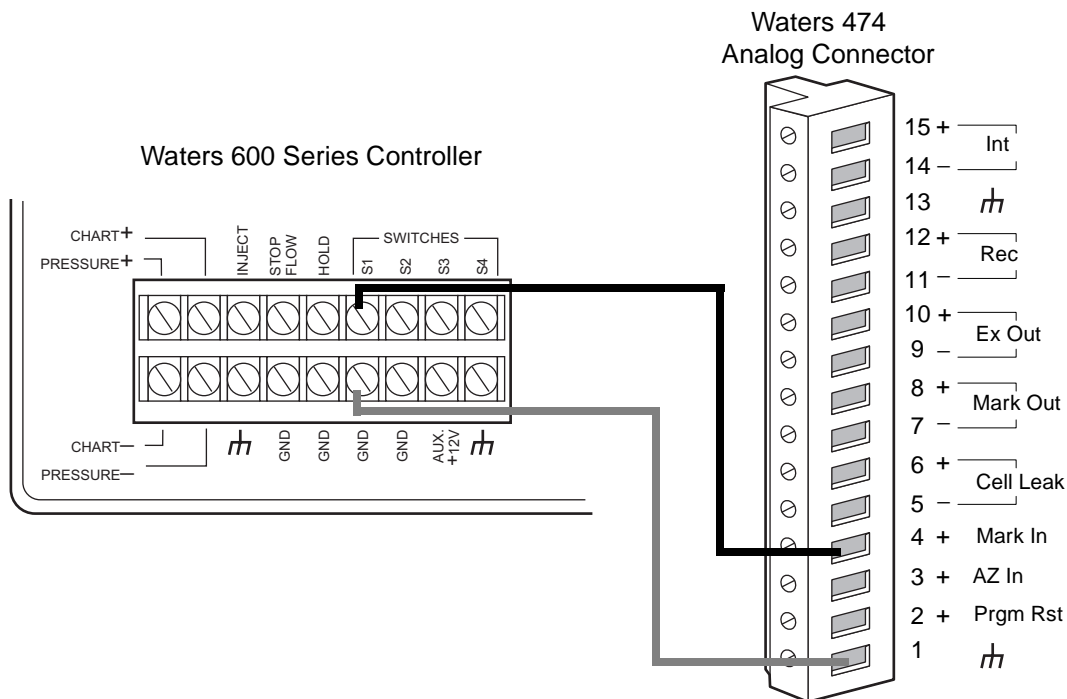


Figure 2-14 Waters 600 Series Controller Chart Mark Connections

- Program the 600 controller to provide a pulse output on the switch at the beginning of each run. Refer to the *Waters 600E Multisolvant Delivery System User's Guide*, Section 5.1.2, for more details.

For a complete description of the Mark In terminal, refer to [Section 3.6.4, Mark In](#).

Method Start

To provide a method start signal to the 474, use a signal cable to make the connections between the 474 and a Waters 600 series controller as summarized in the following table, and as illustrated in [Figure 2-15](#).

474 Analog Connector Terminal	Waters 600 Series Controller Terminal
Prgm Rst (+)	Inject (+)
m	m

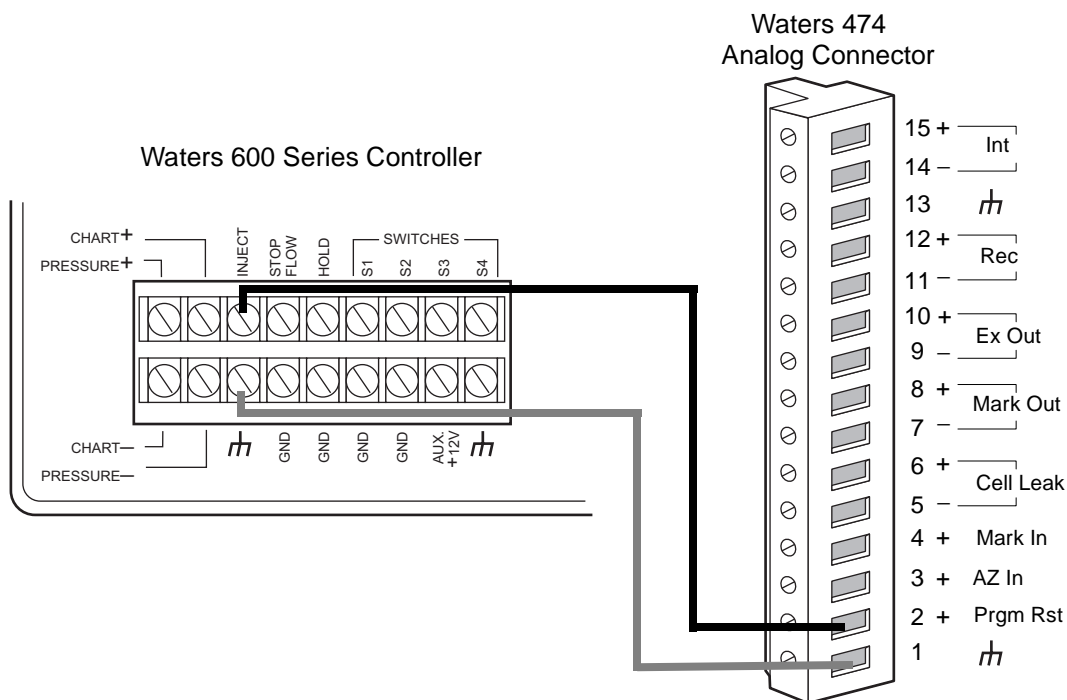


Figure 2-15 Waters 600 Series Controller Connections

Cell Leak

The 474 generates a contact closure signal when a leak occurs in the flow cell cassette. You can use this signal as an alarm signal to the 600 Series Controller.

To provide a signal for an external warning when a leak occurs in the flow cell, make the connections summarized in the following table and illustrated in [Figure 2-16](#).

474 Analog Connector	Waters 600 Series Controller Terminal
Cell Leak (+)	Stop Flow
Cell Leak (-)	GND

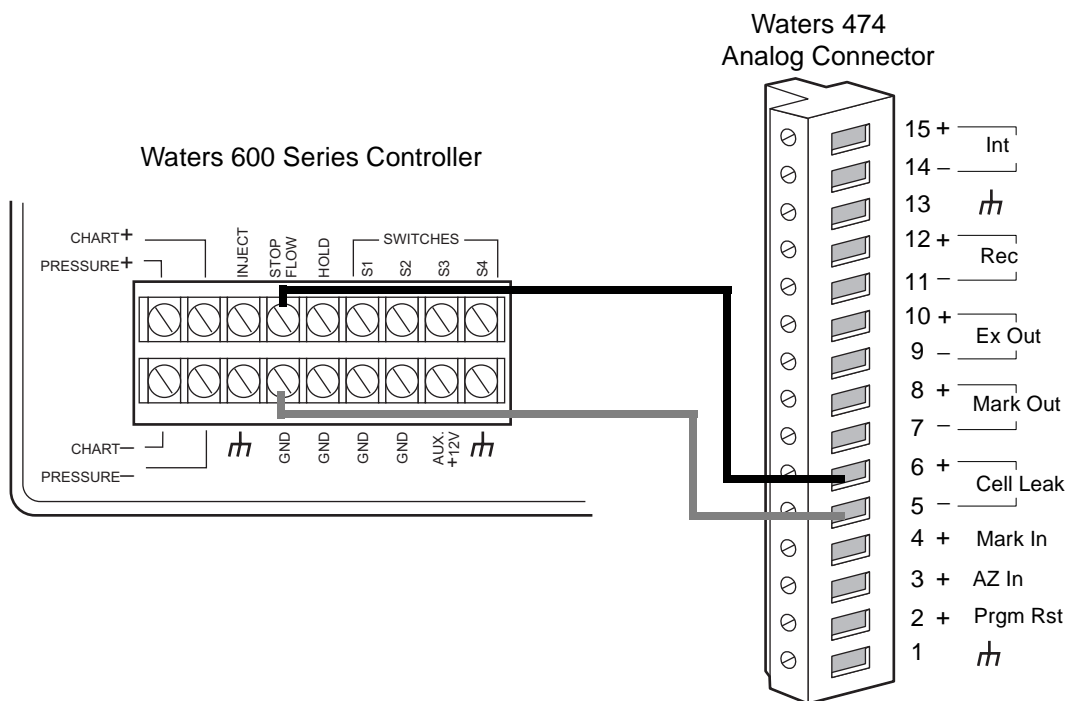


Figure 2-16 Waters 600 Series Controller Cell Leak Connections

For a complete description of the Cell Leak terminals, refer to [Section 3.6.3, Cell Leak](#).

2.6.5 Connecting the 474 to a Waters 745/745B/746 Data Module

The Int (+ and –) terminals on the rear panel analog connector of the 474 provide a 1 V full-scale analog output signal. To send this analog output signal from the 474 to the Waters 745/745B/746 Data Module, use the cable in the 474 Start-Up Kit to make the connections summarized in the following table and illustrated in [Figure 2-17](#).

474 Analog Connector Terminal	745/745B/746 Terminal
Int (+)	+
Int (-)	-

Connect the shield of the cable to chassis ground at one end only. This minimizes the chance of creating a ground loop, which can adversely affect the measurement.

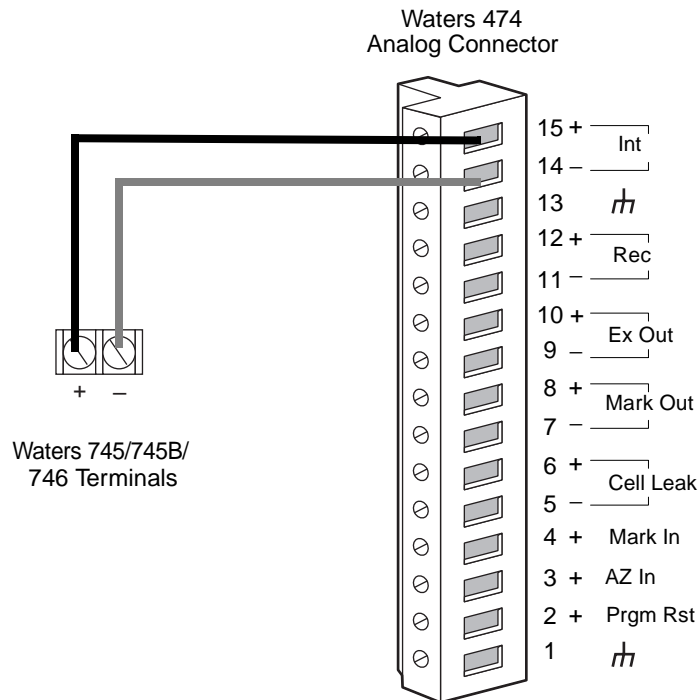


Figure 2-17 Waters 745/745B/746 Connections

2.6.6 Connecting the 474 to a Chart Recorder

The Recorder Output terminals on the analog connector of the 474 provide an analog output signal to a chart recorder. This signal has a maximum full-scale output of 10 mV. The values in the Gain and Attenuation parameters affect the full-scale range (see “Using Gain and Attenuation” in Section 3.4.5).

A chart mark signal is generated each time:

- You press the Chart Mark key on the 474 keypad
- A contact closure signal occurs on the Mark In (+) terminal

To send analog output signals from the 474 to a chart recorder, use the cable in the Start-Up Kit to make the connections summarized in the following table and illustrated in [Figure 2-18](#).

474 Analog Connector Terminal	Chart Recorder Terminal
Rec (+)	Pen 1 (+)
Rec (-)	Pen 1 (-)

Connect the shield of the cable to chassis ground at one end only. This minimizes the chance of creating a ground loop, which can adversely affect the measurement.

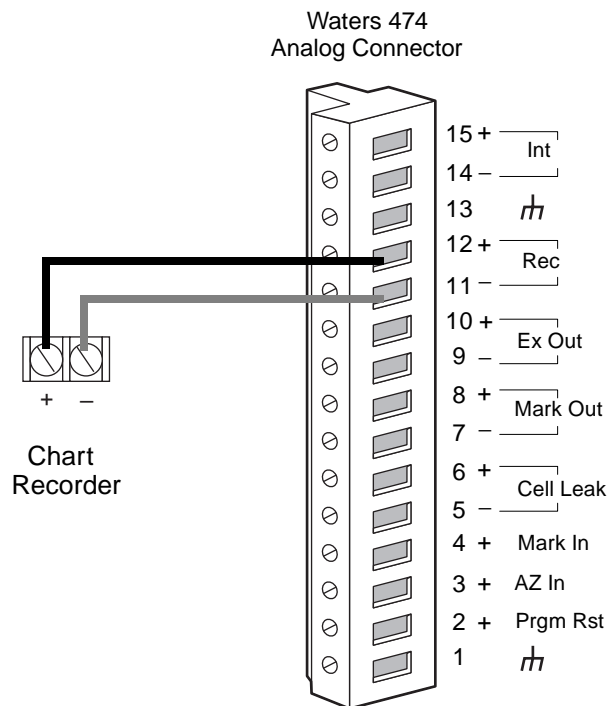


Figure 2-18 Chart Recorder Connections

2.6.7 Connecting the 474 to the Waters 717plus

The Waters 717plus Autosampler signals the start of an injection through a contact closure signal on its Inject Start terminals. You can use this signal to command the 474 to perform an auto zero at the start of an injection.

To autozero the analog voltage on the Rec (+ and –) and Int (+ and –) terminals at the start of an injection, make the connections summarized in the following table and illustrated in [Figure 2-19](#).

474 Analog Connector Terminal	Waters 717plus Connector Terminal
AZ In (+)	Inject Start (+)
\overline{m}	Inject Start (-)

Note: Use any available pair of Inject Start terminals on the 717plus.

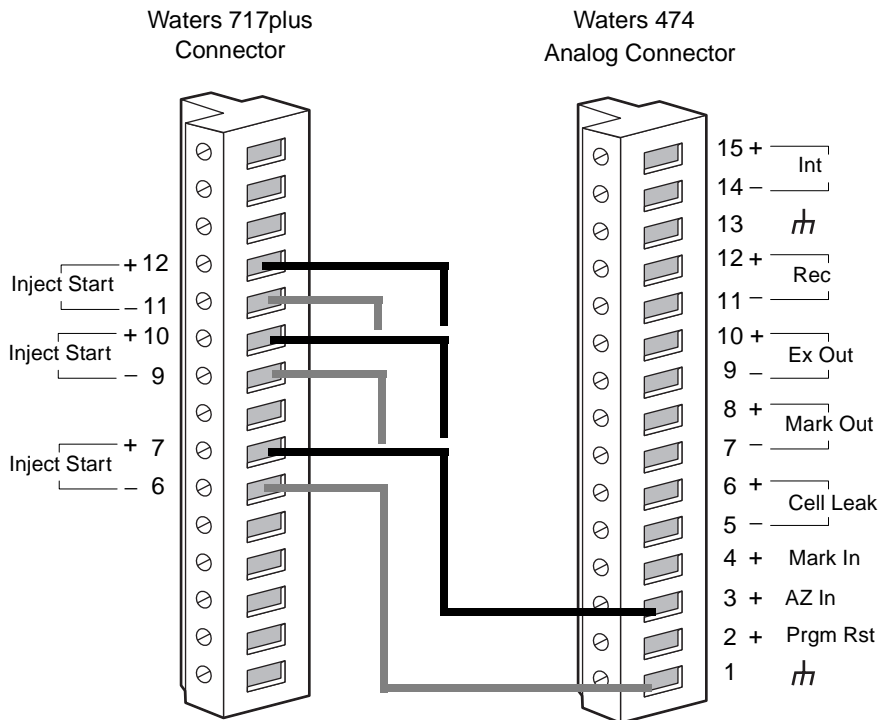


Figure 2-19 Waters 717plus Auto Zero Connections

Refer to [Section 3.6.5, Auto Zero In](#), for a complete description of the AZ In terminal.

2.6.8 Connecting the 474 to the Carbamate System

The Waters Carbamate Analysis System includes a 474 fluorescence detector under Millennium³² version 3.2 or higher software control. To connect the 474 to the Carbamate Analysis System, you must make the following connections:

- Chart Mark
- Inject Start

Chart Mark

Use a signal cable to make the connections between the 474 and the Waters 2690 Separations Module. The connections are summarized in the following table and illustrated in [Figure 2-21](#).

474 Analog Connector Terminal	Waters 2690 Connector B Terminal
Mark In (+)	Inject Start (1)
\overline{m}	Inject Start (2)

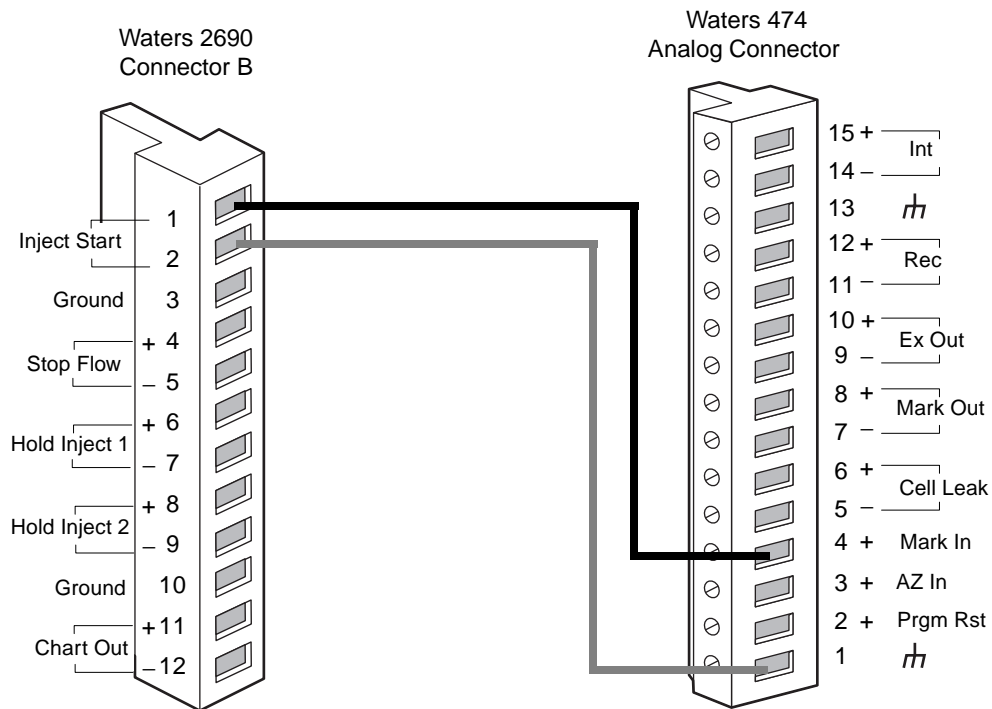


Figure 2-20 Waters Carbamate System Chart Mark Connections

Inject Start

Use a signal cable to make the connections between the 474 and the Waters 2690 Separations Module. The connections are summarized in the following table and illustrated in [Figure 2-21](#).

474 Analog Connector Terminal	Waters 2690 Connector B Terminal
AZ In (+)	Inject Start (1)
h	Inject Start (2)

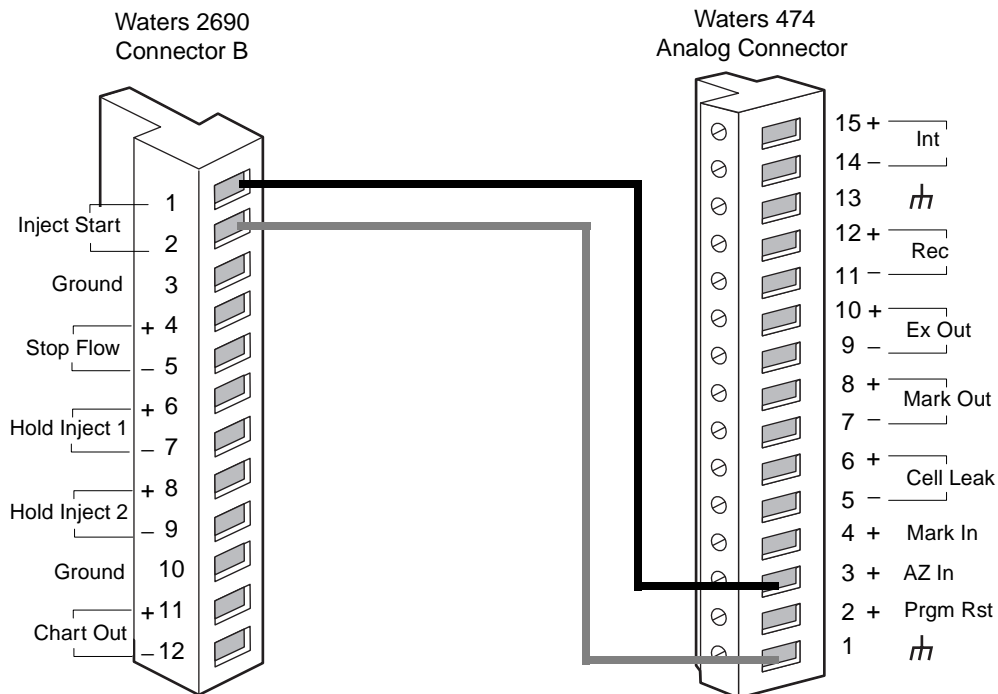


Figure 2-21 Waters Carbamate System Inject Start Connections

2.6.9 Connecting the 474 to the AccQ•Tag System

The Waters AccQ•Tag System includes a 474 fluorescence detector under Millennium³² version 3.2 or higher software control. To connect the 474 to the AccQ•Tag System, you must make the following connections:

- Chart Mark
- Inject Start

Chart Mark

Use a signal cable to make the connections between the 474 and the Waters 2690 Separations Module. The connections are summarized in the following table and illustrated in [Figure 2-22](#).

474 Analog Connector Terminal	Waters 2690 Connector B Terminal
Mark In (+)	Inject Start (1)
\overline{M}	Inject Start (2)

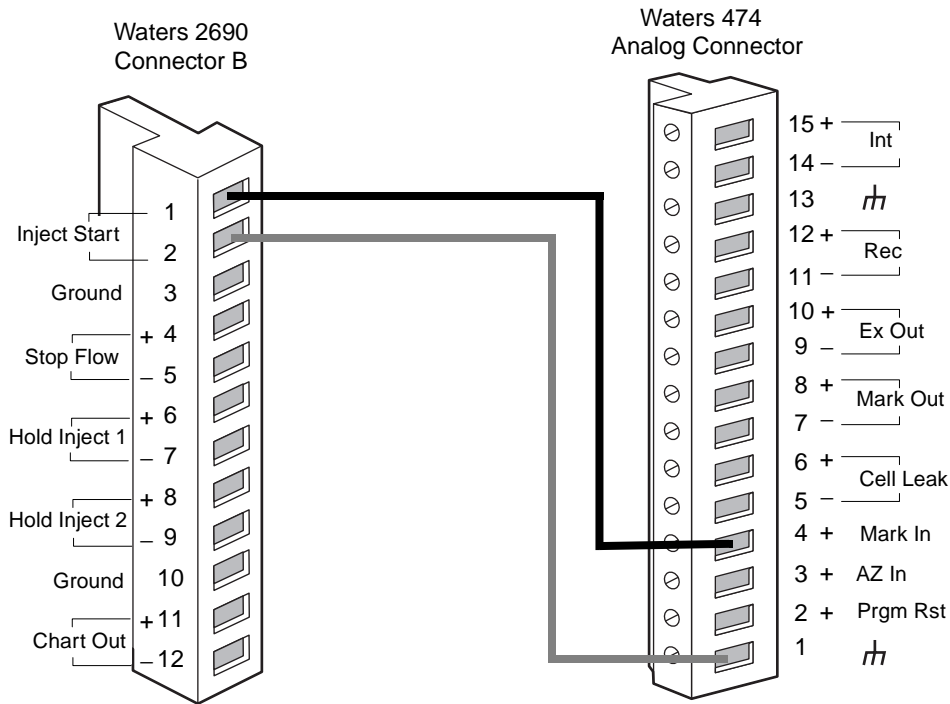


Figure 2-22 AccQ•Tag System Chart Mark Connections

Injct Start

The Waters 2690 Separations Module signals the start of an injection through a contact closure signal on its Inject Start terminals. You can use this contact closure signal to command the 474 to perform an autozero at the start of an injection.

To autozero the analog voltage on the Rec (+ and –) and Int (+ and –) terminals at the start of an injection, make the connections described in the following table and illustrated in [Figure 2-23](#).

474 Analog Connector Terminal	Waters 2690 Connector B Terminal
AZ In (+)	Inject Start (1)
h	Inject Start (2)

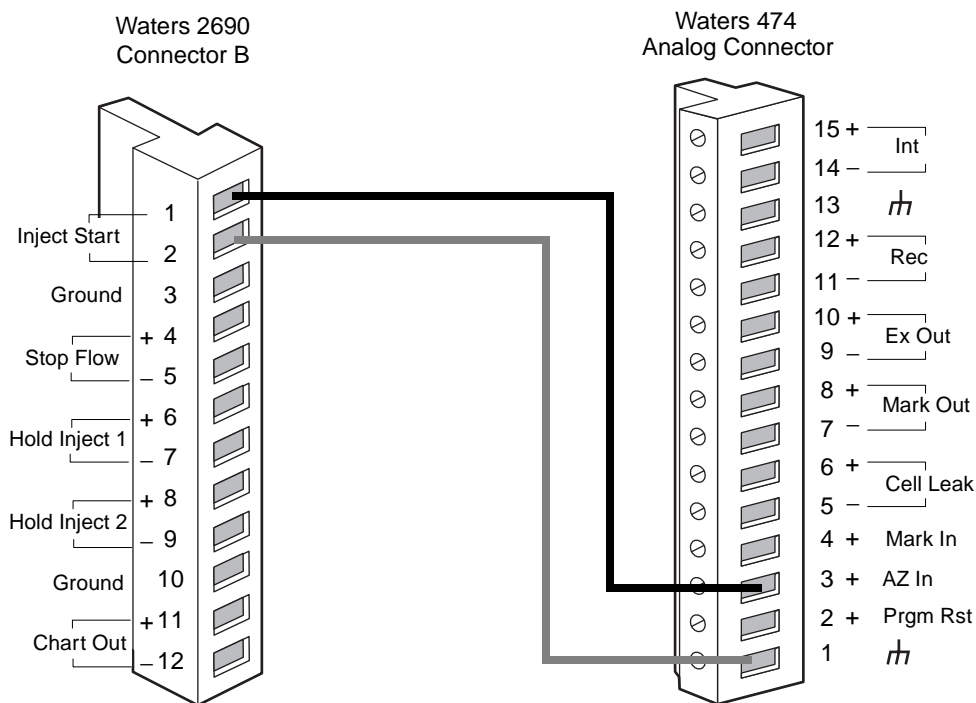


Figure 2-23 Waters AccQ•Tag System Connections

Refer to [Section 3.6.5, Auto Zero In](#) for a complete description of the AZ In terminal.

2.6.10 Connecting a Voltmeter to the Excitation Out Terminals

The Ex Out (+ and –) terminals provide a 1 V full-scale analog signal that corresponds to the lamp excitation energy levels. You can measure this signal with a voltmeter to monitor the lamp energy as the wavelengths change during a scan.

To monitor the analog Ex Out signal from the 474, connect a voltmeter (set to measure 1 VDC full-scale) to the 474 as summarized in the following table.

474 Analog Connector Terminal	Voltmeter DC Voltage Lead
Ex Out (+)	+
Ex Out (-)	-

For a complete description of the Ex Out terminals, refer to [Section 3.6.1, Excitation Energy Out](#).

3

Using the 474 Detector

This chapter describes how to power on, operate, and power off your Waters 474 Scanning Fluorescence Detector.

3.1 Powering On

Before Powering On

Before you power on the 474 detector:

- Verify the position of the voltage selection switch.
- Make sure the correct fuses are installed.
- Make sure the power cable is connected.

Powering On

1. Push the Power switch located on the front panel to the On (I) position.

The unit beeps, the fan turns on, and the display reads:

```
M-474
```

```
WATERS x.xx
```

The x.xx indicates the version of the operating firmware installed in the 474.

Self-Diagnosis

2. The self-diagnosis cycle begins, and the screen displays the following messages:

```
SELF DIAGNOSIS
```

```
ROM CHECK OK!
```

```
SELF DIAGNOSIS
```

```
RAM CHECK OK!
```

```
SELF DIAGNOSIS
```

```
DC POWER OK!
```

```
SELF DIAGNOSIS
```

EX DRIVER OK!
SELF DIAGNOSIS
EM DRIVER OK!
SELF DIAGNOSIS
LAMP ENERGY GOOD
LAMP OPERATION
XXX.X HOURS
BAND WIDTH (nm)
NOW SETTING

When the self-check for each item ends normally, the 474 continues with the next self-check. On successful completion of all self-checks, the 474 enters the Normal mode described in [Section 3.2, Using the Front Panel](#). The display then shows the parameter settings entered before the last powerdown.

If an Error Occurs

If an error is detected, an error code is displayed. [Section 7.1, Error Messages](#), lists each error message and the action needed to correct the error.

If you want to continue with the self-check, press the **Shift** and the **t** key to move past the error. The 474 proceeds with the next self-check. At the end of the self-checks, the display shows the bandwidth setting and the number of hours the lamp has been in use.

Warm-Up

Waters recommends that you allow the 474 to warm up for at least one hour before using it. This warm-up period allows the internal temperature of the 474 to stabilize.

3.2 Using the Front Panel

The 474 front panel ([Figure 3-1](#)) includes the following components:

- Power switch
- 22-key keypad
- Flow cell cassette
- Two-line liquid-crystal display (LCD)

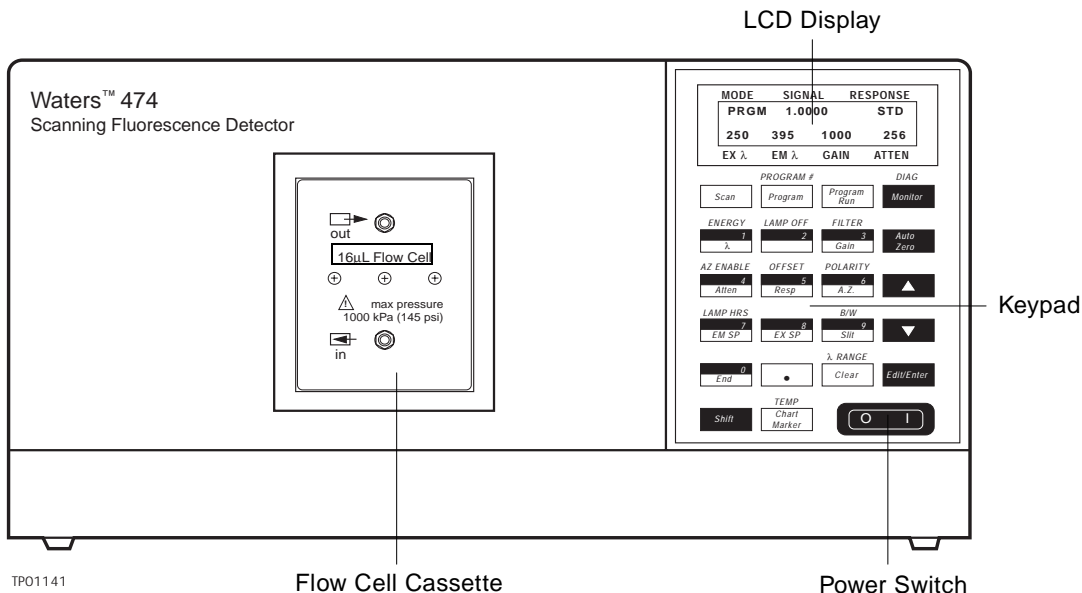


Figure 3-1 474 Detector Front Panel

Power Switch

The power switch turns on the 474 and turns off the 474.

- Off (O) indicates power is off.
- On (I) indicates power is on.

Keypad

The keypad enables you to select parameters, enter numerical settings, create programs, and control the operation of the 474. Each key is described in [Section 3.3, Key Descriptions](#).

Flow Cell Cassette

The flow cell cassette contains the fluid connections for the flow cell. [Section 2.4, Making Fluid Connections](#), describes how to connect the tubing to the flow cell.

Display

The LCD display indicates the operating conditions, fluorescence intensity, parameters, parameter settings, and status messages.

Figure 3-2 shows the display when the 474 is in the Normal mode. The display has seven fields, which are described in Table 3-1.

MODE	SIGNAL	RESPONSE
NORM	0.0000	STD
300	500	1000
16		
EX λ	EM λ	GAIN
ATTEN		

Figure 3-2 Typical Display in Normal Mode

Table 3-1 Display Parameters

Parameter	Description
Mode	Displays the operating mode of the 474 (Normal, Program, or Scan)
Signal	Displays the intensity of the fluorescence emission signal
Response	Displays the response setting (Slow, Standard, or Fast or 3 s, 5 s, 10 s, 20 s, or 40 s)
Ex λ	Displays the excitation wavelength (in nm)
Em λ	Displays the emission wavelength (in nm)
Gain	Displays the gain setting (1, 10, 100, or 1000)
Atten	Displays the attenuation setting (1, 2, 4, 8, 16, 32, 64, 128, 256, or S)

3.3 Key Descriptions

The 474 has 22 keys on the keypad as shown in Figure 3-3. Each key has one, two, or three levels of functions.

- The first-level functions are selected by pressing the key.
- The second-level functions are the ten numeric keys; you can enter the number when a parameter field (Ex λ , Em λ , Gain, Atten, or Resp) is open for editing.

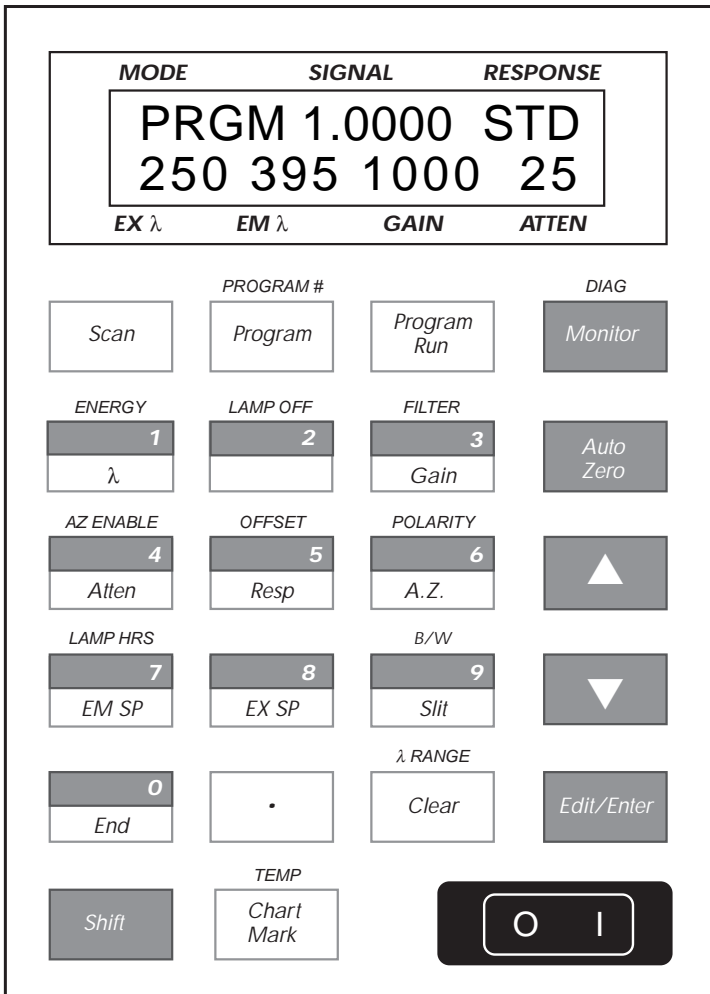
- The third-level functions are activated by pressing the Shift key and then a key with text written above it.

Keystroke Feedback

Each time you press a key, you hear (and feel) a mechanical click followed by a short beep. This lets you know that the 474 processed the keystroke.

A long beep indicates that you pressed a key in the wrong sequence or that you pressed a key that is not valid for the current operation.

[Figure 3-3](#) illustrates the keypad of the 474 detector.



TP01142

Figure 3-3 Keypad Layout

[Table 3-2](#) summarizes the keys in the 474 keypad.

Table 3-2 Key Descriptions

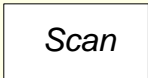

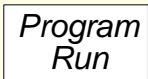

Key	Level 1 Function	Level 2 Function	Level 3 Function (with the Shift key)
	Switches to the Scan mode. See Chapter 5, Using the Scan Mode for details.	_____	_____
<small>PROGRAM #</small> 	Switches to the Program mode. See Chapter 4, Programming the 474 Detector , for details.	_____	Accesses the Program # special parameter. Select one of up to ten user-defined programs.
	Starts and stops a time program: <ul style="list-style-type: none"> • Press this key in the Program mode and the currently selected time program starts. • Press this key when a time program is running to stop the program. 	_____	_____
<small>DIAG</small> 	Returns the 474 to the Normal mode or the previous menu level.	_____	Accesses the Diagnostics special parameter (Waters Service personnel only).

Table 3-2 Key Descriptions (Continued)

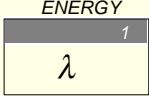
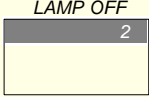
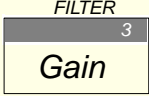
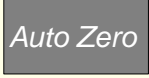
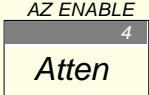
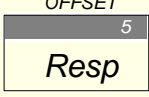
Key	Level 1 Function	Level 2 Function	Level 3 Function (with the Shift key)
 <p>ENERGY 1 λ</p>	Changes the wavelength in the Normal mode. Accesses the Wavelength parameters in the Program mode.	Enters a 1 when a parameter field is open for editing.	Accesses the Energy special parameter.
 <p>LAMP OFF 2</p>	_____	Enters a 2 when a parameter field is open for editing.	Accesses the Lamp Off special parameter.
 <p>FILTER 3 Gain</p>	Changes the gain in the Normal mode. Accesses the Gain parameter in the Program mode.	Enters a 3 when a parameter field is open for editing.	Accesses the Signal Filter special parameter.
 <p>Auto Zero</p>	Sets the voltage to zero on the Int (+ and -) and Rec (+ and -) terminals.	_____	_____
 <p>AZ ENABLE 4 Atten</p>	Changes the attenuation in the Normal mode. Accesses the Attenuation parameter in the Program mode.	Enters a 4 when a parameter field is open for editing.	Accesses the Auto Zero Enable special parameter.
 <p>OFFSET 5 Resp</p>	Changes the response in the Normal mode. Accesses the Response parameter in the Program mode.	Enters a 5 when a parameter field is open for editing.	Accesses the Offset special parameter.

Table 3-2 Key Descriptions (Continued)

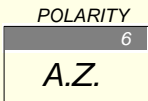
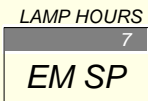




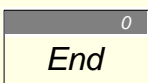
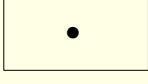
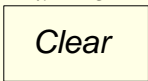

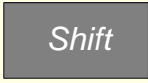

Key	Level 1 Function	Level 2 Function	Level 3 Function (with the Shift key)
	Accesses the Auto Zero parameter in the Program mode.	Enters a 6 when a parameter field is open for editing.	Accesses the Polarity special parameter.
	Accesses the Emission Spectrum parameter in the Program mode.	Enters a 7 when a parameter field is open for editing.	Accesses the Lamp Hours special parameter.
	Accesses the Excitation Spectrum parameter in the Program mode.	Enters an 8 when a parameter field is open for editing.	_____
	Accesses the Spectrum Bandwidth parameter in the Program mode.	Enters a 9 when a parameter field is open for editing.	Accesses the Bandwidth special parameter.
	Moves to the next field, or increments the current parameter setting.	_____	Moves to the first step in a time program.
	Moves to the previous field or decreases the current parameter setting.	_____	Moves to the last step in a time program.
	_____	Enters a 0 when a parameter field is open for editing.	_____
	_____	Enters a decimal point when a field is open for editing.	_____

Table 3-2 Key Descriptions (Continued)

Key	Level 1 Function	Level 2 Function	Level 3 Function (with the Shift key)
λ RANGE 	Clears the contents of the current field or cancels messages such as Lamp Out.	_____	Accesses the Wavelength Range special parameter. In the Scan mode, clears all the spectrum memories.
	Opens the current field for editing or to enter a new setting into the field.	_____	_____
	Accesses the Level 3 functions.	_____	_____
$TEMP$ 	Adds a marker to the Rec (+ and –) output terminals. The voltage on these terminals momentarily increases by approximately 3 mV.	_____	Displays the Temperature Coefficient special parameter.

3

3.4 Operating Parameters

The 474 processes samples based on user-defined settings in five operating parameters:

- Excitation wavelength
- Emission wavelength
- Gain
- Attenuation
- Response

These operating parameters determine the conditions of the analysis and the magnitude of the analog output signal. You must set the parameters before each analysis.

[Table 3-3](#) lists the parameters and their ranges, and the keys used to enter the parameter settings.

Table 3-3 Parameters, Ranges, and Keys

Parameter	Description	Range	Keys
Ex λ	Excitation wavelength	0 to 900 nm ^{1, 2}	Numeric only
Em λ	Emission wavelength	0 to 900 nm ^b	Numeric only
Gain	Gain	1, 10, 100, 1000	▲, ▼, or numeric
Atten	Attenuation	1, 2, 4, 8, 16, 32, 64, 128, S	▲, ▼, or numeric
Resp	Response	SLW, STD, FST 3 s, 5 s, 10 s, 20 s, 40 s ³	▲, ▼ only

¹ Must be less than Em λ

² See [Section 3.5.11, Wavelength Range](#), for different ranges

³ See [Section 3.5.5, Signal Filter](#), for different ranges

At Start-Up

When you power on the 474 and the power-up diagnostics are complete, the 474 enters the Normal mode. The LCD display indicates the parameter settings that were in place when the 474 was last powered down. [Figure 3-4](#) shows a typical display in the Normal mode.

MODE	SIGNAL	RESPONSE	
NORM	0.0110	STD	
300	500	1000	16
EX λ	EM λ	GAIN	ATTEN

Figure 3-4 Typical Display in the Normal Mode

3.4.1 Displaying and Changing Parameter Settings

To display or change the setting of a parameter, the 474 must be in the Normal mode. To access the Normal mode, press either the **Program** key or the **Monitor** key until the Normal mode is displayed.

Displaying a Parameter Setting

To display the current setting of a parameter:

1. Press the key that corresponds to the parameter you wish to display. The current setting flashes on and off.
2. Press the **Monitor** key to confirm the current setting.

Changing a Parameter Setting

To change a parameter setting:

1. Press the key that corresponds to the parameter you wish to change. The current setting flashes on and off.
2. Use the **▲**, **▼**, or numeric keys to enter the new setting. If you make a mistake, press the **Clear** key and then re-enter the correct setting.
3. Press the **Edit/Enter** key to enter the new setting. The new setting is displayed.

3.4.2 Excitation Wavelength

The Excitation Wavelength (Ex λ) parameter determines the wavelength setting of the excitation monochromator. The setting in this parameter determines the wavelengths of light that are used to excite the sample in the flow cell.

The Wavelength Range special parameter (described in [Section 3.5.11, Wavelength Range](#)) affects the settings that you can enter in the Ex λ parameter.

Order Filter

The 474 uses an automatic order filter at excitation wavelengths above 400 nm. This filter reduces the intensity of second-order wavelengths that could interfere with the analysis.

3.4.3 Emission Wavelength

The Emission Wavelength (Em λ) parameter determines the wavelength setting of the emission monochromator. The setting in this parameter determines which wavelengths of fluorescence emitted from the sample in the flow cell are measured.

The Wavelength Range special parameter (described in [Section 3.5.11, Wavelength Range](#)) affects the settings that you can enter in the Em λ parameter.

3.4.4 Gain

The Gain parameter determines how much voltage is applied to the photomultiplier tube (PMT). As you increase the gain setting, the voltage applied to the PMT increases and the sensitivity of the PMT increases correspondingly.

Valid settings for Gain are 1, 10, 100, or 1000.

3.4.5 Attenuation

The Attenuation parameter determines the attenuation on the signal on the Rec (+ and –) terminals. As you increase the attenuation setting, the voltage decreases.

Valid attenuation settings are 1, 2, 4, 8, 16, 32, 64, 128, 256, or S. The S setting stands for Short, and is to be used for test purposes only.

The Attenuation setting has no influence on the signal on the Int (+ and –) terminals on the analog connector.

Using Gain and Attenuation

Use the Gain parameter to maximize the Rec (+ and –) output signal without overloading the recording or measurement device. The Gain parameter is equivalent to the sensitivity control on a UV detector.

Use the Attenuation setting as a fine control of the Rec (+ and –) output signal if it overloads your recording or measurement device. The Attenuation setting performs the same function as the attenuation control on many chart recorders.

3.4.6 Response

The Response parameter determines the settings for the filter mode that you select in the Signal Filter special parameter (described in [Section 3.5.5, Signal Filter](#)).

The settings for each filter mode are listed in [Table 3-4](#). To change the filter mode, refer to [Section 3.5.5, Signal Filter](#).

Table 3-4 Filter Modes and Response Settings

Filter Mode	Response Setting
RC Filter	Slow, Standard, Fast
Digital Filter	3 s, 5 s, 10 s, 20 s, 40 s

3.5 Special Parameters

Function parameters that are infrequently changed once you set them are called special parameters. This section describes how to change the special parameters of the 474.

[Table 3-5](#) summarizes the special parameters and their settings.

Table 3-5 Special Parameter Menu

Name	Settings
Program Number	0 to 9
Diagnostics	See Chapter 7, Error Messages and Diagnostics
Energy	Not adjustable
Lamp Off Timer	0.0 to 9.9 hours
Signal Filter	RC Filter Digital Filter
Auto Zero	Manual Auto
Offset	0 mV, 5 mV, 10 mV, 50 mV, 100 mV
Polarity	+ or –
Lamp Hours	Reset to 0.0 at lamp change
Bandwidth, emission	10, 18, or 40 nm
Wavelength Range	Em > Ex+10 Not Restricted
Temperature Coefficient	Not adjustable

Changing Special Parameter Settings

To change a special parameter setting:

1. Press the **Shift** key and then press the key whose Level 3 function corresponds to the special parameter you want to change.

Note: You do not have to hold down the Shift key while you press the second key. The 474 does not beep when you press the Shift key.

2. Press the **Edit/Enter** key. The current setting of the field flashes on and off.
3. Use the **▲**, **▼**, or numeric keys as appropriate to change the contents of the field.
4. Press the **Edit/Enter** key again to enter the new setting.
5. Press the **Monitor** key to return the 474 to the Normal mode.

3.5.1 Program Number

The Program Number parameter gives you access to the ten user-defined time programs that you can store in the 474. You access this special parameter by pressing the Shift key and then the Program key when the 474 is in the Normal or Program modes. Refer to [Chapter 4, Programming the 474 Detector](#), for complete information on the Program key and the Program mode.

Selecting the Program Number

To select the program number:

1. Press the **Shift** and **Program** keys to access the Program Number parameter.
2. Press the **Edit/Enter** key. The current setting flashes on and off.
3. Use the **▲**, **▼**, or numeric keys to select the program number. If you make a mistake, re-enter the program number.
4. Press the **Edit/Enter** key to enter the program number.
5. Press the **Monitor** key to return to the Normal mode.

3.5.2 Diagnostics

The 474 performs a diagnostic self-check routine every time you power on. Additional diagnostics are available through the Diagnostics parameter, which allows a qualified service technician to run diagnostic self-checks.

Waters strongly recommends that only Waters Service technicians use the Diagnostics functions.



Attention: Unauthorized changes to the parameters in the Diagnostics section can compromise the integrity of the instrument.

If you enter the Diagnostics mode, press the **Monitor** key to return to the Normal mode.

The diagnostics self-check routine is described in [Section 7.2, Diagnostics](#).

3.5.3 Energy Output

Use the Energy Output parameter to check for:

- Deterioration in the lamp or light source mirror
- Gas bubbles in the flow cell
- Lamp misalignment after replacing the Xenon lamp

Displaying the Energy Output Parameter

To display the Energy Output parameter:

1. Press the **Shift** key and then the **I/1** key. [Figure 3-5](#) shows the display screen for this parameter.

MODE	SIGNAL	RESPONSE
EX	: 1.865 V	
EM	: 2.603 V	
EXλ	EMλ	GAIN ATTN

Figure 3-5 Typical Energy Output Display

The Energy Output parameter cannot be changed.

2. Press the **Edit/Enter** key to return to the Normal mode.

3.5.4 Lamp Off Timer

The Lamp Off Timer parameter lets you turn off the Xenon lamp after a preset time, extending its life. The preset time value can vary between 0.1 and 99.9 hours.

Setting the Lamp Off Timer

To set the Lamp Off Timer:

1. Press the **Shift** and **2** keys. [Figure 3-6](#) shows the display screen for this parameter.

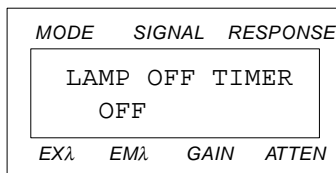


Figure 3-6 Lamp Off Timer Selection Screen

2. Press the **Edit/Enter** key. The Off setting flashes on and off.
3. Use the numeric keys to enter the length of the delay (in hours and tenths) before the lamp turns off. If you make a mistake, press the **Clear** key and re-enter the length of the delay.
4. Press the **Edit/Enter** key to enter the delay. The delay timer begins immediately.
5. Press the **Monitor** key to return to the Normal mode.
6. When the delay expires and the lamp turns off, the display appears as shown in [Figure 3-7](#). Press the **Clear** key to continue.

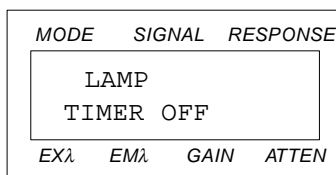


Figure 3-7 Display Screen After Lamp Timer Has Elapsed

7. To turn on the lamp again, press the Power switch to the Off (O) position and wait at least one minute. Then, press the Power switch to the On (I) position.

Note: Occasionally the lamp will not light when turned on shortly after being turned off. If the lamp does not light, repeat step 7 again, waiting several minutes before turning the power back on.

The Lamp Off timer is automatically set to Off when the power is turned on.

3.5.5 Signal Filter

The Signal Filter parameter allows you to select which type of noise reduction method to use on the fluorescence signal:

- RC Filter** – Uses a simulated analog filter
- Digital Filter** – Uses a digital filter

Note: If you experience too much noise with the RC filter method, switch to the digital filter method. The digital filter method reduces baseline noise more than the RC method.

Selecting the Type of Filter

To select the filter type:

1. Press the **Shift** and **Gain/3** keys. [Figure 3-8](#) shows the display screen for this parameter.

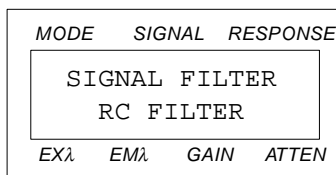


Figure 3-8 Filter Selection Screen

2. Press the **Edit/Enter** key. The current setting flashes on and off.
3. Use the **▲** and **▼** keys to toggle between the RC Filter and Digital Filter settings.
4. Press the **Edit/Enter** key to enter your selection.
5. Press the **Monitor** key to return to the Normal mode.

Filter Response Settings

The Filter Response settings listed in [Table 3-3](#) work with the Signal Filter setting. When you choose response settings, keep in mind that excessively long time constants can adversely affect the chromatogram by:

- Making peaks become asymmetric
- Shifting peak maxima
- Reducing peak height

[Figure 3-9](#) and [Figure 3-10](#) show the effects of the different filter response settings on the same peak. [Figure 3-9](#) shows the effects of the different response settings when using the RC Filter. [Figure 3-10](#) shows the effects of the different response settings when using the Digital Filter. Although the peak shape is different in each case, the integrated peak area is the same.

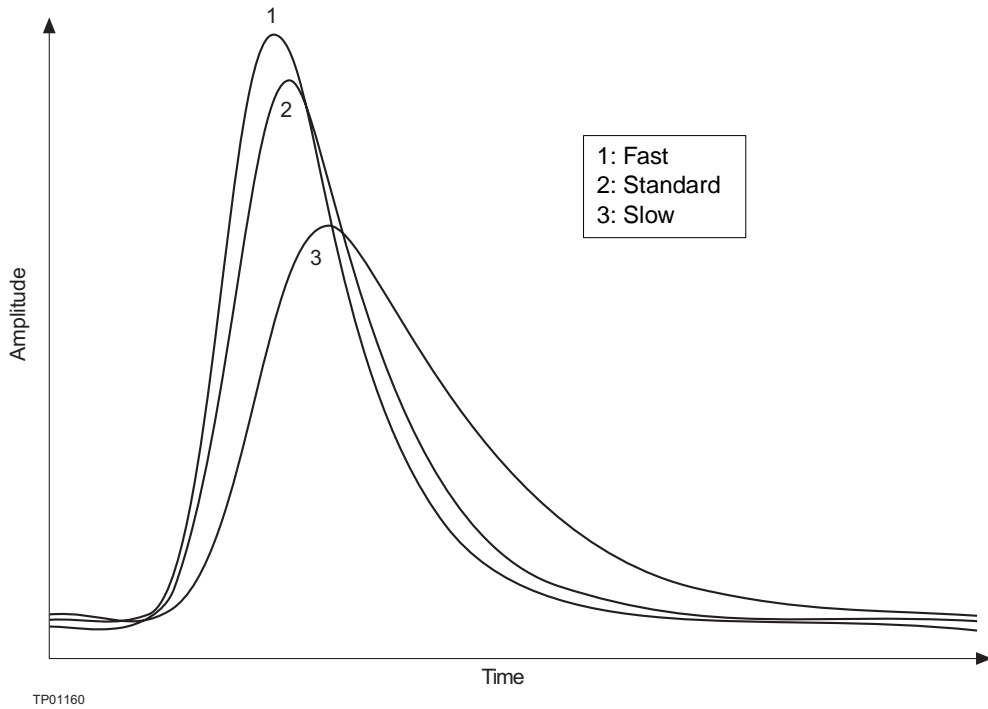


Figure 3-9 Effect of Different Settings in the RC Filter Mode

RC Filter Mode

If you use the RC filter method, you can select FST, STD, or SLW, as appropriate, for the peak width and the degree of noise you expect in the signal.

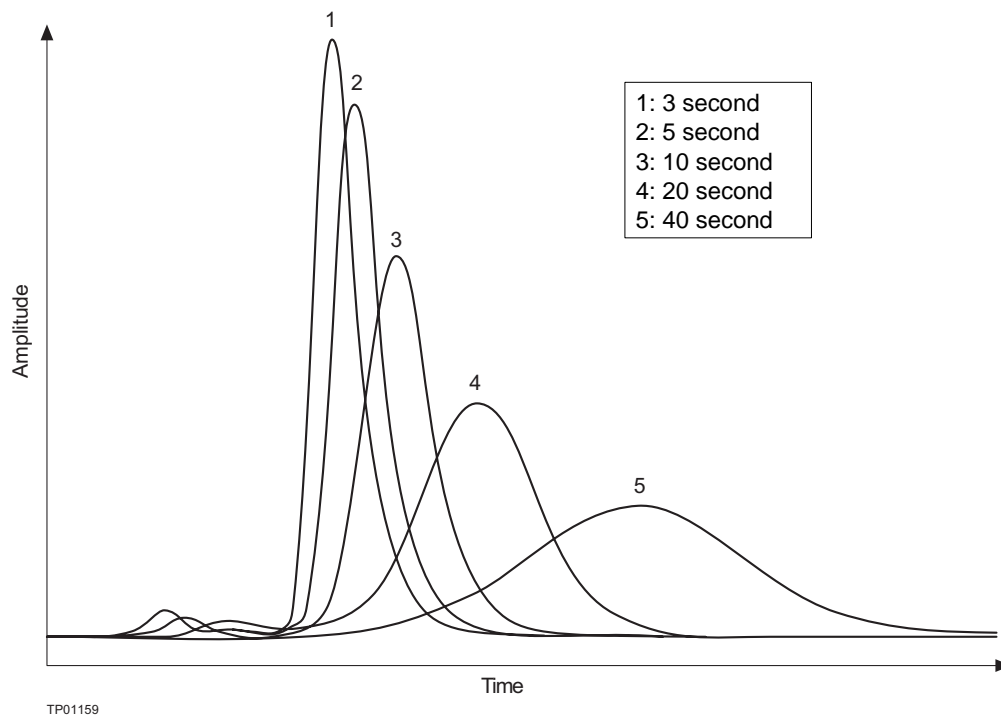


Figure 3-10 Effect of Different Settings in the Digital Filter Mode

Digital Filter Mode

If you use the digital filter method, you can select 3 s, 5 s, 10 s, 20 s, or 40 s, as appropriate, for the peak width and the degree of noise you expect in the signal.

The digital filter method requires approximately three times the amount of time of the Filter Response setting before the filter effect takes place.

The output signal on the Int (+ and –) and Rec (+ and –) terminals is delayed an amount of time approximately 1.5 times the length of the Filter Response setting. For example, with a Filter Response setting of 5 sec, the peak elution time signal is delayed by approximately 8 sec. If you are detecting peaks, be sure to keep the other parameters constant.

3.5.6 Auto Zero Enable

The Auto Zero Enable parameter allows you to specify whether an auto zero is performed automatically after you change a parameter. The auto zero sets the analog output voltage on the Rec (+ and –) and the Int (+ and –) terminals to zero.

You can select one of two settings for this parameter:

- Auto
- Manual

Automatic Auto Zero

If you select Auto, the 474 performs an auto zero every time the following parameters change (manually or in a time program):

- Excitation or emission wavelength
- Gain
- Mark In (on rear panel)
- Auto Zero In (on rear panel)
- Spectrum bandwidth

If you are performing repeated analyses with an autosampler, the 474 automatically performs an auto zero when a mark is added by the autosampler with the Mark In signal.

Manual Auto Zero

If you select Manual, an auto zero is not performed whenever a parameter is changed. The manual mode has the following advantages over the automatic mode:

- If you change the wavelength or gain during a peak, the signal does not go to zero.
- The chromatogram has no steps caused by a Mark In signal.
- The wavelength at which the signal is greatest is easy to find.

[Table 3-6](#) illustrates the effect of each mode during a wavelength change or a Mark In signal.

Table 3-6 Effects of Manual and Automatic Auto Zero Settings

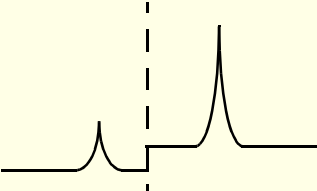
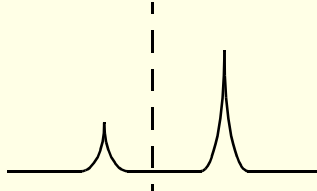
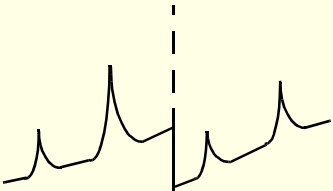
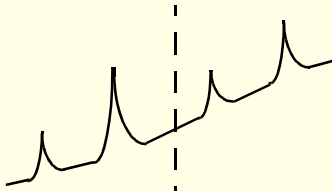
Parameter Changed	Auto Zero Setting	
	Auto	Manual
Wavelength Change		

Table 3-6 Effects of Manual and Automatic Auto Zero Settings (Continued)

Parameter Changed	Auto Zero Setting	
	Auto	Manual
Marker In		

Selecting the Auto Zero Setting

To select the auto zero mode:

1. Press the **Shift** and **Atten/4** keys. [Figure 3-11](#) shows the display screen for this parameter.

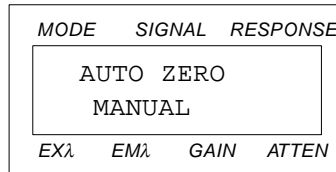


Figure 3-11 Auto Zero Selection Screen

2. Press the **Edit/Enter** key. The current setting flashes on and off.
3. Use the **▲** and **▼** keys to toggle between the Manual and Auto settings.
4. Press the **Edit/Enter** key to enter your selection.
5. Press the **Monitor** key to return to the Normal mode.

3.5.7 Offset

Most integrators can accept an input voltage between -10 mV and $+2$ V. This means that the integrator can not tolerate negative inputs. The Offset special function allows you to shift the voltage on the 474 Int (+ and -) terminals to compensate for negative drift, thus preventing the output from going below the -10 mV limit.

Note: Shifting the zero point does not affect the voltage on the Rec (+ and -) terminals.

The integrator noise level is not affected by shifting the zero point. As the offset setting increases:

- The dynamic range decreases
- The signal resolution of the integrator becomes smaller
- The visible baseline noise becomes larger

You can shift the zero point by 0 mV, 5 mV, 10 mV, 50 mV, or 100 mV.

Selecting the Offset

To select the Offset parameter:

1. Press the **Shift** key and then the **Resp/5** key. [Figure 3-12](#) shows the display screen for this parameter.

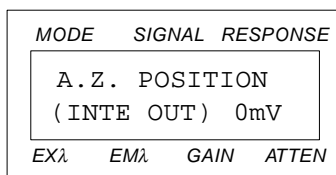


Figure 3-12 Offset Selection Screen

2. Press the **Edit/Enter** key. The current setting flashes on and off.
3. Use the **▲**, **▼**, or numeric keys to select the offset. If you make a mistake, use the **Clear** key and re-enter the offset.
4. Press the **Edit/Enter** key to enter the offset.
5. Press the **Monitor** key to return to the Normal mode.

3.5.8 Polarity

The Polarity parameter allows you to select the polarity of the analog voltage on the Rec (+ and -) terminals.

Note: Changing the polarity does not affect the voltage on the Int (+ and -) terminals.

[Figure 3-13](#) shows the effect on a chromatogram of changing the polarity.

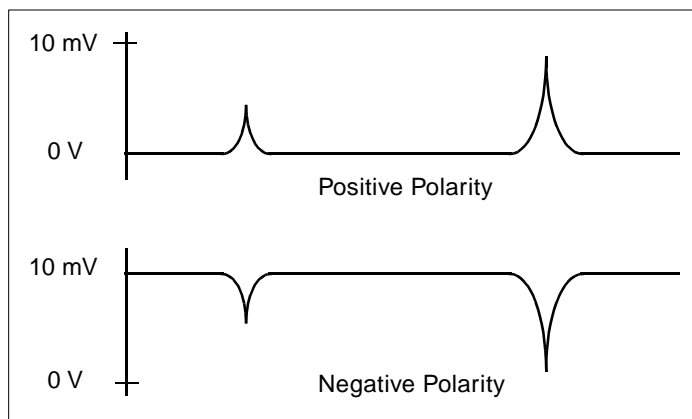


Figure 3-13 Effect of Changing the Polarity Parameter

Selecting the Polarity

To select the polarity:

1. Press the **Shift** and **A.Z./6** keys. [Figure 3-14](#) shows the display screen for this parameter.

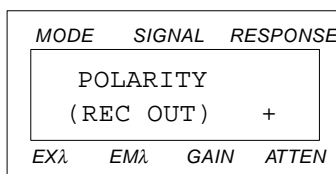


Figure 3-14 Polarity Selection Screen

2. Press the **Edit/Enter** key. The current setting flashes on and off.
3. Use the **▲** or **▼** key to select the polarity.
4. Press the **Edit/Enter** key to enter the polarity.
5. Press the **Monitor** key to return to the Normal mode.

3.5.9 Lamp Hours

The Lamp Hours parameter displays the total number of hours the lamp has been lit. Use this parameter to help determine when the lamp needs to be replaced.

Lamp Life Span

The useful life of the xenon lamp in the 474 is approximately 1000 hours. After 1000 hours, the lamp output gradually diminishes, causing lower signal response. Waters recommends that you change the lamp annually or when noise adversely affects chromatography and peak detection. [Section 6.5, Changing the Lamp](#), contains the procedure for changing the lamp.

Resetting the Lamp Hours

Reset the lamp hours to zero only when you replace the lamp. To reset the lamp hours:

1. Press the **Shift** and **EM SP/7** keys. [Figure 3-15](#) shows the display screen for this parameter.

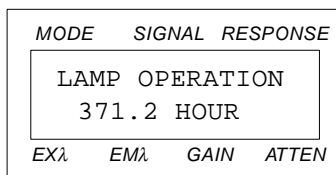


Figure 3-15 Lamp Hours Display Screen

2. Press the **Edit/Enter** key. The current setting flashes on and off.
3. Press the **0**, **.**, and **0** keys to enter 0.0. If you make a mistake, press the **Clear** key and re-enter 0.0.
4. Press the **Edit/Enter** key to enter the setting.
5. Press the **Monitor** key to return to the Normal mode.

3.5.10 Bandwidth

Use the Bandwidth parameter to set the bandwidth of the emission monochromator. The sensitivity and wavelength selectivity of the 474 can in some cases be improved by changing the bandwidth.

Background

The excitation bandwidth is determined by the width of a slit in a shutter (the excitation monochromator) in the light path between the lamp and the flow cell. The emission bandwidth is determined by a slit in a second shutter (the emission monochromator) in the light path between the flow cell and the emission monochromator.

The excitation bandwidth is fixed at 18 nm. The emission bandwidth can be set to 10, 18, or 40 nm. The default emission bandwidth slit is 18 nm.

Adjusting Emission Bandwidth

Adjust the emission bandwidth under the following conditions:

- If the fluorescence wavelength is close to the excitation wavelength, reduce the excitation light and scattered light by setting the emission bandwidth parameter to 10 nm. This improves the selectivity of the measurement.
- When a single component is sufficiently separated from other peaks, set the emission bandwidth parameter to 40 nm. This increases the signal from the fluorescence and increases the sensitivity of the measurement.

Selecting the Bandwidth

To select the bandwidth:

1. Press the **Shift** and **Slit/9** keys. [Figure 3-16](#) shows the display screen for this parameter.

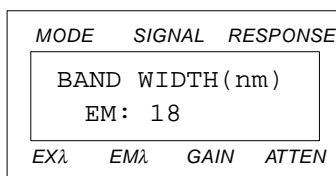


Figure 3-16 Bandwidth Selection Screen

2. Press the **Edit/Enter** key. The current setting flashes on and off.
3. Use the **▲** or **▼** keys to select 10, 18, or 40.
4. Press the **Edit/Enter** key to enter the bandwidth.
While the slit is changing, the display reads **NOW SETTING**. This operation takes up to 16 seconds.
5. Press the **Monitor** key to return to the Normal mode.

Note: While the Now Setting message is displayed, the Integrator Output is held at the current level. When the correct slit is in place, the 474 performs an automatic auto zero (if enabled) and then continues operating.

3.5.11 Wavelength Range

Use the Wavelength Range parameter to set the Excitation and Emission wavelength ranges.

Two wavelength range selections are available:

- EM > EX+10
- Not Restricted

The EM > EX+10 setting ensures that the emission wavelength is at least 10 nm greater than the excitation wavelength. It also limits the excitation and emission wavelengths to between 200 and 900 nm.

Zero Order Operation

The Not Restricted setting allows you to select any excitation and emission wavelength between 0 and 900 nm. Waters recommends that you use this setting only when you are using the optional optical filters described in [Appendix B.2, Using the Optional Optical Filters](#).

When the Not Restricted setting is selected, it is possible to set the excitation wavelength equal to the emission wavelength. In this case, the photomultiplier tube is exposed to scattered light from the lamp plus the fluorescence. This increased exposure to light can shorten the life of the photomultiplier tube.



Attention: To prevent deterioration of the photomultiplier tube when using the Not Restricted setting, set the Gain to 1 (see [Table 3-3](#)).

Selecting the Wavelength Range

To select the wavelength range:

1. Press the **Shift** and **Clear** keys. [Figure 3-17](#) shows the display screen for this parameter.

MODE	SIGNAL	RESPONSE
W.L. RANGE CHECK		
EM > EX+10		
EX λ	EM λ	GAIN ATTN

Figure 3-17 Wavelength Range Selection Screen

2. Press the **Edit/Enter** key. The current setting flashes on and off.

3. Use the ▲ or ▼ keys to select either EM > EX+10 or Not Restricted.
4. Press the **Edit/Enter** key to enter your selection.
5. Press the **Monitor** key to return to the Normal mode.

3.5.12 Temperature Correction

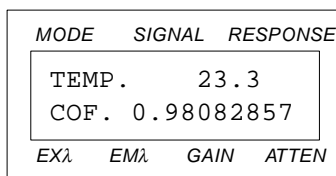
The Temperature Correction function displays the internal temperature (in degrees Centigrade) of the 474 on the top line. The bottom line displays the coefficient used to suppress drift caused by changes in the internal temperature.

The last few digits change randomly; this is normal and shows that the 474 is compensating for changes in temperature.

Displaying the Internal Temperature

To display the temperature correction coefficient:

1. Press the **Shift** and **Chart Marker** keys. [Figure 3-18](#) shows the display screen for this parameter.



MODE	SIGNAL	RESPONSE
TEMP.	23.3	
COF.	0.98082857	
EXλ	EMλ	GAIN ATTEN

Figure 3-18 Temperature Correction Display Screen

The temperature correction coefficient cannot be changed.

2. Press the **Monitor** key to return to the Normal mode.

3.6 Using External Functions

You can integrate the operation of the 474 with the operation of a variety of other HPLC devices. To accomplish this, you need to connect the instruments to the following terminals on the 474 analog connector:

- External Out
- Mark Out
- Cell Leak
- Mark In

- Auto Zero
- Program Reset

Refer to [Section 2.6, Making Connections to Other Devices](#), for instructions on connecting external devices to the 474.

3.6.1 Excitation Energy Out

Use the Ex Out (+ and –) terminals on the analog connector to monitor the excitation energy from the lamp during a scan. The lamp energy is not constant at all wavelengths; the 474 measures fluctuations in the energy using a photodiode in the excitation light path. Control circuits use the signal from the photodiode to compensate for the fluctuations. The Ex Out terminals provide a 1 V full-scale analog signal that corresponds to the signal from the photodiode.

See [Section 2.6.10, Connecting a Voltmeter to the Excitation Out Terminals](#), for instructions on connecting a voltmeter or other device to the Ex Out terminals.

3.6.2 Mark Out

Use the Mark Out (+ and –) terminals on the analog connector to send a temporary 1- to 3-mV marker signal to an external device. The marker is sent when either of the following events occur:

- The Chart Mark key on the front panel is pressed
- The Mark In terminals on the rear panel receive a contact closure signal

You can use this marker signal to automatically start an external device such as an integrator. During spectrum outputs, chart marks automatically appear every 100 nm on the Mark Out and Rec Out terminals; see [Section 5.3, Spectrum Output](#).

3.6.3 Cell Leak

The Cell Leak (+ and –) terminals on the analog connector provide a contact closure signal if a leak is detected inside the flow cell. You can use these terminals to trigger a remote alarm.

Note: *The Cell Leak sensor works only when you are using conductive mobile phases*

If a leak occurs, the 474 stops all operations and the display appears as shown in [Figure 3-19](#).

MODE	SIGNAL	RESPONSE
TROUBLE		
LEAK IN CELL		
EXλ	EMλ	GAIN ATTEN

Figure 3-19 Error Message for a Cell Leak

Refer to [Section 7.1, Error Messages](#), for procedures on correcting the leak.

See [Section 2.6.3, Connecting the 474 to the Waters 2690 Separations Module](#) and [Section 2.6.4, Connecting the 474 to the Waters 600 Series Controller](#), for instructions on connecting an external device to the Cell Leak terminals.

3.6.4 Mark In

A contact closure signal between the Mark In (+) and the (–) terminal performs the same function as pressing the Chart Mark key. An external device (such as an autosampler) can provide this signal.

A contact closure on this terminal momentarily increases the voltage on the Rec (+ and –) terminals by 1 to 3 mV. This places a chart mark on the chart recorder graph.

3.6.5 Auto Zero In

A contact closure signal between the AZ In (+) and the (–) terminal performs the same function as pressing the Auto Zero key. An external device (such as an autosampler) can provide this signal.

A contact closure on this terminal sets the analog voltage on the Rec (+ and –) and Int (+ and –) terminals on the analog connector to zero.

3.6.6 Program Reset

A contact closure signal between the Program Reset (+) and the (–) terminal causes the following actions:

- Stops a currently-running time program
- Resets the initial conditions
- Restarts the currently-selected time program

3.7 Calibration Check

Two procedures check the calibration of the 474:

- Raman band of water test
- Signal-to-noise test

3.7.1 Excitation Wavelength Accuracy (Raman Band of Water)Test

The Excitation wavelength accuracy (Raman band of water) test checks the calibration of the 474 excitation monochromator. Water emits light at a wavelength peak of approximately 397 nm when the wavelength of the excitation light is 350 nm. Water can be used to check the accuracy of the monochromator by looking for a peak in the emission scan at 397 nm.

Procedure

To perform the excitation wavelength accuracy test:

1. Flush the flow cell with approximately 5 mL of HPLC-quality water.

Note: *In order for the test to be valid, the flow cell must be absolutely clean and the water must be HPLC-quality.*

2. Connect a chart recorder to the Rec (+ and –) terminals, using the procedure in [Section 2.6.6, Connecting the 474 to a Chart Recorder](#). Set the chart speed to approximately 10 cm/min.
3. Enter the following parameter values in the 474:
 - Ex λ = 350 nm
 - Em λ = 397 nm
 - Gain = 1000
 - Atten = 32
 - Response = Standard
4. Press the **Scan** key to enter the Scan mode.
5. Press the **Edit/Enter** key. The display appears as shown in [Figure 3-20](#) with the Memory Location field flashing on and off.

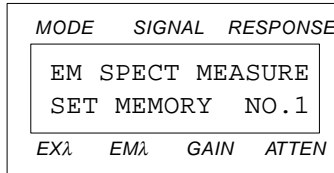


Figure 3-20 Entering the Emission Spectrum Memory Location

Selecting the Memory Location

6. Use the numeric keys to select the emission memory location you want to use. Record this number.
7. Press the **Edit/Enter** key to enter your selection. The display appears as shown in [Figure 3-21](#) with the Excitation Wavelength field flashing on and off.

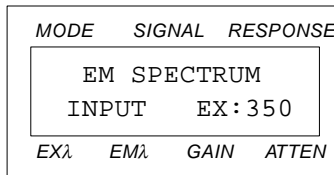


Figure 3-21 Entering the Excitation Wavelength for an Emission Spectrum

Selecting the Excitation Wavelength

8. Press the **3**, **5**, and **0** keys to enter 350 nm.
9. Press the **Edit/Enter** key to enter your selection. The display appears as shown in [Figure 3-22](#).

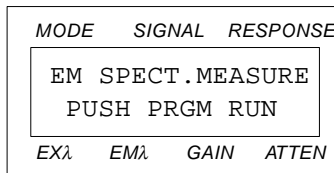


Figure 3-22 Prompt to Begin an Emission Spectrum Scan

10. Press the **Program Run** key. The lower line of the display changes to `RUNNING`. When the scan is complete, the spectrum is stored in the location you selected in step 3 above. The display appears as shown in [Figure 3-20](#).
11. Press the **Monitor** key to return to the Scan mode.

Outputting the Scan

12. Use the ▲ or ▼ keys or press the **Gain/3** key to select the EM D.OUT parameter. The display appears as shown in [Figure 3-23](#).

MODE	SIGNAL	RESPONSE
SPECTRUM MEASURE		
No. 3 EM D.OUT		
EXλ	EMλ	GAIN ATTN

Figure 3-23 Starting an Emission Spectrum Output

13. Press the **Edit/Enter** key. The display appears as shown in [Figure 3-24](#) with the Memory Location field flashing on and off.

MODE	SIGNAL	RESPONSE
EM SPECT NO. 0		
EX : 350 nm		
EXλ	EMλ	GAIN ATTN

Figure 3-24 Entering the Emission Spectrum Memory Location

Accessing the Stored Spectrum

14. Use the numeric keys to select the excitation memory location you used in step 6. The lower line displays the wavelength of the excitation spectrum that was used when the spectrum was stored.
15. Press the **Edit/Enter** key to enter your selection. The display appears as shown in [Figure 3-25](#) with the Start field flashing on and off.

MODE	SIGNAL	RESPONSE
EM SPECT. EX:350		
STRT:360 END:700		
EXλ	EMλ	GAIN ATTN

Figure 3-25 Entering the Start and End Wavelengths in the Emission Spectrum

Selecting Starting and Ending Wavelength

The Range setting in the Scan Parameter determines the minimum starting and maximum ending wavelength of the stored spectrum. You cannot enter a starting wavelength below the minimum or an ending wavelength greater than the maximum. See [Section 5.1, Scan Parameters](#), for details.

16. Press the **3**, **6**, and **0** keys to select a starting wavelength of 360 nm.
17. Press the **Edit/Enter** key to enter your selection. The End field flashes on and off.
18. Press the **7**, **0**, and **0** keys to select an ending wavelength of 700 nm.
19. Press the **Edit/Enter** key to enter your selection. The display appears as shown in [Figure 3-26](#).

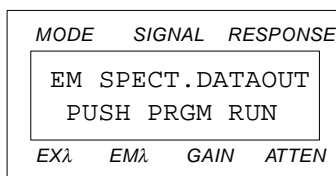


Figure 3-26 Prompt to Start Output of a Spectrum

Start the Output

20. Press the **Program Run** key. When the output of the spectrum is complete, the display appears as shown in [Figure 3-24](#).

Check that the resulting plot is similar to the one shown in [Figure 3-27](#). If the plot goes off-scale, change the value in the Atten parameter to **64** and run the scan again. A chart mark appears at each 100 nm point.

If the peak occurs at 397 nm \pm 2 nm, the test is successful.

If the peak fall outside this range, the optics bench is faulty. If this occurs, contact Waters service.

21. Press the **Monitor** key to return to the Scan mode. Proceed to [Section 3.7.2, Signal-to-Noise Test](#).

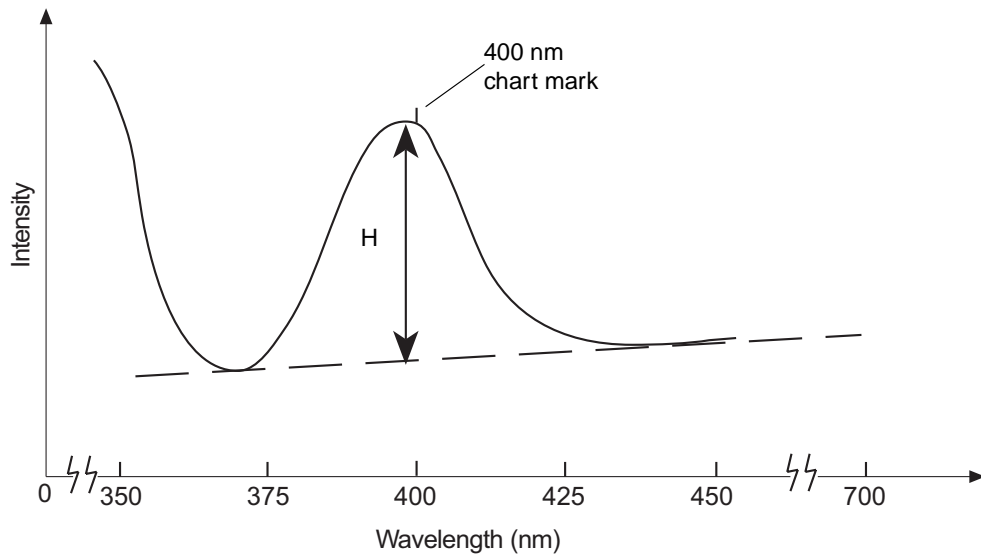


Figure 3-27 Typical Raman Band Test Results

3.7.2 Signal-to-Noise Test

Perform the signal-to-noise test in conjunction with the Excitation wavelength accuracy (Raman band of water) test. The signal-to-noise test shows the long-term noise occurring over the range of wavelengths used in the Excitation wavelength accuracy (Raman band of water) test.

1. With Milli-Q or equivalent water still in the flow cell, enter the following parameters:
 - Ex λ = 350 nm
 - Em λ = 397 nm
 - Atten = 1
 - Gain = 1000
 - Filter = Standard
2. Adjust the recorder pen to mid-scale and record the plot for 30 minutes. [Figure 3-28](#) shows a typical noise plot.
3. Measure the width (W) of the maximum pen deflection as shown in [Figure 3-28](#).

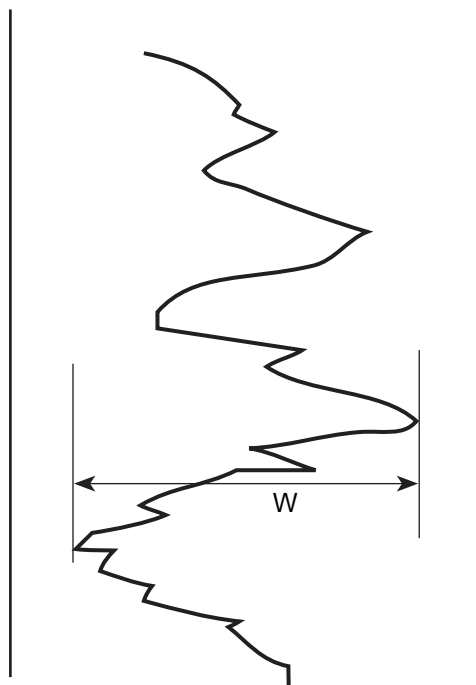


Figure 3-28 Measuring the Maximum Pen Deflection

4. Draw a line tangent to the lowest two points on the Raman curve, as shown in [Figure 3-27](#).
5. Draw a vertical line from the peak to the tangent line.
6. Measure the line. The length is the height (H) of the Raman peak.
7. Insert these values into the following equation to obtain the signal-to-noise (S/N) ratio.

$$S/N = (H \times \text{Attenuation}) / W$$
8. The minimum acceptable S/N ratio is 320.

3.8 Powering Off

Waters recommends that you power off the 474 when you do not plan to use it within the next four hours.

To power off the 474, press the Power switch to the Off (⏻) position. The current parameter settings and all programs are stored until the power is turned on again.

Considerations

Be aware of the following considerations when you power off the 474 for a period of time:

- Do not leave buffered solvent in the flow cell.
- Clean the lines completely by flushing them with filtered HPLC-quality water.
- Fill the lines with water containing 10 percent methanol or other miscible organic solvent.

4

Programming the 474 Detector

Overview

The 474 features a Program mode that allows you to set up and run automatic, time-based analysis sequences of up to a maximum of 64 steps. You can store up to ten of these sequences, called *time programs*, in the 474 nonvolatile memory. Time programs are not erased when you turn off the power.

This chapter describes how to use the Program mode to:

- Write a time program
- Run a previously stored time program
- Modify an existing time program

4.1 Using the Program Mode

Use the Program mode when you want to perform one or more of the following actions automatically during a run:

- Change the excitation or emission wavelength as a function of the elution time
- Use the auto zero
- Change the gain
- Change the attenuation
- Change the response
- Store an excitation or emission spectrum
- Change the emission bandwidth

Entering the Program Mode

To change to the Program mode from the Normal mode, press the **Program** key. To return to the Normal mode, press the **Program** key again.

The Program mode has two display screens:

- Time screen ([Figure 4-1](#))
- Fluorescence intensity screen ([Figure 4-2](#))

Press the **Monitor** key to switch between the two screens.

<i>MODE</i>	<i>SIGNAL</i>	<i>RESPONSE</i>	
PRG1	MON T:	0.0	
300	500	100	16
<i>EXλ</i>	<i>EMλ</i>	<i>GAIN</i>	<i>ATTEN</i>

Figure 4-1 Typical Time Screen in the Program Mode

<i>MODE</i>	<i>SIGNAL</i>	<i>RESPONSE</i>	
PRG1	0.0000	STD	
300	500	100	16
<i>EXλ</i>	<i>EMλ</i>	<i>GAIN</i>	<i>ATTEN</i>

Figure 4-2 Typical Fluorescence Intensity Screen in the Program Mode

Upper Line of Display

In both Program mode screens, *PRG1* in the upper line of the display indicates that program 1 is selected. The two screens differ in the remaining part of the upper line.

- In [Figure 4-1](#), *MON T: 0.0* indicates the elapsed monitored time in minutes and tenths.
- In [Figure 4-2](#), *0.0000* indicates the fluorescence intensity and *STD* indicates the filter response setting.

Lower Line of Display

The lower line in both figures contains the same information:

- 300** – The excitation wavelength
- 500** – The emission wavelength
- 100** – The gain setting
- 16** – The attenuation setting

4.2 Writing a Multi-Step Time Program

You can store up to ten time programs in locations 0 through 9 using the Program Number special function. The 474 stores the programs in memory that is not erased when power is turned off.

Overview

Before you start to write a time program, make a list of what events must occur and when they must occur during a run. This sequence of events determines the structure of the time program.

Write the time program in steps. Each step specifies the settings for certain parameters and the time during which those parameter settings remain in effect.

You can specify the settings of the following parameters in a time program:

- Time
- Excitation and emission wavelengths
- Gain
- Attenuation
- Response
- Auto Zero
- Excitation and emission spectra
- Emission bandwidth

To write a time program:

1. Select the memory location you want to store your program in using the Program Number special function (see [Section 3.5.1, Program Number](#)).
2. Set the initial conditions. These conditions define the 474 setup when the time program begins. See [Section 4.2.1, Setting the Initial Conditions of a Time Program](#).
3. Select the parameter settings and time for each event. You can program up to 64 events. Do not attempt to program more than 64 events. See [Section 4.2.2, Setting Steps in a Time Program](#).

[Figure 4-3](#) is a flowchart of the steps in writing a program.

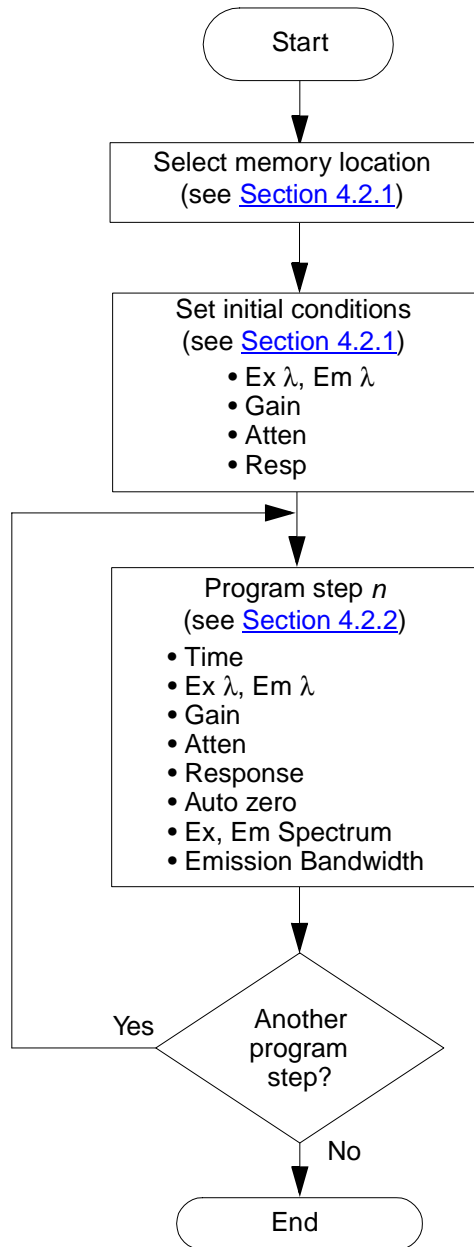


Figure 4-3 Flowchart for Writing a Time Program

4.2.1 Setting the Initial Conditions of a Time Program

The initial conditions of a time program specify the parameter values you want when the program starts. These values remain in effect until they are changed in a subsequent program step.

You set the initial conditions by setting parameter values with the initial parameters display screen. [Figure 4-4](#) shows a typical display for the initial conditions. The values you see depend on the previous settings.

<i>MODE</i>	<i>SIGNAL</i>	<i>RESPONSE</i>	
P1	ST 0	INIT	FST
400	500	100	32
<i>EXλ</i>	<i>EMλ</i>	<i>GAIN</i>	<i>ATTEN</i>

Figure 4-4 Typical Initial Parameter Settings

Upper Line of Display

The upper line of the display in [Figure 4-4](#) includes the following information:

- P1** – Program stored in program memory 1
- ST 0** – Initial step of the program
- INIT** – Initial parameters
- FST** – Filter response setting

Lower Line of Display

The lower line of the display shows the current values for the following parameters:

- 400** – Excitation wavelength
- 500** – Emission wavelength
- 100** – Gain
- 32** – Attenuation

Entering Initial Conditions

To set the initial conditions of a time program:

1. Check that the 474 is in the Program mode. If it is not, press the **Program** key.

Selecting the Program Number

2. Press the **Shift** and the **Program** keys to select the Program Number special function. If the desired program number is currently selected, skip to step 6 of this procedure.

3. Press the **Edit/Enter** key. The Program Number field flashes on and off.
4. Use the **▲**, **▼**, or numeric keys to select the number of the program that you want.
5. Press the **Edit/Enter** key to enter your selection.
6. Press the **Monitor** key to return to the time or intensity display.
7. Press the **▼** key. The display changes to the initial parameter setting screen as shown in [Figure 4-4](#).
8. Press the **Edit/Enter** key. The Excitation Wavelength field flashes on and off.

Selecting the Excitation Wavelength

9. Use the numeric keys to select the excitation wavelength.
10. Press the **Edit/Enter** key to enter your selection. The Emission Wavelength field flashes on and off.

Selecting the Emission Wavelength

11. Use the numeric keys to select the emission wavelength.
12. Press the **Edit/Enter** key to enter your selection. The Gain field flashes on and off.

Selecting the Gain

13. Use the **▲**, **▼**, or numeric keys to select the gain.
14. Press the **Edit/Enter** key to enter your selection. The Attenuation field flashes on and off.

Selecting the Attenuation

15. Use the **▲**, **▼**, or numeric keys to select the attenuation.
16. Press the **Edit/Enter** key to enter your selection. The Response field flashes on and off.

Selecting the Response

17. Use the **▲**, **▼**, or numeric keys to select the response.
You can select the response times for the filter mode you selected in the Signal Filter special parameter. To change the filter type, see [Section 3.5.5, Signal Filter](#). You cannot change the filter mode within a time program.
18. Press the **Edit/Enter** key to enter your selection.
19. The initial conditions are now complete. If you find any errors, start again at step 1 of this procedure.

Note: You can press the **Edit/Enter** key repeatedly until the parameter you want to change is flashing on and off. Make the correction and press **Edit/Enter** again until no parameters are flashing on and off.

4.2.2 Setting Steps in a Time Program

Overview

Each step in a time program specifies a parameter that changes and the time at which the parameter changes.

You enter the parameters for the first step of a time program by pressing the ▼ key when the initial conditions screen is displayed ([Figure 4-4](#)). [Figure 4-5](#) shows the display for the first step in a new time program.

MODE	SIGNAL	RESPONSE
P1	ST 1	T: 0.0
EXλ	EMλ	GAIN ATTEN

Figure 4-5 First Step of a New Time Program

If a program is stored in the memory number you selected, the display appears as shown in [Figure 4-6](#).

MODE	SIGNAL	RESPONSE
P1	ST 1	T: 1.0
EX: 310	EM: 500	
EXλ	EMλ	GAIN ATTEN

Figure 4-6 Typical First Step of an Existing Time Program

See [Section 4.4, Modifying a Time Program](#), for details on modifying an existing program. If you do not want to change the existing program, select another memory location using the Program Number special parameter.

You enter the parameters for subsequent steps of a time program by pressing the ▼ key when you have completed the current step.

Entering Step Parameters

To enter a step in a new time program:

1. With the initial conditions screen or the previous step displayed, press the ▼ key. An empty program step is displayed on the screen, as shown in [Figure 4-7](#).

MODE	SIGNAL	RESPONSE
P1	ST	2 T: 0.0
EXλ	EMλ	GAIN ATTN

Figure 4-7 Typical Empty Program Step

2. Press the **Edit/Enter** key. The value in the Time field flashes on and off.
3. Use the numeric keys to select the time when the change takes place. Enter this number in minutes and tenths.
4. Press the **Edit/Enter** key to enter your selection. The display appears as shown in [Figure 4-8](#).

MODE	SIGNAL	RESPONSE
P1	ST	1 T: 0.5
No.1	EX,EM	W.L.
EXλ	EMλ	GAIN ATTN

Figure 4-8 First Line of the Program Menu

Program Menu

The lower line of the display shows the first line of the Program menu. [Table 4-1](#) lists the parameters and the parameter settings that you can use in each program step.

Table 4-1 Program Menu

Parameter Number	Parameter	Description	Valid Ranges
1	EX, EM W.L.	Excitation and emission wavelength	200 to 900 nm ¹
3	GAIN	Gain	1, 10, 100, 1000
4	ATTEN	Attenuation	1, 2, 4, 8, 16, 32, 64, 128, 256, S
5	RSPNS	Response	Slow, Standard, Fast
6	A.Z.	Auto zero	On or off
7	EM SPECT	Emission spectrum	See Section 4.5, Performing a Wavelength Scan in a Time Program
8	EX SPECT	Excitation spectrum	See Section 4.5, Performing a Wavelength Scan in a Time Program
9	B. WIDTH	Emission bandwidth	10, 18, 40

¹ Wavelength is limited by the Wavelength Range parameter. See [Section 3.5.11, Wavelength Range](#).

5. Use the ▲, ▼, or numeric keys to select the parameter number that you want to use in the program step.
6. Press the **Edit/Enter** key to enter your selection.
7. Use the ▲, ▼, or numeric keys to select the appropriate parameter value.
8. Press the **Edit/Enter** key to enter your selection.
9. Press the ▼ key to move to the next program step.

Repeat this procedure until all steps are entered.

4.2.3 Saving the Time Program

Press the **Monitor** key when you are done entering the program. This returns the 474 to the Program mode and also stores the program you just wrote into the nonvolatile memory.

As you enter each step in the time program, the 474 stores the settings in temporary memory. If power is interrupted before you press the **Monitor** key, or if you forget to press the **Monitor** key before you power off, the program is deleted.

4.2.4 Example Time Program

This section contains an example time program that illustrates the procedure for writing a time program. The example program is summarized in [Table 4-2](#).

Table 4-2 Example Program

Step	Time (minutes)	Description
0	0.0	Set initial conditions: <ul style="list-style-type: none">• Ex λ to 355• Em λ to 512• Gain to 10• Atten to 1• Resp to Fst
1	0.5	Change Ex λ to 310 nm Change Em λ to 500 nm
2	2.0	Change gain to 1
3	2.0	Perform an auto zero
4	3.0	Record an emission spectrum

Setting Initial Conditions for the Example Program

To set the initial conditions for the example program, access the initial parameter setting screen ([Figure 4-4](#)).

The initial conditions for the example time program are as follows:

- Excitation wavelength: 355
- Emission wavelength: 512
- Gain setting: 10
- Attenuation setting: 1
- Response setting: Fst

To set the initial conditions for the example program:

1. Press the **Edit/Enter** key. The Excitation Wavelength field flashes on and off.

Setting the Excitation Wavelength

2. Press the **3**, **5**, and **5** keys to select an excitation wavelength of 355 nm.
3. Press the **Edit/Enter** key to enter this value. The Emission Wavelength field flashes on and off.

Setting the Emission Wavelength

4. Press the **5**, **1**, and **2** keys to select an emission wavelength of 512 nm.
5. Press the **Edit/Enter** key to enter this value. The Gain field flashes on and off.

Setting the Gain

6. Use the **▲** or **▼** keys, or press the **1** and the **0** keys to select a gain of 10.
7. Press the **Edit/Enter** key to enter this value. The Attenuation field flashes on and off.

Setting the Attenuation

8. Use the **▲** or **▼** keys, or press the **1** key to select an attenuation of 1.
9. Press the **Edit/Enter** key to enter this value. The Response field flashes on and off.

Setting the Response

10. Use the **▲** or **▼** keys to select the Fst response.

If you cannot select the Fst setting, change the Signal Filter special parameter to RC using the procedure in [Section 3.5.5, Signal Filter](#).

11. Press the **Edit/Enter** key to enter this value.
12. The display appears as shown in [Figure 4-9](#). If you made a mistake, start again at step 1 of this procedure. This example program is continued in the next section.

MODE	SIGNAL	RESPONSE
P1	ST 0	INIT FST
355	512	10 1
EXλ	EMλ	GAIN ATTEN

Figure 4-9 Initial Conditions of the Example Time Program

Step 1 of Example Program

The first step of the example time program (see [Table 4-2](#)) takes place at 0.5 minutes, and changes the excitation and emission wavelengths as follows:

- Excitation wavelength changes to 310 nm
- Emission wavelength changes to 500 nm

To set the parameters for the first program step:

1. With the initial conditions screen of the example time program displayed, press the ▼ key to access the first program step.
2. Press the **Edit/Enter** key. The value in the Time field flashes on and off.
3. Press the **0**, **.**, and **5** keys to select a time of 0.5 minutes.
4. Press the **Edit/Enter** key. The display shows the first line of the program menu ([Figure 4-8](#)).
5. Press the **Edit/Enter** key again to select the Ex, Em W.L. parameters. The value in the Excitation Wavelength field flashes on and off.
6. Press the **3**, **1**, and **0** keys to select a wavelength of 310 nm. If you make a mistake, press the **Clear** key to reset the wavelength. Re-enter the correct value.
7. Press the **Edit/Enter** key to enter this value. The value in the Emission Wavelength field flashes on and off.
8. Press the **5**, **0**, and **0** keys to select a wavelength of 500 nm. If you make a mistake, press the **Clear** key to reset the wavelength. Re-enter the correct value.
9. Press the **Edit/Enter** key to enter this value. The display appears as shown in [Figure 4-10](#).

MODE	SIGNAL	RESPONSE
P1	ST 1	T: 0.5
EX: 310	EM: 500	
EXλ	EMλ	GAIN ATTN

Figure 4-10 Step 1 of the Example Time Program

Step 2 of Example Program

The second step of the example time program takes place at 2.0 minutes and changes the gain to 1 (see [Table 4-2](#)).

To set the parameters for step 2 of the example program:

1. With program step 1 displayed on the screen, press the ▼ key to access program Step 2.
2. Press the **Edit/Enter** key. The value in the Time field flashes on and off.
3. Press the **2** key to select a time of 2.0 minutes.
4. Press the **Edit/Enter** key. The display shows the first line of the program menu.
5. Press the ▼ key, or press the **Gain/3** key to select the gain screen.
6. Press the **Edit/Enter** key. The value in the Gain field flashes on and off.
7. Press the **1** key to select a gain of 1.
8. Press the **Edit/Enter** key to enter this value. The display appears as shown in [Figure 4-11](#).

MODE	SIGNAL	RESPONSE
P1 ST	2 T:	2.0
GAIN		1
EXλ	EMλ	GAIN ATTN

Figure 4-11 Step 2 of the Example Time Program

Step 3 of Example Program

The third step of the example time program also takes place at 2.0 minutes, and performs an auto zero (see [Table 4-2](#)).

To set the parameters for step 3 of the example program:

1. With program step 2 displayed on the screen, press the ▼ key to access the next program step.
2. Press the **Edit/Enter** key. The value in the Time field flashes on and off.
3. Press the **2** key to select a time of 2.0 minutes.
4. Press the **Edit/Enter** key. The display shows the first line of the program menu.
5. Press the **A.Z./6** key, or press the ▼ key four times to select the Auto Zero screen.
6. Press the **Edit/Enter** key. The display appears as shown in [Figure 4-12](#).

MODE	SIGNAL	RESPONSE
P1 ST	3 T:	3.0
AUTO ZERO		
EXλ	EMλ	GAIN ATTEN

Figure 4-12 Step 3 of the Example Time Program

Step 4 of Example Program

The fourth step of the example time program takes place at 3.0 minutes, and stores an emission spectrum (see [Table 4-2](#)). The emission spectrum parameter is explained in detail in [Section 4.5, Performing a Wavelength Scan in a Time Program](#).

To set the parameters for step 4 of the example program:

1. With program step 3 displayed on the screen, press the ▼ key to access the next program step.
2. Press the **Edit/Enter** key. The value in the Time field flashes on and off.
3. Press the **3** key to select a time of 3.0 minutes.
4. Press the **Edit/Enter** key. The display shows the first line of the program menu.
5. Press the **EM SP/7** key, or press the ▼ key five times to select the Emission Spectrum screen.
6. Press the **Edit/Enter** key. The value in the W: field flashes on and off. The value in this field is the interval on either side of the value in the time field (the elution time, in this case 3.0 minutes) during which a peak is expected.
7. Press the **0**, **.**, and **5** keys to select an interval of 0.5 minutes.
A peak detected between 2.75 and 3.25 minutes after the start of the program fulfills the first requirement for storing a scan.
8. Press the **Edit/Enter** key. The value in the L: field flashes on and off. This value in this field sets the level that the fluorescence intensity must exceed for the spectrum to be stored.
9. Press the **7** and **0** keys to select a level of 70.
A peak with a fluorescence intensity above 0.7 and detected between 2.75 and 3.25 minutes fulfills the second requirement for storing a scan.
10. Press the **Edit/Enter** key. The value in the M: field flashes on and off. This value in this field selects the memory location where the spectrum is to be stored.
11. Press the **1** key to select memory location 1.

If a peak that meets the requirements in the L: and W: fields is detected, the 474 stores the scan in memory location 1.

12. Press the **Edit/Enter** key. The display appears as shown in [Figure 4-13](#).
13. Press the **Monitor** key to return to the Program mode. This also saves the program.

MODE	SIGNAL	RESPONSE
P1	ST 4	T: 3.0
7	W:0.5L:70.0M:1	
EXλ	EMλ	GAIN ATTEN

Figure 4-13 Step 4 of the Example Time Program

Running the Example Program

Run the example program from the front panel by following the procedure in [Section 4.3.1, Manual Time Program Start](#).

4.3 Running a Time Program

You can run a time program only when the 474 is in the Program mode. Press the **Program** key to put the 474 into the Program mode.

You can start a time program by one of two methods:

- Manually, using a keystroke
- Externally, using a contact closure signal

4.3.1 Manual Time Program Start

To manually start a time program stored in memory, perform the following steps:

1. Press the **Program** key to put the 474 into the Program mode.
2. Press the **Shift** and **Program** keys to select the Program Number special function.
3. Press the **Edit/Enter** key. The current program number flashes on and off.
4. Use the **▲**, **▼**, or numeric keys to select the program number you desire.
5. Press the **Edit/Enter** key again to enter the program number.
6. Press the **Monitor** key to return to the Program mode.

7. Press the **Program Run** key. The selected program starts running as soon as you press this key.
8. To monitor the progress of the program on the display, press the **Monitor** key to toggle between the monitored time and fluorescence intensity screens.
The Rec (+ and –) and the Int (+ and –) terminals on the analog connector provide an analog output signal that corresponds to the fluorescence intensity.
When the program is finished, the lower line of the display reads PUSH PRGM RUN ([Figure 4-14](#)).
9. Press the **Program Run** key. The 474 returns to the Program mode.

MODE	SIGNAL	RESPONSE
PRG1	0.0000	STD
PUSH PRGM RUN		
EXλ	EMλ	GAIN ATTEN

Figure 4-14 Fluorescence Intensity Screen at the End of a Program Run

4.3.2 External Time Program Start

You can start a time program using an external contact closure signal. Apply the signal between the Prgm Rst (+) and the (–) terminals on the 474 rear panel. To connect the cable, refer to the Method Start discussions in [Section 2.6.3, Connecting the 474 to the Waters 2690 Separations Module](#), and in [Section 2.6.4, Connecting the 474 to the Waters 600 Series Controller](#) to connect the cable.

To start a time program from an external contact closure:

1. Press the **Program** key to enter the Program mode.
2. Press the **Shift** and **Program** keys to select the Program Number special function.
3. Press the **Edit/Enter** key. The current program number flashes on and off.
4. Use the **▲**, **▼**, or numeric keys to select the program number you desire.
5. Press the **Edit/Enter** key again to enter the program number.
6. Press the **Monitor** key to return to the Program mode.

When the contact closure occurs, the time program starts.

If a time program is running when the contact closure occurs:

- The program stops.
- The initial conditions are set.
- The program starts.

7. To monitor the progress of the program on the display, press the **Monitor** key to toggle between the monitored time and fluorescence intensity screens.
The Rec (+ and –) and the Int (+ and –) terminals on the analog connector provide an analog output signal that corresponds to the fluorescence intensity.
When the program is finished, the lower line of the display reads `PUSH PRGM RUN` (Figure 4-14).
8. Press the **Program Run** key. The 474 returns to the Program mode.

4.4 Modifying a Time Program

At times it may be easier to modify an existing program than to write a new program. This section shows you how to:

- Access the correct program number
- Modify a program step
- Add a program step
- Delete a program step
- Delete a group of program steps

4.4.1 Accessing the Program Number

Before making any changes to a program, you must first access the program you want to modify. To access the program you want to modify:

1. Press the **Shift** and **Program** keys to access the Program Number special parameter. This displays the current program number.
2. Press the **Edit/Enter** key to change the program number, if necessary.
3. Press the **▲** or **▼** keys to select the number of the program you want to modify.
4. Press the **Edit/Enter** key again to enter your selection.
5. Press the **Monitor** key. The display shows the initial conditions screen for the selected program.

4.4.2 Modifying a Program Step

You can change the time, the parameter, and/or the parameter values in any program step.

Accessing the Step

To modify a program step:

1. Access the program you wish to modify as described in [Section 4.4.1, Accessing the Program Number](#).
2. Use the ▲ and ▼ keys to move to the program step you wish to change.
Press the Shift and ▼ keys to move to the last step of the program. Press the Shift and ▲ keys to move to the first step of the program.
3. Press the Edit/Enter key. The Time field flashes on and off.
If you wish to change the setting in the time field, continue with step 4. If you do not wish to change the value in the time field, go to step 11.

Changing the Time Value

To change the time value in a program step:

4. Use the numeric keys to select the time when the change is to take place. This number is in minutes and tenths.

Note: If you wish to change the parameter and the time setting, enter the new time setting. If you wish to change only the parameter, enter the same time setting.
5. Press the Edit/Enter key to enter your selection. The display appears as shown in [Figure 4-15](#). This is the first line of the Program menu (see [Table 4-1](#)).

MODE	SIGNAL	RESPONSE
P1	ST 1	T: 0.5
No.1	EX, EM	W.L.
EXλ	EMλ	GAIN ATTN

Figure 4-15 Editing the Parameters in a Time Program

6. Press the ▲ and ▼ keys to select the parameter you wish to use in the program step. [Table 4-1](#) lists the parameters that are available and their valid ranges.
7. Press the Edit/Enter key to enter the parameter you selected.
8. Use the ▲, ▼, or numeric keys to select the appropriate parameter value.
9. Press the Edit/Enter key to enter your selection.

If you are changing parameters in more than one program step, use the ▲, ▼, or numeric keys to select the next step you want to modify and start at step 1 of this procedure.

10. Press the **Monitor** key to store the change(s) and return to the Program mode.

If you changed the time parameter in any step of the program, the 474 automatically rearranges the program steps in the correct time sequence.

Changing a Parameter Value

If you wish to change a parameter value but not the time setting, continue as follows:

11. Press the **Edit/Enter** key. The previously entered parameter is displayed, and the parameter value flashes on and off.
12. Use the ▲, ▼, or numeric keys to select the appropriate parameter value.
13. Press the **Edit/Enter** key to enter your selection.

If you are changing parameters in more than one program step, access the next step you want to modify and start at step 1 of this procedure.

14. Press the **Monitor** key to store the change(s) and return to the Program mode.

If you changed the time parameter in any step, the 474 automatically rearranges the program steps in the correct time sequence.

4.4.3 Adding a Program Step

The 474 arranges the steps in a time program in sequence according to the value set in the Time field. You can enter a new step at any point in time within the time program. When you press the **Monitor** key the 474 rennumbers all the steps in the correct time sequence.

To add a program step:

1. Access the program number you wish to change as described in [Section 4.4.1. Accessing the Program Number](#).
2. Use the ▼ key to locate the first step that has no entries (the lower line of the display is blank).
3. Follow the procedure in [Section 4.2.2. Setting Steps in a Time Program](#), to enter the time and parameter values.
4. Press the **Monitor** key to save the change and return to the Program mode. The 474 automatically rearranges the program steps in the correct time sequence.

4.4.4 Deleting a Program Step

You can delete a step in a time program. The remaining steps are arranged according to the values in the Time fields.

Note: *This operation cannot be undone.*

To delete a step from a program:

1. Access the program number you wish to change as described in [Section 4.4.1. Accessing the Program Number](#).
2. Press the ▼ key until the step that you want to delete appears on the display.
3. Press the **Edit/Enter** key. The Time field flashes on and off.
4. Press the **0** key to set the time to zero.
5. Press the **Edit/Enter** key to enter this value, which deletes the step. The 474 renumbers the program steps according to the time sequence in each of the remaining steps.

4.4.5 Deleting a Group of Steps

Note: *This operation cannot be undone.*

To delete a step and all following steps in a time program:

Deleting Multiple Steps in a Time Program

1. Access the program number you wish to change as described in [Section 4.4.1. Accessing the Program Number](#).
2. Press the ▼ key until the first of the program steps that you want to delete appears on the display.
3. Press the **Shift** and then the **Clear** keys to delete the selected line and all following lines.

Deleting an Entire Time Program

To delete an entire time program:

1. Access the program number you wish to change as described in [Section 4.4.1. Accessing the Program Number](#).
2. Press the ▼ key once to display the initial conditions.
3. Press the **Shift** and then the **Clear** keys to delete the entire time program.

4.5 Performing a Wavelength Scan in a Time Program

The 474 can perform a *wavelength scan* during a time program. A wavelength scan produces a spectrum, which is a graph of the intensity versus the wavelength. [Figure 4-16](#) shows a typical spectrum. The spectrum is stored during the time program in one of 20 memory locations. You can send the spectrum to an output device when the 474 is in the Scan mode (see [Chapter 5. Using the Scan Mode](#)).

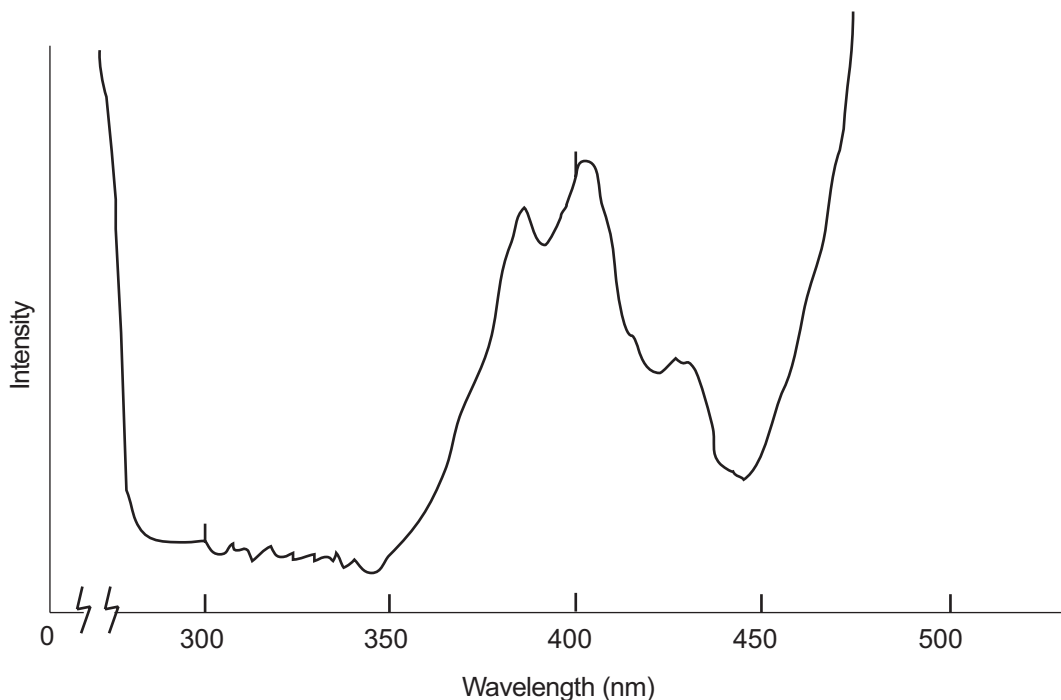


Figure 4-16 Typical Spectrum Output by the 474 Detector

You can measure and store emission and excitation spectra in a time program whenever a preset fluorescence level is exceeded within a preset time frame. To do this, you enter the Em Spect or Ex Spect functions (lines 7 or 8 of the Program menu in [Table 4-1](#)) in a program step.

Four parameters define the conditions necessary to measure and store a spectrum:

- The peak elution time
- The interval (or window) of time around the peak time
- The preset level or threshold for fluorescence intensity
- The memory location for storing the spectrum

[Figure 4-17](#) shows a peak and the conditions for storing a spectrum. The 474 stores a spectrum when the intensity level exceeds L during the interval around T , indicated by the shaded area.

The flat area in the peak is the time the 474 takes to store the spectrum, which takes approximately 20 seconds. While the spectrum is being stored, the output on the Rec (+ and -) terminals remains at the trigger level. When the 474 is done storing the spectrum, the output returns to the current level. The dashed line indicates the actual peak.

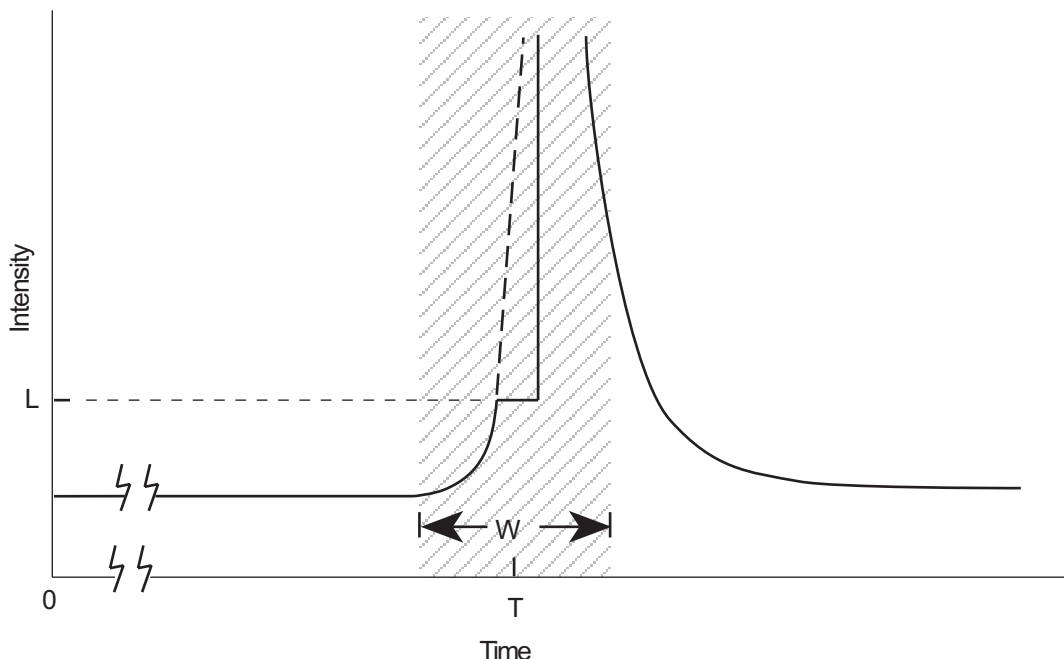


Figure 4-17 Conditions for Storing a Spectrum

4.5.1 Storing a Spectrum

To measure and store emission and excitation spectra in a time program, you need to enter the Em Spect or Ex Spect functions (lines 7 or 8 of the Program menu in [Table 4-1](#)) in a program step.

See [Section 4.2, Writing a Multi-Step Time Program](#), for details on selecting parameters. Before executing the program, set the scan rate to either 30 nm/sec or 100 nm/sec using the Scan Parameter function described in [Section 5.1, Scan Parameters](#).

When you select the Em Spect or Ex Spect parameter, the display appears as shown in [Figure 4-18](#).

MODE	SIGNAL	RESPONSE	
P1	ST 6	T: 7.4	
7	W:0.2	L:70.0	M:1
EXλ	EMλ	GAIN	ATTEN

Figure 4-18 Typical Values for Storing an Emission Spectrum

Upper Line of Display

The upper line of the display in [Figure 4-18](#) includes the following information:

P1 – Time program stored in program memory 1.

ST 6 – Step 6 of the program.

T: 7.4 – Peak elution time, 7.4 minutes.

Lower Line of Display

The lower line of the display shows the current values for the following parameters:

7 – The Emission Spectrum function (item 7 in the Program menu, see [Table 4-1](#)).
The Excitation Spectrum function is item 8 in the Program menu.

W:0.2 – Allowed time interval, 0.2 minutes

L:70.0 – Minimum fluorescence intensity (or threshold) for measuring a spectrum.

M:1 – Emission spectrum memory location 1, where the spectrum is stored.

Time T:

The Time value is the peak elution time in minutes and tenths. The Time value can be set from 0.0 to 999.9 minutes in 0.1 minute increments.

Interval W:

The Interval defines the period during which a peak can trigger a spectrum measurement. The period is extended by one-half of the interval on either side of the peak elution time. In other words, the 474 records a spectrum if the fluorescence intensity exceeds the minimum level between $T - W/2$ and $T + W/2$. The interval value can be set from 0.0 to 9.9 minutes in 0.1 minute increments

For example, if **T:** is set to 7.4 and **w:** is set to 0.2, a spectrum measurement is recorded if the fluorescence intensity exceeds the minimum level at 7.4 ± 0.1 min.

Level L:

The Level is the minimum level of fluorescence intensity at which a spectrum is to be stored. The level value can be set from 0.0 to 99.9 in increments of 0.1, and is expressed as a proportion of the full-scale reading (100.0) in the Signal field.

For example, if L: is set to 70.0, the 474 records a spectrum if the fluorescence intensity exceeds 0.7000 during the interval. You may need to empirically determine the proper value for the L: parameter.

Memory M:

The 474 has ten locations available for storing emission spectra and ten locations for storing excitation spectra. The value specifies the memory location where the spectrum is to be stored. The memory value can be set from 0 to 9 in increments of 1.

4.5.2 Entering the Spectrum Parameters

You can enter a scan step in a new program or you can modify an existing program.

To enter a spectrum scan step in a program:

1. Press the **Edit/Enter** key. The Time field flashes on and off.
2. Use the numeric keys to select the peak elution time. This number is in minutes and tenths.
3. Press the **Edit/Enter** key to enter your selection.
4. Press the **7** or **8** key to select the Emission Spectrum function or the Excitation Spectrum function, respectively.
5. Press the **Edit/Enter** key to enter your selection. The W: field flashes on and off.
6. Use the numeric keys to select the interval.
7. Press the **Edit/Enter** key to enter your selection. The L: field flashes on and off.
8. Use the numeric keys to select the level.
9. Press the **Edit/Enter** key to enter your selection. The M: field flashes on and off.
10. Use the numeric keys to select the memory location for the spectrum.
11. Press the **Edit/Enter** key to enter the memory location number.
12. Press the **Monitor** key to store the program and return to the Program mode.

Using Spectra

You can perform several operations on stored spectra when the 474 is in the Scan mode. See [Chapter 5, Using the Scan Mode](#), for more information.

5

Using the Scan Mode

In addition to the Normal and Program modes, the 474 features a Scan mode. This chapter describes how to use the scan mode to:

- Set scan parameters
- Manually perform a spectral scan
- Output stored spectra
- Perform difference spectra

Accessing the Scan Mode

To access the Scan mode, press the **Scan** key. The display appears with the first line of the Scan menu, as shown in [Figure 5-1](#).

To return to the Normal mode, press the **Monitor** key.

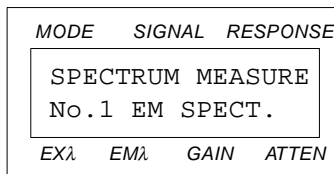


Figure 5-1 Initial Display in the Scan Mode

[Table 5-1](#) lists the functions in the Scan menu.

Table 5-1 Scan Menu

Line No.	Function	Description	Reference
1	EM SPECT	Emission spectrum	Section 5.2.1
2	EX SPECT	Excitation spectrum	Section 5.2.2
3	EM D.OUT	Output emission spectrum	Section 5.3.1

Table 5-1 Scan Menu (Continued)

Line No.	Function	Description	Reference
4	EX D.OUT	Output excitation spectrum	Section 5.3.2
5	DIFFER EM	Difference between two emission spectra	Section 5.4.1
6	DIFFER EX	Difference between two excitation spectra	Section 5.4.2
0	SCAN PARAM.	Scan parameters	Section 5.1

5.1 Scan Parameters

The Scan parameter function allows you to specify the wavelength range and speed of a scan, using two parameters:

Range (Rng) – The scan width, which sets the starting and ending bandwidth of the output spectrum. The Range can be set to Wide, Standard, or Narrow.

Speed (Spd) – The scan rate, which sets the rate (in nm/sec) of the scan. The Speed can be set to 100 nm/sec or 30 nm/sec.

[Table 5-2](#) summarizes the scan parameters and their settings.

Table 5-2 Scan Parameters

Scan Parameter	Settings	Excitation Scan	Emission Scan
Range (Rng)	WIDE	0 nm < Ex < 900 nm	0 nm < Em < 900 nm
	STD	200 nm to Em – 10 nm	Ex + 10 nm to 900 nm
	NARROW	Em/2 nm to Em – 10 nm (or 220 nm, whichever is longer)	Ex + 10 nm to 2 x Ex nm (or 650 nm, whichever is shorter)
Speed (Spd)	1	100 nm/sec	100 nm/sec
	2	30 nm/sec	30 nm/sec

Setting Scan Parameters

To set the scan parameters:

1. Press the **Scan** key to enter the Scan mode if the 474 is not already in the Scan mode.
2. Use the **▲** or **▼** keys, or press the **End/0** key to select the Scan parameter function. The display appears as shown in [Figure 5-2](#).

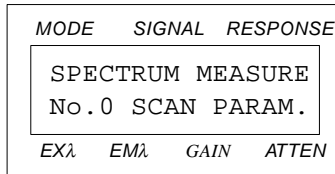


Figure 5-2 Selecting the Scan Parameters

3. Press the **Edit/Enter** key. The display appears as shown in [Figure 5-3](#).

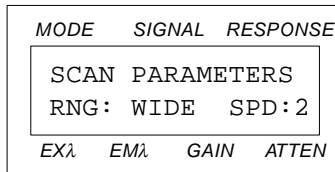


Figure 5-3 Entering the Scan Parameters

4. Press the **Edit/Enter** key again. The value in the Range field flashes on and off.
5. Use the **▲** or **▼** keys to select the appropriate range setting.
6. Press the **Edit/Enter** key to enter your selection. The value in the Speed field flashes on and off.
7. Use the **▲** or **▼** keys or the numeric keys to select the appropriate speed setting.
8. Press the **Edit/Enter** key to enter your selection.
9. Press the **Monitor** key to return to the Scan mode.

5.2 Performing Spectral Scans

The 474 can perform and store spectral scans manually and under a time program. [Section 4.5, Performing a Wavelength Scan in a Time Program](#), describes how to perform a spectral scan under a time program. This section describes how to perform a manual spectral scan.

The 474 can perform two types of manual scans:

- Emission spectra
- Excitation spectra

Emission Spectra

During an emission spectrum scan, the excitation wavelength is held constant while a range of emission wavelengths is scanned. The range of wavelengths is determined by the Range setting in the Scan parameters. See [Table 5-2](#).

As the scan is performed, the emission spectrum is stored in the memory location you select in preparing to run a spectrum. Up to 10 emission spectra can be stored in the 474 memory. The memory is not erased when the 474 is powered off.

Excitation Spectra

During an excitation spectrum scan, the emission wavelength is held constant while a range of excitation wavelengths is scanned. The range of wavelengths is determined by the Range setting in the Scan parameters. See [Table 5-2](#).

As the scan is performed, excitation spectrum is stored in the memory location you select when preparing to run a spectrum. Up to 10 excitation spectra can be stored in the 474 memory. The memory is not erased when the 474 is powered off.

Spectrum Memory

The 474 has two sets of ten memory locations for storing spectra:

- One set for emission spectra
- One set for excitation spectra

Emission spectra that you perform under manual control or under a time program are stored in the emission spectrum locations. Likewise, excitation spectra that you measure under manual control or under a time program are stored in the excitation spectrum locations.

Note: Both emission and excitation spectra use the same memory location numbers even though they are stored separately. Be sure to record the memory number and the spectrum type when you store a spectrum.

Erasing the Spectrum Memory Locations

You can erase all 20 spectrum memory locations when you are in the Scan mode. Press the **Shift** and **Clear** keys to erase all memory locations.

Note: You cannot undo the erase operation.

5.2.1 Manual Emission Spectrum Scans

The manual mode for scanning emission spectra is convenient when you have only one or a few samples to scan. You can set the parameters quickly, and the scan begins when you press a key. You do not have to write a time program to run a scan.

The emission spectrum covers the wavelength range in the Range setting in the Scan parameter function (see [Section 5.1, Scan Parameters](#)).

To manually run an emission spectrum scan:

1. Press the **Scan** key to access the Scan mode. The display appears as shown in [Figure 5-1](#).
2. Set the Scan parameters you desire, as described in [Section 5.1, Scan Parameters](#).
3. Press the **Edit/Enter** key. The display appears as shown in [Figure 5-4](#) with the Memory Location field flashing on and off.

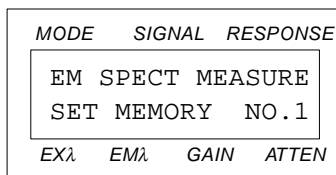


Figure 5-4 Entering the Emission Spectrum Memory Location

Selecting the Memory Location

To select the memory location

4. Use the numeric keys to select the emission memory location you want to use. Record this number.
5. Press the **Edit/Enter** key to enter your selection. The display appears as shown in [Figure 5-5](#) with the Excitation Wavelength field flashing on and off.

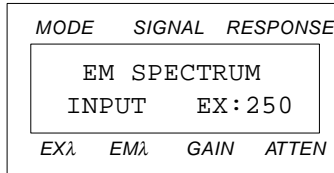


Figure 5-5 Entering the Excitation Wavelength for an Emission Spectrum

Selecting the Excitation Wavelength

- Use the numeric keys to select the excitation wavelength you want to use. Record this number.
- Press the **Edit/Enter** key to enter your selection. The display appears as shown in [Figure 5-6](#).

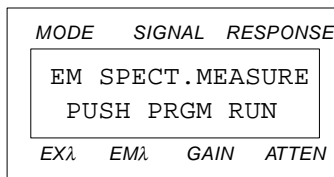


Figure 5-6 Prompt to Begin an Emission Spectrum Scan

Starting the Scan

- Press the **Program Run** key. The scan begins and the lower line of the display changes to `RUNNING`.

When the scan is complete, the spectrum is stored in the location you selected in step 3 above. The display appears as shown in [Figure 5-4](#).

To make another spectrum scan at this time, start at step 2.

- Press the **Monitor** key to return to the Scan mode.

Refer to [Section 5.3, Spectrum Output](#), to output the spectrum, or to [Section 5.4, Difference Spectra](#), to make a difference spectrum.

5.2.2 Manual Excitation Spectrum Scans

The manual mode for scanning excitation spectra is convenient when you have only one or a few samples to scan. You can set the parameters quickly, and the scan begins when you press a key. You do not have to write a time program to run a scan.

The excitation spectrum covers the wavelength range in the Range setting in the Scan parameter function (see [Section 5.1, Scan Parameters](#)).

To manually run an excitation spectrum scan:

1. Press the **Scan** key to access the Scan mode. The display appears as shown in [Figure 5-1](#).
2. Set the Scan parameters you desire, as described in [Section 5.1, Scan Parameters](#).
3. Use the **▲** or **▼** keys, or press the **2** key to select the Ex Spect. function. The display appears as shown in [Figure 5-7](#).

MODE	SIGNAL	RESPONSE
SPECTRUM MEASURE		
No. 2 EX SPECT.		
EXλ	EMλ	GAIN ATTN

Figure 5-7 Starting an Excitation Spectrum Scan

4. Press the **Edit/Enter** key. The display appears as shown in [Figure 5-8](#) with the Memory Location field flashing on and off.

MODE	SIGNAL	RESPONSE
EX SPECT MEASURE		
SET MEMORY NO. 1		
EXλ	EMλ	GAIN ATTN

Figure 5-8 Entering the Excitation Spectrum Memory Location

Selecting the Memory Location

5. Use the numeric keys to select the excitation memory location you want to use. Record this number.
6. Press the **Edit/Enter** key to enter your selection. The display appears as shown in [Figure 5-9](#) with the Emission Wavelength field flashing on and off.

MODE	SIGNAL	RESPONSE
EX SPECTRUM		
INPUT EM: 512		
EXλ	EMλ	GAIN ATTN

Figure 5-9 Entering the Emission Wavelength for an Excitation Spectrum

Selecting the Emission Wavelength

7. Use the numeric keys to select the emission wavelength you want to use. Record this number.
8. Press the **Edit/Enter** key to enter your selection. The display appears as shown in [Figure 5-10](#).

MODE	SIGNAL	RESPONSE
EX	SPECT	MEASURE
PUSH PRGM RUN		
EX λ	EM λ	GAIN ATTN

Figure 5-10 Prompt to Begin an Excitation Spectrum Scan

Starting the Scan

9. Press the **Program Run** key. The lower line of the display changes to `RUNNING`.
When the scan is complete, the spectrum is stored in the location you selected in step 4 and the display appears as shown in [Figure 5-7](#).
To make another spectrum scan at this time, start at step 2.
10. Press the **Monitor** key to return to the Scan mode.
Refer to [Section 5.3, Spectrum Output](#), to output the spectrum, or to [Section 5.4, Difference Spectra](#), to make a difference spectrum.

5.3 Spectrum Output

Once you have performed a spectral scan (manually or through a time program) you can send the stored spectra to an output device.

Output Connections

A spectrum stored in memory can be sent to an external device (such as a strip chart recorder) as an analog voltage signal. This signal is placed on both the Rec (+ and –) and the Int (+ and –) terminals when you use the Em D.Out or Ex D.Out functions of the Scan mode. [Section 2.6.1, Analog Connector Overview](#), describes the terminals, and [Section 2.6, Making Connections to Other Devices](#), describes how to connect the 474 to output devices that can plot the spectra.

Varying the Output Signal

You can vary the magnitude of the Recorder output using the Attenuation parameter. See [Section 3.4.5, Attenuation](#), the discussion “Using Gain and Attenuation” for details on how the value in this parameter affects the signal levels on the Rec (+ and –) terminals.

Wavelength Markers

The 474 adds a chart mark to the output signal on the Rec (+ and –) terminals every 100 nm to help identify peaks in the spectrum.

Data Rate

The 474 outputs data at a rate of 200 nm/min. If the chart recorder is set to a chart speed of 100 mm/min, a 100-nm long spectrum produces a chart 50 mm long.

5.3.1 Outputting Emission Spectra

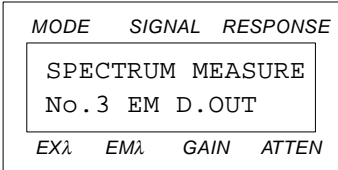
To output an emission spectrum, connect an appropriate data acquisition or recording device to either the Rec (+ and –) or the Int (+ and –) terminals of the 474. See [Section 2.6.5, Connecting the 474 to a Waters 745/745B/746 Data Module](#), and [Section 2.6.6, Connecting the 474 to a Chart Recorder](#), for details on connecting devices to these terminals.

To send an emission spectrum to an output device:

1. Press the **Scan** key to enter the Scan mode if the 474 is not already in the Scan mode.

Selecting the Emission Spectrum Output Function

2. Use the **▲** or **▼** keys, or press the **Gain/3** key to select the Em D.Out function. The display appears as shown in [Figure 5-11](#).



The image shows a rectangular display window with a double border. The text inside is as follows:

MODE	SIGNAL	RESPONSE
SPECTRUM MEASURE		
No. 3 EM D. OUT		
EXλ	EMλ	GAIN ATTEN

Figure 5-11 Starting an Emission Spectrum Output

3. Press the **Edit/Enter** key. The display appears as shown in [Figure 5-12](#) with the Memory Location field flashing on and off.

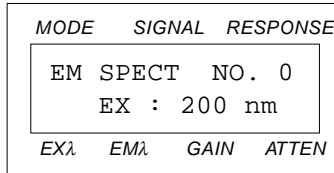


Figure 5-12 Entering the Emission Spectrum Memory Location

Accessing the Stored Spectrum

4. Use the numeric keys to select the emission memory location you want to use. The lower line displays the excitation wavelength used when the spectrum was stored.

If a spectrum is not stored in the location, the lower line displays `EMPTY DATA`.

5. Press the **Edit/Enter** key to enter your selection. The display appears as shown in [Figure 5-13](#) with the Start field flashing on and off.

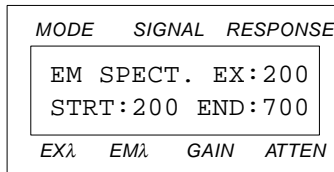


Figure 5-13 Entering the Start and End Wavelengths in the Emission Spectrum

Selecting the Starting and Ending Wavelengths

The Range setting in the Scan parameter function determines the minimum starting and maximum ending wavelength of the stored spectrum. You cannot enter a starting wavelength below the minimum or an ending wavelength greater than the maximum. See [Section 5.1, Scan Parameters](#), for details.

6. Use the numeric keys to select the starting wavelength for the output spectrum.
7. Press the **Edit/Enter** key to enter your selection. The End field flashes on and off.
8. Use the numeric keys to select the ending wavelength for the output spectrum.
9. Press the **Edit/Enter** key to enter your selection. The display appears as shown in [Figure 5-14](#).

MODE	SIGNAL	RESPONSE
EM SPECT. DATAOUT		
PUSH PRGM RUN		
EXλ	EMλ	GAIN ATTEN

Figure 5-14 Prompt to Start Output of a Spectrum

Starting the Emission Spectrum Output

10. Press the **Program Run** key. The display appears as shown in [Figure 5-15](#). The value in the EM: field increments as the emission wavelength increases.

MODE	SIGNAL	RESPONSE
EM SPECT. EX: 200		
EM: 348 S: 0.578		
EXλ	EMλ	GAIN ATTEN

Figure 5-15 Typical Display During Output of a Spectrum

When the output of the spectrum is complete, the display appears as shown in [Figure 5-12](#).

To send another spectrum to the output device, start at step 4.

11. Press the **Monitor** key to return to the Scan mode.

5.3.2 Outputting Excitation Spectra

To output an excitation spectrum, connect an appropriate data acquisition or recording device to either the Rec (+ and –) or the Int (+ and –) terminals. See [Section 2.6.5, Connecting the 474 to a Waters 745/745B/746 Data Module](#), and [Section 2.6.6, Connecting the 474 to a Chart Recorder](#), for details on connecting devices to these terminals.

Selecting the Excitation Spectrum Output Function

To send an excitation spectrum to an output device, press the **Scan** key to enter the Scan mode if the 474 is not already in the Scan mode.

1. Use the **▲** or **▼** keys or press the **Atten/4** key to select the Ex D.Out parameter. The display appears as shown in [Figure 5-16](#).

MODE	SIGNAL	RESPONSE
SPECTRUM MEASURE		
No. 4 EX D.OUT		
EXλ	EMλ	GAIN ATTEN

Figure 5-16 Starting an Excitation Spectrum Output

2. Press the **Edit/Enter** key. The display appears as shown in [Figure 5-17](#) with the Memory Location field flashing on and off.

MODE	SIGNAL	RESPONSE
EX SPECT NO. 0		
EM : 200 nm		
EXλ	EMλ	GAIN ATTEN

Figure 5-17 Entering the Excitation Spectrum Memory Location

Accessing the Stored Spectrum

3. Use the numeric keys to select the excitation memory location you want to use. The lower line displays the emission wavelength used when the spectrum was stored.
If a spectrum is not stored in the location, the lower line displays **EMPTY DATA**.
4. Press the **Edit/Enter** key to enter your selection. The display appears as shown in [Figure 5-18](#) with the Start field flashing on and off.

MODE	SIGNAL	RESPONSE
EX SPECT. EM:200		
STRT:200 END:700		
EXλ	EMλ	GAIN ATTEN

Figure 5-18 Entering the Start and End Wavelengths in the Excitation Spectrum

Selecting the Starting Wavelength

The Range setting in the Scan parameter function determines the minimum starting and maximum ending wavelength of the stored spectrum. You cannot enter a starting wavelength below the minimum or an ending wavelength greater than the maximum. See [Section 5.1, Scan Parameters](#), for details.

5. Use the numeric keys to select the starting wavelength for the output spectrum.
6. Press the **Edit/Enter** key to enter your selection. The End field flashes on and off.

Selecting the Ending Wavelength

7. Use the numeric keys to select the ending wavelength for the output spectrum.
8. Press the **Edit/Enter** key to enter your selection. The display appears as shown in [Figure 5-19](#).

```
MODE  SIGNAL  RESPONSE
-----
EX SPECT.DATAOUT
PUSH PRGM RUN
EXλ  EMλ  GAIN  ATTEN
```

Figure 5-19 Prompt to Start Output of a Spectrum

Starting the Excitation Spectrum Output

9. Press the **Program Run** key. The display appears as shown in [Figure 5-20](#). The value in the Ex: field increments as the excitation wavelength increases.

```
MODE  SIGNAL  RESPONSE
-----
EX SPECT. EM:200
EX:348  S: 0.578
EXλ  EMλ  GAIN  ATTEN
```

Figure 5-20 Typical Display During Output of a Spectrum

When the output of the spectrum is complete, the display appears as shown in [Figure 5-17](#). To send another spectrum to the output device, start at step 4.

10. Press the **Monitor** key to return to the Scan mode.

5.4 Difference Spectra

The 474 permits you to subtract one stored spectrum from another to obtain:

- The difference spectrum between two samples
- The difference spectrum between a sample and the mobile phase solvent

Background

You can subtract one stored spectrum from another stored spectrum. Both spectra must be the same type, either both excitation spectra or both emission spectra. Also, to obtain an excitation difference spectrum, the emission wavelength must be the same in both spectra. Likewise, to obtain an emission difference spectrum, the excitation wavelength must be the same in both spectra.

You select the wavelength of the difference spectrum by setting the Range in the Scan parameter function (see [Section 5.1, Scan Parameters](#)).

Difference Output

A difference spectrum is sent to an external device (such as a strip chart recorder or integrator) as an analog voltage signal. This signal is placed on the Rec (+ and –) and the Int (+ and –) terminals of the 474 when you use the Differ Em or Differ Ex functions. [Section 2.6.1, Analog Connector Overview](#), describes the terminals, and [Section 2.6, Making Connections to Other Devices](#), describes how to connect the 474 to output devices that can plot the spectra.

Varying the Output Signals

The magnitude of the Recorder output can be varied using the Attenuation parameter. See [Section 3.4.5, Attenuation](#), the discussion “Using Gain and Attenuation” for details on how the value in this parameter affects the signal levels on the Rec (+ and –) terminals.

Wavelength Markers

The 474 adds a mark to the output signal on the Rec (+ and –) terminals every 100 nm to help identify the wavelength of peaks in the spectrum.

Data Rate

Scan data is output at a rate of 200 nm/min, or approximately 3.3 nm/sec. If the chart speed on the recorder is set to 100 mm/min, a 100 nm spectrum will yield a chart 50 mm long.

5.4.1 Emission Difference Spectrum

To output an emission difference spectrum, connect an appropriate data acquisition or recording device to either the Rec (+ and –) or the Int (+ and –) terminals. See [Section 2.6.5, Connecting the 474 to a Waters 745/745B/746 Data Module](#), and [Section 2.6.6, Connecting the 474 to a Chart Recorder](#), for details on connecting devices to these terminals.

To send an emission difference spectrum to an output device:

1. Press the **Scan** key to enter the Scan mode if the 474 is not already in the Scan mode.

Selecting the Emission Difference Spectrum Function

2. Use the **▲** or **▼** keys, or press the **Resp/5** key to select the Differ Em function. The display appears as shown in [Figure 5-21](#).

MODE	SIGNAL	RESPONSE
SPECTRUM MEASURE		
No. 5 DIFFER EM		
EXλ	EMλ	GAIN ATTN

Figure 5-21 Starting an Emission Difference Spectrum Output

3. Press the **Edit/Enter** key. The display appears as shown in [Figure 5-22](#) with the current value in the first memory location field flashing on and off.

MODE	SIGNAL	RESPONSE
SUB. 2 FROM 4		
EX : 200 nm		
EXλ	EMλ	GAIN ATTN

Figure 5-22 Entering the Memory Locations for an Emission Difference Spectrum

The display indicates that the emission spectrum in the first memory location in [Figure 5-22](#) is to be subtracted from the emission spectrum in the second memory location. The equation for the difference spectrum in this example is:

$$(\text{spectrum } 4) - (\text{spectrum } 2) = \text{difference spectrum}$$

Selecting the First Memory Location

- Use the numeric keys to select the memory location of the spectrum that you want to subtract *from* the other spectrum. The lower line displays the excitation wavelength that was used when the emission spectrum was performed.

If a spectrum is not stored in the first memory location, the lower line displays `EMPTY DATA`. You cannot use an empty location. Use the numeric keys to select a valid memory location.

- Press the **Edit/Enter** key to enter your selection. The next memory location flashes on and off.

Selecting the Second Memory Location

- Use the numeric keys to select the memory location of the emission spectrum *from which* you want to subtract the first spectrum.

If an emission spectrum is not stored in the second memory location, the lower line displays `EMPTY DATA`. You cannot use an empty location. Use the numeric keys to select a valid memory location.

If the emission spectrum in the second memory location does not have the same excitation wavelength as the spectrum in the first memory location, the display appears as shown in [Figure 5-23](#).

MODE	SIGNAL	RESPONSE
NOT SAME EX W.L.		
ENTER MONIT		
EX λ	EM λ	GAIN ATTEN

Figure 5-23 Incorrect Wavelengths for a Difference Spectrum

Press the **Monitor** key. The display appears as shown in [Figure 5-22](#). Continue at step 4.

- Press the **Edit/Enter** key to enter your selection. The display appears as shown in [Figure 5-24](#). The value in the Start field flashes on and off.

MODE	SIGNAL	RESPONSE
D. EM	EX:200	
STRT:200		END:700
EX λ	EM λ	GAIN ATTEN

Figure 5-24 Entering the Start and End Wavelengths for an Emission Difference Spectrum

Entering the Starting and Ending Wavelengths

You need to specify the range of wavelengths over which you want the difference spectrum to be performed. The Range setting in the Scan parameter function ([Section 5.1, Scan Parameters](#)) determines the minimum starting and maximum ending wavelength of the stored spectra. For the difference spectrum, you cannot enter a starting wavelength below the minimum, or an ending wavelength greater than the maximum for the stored spectra. See [Section 5.1, Scan Parameters](#), for details.

8. Use the numeric keys to enter the starting wavelength.
9. Press the **Edit/Enter** key to enter your selection. The value in the End field flashes on and off.
10. Use the numeric keys to enter the ending wavelength.
11. Press the **Edit/Enter** key to enter your selection. The display appears as shown in [Figure 5-25](#).

MODE	SIGNAL	RESPONSE
D. EM	DATAOUT	
PUSH PRGM RUN		
EX λ	EM λ	GAIN ATTN

Figure 5-25 Prompt to Begin an Emission Difference Spectrum

Starting the Emission Difference Spectrum

12. Press the **Program Run** key to begin the emission difference spectrum output.

While the 474 is outputting the difference spectrum data, the display appears as shown in [Figure 5-26](#). The value in the Em: field increments as the data is sent. The value in the S: field is the difference between the spectra at each value in the Em: field.

MODE	SIGNAL	RESPONSE
D. EM	EX:200	
EM :234	S:0.0828	
EX λ	EM λ	GAIN ATTN

Figure 5-26 Typical Display During Output of an Emission Difference Spectrum

When the difference spectrum is complete, the display returns as shown in [Figure 5-22](#). To perform another difference spectrum, repeat the procedure beginning at step 4.

- Press the **Monitor** key to return to the Scan mode.

5.4.2 Excitation Difference Spectra

To output an excitation difference spectrum, connect an appropriate data acquisition or recording device to either the Rec (+ and –) or the Int (+ and –) terminals. See [Section 2.6.5, Connecting the 474 to a Waters 745/745B/746 Data Module](#), and [Section 2.6.6, Connecting the 474 to a Chart Recorder](#), for details on connecting devices to these terminals.

To send an excitation difference spectrum to an output device:

- Press the **Scan** key to enter the Scan mode if the 474 is not already in the Scan mode.

Selecting the Excitation Difference Spectrum Function

- Use the **▲** or **▼** keys, or press the **A.Z./6** key to select the Differ Ex function. The display appears as shown in [Figure 5-27](#).

MODE	SIGNAL	RESPONSE
SPECTRUM MEASURE		
No. 6 DIFFER EX		
EXλ	EMλ	GAIN ATTN

Figure 5-27 Starting an Excitation Difference Spectrum Output

- Press the **Edit/Enter** key. The display appears as shown in [Figure 5-28](#) with the current value in the first memory location field flashing on and off.

MODE	SIGNAL	RESPONSE
SUB. 3 FROM 6		
EX : 200 nm		
EXλ	EMλ	GAIN ATTN

Figure 5-28 Entering the Memory Locations for an Excitation Difference Spectrum

The display indicates that the excitation spectrum in the first memory location in [Figure 5-28](#) is to be subtracted from the excitation spectrum in the second memory location. The equation for the difference spectrum in this example is:

$$(\text{spectrum } 6) - (\text{spectrum } 3) = \text{difference spectrum}$$

Selecting the First Memory Location

4. Use the numeric keys to select the memory location of the spectrum that you want to subtract *from* the other spectrum. The lower line displays the emission wavelength that was used when the excitation spectrum was performed.

If a spectrum is not stored in the first memory location, the lower line displays `EMPTY DATA`. You cannot use an empty location. Use the numeric keys to enter a valid memory location.

5. Press the **Edit/Enter** key to enter your selection. The next memory location flashes on and off.

Selecting the Second Memory Location

6. Use the numeric keys to select the memory location of the excitation spectrum *from which* you want to subtract the first excitation spectrum.

If an excitation spectrum is not stored in the second memory location, the lower line displays `EMPTY DATA`. You cannot use an empty location. Use the numeric keys to enter a valid memory location.

If the excitation spectrum in the second memory location does not have the same emission wavelength, the display appears as shown in [Figure 5-29](#).

MODE	SIGNAL	RESPONSE
NOT SAME EM W.L.		
ENTER MONIT		
EX λ	EM λ	GAIN ATTN

Figure 5-29 Incorrect Wavelengths for a Difference Spectrum

Press the **Monitor** key. The display appears as shown in [Figure 5-28](#). Enter the correct memory location number and continue at step 4.

7. Press the **Edit/Enter** key to enter your selection. The display appears as shown in [Figure 5-30](#). The value in the Start field flashes on and off.

MODE	SIGNAL	RESPONSE
D. EX	EM: 200	
STRT: 200	END: 700	
EX λ	EM λ	GAIN ATTN

Figure 5-30 Entering the Start and End Wavelengths for an Excitation Difference Spectrum

Entering the Starting and Ending Wavelength

You need to specify the range of wavelengths over which you want the difference spectrum to be performed. The Range setting in the Scan parameter function ([Section 5.1, Scan Parameters](#)) determines the minimum starting and maximum ending wavelength of the stored spectra. For the difference spectrum, you cannot enter a starting wavelength below the minimum, or an ending wavelength greater than the maximum for the stored spectra. See [Section 5.1, Scan Parameters](#), for details.

8. Use the numeric keys to enter the starting wavelength.
9. Press the **Edit/Enter** key to enter your selection. The value in the End field flashes on and off.
10. Use the numeric keys to enter the ending wavelength.
11. Press the **Edit/Enter** key to enter your selection. The display appears as shown in [Figure 5-31](#).

MODE	SIGNAL	RESPONSE
D. EX	DATAOUT	
PUSH PRGM RUN		
EX λ	EM λ	GAIN ATTN

Figure 5-31 Prompt to Begin an Excitation Difference Spectrum

Starting the Excitation Difference Spectrum

12. Press the **Program Run** key to begin the excitation difference spectrum output. While the 474 is outputting the difference spectrum data, the display appears as shown in [Figure 5-32](#). The value in the Ex: field increments as the data is sent. The value in the S: field is the difference between the spectra at each value in the Ex: field.

MODE	SIGNAL	RESPONSE
D. EX	EM: 200	
EX : 504	S: 0.0423	
EX λ	EM λ	GAIN ATTN

Figure 5-32 Typical Display During Output of an Excitation Difference Spectrum

When the difference spectrum is complete, the display returns as shown in [Figure 5-28](#). To perform another difference spectrum, repeat the procedure beginning at step 4.

13. Press the **Monitor** key to return to the Scan mode.

6

Maintenance Procedures

This chapter contains the following information on maintaining the Waters 474 detector:

- Performing routine maintenance
- Cleaning the air filters
- Cleaning the flow cell
- Replacing the flow cell
- Replacing the lamp
- Replacing fuses

Note: Read the appropriate maintenance instructions before starting any of the procedures listed above. If you are uncertain about how to perform the procedures, call the Waters Service Department to have a trained service representative perform the procedure.

Waters Service Department

The Service Department of Waters maintains a staff of highly trained service specialists to provide preventive and corrective maintenance of your 474 detector.

If you have any questions regarding the repair or performance of your 474, contact Waters Technical Service at (800) 252-4752, U.S. and Canadian customers only. Other customers, call your local Waters subsidiary or your local Waters Technical Service Representative, or call Waters corporate headquarters for assistance at (508) 478-2000 (U.S.).

Spare Parts

Waters recommends that you keep a stock of the spare parts listed in [Appendix B, Spare Parts and Accessories](#), to minimize downtime. Parts not included on this list may require replacement by a trained service representative.

Safety and Handling

The printed circuit (PC) boards inside the 474 are sensitive to static electricity. To avoid exposing the 474 circuitry to static electricity, observe the following precautions:

- When you handle a PC board, use an anti-static mat and wear an anti-static wrist strap to minimize static discharge.
- Do not handle the integrated circuits or other components that do not specifically require manual adjustment.



Caution: To avoid the possibility of electric shock, power off the 474 and remove the power cord from the rear panel before working on the rear panel or inside the unit.

Removing and Reinstalling the 474 Cover

Some of the procedures in this chapter require that you remove the 474 cover.

To remove and reinstall the 474 cover:

1. Power off the 474 and remove the power cord from the receptacle on the rear panel.
2. Remove the four screws located on the lower four corners of the cover using a Phillips screwdriver. Remove the two screws on the top of the cover.
3. Slide the cover straight up and off the chassis.

To reinstall the cover:

1. Slide the cover onto the chassis and align the screw holes.
2. Replace the four screws located on the lower four corners. Replace the two screws on the top of the cover.
3. Replace the power cord in the receptacle on the rear panel.

6.1 Routine Maintenance

To keep your 474 operating at optimal performance levels, perform the following routine maintenance checks:

Routine Maintenance Checks

1. Replace solvent reservoir filters regularly.
2. Degas solvents before use. Filtering and degassing solvents provide the following benefits:
 - Prolonged column life
 - Reduced pressure variations
 - Decreased baseline noise
3. Flush buffered mobile phases out of the 474 with a 5 to 10 percent methanol solution each time you power off the 474. This prevents:
 - Plugging of the fluidic lines
 - Damage to the components
 - Microbial growth
4. Inspect the ventilation filters on the rear panel weekly. If the filters require cleaning, follow the procedure in [Section 6.2, Cleaning the Ventilation Filters](#).

6.2 Cleaning the Ventilation Filters

Inspect the ventilation filters on a weekly basis. Clean the filters as necessary. To clean the ventilation air filters:

Procedure

1. Power off the 474 and unplug the power cord from the rear panel.
2. Remove the cover as described the “Removing and Reinstalling the 474 Cover” discussion in [Section 6.1, Routine Maintenance](#).
3. Remove the air filters from the side and rear panels.
4. Shake them to remove loose dust.

If the filters are visibly dirty and do not shake clean, replace them with new filters.

5. Reinstall the cover as described in the “Removing and Reinstalling the 474 Cover” discussion in [Section 6.1, Routine Maintenance](#).
6. Insert the power cord into the receptacle on the rear panel.

6.3 Cleaning the Flow Cell

A dirty flow cell can cause several problems, including:

- Baseline noise
- Low fluorescence intensity levels

To ensure that the flow cell is clean and operating correctly, you can either:

- Flush the flow cell
- Replace the flow cell

Flushing the Flow Cell

After the flow cell has been in use for a period of time, the cell wall may become contaminated. Flushing the flow cell with appropriate solvents can clean the flow cell.

To flush the flow cell:

1. Power off the 474 and remove the power cord from the rear panel.
2. Remove the tubing from the inlet on the flow cell cassette.
3. Attach a tube from the pump to the 474 inlet.
4. Attach a suitable waste container to the tube on the 474 outlet.
5. Flush the mobile phase from the 474 with a miscible solvent and then with water (unless your mobile phase is miscible with water).
6. Flush the 474 with HPLC-quality water to remove contaminants from the flow path. Use a flow rate of 2.0 mL/min for 20 minutes.

If an aqueous solvent or a buffer solution has been used, pump 30 percent nitric acid through the 474 at 2.0 mL/min for 30 minutes. Then pump HPLC-quality water at 2.0 mL/min for 30 minutes.

If an organic solvent has been used, pump HPLC-quality acetone through the 474 at 2.0 mL/min for 30 minutes. Then pump HPLC-quality water at 2.0 mL/min for 30 minutes.



Attention: To avoid damage to the flow cell, do not allow backpressure to exceed 500 psi for the 5- μ L cell or 145 psi for the 16- μ L cell. If the backpressure rises because of blocked tubing, replace the tubing.

7. Reattach the original tubing to the 474 inlet.
8. Insert the power cord into the receptacle on the rear panel.

6.4 Replacing the Flow Cell

Replacing the flow cell is necessary when:

- Flushing does not adequately clean the flow cell
- Your application calls for a different size cell
- There is a leak in the flow cell cassette

In each of the above instances, complete the following steps:

1. Remove the flow cell cassette
2. Disassemble the flow cell cassette
3. Disassemble the flow cell
4. Reassemble the flow cell
5. Reassemble and install the flow cell cassette

Required Materials

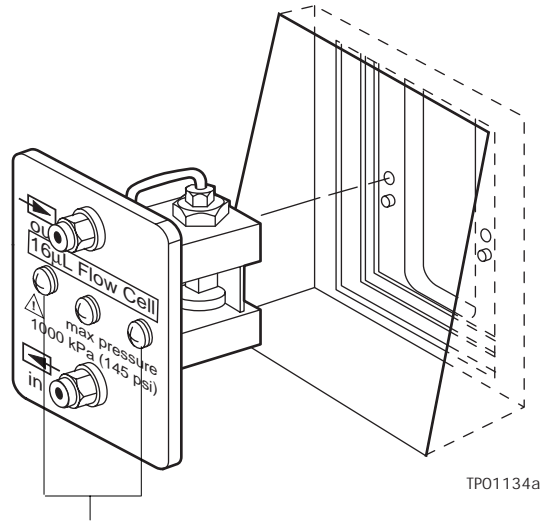
To remove and replace the flow cell, you need the following tools:

- Phillips screwdriver
- Adjustable wrench, or 5/16-inch, 6 mm, and 12 mm open-end wrenches
- Tweezers with the tips covered with Teflon tubing

6.4.1 Removing the Flow Cell Cassette

To remove the flow cell cassette:

1. Power off the 474 and remove the power cord from the rear panel.
2. Drain the tubing.
3. Remove the inlet and outlet tubes from the 474.
4. Remove the two outside screws on the flow cell cassette using the Phillips screwdriver ([Figure 6-1](#)).
5. Slide the flow cell cassette out of the chassis.



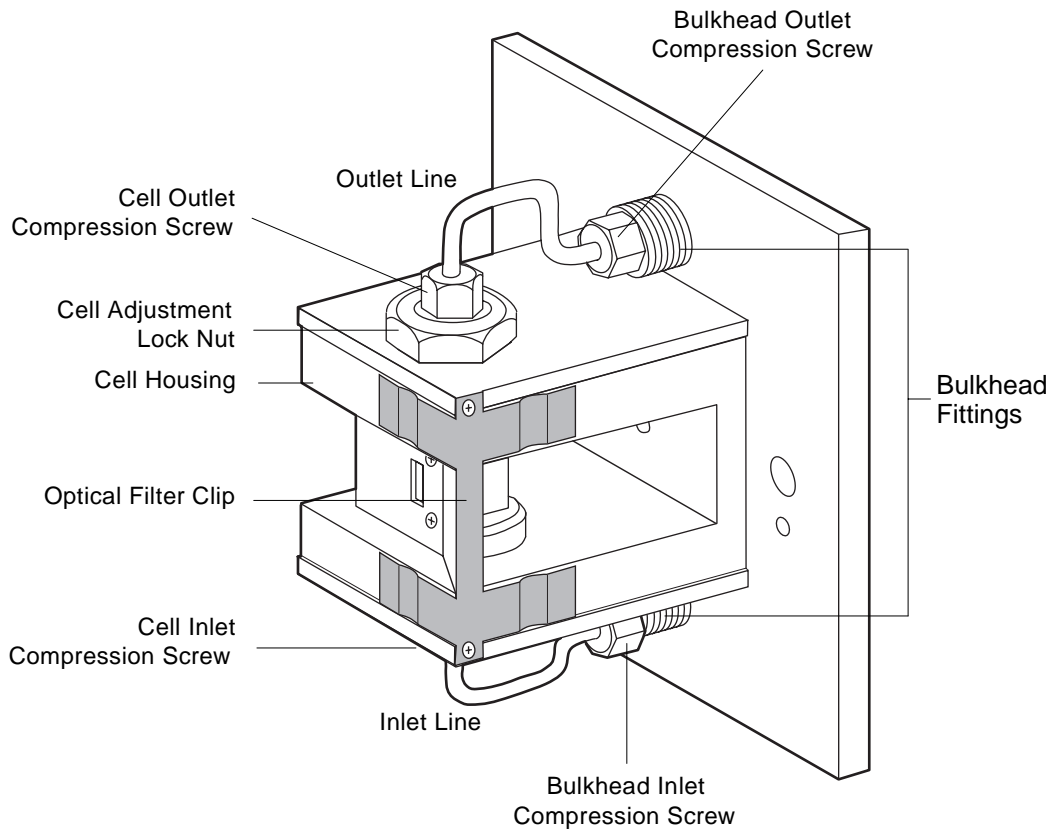
Remove Two Screws

Figure 6-1 Removing the Flow Cell Cassette

6.4.2 Disassembling the Flow Cell Cassette

To disassemble the flow cell cassette, perform the following steps. Refer to [Figure 6-2](#) and [Figure 6-3](#) for part names and locations.

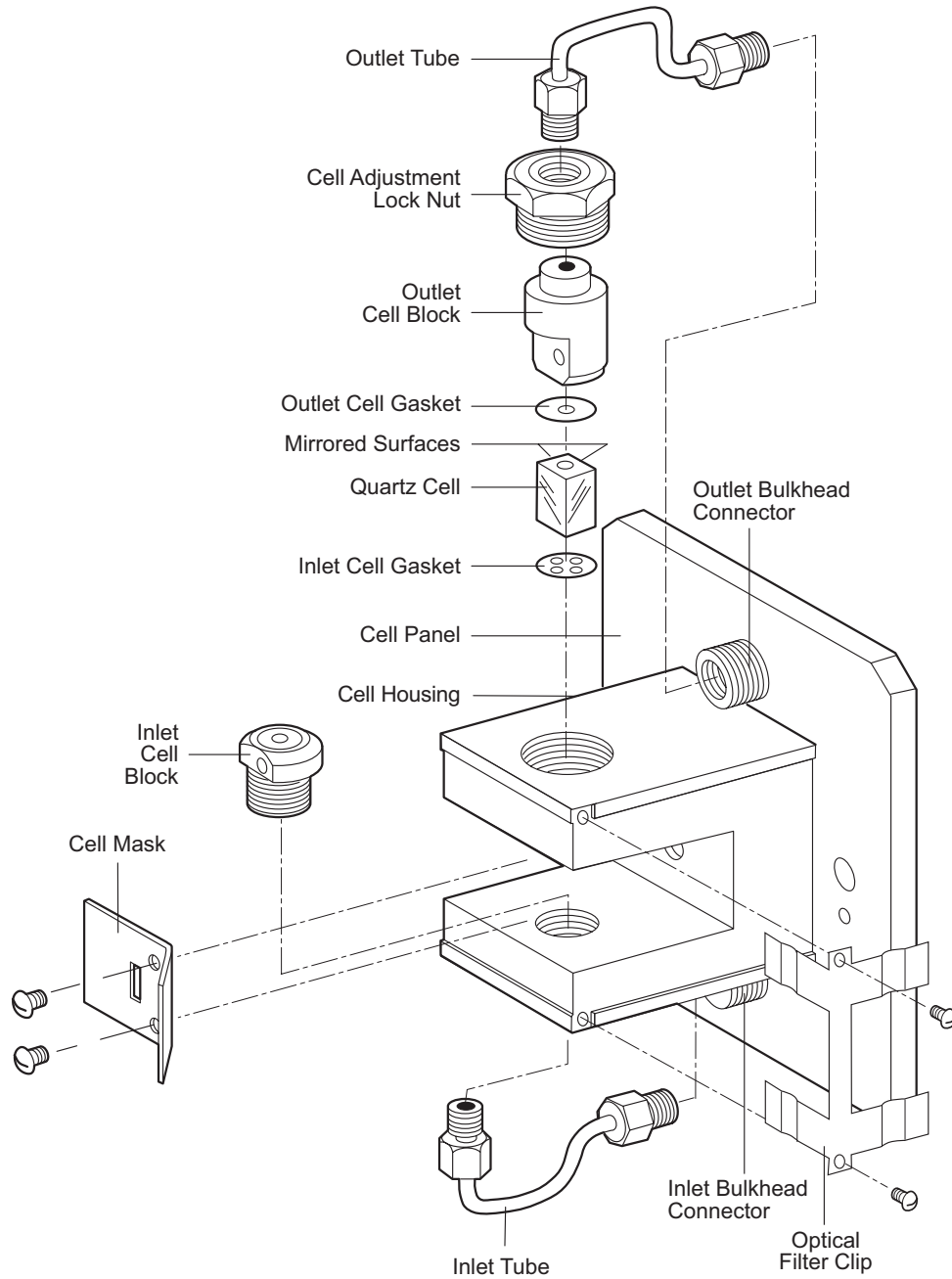
1. Loosen the bulkhead inlet and outlet compression screws on the inside of the flow cell panel. Use the adjustable wrench or the 6-mm wrench.
2. Remove the bulkhead fittings from the front panel. Use the adjustable or the 5/16-inch wrench.



TP01133b

Figure 6-2 Flow Cell Cassette

3. Loosen the cell inlet and outlet compression screws above and below the flow cell. Use the adjustable or the 6-mm wrench.
4. Remove the inlet and outlet lines.
5. Loosen and remove the cell adjustment lock nut located at the top (outlet) of the cell housing. Use the adjustable or the 12-mm wrench.
6. Remove the two screws holding the cell mask in place ([Figure 6-3](#)).



TP01132a

Figure 6-3 Exploded View of Flow Cell Parts

7. Lift the outlet cell block slightly to create a clearance for removing the cell gaskets and quartz cell.
8. Remove the quartz cell and two gaskets using tweezers with Teflon tubing covering the tips.



Attention: To avoid contamination or breakage, do not handle the quartz cell with fingers or a sharp instrument.

9. Using methanol and a lint-free cloth, thoroughly clean the inlet and outlet cell blocks and other reusable parts.

6.4.3 Reassembling the Flow Cell

To reassemble the flow cell:

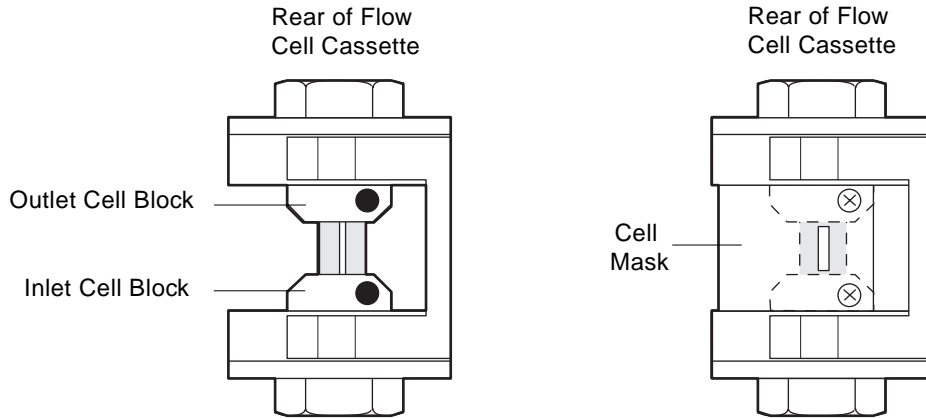
1. Install the inlet cell block into the cell housing ([Figure 6-3](#)). Align the block so that the screw hole for the cell mask faces the rear of the cassette.
2. Place the inlet flow cell gasket (with four holes) into position on the inlet cell block. Align the holes in the gasket with the hole in the cell block.
3. Insert the quartz cell using a pair of tweezers with Teflon tubing covering the tips.

Position the mirrored surfaces as shown in [Figure 6-3](#).



Attention: To avoid contamination or breakage, do not handle the quartz cell with fingers or a sharp instrument.

4. Install the outlet cell gasket into position on the quartz cell. Align the hole in the gasket with the hole in the cell block.
5. Install the outlet cell block. Align the block so that the screw hole for the cell mask faces the rear of the cassette.
6. Install the cell mask. Make sure the slot in the cell mask is centered over the quartz cell ([Figure 6-4](#)).



TP01131a

Figure 6-4 Position of Cell Mask

7. Install the cell adjustment lock nut. Tighten the cell adjustment lock nut so that it is finger-tight plus one-quarter turn.

Wait 30 minutes and check the lock nut. If necessary, tighten 1/16 turn. The maximum recommended torque is 10 kg/cm.



Attention: To avoid cracking the quartz cell, do not over-tighten the lock nut.

6.4.4 Reassembling the Flow Cell Cassette

To reassemble the flow cell cassette:

1. Insert the bulkhead compression screw on the inlet line into the hole in the front panel.
2. Install the inlet line on the inlet cell block. Tighten the cell block compression screw so that it is finger-tight.
3. Install the inlet bulkhead connector, lining up the bulkhead compression screw with the opening. Tighten the bulkhead connector with the 5/16-inch or adjustable wrench.
4. Tighten the bulkhead compression screw so that it is finger-tight.
5. Repeat steps 1 through 4 for the outlet line.
6. Tighten all the inlet and outlet compression screws so that they are leak-proof.

Test the Connections

7. Connect the cell inlet line to a pump and the cell outlet line to a suitable waste container.
8. Flush the flow cell cassette with methanol to ensure that there are no leaks. The cassette can withstand approximately 500 psi for 5- μ L cells and 145 psi for 16- μ L cells.
9. If a leak occurs, slightly tighten the leaking compression screw. Retest the cassette for leaks.
10. Drain the flow cell cassette and remove the inlet and outlet lines.

6.4.5 Reinstalling the Flow Cell Cassette

To reinstall the flow cell cassette:

1. Install the flow cell cassette into the chassis. Make sure the flow cell cassette is all the way into the chassis and seated securely on the alignment pins.

Aligning the Cassette

If the alignment pins do not line up with the holes in the chassis, loosen the center cell alignment screw and adjust the cell block so that the cassette seats securely. Tighten the center cell alignment screw.

2. Install and tighten the outer two cell panel screws. Make sure they are tightened evenly.
3. Connect the inlet and outlet lines.
4. Connect the power cord to the receptacle on the rear panel.

6.5 Changing the Lamp

The lamp output energy decreases with age. Waters recommends that you change the xenon lamp annually. If sensitivity is a high priority, you may want to replace the lamp more often.

Note: *Excessive vibration and frequently turning the lamp on and off while it is still hot can considerably shorten the lifetime of the lamp.*

The number of hours that the lamp has been lit is displayed in the Lamp Hours special parameter (see [Section 3.5.9, Lamp Hours](#)). Use this number as a guide to help you determine when to replace the lamp.

The procedure for replacing the lamp consists of:

- Removing the lamp housing
- Replacing the lamp housing
- Adjusting the lamp



Attention: To avoid damage to the lamp, do not touch the glass lamp. Replace the lamp housing as a complete assembly.



Caution: To avoid burn injury, allow components to cool before you replace the lamp.



Caution: To avoid possible injury due to explosion, do not touch the glass lamp. The lamp contains Xenon gas at high pressure. Replace the lamp housing as a complete assembly.

Required Tools

To change the lamp you need the following tools:

- Phillips screwdriver
- Flat-blade screwdriver

6.5.1 Removing the Lamp Housing

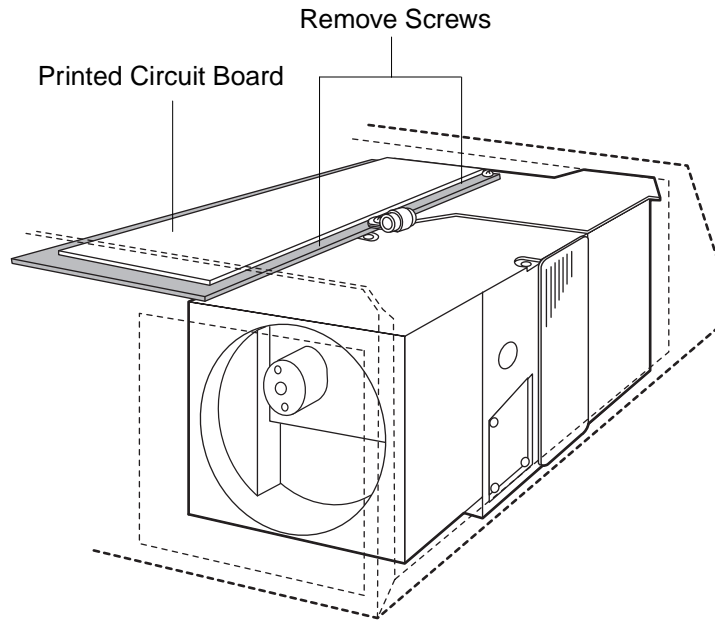
To remove the lamp housing:

1. Turn the Power switch to the Off (O) position, then remove the power cord from the receptacle on the rear panel.



Caution: To prevent the possibility of electrical shock, be sure the power is off and the power cord is disconnected before continuing.

2. Remove the fluidic connections from the flow cell cassette.
3. Move the 474 to a location where you can access the front and rear panels of the unit.
4. Remove the cover, following the procedure in [Section 6.1, Routine Maintenance](#).
5. Remove the two Phillips screws securing the printed circuit board panel, as shown in [Figure 6-5](#).
6. Lift the hinged printed circuit board panel out of the way, as shown in [Figure 6-6](#). Let the panel rest on the side of the 474.



TP01146a

Figure 6-5 Accessing the Lamp Housing



Attention: To avoid damage to the printed circuit board, lift the panel by the edges only. Do not handle the printed circuit board. Static electricity discharges can damage the board. Follow the procedures at the beginning of [Chapter 6. Maintenance Procedures](#), to minimize the risk of damage from static electricity.

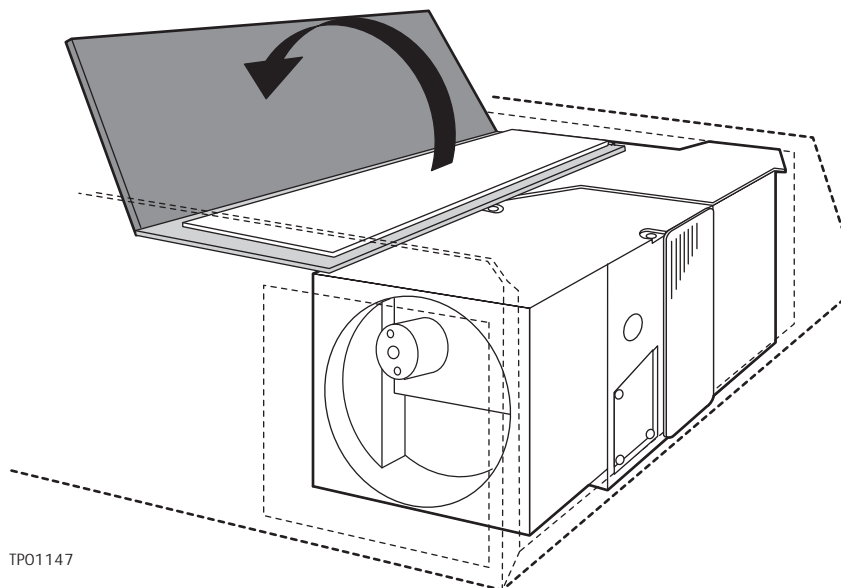


Figure 6-6 Moving the Printed Circuit Board Panel

7. Remove the two Phillips screws securing the fan access door on the rear panel, as shown in [Figure 6-7](#). Open the door.

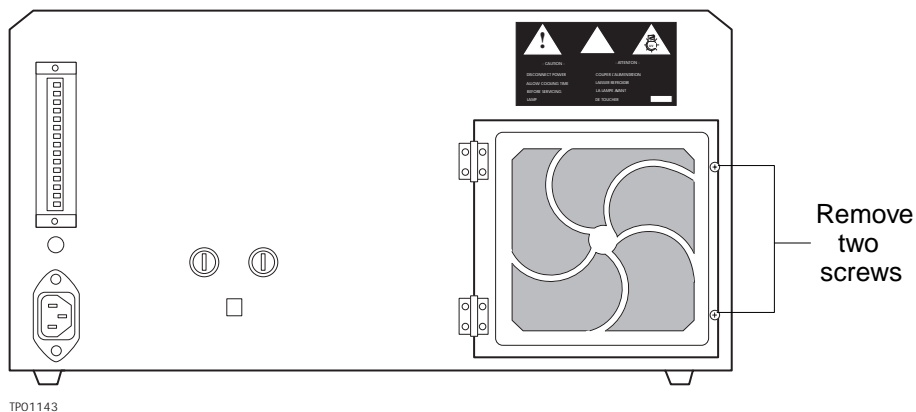
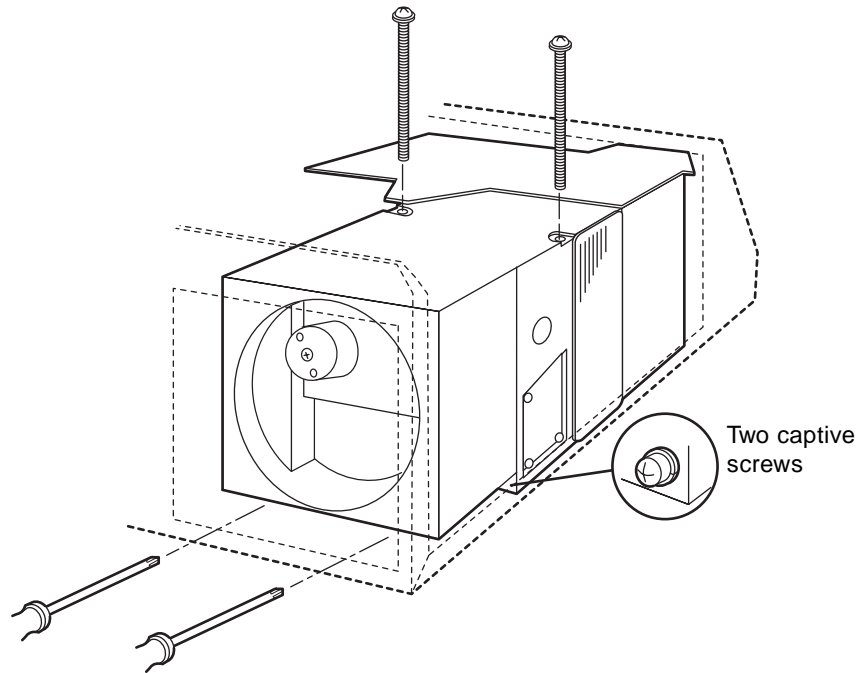


Figure 6-7 Removing the Screws That Secure the Fan Access Door

8. Remove the two large Phillips screws on the top of the lamp housing, as shown in [Figure 6-8](#).



TP01144

Figure 6-8 Removing the Securing Screws on the Lamp Housing

9. Remove the two large Phillips screws from the underside of the lamp housing, as shown in [Figure 6-8](#).

Note: Access these screws through the fan access door. These screws are captive, and remain attached to the housing when they are fully loosened.

10. Lift the lamp housing up and to the side to expose the lamp power supply terminals.
11. Disconnect the two wires from the housing and the power supply. One wire disconnects at the power supply, and the other disconnects at the lamp housing.

Note: Disconnect the bayonet-style terminals by pulling on them. Do not unscrew the screws on the power supply or lamp housing.

12. Remove the lamp housing from the chassis.

6.5.2 Replacing the Lamp Housing

Once you have removed the lamp housing, install the new lamp housing.



Attention: Before installing the lamp housing, remove the transparent tape used to protect the lamp during shipping.

To replace the lamp housing:

1. Lower the replacement lamp housing into the chassis.
2. Connect the two wires to the lamp power supply.
3. Install and tighten the two Phillips screws on the top of the lamp housing.
4. Tighten the two Phillips screws on the underside of the lamp housing.

Note: Do not close and secure the fan access door.

5. Return the printed circuit board panel to its original position. Install and tighten the two Phillips screws.
6. Replace the protective cover over the side panel. Install and tighten the two Phillips screws.

6.5.3 Adjusting the Lamp

Once you have installed the lamp, you need to adjust it for optimum intensity.

To adjust the lamp:

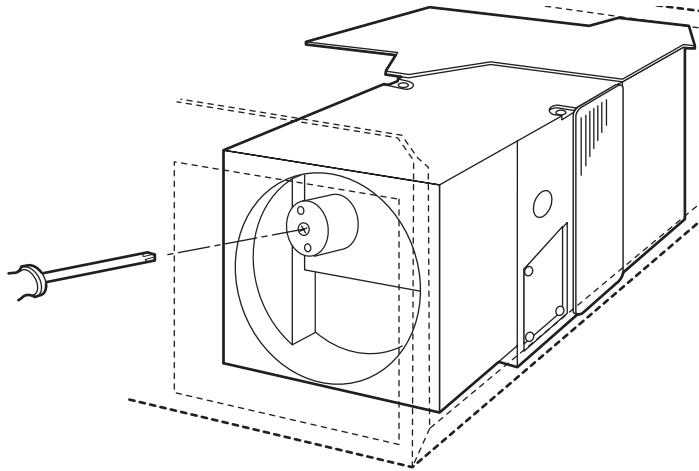
1. Attach the power cord to the receptacle on the rear panel of the 474.
2. Turn the Power switch to the On (I) position.

Note: The power-on diagnostics may indicate poor lamp intensity. This is normal because the lamp is not yet adjusted.

3. Set the Excitation λ parameter to **475** nm by performing the following steps:
 - Press the λ 1 key.
 - Press the **4**, **7**, and **5** keys to select 475.
 - Press the **Edit/Enter** key to enter the selection.
4. Press the **Shift** and the λ 1 keys to display the excitation energy.
5. Allow the 474 to warm up for approximately three minutes. The excitation energy will stabilize enough to adjust the position.

Note: If you increased the gain setting to compensate for the reduced output of the older lamp, an auto zero error message may appear when you power up the 474 with the new lamp installed. In this case, lower the gain setting (see [Section 3.4.4. Gain](#)) then press **Shift+▼** to clear the error message.

6. Set the Lamp Hours to zero. Follow the procedure in [Section 3.5.9. Lamp Hours](#).
7. Adjust the horizontal position of the lamp using the adjustment screw shown in [Figure 6-9](#). Adjust the screw to obtain the maximum excitation energy reading on the display.



TP01145

Figure 6-9 Horizontal Adjustment of the Lamp

8. Close the fan access door. Replace and tighten the two Phillips screws.
9. Turn the Power switch to the Off (O) position.
10. Replace the cover.

Reinstalling the 474

11. Return the 474 to its proper position. Be sure to observe the installation site requirements described in [Section 2.1. Site Selection and Power Requirements](#).
12. Reconnect the fluidic connections on the flow cell cassette.
13. Turn the Power switch to the On (I) position.
14. Allow the 474 to stabilize for one hour before you use it.

6.6 Replacing AC Fuses



Caution: To avoid the risk of electric shock, carefully observe the following instructions when replacing the AC fuses.

Procedure

To remove and replace the fuses:

1. Power off the 474. Make sure that the Power switch is in the Off (○) position.
2. Remove the power cord from the rear panel.
3. Use a small, flat-blade screwdriver to press in the fuse holder and turn it counterclockwise one-quarter turn. The spring-loaded fuse holder pops out when you withdraw the screwdriver.
4. Remove the old fuse from the fuse holder and replace it with a new fuse of the appropriate type, as shown in [Table 6-1](#).

Table 6-1 AC Fuses

Nominal Voltage (VAC)	Voltage Range (VAC)	Fuse Required
115	90 to 132	5 A, fast-blow
230	187 to 264	2.5 A, fast-blow



Warning: To reduce the risk of fire hazard, always replace fuses with the same type and rating.

5. Insert the fuse holder into the receptacle.
6. Use the screwdriver to press in and turn the holder clockwise until it locks into place.

7

Error Messages and Diagnostics

This chapter covers the error messages that can occur during powerup diagnostics and routine operation. It also describes some of the actions that you can take in response to the messages.

In addition, the chapter briefly describes the Diagnostics function available for use by Waters Service representatives.

7.1 Error Messages

The 474 detector displays error messages to alert you to any problems that may occur within the unit. The 474 displays two types of error messages:

- Powerup diagnostic failures
- Operating errors

7.1.1 Power-On Diagnostic Tests

At each power-on, the 474 detector performs a series of diagnostic tests to verify the performance of various subsystems. These tests are listed in [Table 7-1](#), along with the pass and fail messages.

If any of the tests fail:

- The diagnostic routine stops
- The display shows an appropriate error message

Table 7-1 Power-On Diagnostic Tests

Test	Description	Pass Message	Fail Message
ROM Check	Read-only memory check	OK!	ROM Check Error
RAM Check	Random-access memory check	OK!	RAM Check Error
DC Power	DC Voltage check	OK!	DC Power Error
EX Driver	Excitation monochromator driver check	OK!	EX Driver Error
EM Driver	Emission monochromator driver check	OK!	EM Driver Error
Lamp Energy	Excitation energy check	OK!	EM Energy Poor Lamp Energy Off
Lamp Operation	Accumulated hours of lamp use	Hours of operation	None
Bandwidth	Monochromator check Report of bandwidth settings	EX: 18 EM: Selected bandwidth	None

Restarting Tests After a Failure

If a failure occurs during the powerup diagnostic tests, press the **Shift** key and then the **▼** key to continue with the diagnostic tests.

7.1.2 Operating Errors

Two errors generate an error message while the 474 is running:

- Fluid leak in the flow cell cassette
- Auto zero out of range

Fluid Leak Error

The 474 has a small tray under the flow cell cassette. The tray contains a sensor that activates if fluid collects in the tray. If mobile phase leaks into the tray, the circuit activates and two events occur:

- The Cell Leak terminals on the analog connector close
- The display appears, as shown in [Figure 7-1](#)

MODE	SIGNAL	RESPONSE
TROUBLE LEAK IN CELL		
EXλ	EMλ	GAIN ATTN

Figure 7-1 Error Message for Fluid Leak

Note: *The fluid leak sensor works only with conductive mobile phases.*

Press the **Clear** key to reset the error message. If the condition still exists, the 474 displays the error message again after about 10 seconds.

Refer to [Section 6.4, Replacing the Flow Cell](#), for the procedure to check the flow cell.

Auto Zero Error

The auto zero can compensate for drift and baseline changes that are up to 70 percent of the full-scale analog output signal. If an auto zero is requested (manually or automatically) when the emission intensity is greater than 70 percent of the full-scale level, the 474 displays the message shown in [Figure 7-2](#).

MODE	SIGNAL	RESPONSE
WARNING AUTO ZERO		
EXλ	EMλ	GAIN ATTN

Figure 7-2 Auto Zero Error Message

The most common reason for the auto zero error message is excessive light in the emission beam from the flow cell. [Table 7-2](#) lists three possible causes of excessive light from the flow cell and their remedies.

Table 7-2 Possible Causes of the Auto Zero Error Message

Possible Cause	Remedy
High fluorescence intensity	Decrease the Gain setting.
Scattered light from solvent or sample	Investigate the solvent and the Ex and Em settings.
Air bubbles in the solvent or sample	Remove bubbles.

To resolve the cause of an auto zero error message:

1. Perform the most likely remedy listed in [Table 7-2](#).
2. Press the **Clear** key to reset the error message.

If the condition still exists, the 474 displays the error message again after about 10 seconds.

7.2 Diagnostics

The 474 has a diagnostics function with a series of tests that allows Waters service technicians to:

- Check the operating status of the 474
- Troubleshoot the 474

Waters strongly recommends that only Waters service technicians use the Diagnostics functions.



Attention: To avoid compromising the integrity of the 474, do not make unauthorized changes to the parameters in the Diagnostics section.

8

Troubleshooting

This chapter contains:

- General procedures for troubleshooting the 474.
- A troubleshooting table of probable causes and recommended corrective actions.

Keep in mind that the source of apparent detector problems may lie within the chromatography or hardware of your system. Consult the *Waters Guide to Successful Operation of Your LC System* for detailed chromatographic troubleshooting tables.

Most problems are easily corrected. If you cannot correct a problem, contact Waters Technical Service at (800) 252-4752, U.S. and Canadian customers only. Other customers, call your local Waters subsidiary or your local Waters Technical Service Representative, or call Waters corporate headquarters for assistance at (508) 478-2000 (U.S.).

When You Call Waters Technical Service

To expedite your request for service, have the following information available when you call Waters regarding a 474 problem:

- Symptom
- Operating wavelengths (excitation and emission)
- Gain setting
- Attenuation setting
- Filter setting
- Lamp hours
- Type of column
- Operating pressure
- Solvents
- Diagnostic message
- System configuration

Diagnostics

The 474 performs powerup diagnostics to help you to troubleshoot basic detector problems. Refer to [Chapter 7, Error Messages and Diagnostics](#) for descriptions of the diagnostic tests.

Power Supply

The following factors can adversely affect the operation of the 474:

- Power surges
- Line spikes
- Transient energy sources

Be sure that the electrical supply used for the 474 is properly grounded and free from any of these conditions.

8.1 General Troubleshooting Hints

This section describes general troubleshooting procedures tables to help identify and isolate possible causes.

Keep a written record of system conditions under normal operating conditions.

Troubleshooting Procedures

As soon as you realize that a problem may exist:

1. Examine the system. Determine if something obvious is causing the problem.

For example, if the 474 does not power up when you turn it on, check that the unit is plugged in. This may seem trivial, but always eliminate simple causes first.

2. Compare the current system operation with the system operation before you identified the problem.

For example, if your system usually runs at a certain pressure with a certain method:

- Is the system pressure in the same range, or is it drastically higher (possibly caused by a blocked line) or lower (possibly caused by a leak)?
- Are pressure fluctuations in the same range as during normal operation?

Note: This step emphasizes the importance of keeping track of system parameters and the results of your chromatography during normal operation. Troubleshooting is easier if you know the normal operating conditions.

3. Isolate the parameter that varies from normal operation. The parameters to observe include:

- Baseline noise
- Peak retention time
- Peak resolution
- Qualitative/quantitative chromatographic results
- System pressure

Evaluate the parameters in the order presented above to rule out simple causes of the problem.

4. Use the troubleshooting table in the following section to determine corrective actions for the problems that you identify.

8.2 Hardware Troubleshooting

This section contains general system troubleshooting guides for the Waters 474 Scanning Fluorescence Detector.

Table 8-1 General System Troubleshooting

Symptom	Possible Cause	Corrective Action
Detector inoperative	Fuse blown	Check that the fan and the LCD displays are operational; if neither operates, replace the AC fuses on rear panel. Be sure to use fuses of the same rating.
	No power at outlet	Check outlet by connecting another electrical unit known to be in working order and see if it operates.

Table 8-1 General System Troubleshooting (Continued)

Symptom	Possible Cause	Corrective Action
Front panel LCD fails to illuminate	Broken electrical connection Fuse blown Bad LCD control board	Check electrical connections. Replace any faulty fuses on the lamp or power supply board. Call Waters Technical Service.
Front panel displays odd characters	Bad LCD control board	Call Waters Technical Service.
Keypad not functioning	Keypad defective	Call Waters Technical Service.
Xenon source lamp does not light	Faulty fuse Faulty lamp connections Bad lamp power supply board	Replace the lamp power supply board fuse. Plug in the lamp connector. Call Waters Service.
Unit overheating	Dirty ventilation filters Faulty fan	Clean the ventilation air filters. Refer to Section 6.2, Cleaning the Ventilation Filters . Call Waters Technical Service.
No excitation energy	Lamp burned out	Replace the lamp. Refer to Section 6.5, Changing the Lamp .

Appendix A

Specifications

This appendix includes information on operational specifications.

Table A-1 Operational Specifications

Condition	Specification
Monochromator	Holographic concave diffraction grating (for both excitation and emission)
Light source	150 W Xenon lamp
Wavelength range	200 to 700 nm (for excitation and emission). Can be set from 0 to 900
Spectrum band width	Excitation: 18 nm Emission: 10, 18, or 40 nm (set on keypad)
Wavelength accuracy	±2 nm
Wavelength repeatability	±0.3 nm
Detector	Excitation: Photodiode Emission: Photomultiplier tube
Second order filter	Automatically inserted into light path when Ex > 400 nm
Flow cell	16 µL (standard) or 5 µL
Maximum flow cell pressure	16 µL: 145 psi (10 bar) 5 µL: 500 psi (34.5 bar)
Wetted surfaces	Synthetic silica, fluororesin, PEEK, stainless steel SXS-316

Table A-1 Operational Specifications (Continued)

Condition	Specification
Sensitivity	S/N for the Raman peak of water is 320 or above, where $\text{Ex } \lambda = 350 \text{ nm}$ and time constant is 1.5 sec, 16 μL cell (Instruments shipped prior to June 1997, S/N for the Raman peak of water is 200 or above)
Attenuation range (Recorder output)	Ten steps, including 1, 2, 4, 8, 16, 32, 64, 128, 256, S
Gain range	1, 10, 100, 1000
Response	Fast, Standard, Slow, and digital filter
Signal processing	Digital processing by A/D and D/A converters (with surrounding temperature compensation circuits)
Outputs	<ul style="list-style-type: none"> • Recorder: 10 mV full-scale (can be inverted) • Integrator: 1 V full-scale • Marker output • Leak output
Inputs	<ul style="list-style-type: none"> • Marker input • Auto Zero input • Program reset
Program functions	Time program with following variables: <ul style="list-style-type: none"> • Wavelength change (excitation and emission) • Gain • Attenuation • Wavelength scan • Ex, Em Spectra
Wavelength scan functions	Manual and time program parameters: <ul style="list-style-type: none"> • Time • Interval • Amplitude • Memory location • Ex, Em Spectra

Table A-1 Operational Specifications (Continued)



Condition	Specification
Spectrum storage	Ten locations for both emission and excitation spectra
Self-test diagnostics	<ul style="list-style-type: none"> • Memory (ROM and RAM) • DC power • Excitation energy decrease • Leak in cell • Lamp use time
Lamp use timer	Internal timer for accumulating the total hours on the Xenon lamp
Dimensions	Width: 11.81 inches (300 mm) Height: 5.90 inches (150 mm) Depth: 18.11 inches (460 mm)
Weight	41.8 pounds (19 kg)
Power requirement	115 VAC (90 to 132 V), 50/60 Hz 230 VAC (187 to 264 V), 50/60 Hz
Power consumption	300 W maximum
Ambient temperature	10 °C to 32 °C (50 °F to 90 °F) operation 10 °C to 60 °C (50 °F to 140 °F) storage
Relative humidity	20 to 80%, non-condensing

Appendix B

Spare Parts and Accessories

The following spare parts and accessories are recommended for customer installation. Any parts not listed in this section may require installation by a Waters service representative.

Table B-1 Spare Parts and Accessories

Item	Part Number
Fuse 230 V, 2.5 A	WAT072919
Fuse 115 V, 5 A	WAT072774
Flow Cell Repair Kit, 5 μ L	WAT047494
Flow Cell Repair Kit, 16 μ L	WAT047491
Flow Cell Gaskets (kit of 2)	WAT047477
Cell Mask	WAT031326
Cell Inlet/Outlet Tube Assembly	WAT031328
Start-up Kit	WAT047434
Panel Bulkhead Connectors	WAT031317
Signal Cable	WAT057235
Power Cable	WAT097194
Compression Screws (pack of 10)	WAT005070
Ferrules (pack of 10)	WAT005063
Compression Screws and Ferrules (pack of 5 each)	WAT025604
Optical Filter Kit	WAT047448



Table B-1 Spare Parts and Accessories (*Continued*)

Item	Part Number
Xenon Lamp Assembly	WAT047447
Flow Cell Assembly, 5- μ L	WAT047479
Flow Cell Assembly, 16- μ L	WAT047478
Cuvette Holder Option	WAT047475
Photomultiplier Tube R3788, High Sensitivity (Standard on instruments shipped beginning June, 1997)	WAT047416
Photomultiplier Tube R928, Red Sensitive	WAT031336
Photomultiplier Tube P28, Standard (Standard on instruments shipped before June, 1997)	WAT031313

B

B.1 Using the Optional Cuvette Cell

The optional cuvette cell allows you to measure the fluorescence of a sample without introducing the sample into the fluidic path of your chromatographic system. The cuvette cell simplifies sample handling and is especially useful when only a small amount of sample is available or during instrument verification and qualification.

You fill the quartz cuvette cell with sample and mount it in the cuvette cell cassette, which is then installed in place of the standard flow cell cassette. You can use all normal functions of the detector to analyze samples in the cuvette cell.



Attention: Use a quartz cuvette cell with four clear sides. Cuvette cells with frosted sides are not suitable for use in the 474.

Installing the Cuvette Cell Cassette



Attention: Handle the cuvette by the top and bottom only. Fingerprints on the sides of the cell interfere with the light path and compromise the integrity of your results.

To install the cuvette cell cassette:

1. Remove the standard flow cell cassette as described in [Section 6.4.1, Removing the Flow Cell Cassette](#).
2. Fill the cuvette cell with the desired sample and screw on the cuvette cell cap.

Note: The sample level in the cuvette cell must be high enough to completely fill the oval source-light window in the cuvette cell cassette. To ensure the source light window is full, always fill the cuvette cell with at least 3 mL of sample.

3. To ensure a clear and clean cuvette cell, wipe the sides of the cuvette cell with a low lint, nonabrasive tissue.
4. Gently slide the cuvette cell straight down into the holder in the cuvette cell cassette.
5. Turn the the cuvette cell cassette lock lever to the **Unlocked** position.
6. Install the cuvette cell cassette into the chassis ([Figure B-1](#)). Make sure the cuvette cell cassette is all the way into the chassis and seated securely on the alignment pins.
7. Turn the cuvette cell cassette lock lever clockwise to the **Locked** position.

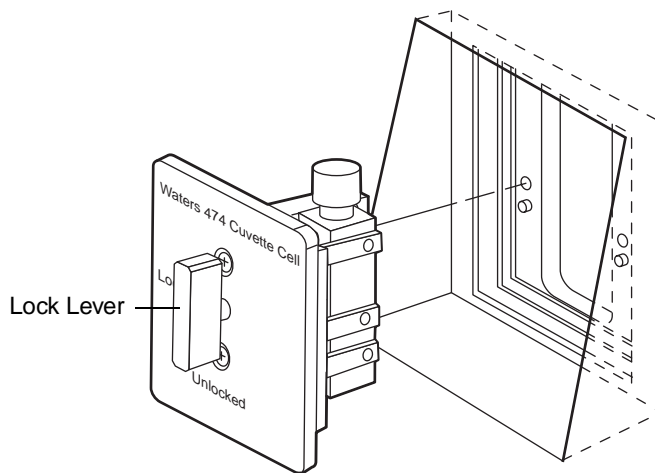


Figure B-1 Installing the Optional Cuvette Cell Cassette

B

Removing the Cuvette Cell Cassette

To remove the cuvette cell cassette:

1. Turn the cuvette cell cassette lock lever counterclockwise to the **Unlocked** position.
2. Slide the cuvette cell cassette out of the chassis.

B.2 Using the Optional Optical Filters

You can attach optional optical filters to the 474 flow cell for additional flexibility in making measurements. Use the filters only when the 474 is in the zero order mode, which eliminates the effects of the monochromators on the light path.

To select the zero order mode:

1. Set the Wavelength Range parameter to “**Not Restricted**”, as described in [Section 3.5.11, Wavelength Range](#).
2. Use the band pass filters on the excitation side of the flow cell to select a wide band of excitation energy.
3. Use the long-pass filters on the emission side of the flow cell to collect all light above the cutoff wavelength of the filter.

[Table B-2](#) describes the optional filters available.

Table B-2 Optional Optical Filter Characteristics

Filter Type	Use	Characteristics
UV-D36C	Excitation	360 nm bandpass filter
C-39B	Excitation	390 nm bandpass filter
B-460	Excitation	460 nm bandpass filter
UV-32	Emission	Sharp cut long pass filter, 50% transmittance near 320 nm
UV-36	Emission	Sharp cut long pass filter, 50% transmittance near 360 nm
L-39	Emission	Sharp cut long pass filter, 50% transmittance near 390 nm
Y-46	Emission	Sharp cut long pass filter, 50% transmittance near 460 nm

The characteristics of the three excitation filters are shown in [Figure B-2](#). The characteristics of the four emission filters are shown in [Figure B-3](#).

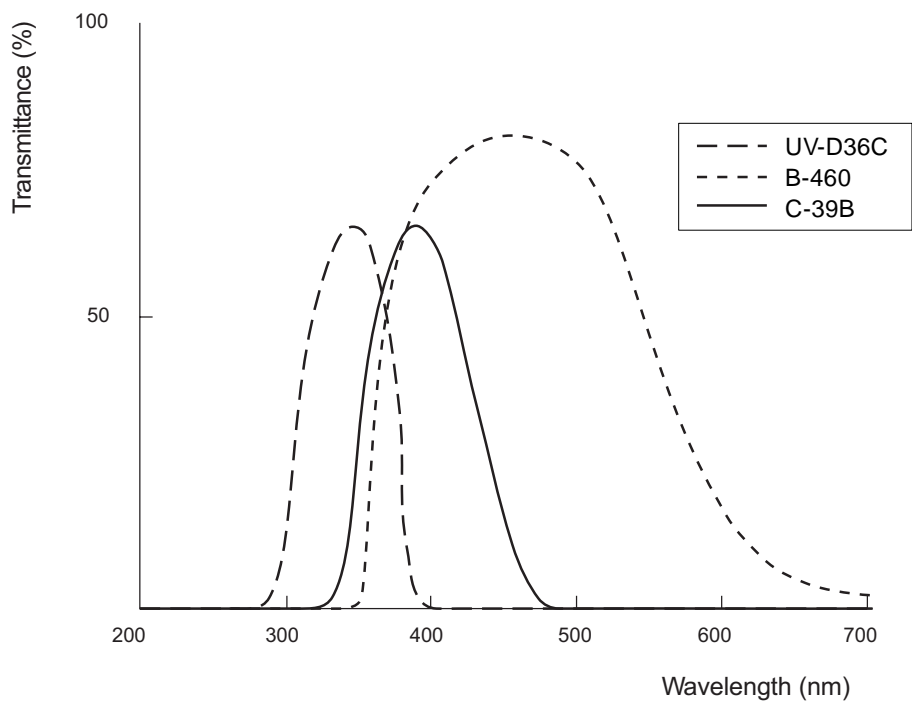


Figure B-2 Characteristics of Excitation Filters

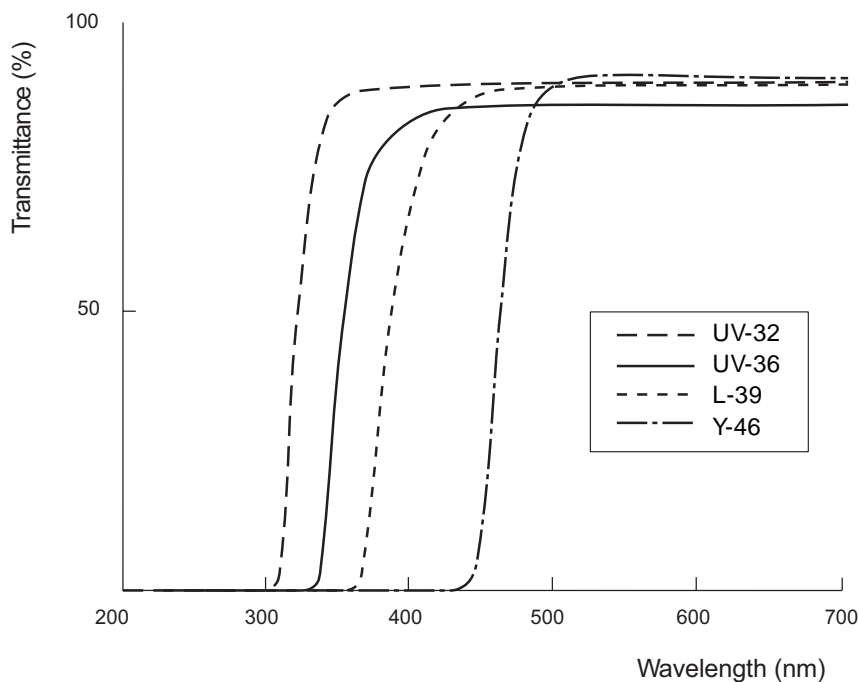
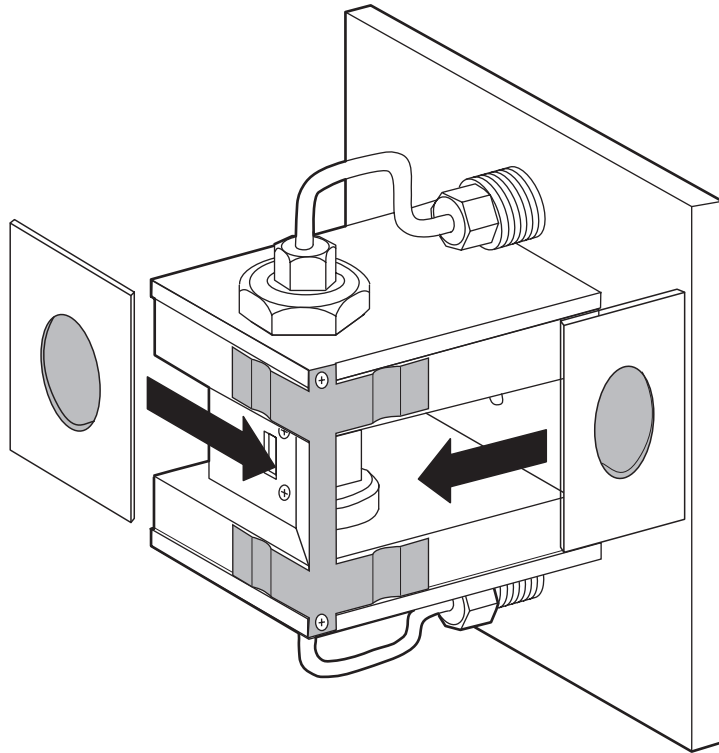


Figure B-3 Characteristics of Emission Filters

Attaching a Filter

To attach a filter to the flow cell:

1. Remove the two Phillips screws that hold the flow cell cassette to the front panel of the 474.
2. Remove the flow cell cassette. Be careful not to kink the inlet or outlet tubing.
3. Slide the filter under the clips on the appropriate side of the flow cell. See [Figure B-4](#) for details.



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Figure B-4 Installing Optical Filters on the Flow Cell



Attention: To avoid damage to the filters, handle them by the edges only.

4. Install the flow cell cassette in the front panel.
5. Reinstall the two Phillips screws that hold the flow cell in place.

Removing a Filter

To remove a filter from the flow cell:

1. Remove the two Phillips screws that hold the flow cell cassette to the front panel of the 474.
2. Remove the flow cell cassette. Be careful not to kink the inlet or outlet tubing.
3. Slide the filter out from under the clips on the flow cell. See [Figure B-4](#) for details.



Attention: To avoid damage to the filters, handle them by the edges only.

4. Install the flow cell cassette in the front panel.
5. Reinstall the two Phillips screws that hold the flow cell in place.
6. Set the Wavelength Range parameter to **EM > EX+10** if you are finished using the optical filters. See [Section 3.5.11, Wavelength Range](#) for instructions on changing the Wavelength Range parameter.

2.3 Optional Photomultiplier Tubes

The Waters 474 Scanning Fluorescence Detector has two optional photomultiplier tubes (PMTs). One can be used for high-sensitivity measurements and is now supplied standard, the other is designed for long-wavelength (red-sensitive) measurements.

The high-sensitivity PMT is supplied standard in instruments shipped beginning June 1997. The maximum wavelength is approximately 750 nm.

The red-sensitive PMT has a maximum wavelength of approximately 900 nm, while the standard PMT has a maximum wavelength of approximately 650 nm. [Table B-3](#) summarizes the designations and maximum wavelength limits of the three PMTs.

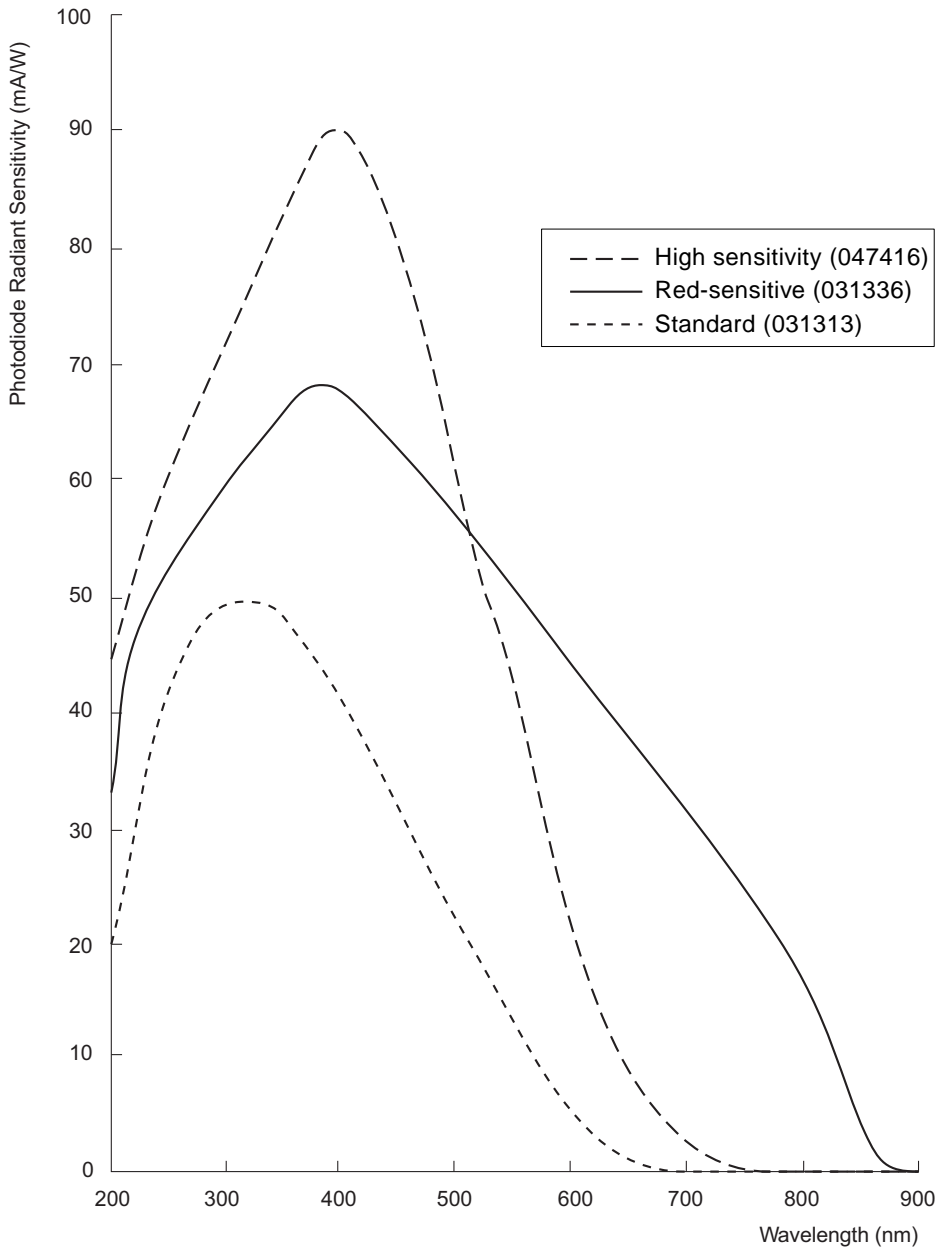
Table B-3 Photomultiplier Tube Type Numbers

Photomultiplier Tube	Type Number	Maximum Wavelength (nm)
Standard (WAT031313)	1P28	650
High-sensitivity (WAT047416)	R3788	750
Red-sensitive (WAT031336)	R928	900

Note: The PMT should only be replaced by a Waters Service representative.

[Figure B-5](#) shows the spectral response characteristics of the three photomultiplier tubes.

Note: The sensitivity of the 474 also depends on factors other than the photomultiplier characteristics, such as stray light from the monochromator and background noise. Replacing the photomultiplier may not always provide the desired improvement in PMT performance.



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Figure B-5 Sensitivity of Optional Photomultiplier Tubes

Appendix C

Warranty Information

This appendix includes information on:

- Limited express warranty
- Shipments, damages, claims, and returns

C.1 Limited Express Warranty

Waters Corporation provides this limited express warranty (the Warranty) to protect customers from nonconformity in the product workmanship and materials. The Warranty covers all new products manufactured by Waters.

Waters warrants that all products that it sells are of good quality and workmanship. The products are fit for their intended purpose(s) when used strictly in accordance with Waters' instructions for use during the applicable warranty period.

Limited Warranty

WATERS Corporation warrants that the Waters 474 Scanning Fluorescence Detector is a Class I medical device under 21 CFR 862.2260, as now in effect, and is for general purpose use and is not for use in clinical diagnostic procedures, and that during the Warranty period, the performance of all components of the Waters 474 Scanning Fluorescence Detector [other than Third-Party Components], will not deviate materially from the Specifications for such Waters 474 Scanning Fluorescence Detector. Warranties, if any, that may be applicable to Third-Party Components shall be provided by the respective manufacturers or suppliers of such Third-Party Components, and WATERS Corporation shall use reasonable efforts to assist Customer in securing the benefits of any such warranties.

Exclusions

The foregoing warranty does not apply to any material deviation from the Specifications by any component of the Waters 474 Scanning Fluorescence Detector that results from (a) use of the Waters 474 Scanning Fluorescence Detector for any purpose other than general purpose use and specifically excluding use of the Waters 474 Scanning Fluorescence Detector in clinical diagnostic procedures, or use of the Waters 474

Scanning Fluorescence Detector for investigational use with or without confirmation of diagnosis by another, medically established diagnostic product or procedure, (b) errors or defects in any Third-Party Component, (c) modification of the Waters 474 Scanning Fluorescence Detector by anyone other than WATERS Corporation, (d) failure by Customer to install any Standard Enhancement in accordance with an update procedure, release of firmware or any operating system release, (e) any willful or negligent action or omission of Customer, (f) any misuse or incorrect use of the Waters 474 Scanning Fluorescence Detector, (g) any malfunction of any information system or instrument with which the Waters 474 Scanning Fluorescence Detector may be connected, or (h) failure to establish or maintain the operating environment for the Waters 474 Scanning Fluorescence Detector in accordance with the Operator's Manual.

Exclusive Remedy

In the event of any failure of the Waters 474 Scanning Fluorescence Detector to perform, in any material respect, in accordance with the warranty set forth herein, the only liability of WATERS Corporation to Customer, and Customer's sole and exclusive remedy, shall be the use, by WATERS Corporation, of commercially reasonable efforts to correct for such deviations, in WATERS Corporation's sole discretion, replacement of the purchased Waters 474 Scanning Fluorescence Detector, or refund of all amounts theretofore paid by Customer to WATERS Corporation for the Waters 474 Scanning Fluorescence Detector.

Disclaimers

THE LIMITED WARRANTY SET FORTH HEREIN IS EXCLUSIVE AND IN LIEU OF, AND CUSTOMER HEREBY WAIVES, ALL OTHER REPRESENTATIONS, WARRANTIES AND GUARANTEES, EXPRESS OR IMPLIED, INCLUDING WITHOUT LIMITATION ANY IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS OF THE WATERS 474 SCANNING FLUORESCENCE DETECTOR FOR A PARTICULAR PURPOSE, INCLUDING FITNESS FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES OR FOR INVESTIGATIONAL USE WITH OR WITHOUT CONFIRMATION OF DIAGNOSIS BY ANOTHER MEDICALLY ESTABLISHED DIAGNOSTIC PRODUCT OR PROCEDURE, OR NONINFRINGEMENT, AND ANY WARRANTIES ARISING OUT OF COURSE OF DEALING OR COURSE OF PERFORMANCE. CUSTOMER EXPRESSLY ACKNOWLEDGES THAT BECAUSE OF THE COMPLEX NATURE OF THE WATERS 474 SCANNING FLUORESCENCE DETECTOR AND ITS MANUFACTURE, WATERS CORPORATION CANNOT AND DOES NOT WARRANT THAT THE OPERATION OF THE WATERS 474 SCANNING FLUORESCENCE DETECTOR WILL BE WITHOUT DEFECT. CUSTOMER EXPRESSLY ACKNOWLEDGES THAT CUSTOMER IS SOLELY RESPONSIBLE FOR USE OF THE WATERS 474 SCANNING FLUORESCENCE DETECTOR IN CLINICAL DIAGNOSTIC PROCEDURES OR FOR INVESTIGATIONAL USE WITH OR WITHOUT CONFIRMATION OF DIAGNOSIS BY ANOTHER MEDICALLY ESTABLISHED DIAGNOSTIC PRODUCT OR PROCEDURE.

Warranty Service

Warranty service is performed at no charge and at Waters' option in one of three ways:

- A service representative is dispatched to the customer facility.
- The product is repaired at a Waters repair facility.
- Replacement parts with appropriate installation instructions are sent to the customer.

Nonconforming products or parts are repaired, replaced with new or like-new parts, or refunded in the amount of the purchase price, when the product is returned. Warranty service is performed only if the customer notifies Waters during the applicable warranty period.

Unless otherwise agreed at the time of sale, warranty service is not provided by dispatching a service representative when the equipment has been removed from the initial installation location to a new location outside the home country of the selling company.

Warranty service is provided during business hours (8 AM to 5 PM, Monday through Friday). Service is not available when Waters offices are closed in observance of legal holidays.

Warranty Service Exceptions

Warranty service is not performed on:

- Any product or part that has been repaired by others, improperly installed, altered, or damaged in any way.
- Products or parts identified prior to sale as not manufactured by Waters. In such cases, the warranty of the original manufacturer applies.
- Products that malfunction because the customer has failed to perform maintenance, calibration checks, or observe good operating procedures.
- Products that malfunction due to the use of unapproved parts and supplies.

Repair or replacement is not made:

- For expendable items such as filament devices, panel lights, fuses, batteries, and seals, if such items were operable at the time of initial use.
- Because of decomposition due to chemical action.
- For used equipment.
- Because of poor facilities, operating conditions, or utilities.

Warranty Period

This instrument is warranted against defects in workmanship and materials for a period of twelve months (the “Warranty Period”), excluding assemblies, modules, serviceable parts, and components that may have different warranty periods. Refer to [Table C-1](#) below for the warranty periods of such assemblies, modules, serviceable parts, and components.

The Warranty Period commences at the date of product shipment. The Warranty Period may be extended for such time, not to exceed one month, required to deliver and install the product at the customer’s site. In no case does the Warranty Period extend beyond **13 months** from date of shipment. If an item is repaired or replaced during the Warranty Period, the replacement part or repair is warranted for the balance of the original warranty period.

[Table C-1](#) summarizes the warranty periods for the Waters 474 Scanning Fluorescence Detector and its components.

Table C-1 Waters 474 Scanning Fluorescence Detector Warranty Periods

Component	Warranty Period
Waters 474 Scanning Fluorescence Detector, excluding the components listed below	1 year
Normal Wear and Maintenance Parts in Performance Maintenance Kit, part number WAT052665* (excluding xenon source lamp) <ul style="list-style-type: none">Lamp mirror assemblyHigh voltage lamp electrode	90 days
Xenon Source Lamp	1000 hours of lamp usage (as indicated by the lamp operation parameter) or 1 year, whichever comes first
Expendables, Consumables, and Operating Supplies <ul style="list-style-type: none">FusesTubing and fittings	Warranted to function properly when delivered

* Instrument improvements may result in changes to the contents of this kit.

Lamp Replacement Warranty

The 474 Scanning Fluorescence Detector xenon source lamp is warranted to light and pass powerup verification tests for 1000 hours. Under normal operating conditions, the functional life of the source lamp is expected to exceed the warranty coverage period.

C.2 Shipments, Damages, Claims, Returns

Shipments

Since all shipments are made free on board (FOB) shipping point, Waters suggests that you authorize insurance on all shipments. Instruments and major components are shipped by surface, unless otherwise required. Supplies and/or replacement parts are shipped via UPS, UPS Blue, air parcel post, or parcel post unless otherwise requested.

Damages

The Interstate Commerce Commission has held that carriers are as responsible for concealed damage as for visible damage that occurs in transit. Unpack shipment promptly after receipt as there may be concealed damage. When concealed damage is discovered, cease further unpacking of the unit involved and request immediate inspection by the local agent or carrier. Secure a written report of the inspector's findings to support the claim. The request for inspection must be made within 15 days of shipment receipt. Otherwise, the claim will not be honored by the carrier. Do not return damaged goods to the factory without first securing an inspection report and contacting Waters for a return merchandise authorization (RMA) number.

Claims

After a damage inspection report is secured, Waters cooperates fully in supplying replacements and handling of a claim, which may be initiated by either party.

Returns

No returns may be made without prior notification of, and authorization from, Waters. If for any reason it is necessary to return material to Waters, please contact Waters Customer Service or your nearest Waters subsidiary or representative for a return merchandise authorization (RMA) number and forwarding address.

Index

A

- AC power connections [39](#)
- Accessories [178](#)
- Air filters, cleaning [151](#)
- Analog connector
 - making connections [40](#)
 - overview [40](#)
 - pin assignments [41](#)
 - pin descriptions [42–43](#)
- Attenuation and gain, using [80](#)
- Attenuation parameter [80](#)
- Audible feedback [72](#)
- Auto zero
 - automatic [88](#)
 - manual [88](#)
- Auto zero enable special parameter [87–89](#)
- Auto zero error [169](#)
- Auto Zero In (AZ IN) terminal
 - connections [48](#), [53](#), [60](#), [61](#)
 - description [97](#)

B

- Bandwidth special parameter [92–93](#)
- Beam splitter [29](#)
- Beeps *see* Audible feedback
- Bus LAC/E connections [44–45](#)
- Bus SAT/IN connections [43–46](#)

C

- Carbamate system connections [62–64](#)
- CELL LEAK (+ and –) terminals
 - connections [52](#), [57](#)
 - description [96](#)

- Chart marks [59](#)
- Chart recorder connections [59–60](#)
- Clearing an error [69](#)
- Compression screw [38](#)
- Connections
 - AccQ•Tag system [66–67](#)
 - Auto Zero In (AZ IN) terminals [48](#), [53](#), [60](#), [63](#), [66](#)
 - Bus SAT/IN to Bus LAC/E [44–45](#)
 - Carbamate system [62–64](#)
 - CELL LEAK (+ and –) terminals [52](#), [57](#)
 - chart recorder [59–60](#)
 - EX OUT (+ and –) terminals [67](#)
 - INT (+ and –) terminal
 - connections [58–58](#)
 - MARK IN terminal [48](#), [49](#), [54](#), [62](#), [65](#)
 - Millennium Data System [43–46](#)
 - REC (+ and –) terminals [59–60](#)
 - voltmeter [67](#)
 - Waters 2690 Separations Module [48](#)
 - Waters 600 Series Controller [53](#)
 - Waters 717plus [60–61](#)
 - Waters 745/745B/746 Data
 - Module [58–58](#)
 - Waters Bus SAT/IN Module [43–46](#)

- Cover, removing [150](#)
- Cuvette cell
 - description [179](#)
 - installing [180](#)
 - removing [181](#)

D

- Data rate in spectrum output [136](#)
- Difference spectrum
 - emission [143–146](#)
 - excitation [146–148](#)
 - overview [142](#)

Display [70](#)
 Display parameters [71–71](#)

E

Electrical connections
 ac power [39](#)
 analog connector [40](#)
 see also Connections

Emission spectrum
 difference [143–146](#)
 measurement [133–134](#)
 output [137–139](#)
 storing [132](#)

Emission wavelength parameter [79](#)

Error
 auto zero [169](#)
 fluid leak [169](#)
 recovery [168](#)

Error messages
 diagnostic tests [167–168](#)
 operating errors [168–170](#)

EX OUT (+ and –) terminals
 connections [67](#)
 description [96](#)

Excitation spectrum
 difference [146–148](#)
 measurement [134–136](#)
 output [139–141](#)
 storing [132](#)

Excitation wavelength parameter [79](#)

F

Ferrule [38](#)
 Filter [25](#)
 digital mode [87](#)

installing optical [183–184](#)
 long-wavelength [26](#)
 monochromator [25](#)
 optional [181](#)
 RC mode [86](#)
 removing optical [184](#)

Flow cell
 description [26](#)
 flow cell cassette [70](#)
 flushing [152–153](#)
 installing optical filters [183–184](#)
 pressure rating [36](#)
 removing optical filters [184](#)
 replacing [153–159](#)

Fluid connections [36–38](#)
 assembling fittings [38](#)
 cutting tubing [37](#)
 flushing the system [38](#)
 making connections [38](#)

Fluid leak error [169](#)

Fluorescence
 detection [24](#)
 filtering [25](#)
 light sources [25](#)
 measuring [26](#)
 overview [24](#)

Flushing
 flow cell [152–153](#)
 system [38](#)

Fuses
 replacing [166](#)
 requirements [33](#)

G

Gain and attenuation, using [80](#)
 Gain parameter [79](#)

H

How to use this guide [19](#)

I

Initial conditions of Time Program [109–110](#)

Inject Start connections [60](#), [63](#), [65](#), [66](#)

INT (+ and –) terminals, connections [46](#), [58](#)

K

Key descriptions [74–77](#)

Keypad [70](#)

L

LAC/E connections. See Bus LAC/E connections

Lamp hours special parameter [91–92](#)

Lamp off timer special parameter [83–84](#)

Lamp, changing [159–165](#)

LCD. see Display

M

Maintenance, see Routine Maintenance

MARK IN terminal

connections [48](#), [49](#), [54](#), [62](#), [65](#)

description [97](#)

MARK OUT (+ and –) terminals

description [96](#)

Menu

program [112](#)

scan [129](#)

special parameter [81](#)

Method Start connections [51](#), [56](#)

Millennium Data System
connections [43–46](#)

Monochromator

emission [26](#), [27](#), [29](#)

excitation [26](#), [29](#)

overview [25](#)

N

Noise test [98–102](#)

Normal mode [78](#)

O

Offset special parameter [89](#)

Operating parameters [77–78](#)

Optical bench [28–29](#)

Optical filters, optional [181](#)
wavelength range parameter [94](#)

Order filter [79](#)

Orders [26](#)

P

Parameter settings

changing [78](#)

displaying [78](#)

Photomultiplier tube [27](#)

optional [185](#)

type numbers [185](#)

PMT see Photomultiplier tube

Polarity special parameter [90–91](#)

Power requirements [33](#)

Power switch [70](#)

Powering down [103](#)

Powering up [68](#)
 Preamplifier output special parameter [83](#)
 Pressure rating, flow cells [36](#)
 Program Menu [112](#)
 Program mode [105](#)
 Program number special parameter [82](#)
 Program Reset (PRGM RST) terminal
 description [97](#)

R

Raman band of water test [98–102](#)
 REC (+ and –) terminals
 connections [59–60](#)
 description [59–60](#)
 Removing the cover [150](#)
 Response parameter [80](#)
 Returning damaged shipments [191](#)
 Routine maintenance
 checks [151](#)
 cleaning air filters [151](#)
 flow cell [152–153](#)
 RS-232 Control Option kit [47](#)

S

SAT/IN connections. See Bus SAT/IN
 connections
 Scan mode
 accessing [129–130](#)
 Scan menu [129](#)
 Scan parameters
 output rate [142](#)
 rate [130](#)
 setting [131–131](#)
 width [130](#)

Scans [27](#)
 Selectivity [26](#)
 Self-diagnosis screen messages [68](#)
 Sensitivity [26](#)
 Shift key [82](#)
 Signal filter special parameter [84–87](#)
 Signal-to-noise test [102–103](#)
 Site selection [32](#)
 Spare parts [178](#)
 Special parameter
 auto zero enable [87–89](#)
 bandwidth [92–93](#)
 changing [82](#)
 lamp hours [91–92](#)
 lamp off timer [83–84](#)
 menu [81](#)
 offset [89](#)
 polarity [90–91](#)
 preamplifier output [83](#)
 program number [82](#)
 signal filter [84–87](#)
 temperature correction [95](#)
 wavelength range [94–95](#)
 Specifications [175](#)
 Spectrum memory [132](#)
 Spectrum output
 connections [136](#)
 data rate [136](#)
 wavelength markers [136](#)
 Static electricity considerations [150](#)
 Storing spectra in a Time Program [126–128](#)

T

- Temperature correction special parameter [95](#)
- Time program
 - adding a step [123](#)
 - deleting a step [124–124](#)
 - deleting an entire program [124](#)
 - description [105](#)
 - external start [120–121](#)
 - initial conditions [109–110](#)
 - manual start [119–120](#)
 - modifying [121–124](#)
 - Program Menu [112](#)
 - programming flowchart [108](#)
 - storing [113](#)
 - storing spectra [126–128](#)
 - wavelength scan [125–126](#)
 - writing programs [107–108](#)
 - writing steps [112–113](#)
- Tools
 - analog cable connections [40](#)
 - changing the lamp [160](#)
 - cutting tubing [37](#)
 - replacing the flow cell [153](#)

U

- Unpacking the 474 [34](#)

V

- Voltage selection switch [35](#)
- Voltmeter, connecting [67](#)

W

- Warm-up period [69](#)
- Warranty information [187](#)
- Waters 2690 Separations Module connections [48](#)
- Waters 600 Series Controller connections [53](#)
- Waters 717plus connections [60–61](#)
- Waters 745/745B/746 Data Module connections [58–58](#)
- Waters AccQ•Tag system connections [66–67](#)
- Waters Bus SAT/IN Module connections [43–46](#)
- Wavelength markers [142](#)
- Wavelength range special parameter [94–95](#)
- Wavelength scan in a Time Program [125–126](#)
- Wavelength scans [27](#)

Z

- Zero order operation [94](#)