
HP 1046A Fluorescence Detector

User's Guide

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Warranty

For details of warranty, see
"Warranty Statement" in Chapter 8.

Safety

For details of safety, see "Safety
Information" in Chapter 8.



The apparatus is marked with this
symbol when the user should refer
to the instruction manual in order to
protect the apparatus against
damage.



Indicates dangerous voltages.



Indicates a protected ground
terminal.

About This Guide

This guide is for the HP 1046A Programmable Fluorescence Detector. It describes installation, operation, maintenance and troubleshooting of your detector and is designed to be used as both a beginner's self-teaching guide and a day-to-day reference guide.

Chapter 1 "Getting Started" describes a few basic functions of your detector so that you can get chromatographic results as quickly as possible.

Chapter 2 "Optimizing Detection" describes the parameters you can use to optimize detection. The chapter gives you examples of how to optimize the settings of the detector's parameters.

Chapter 3 "Troubleshooting Your Detector" helps you to investigate problems, find the cause and take appropriate action.

Chapter 4 "Maintaining Your Detector" describes the tasks you can perform to keep your detector in good working order.

Chapter 5 "Touring Your Detector" describes the theory behind fluorescence and phosphorescence detection, as well as the design of the optical unit, data acquisition and data handling in the electronic firmware.

Chapter 6 "Detector Reference" describes each of the detector's functions in detail. The functions are arranged logically according to the keyboard for easy access.

Chapter 7 "Installing Your Detector" describes how to install your detector and how to verify that it is working correctly.

Chapter 8 "Legal, Safety and Regulatory Information" gives you details of warranty, safety, and other regulatory information.

What Other Guides are Available?. Your detector is also supplied with a *Quick Reference Guide*. Keep your *Quick Reference Guide* near to your detector so that day-to-day reference information is at your fingertips. The *Quick Reference Guide* is available separately should you require further copies.

A *Service Handbook* is available for detailed reference. It contains information on service and repair, with descriptions of all components down to printed-circuit board level. It is not shipped with the detector. You can order the service handbook by contacting your local Hewlett-Packard office.

Ordering Information.

Title	Hewlett-Packard Part Number
<i>User's Guide</i>	01046-90004
<i>Quick Reference Guide</i>	01046-90002
<i>Service Handbook</i>	01046-90100

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Getting Started

Getting Started

This chapter describes how to start your detector and explains examples of fluorescence, phosphorescence, and chemiluminescence detection.

Starting Your Detector

1. Install your detector as described in Chapter 7.
2. Turn on line power to detector at LINE switch on rear panel.

The detector does a self-test and after about 1 minute displays:

HP 1046A detector on (X-2718)

If anything else is displayed (the text in parentheses may vary) or if the *Not ready* or *Error* lamps are on, see Chapter 3.

3. Press    to turn on the xenon lamp.

You are now ready to make measurements with your detector.

Using Fluorescence Detection

To follow this example you need a dilute solution of anthracene, benzo[a]fluoranthrene, and cyclopentapyrene in methanol. To separate this mixture you need a mobile phase of 20% bidistilled water and 80% methanol, and an MOS-Hypersil column, 100 mm × 4.6 mm i.d. with 5 μ m particles (available from Hewlett-Packard as order number 79916MO-554).

Stage 1: Getting a Chromatogram

1. Press (λ Ex) (0) (Enter) to illuminate flow cell with full spectrum of light from xenon lamp.
2. Press (λ Em) (0) (Enter) to measure light intensity at all wavelengths.
3. Press (Pmtgain) (7) (Enter) to set signal amplification to an intermediate value.
4. If you have not already done so, press (Lamp) (1) (Enter) to turn on the xenon lamp.
5. Start your liquid chromatograph and recording device. The chromatogram will be similar to Figure 1-1.

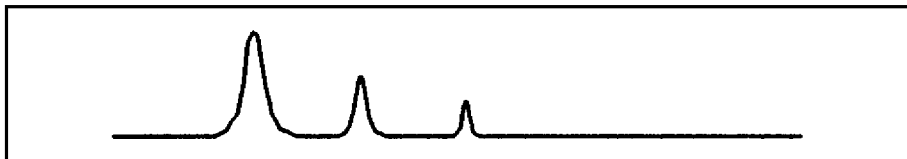


Figure 1-1. Fluorescence Detection With PMTGAIN=7

6. Press (Pmtgain) (8) (Enter) to set signal amplification to a higher value.
7. Start your liquid chromatograph and recording device. The chromatogram will now be similar to Figure 1-2.



Figure 1-2. Fluorescence Detection With PMTGAIN=8

8. Optimize chromatographic separation using a solvent gradient or other mobile phase combination.

Stage 2: Trapping the Peak

In this stage you will trap the peak in the flow cell. Do this either by stopping your solvent pump, or by diverting the solvent flow before the flow cell using a switching valve.

1. Start your liquid chromatograph and recording device.
2. Stop or divert the solvent flow just before the first peak's retention time.
3. Allow the pressure to equilibrate. The first peak is now trapped in the cell, see Figure 1-3.

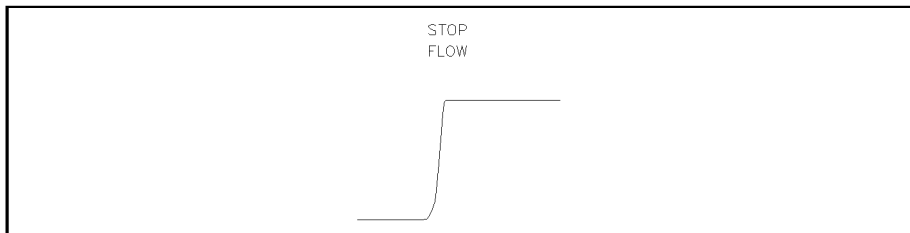


Figure 1-3. First Peak Trapped in Flow Cell

Stage 3: Finding the Optimum Excitation Wavelength

In this stage you will find the optimum excitation wavelength by scanning the contents of the flow cell.

1. Press **(λ Ex) (1) (9) (0) (Enter)** to set the excitation wavelength to 190 nm.
2. Press **(Ctrl) (0)** to display the SCANSPEED function.
3. Press **(3) (Enter)** to set the scanning speed of the excitation grating to 6 nm/s.
4. Press **(Monitor)** to display the luminescence monitor.

EX= 190nm EM= 0 nm F= 5.63

5. Press and hold down the **(▲)** key to increase the excitation wavelength. As you increase the excitation wavelength (EX) the intensity of the fluorescence (F) increases or decreases. The plot will be similar to Figure 1-4.

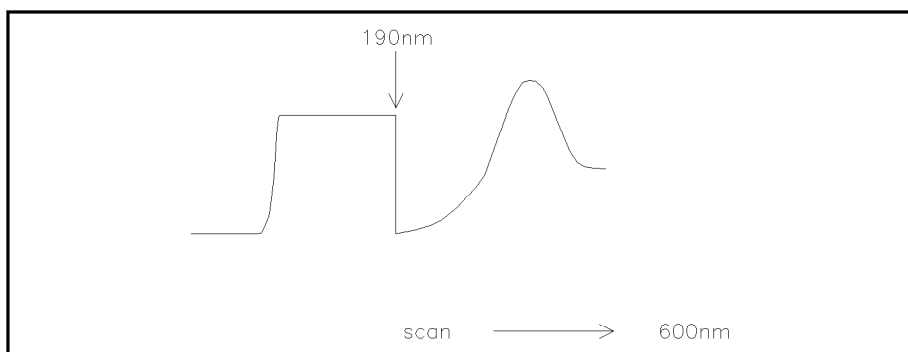


Figure 1-4. Scanning First Peak Over Full Wavelength Range

6. Press **(2) (4) (0) (Enter)** to set the excitation wavelength near to the optimum.
7. Press the **(▲)** key repeatedly to increase the excitation wavelength in steps of 1 nm. The plot will be similar to Figure 1-5.

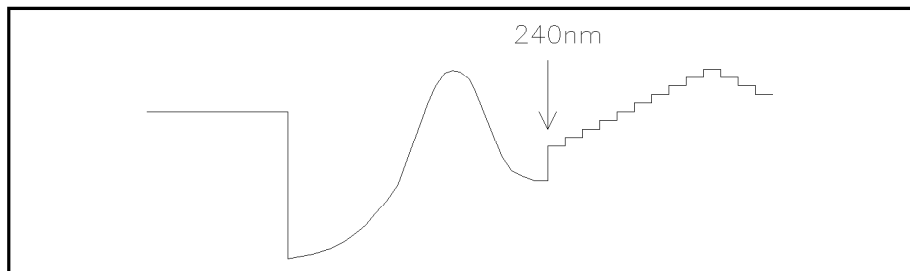


Figure 1-5. Scanning First Peak Near Fluorescence Maximum

Now you can see the optimum excitation wavelength, where this compound absorbs most light and emits most fluorescence, is 250 nm.

8. Press **[2]** **[5]** **[0]** **[Enter]** to set the excitation wavelength to 250 nm.

Stage 4: Finding the Optimum Emission Wavelength

In this stage you will find the optimum emission wavelength by scanning the contents of the flow cell.

1. Press **[λ Em]** **[3]** **[5]** **[0]** **[Enter]** to set the emission wavelength to 350 nm.
2. Press **[Monitor]** to display the luminescence monitor.
3. Press **[▶]** to move the cursor to the entry field for the emission wavelength (EM).

EX= 250nm EM= 350 nm F= 5.63

4. Press and hold down the **[▲]** key to increase the excitation wavelength. As you increase the emission wavelength (EM) the intensity of the fluorescence (F) increases or decreases. The plot will be similar to Figure 1-6.

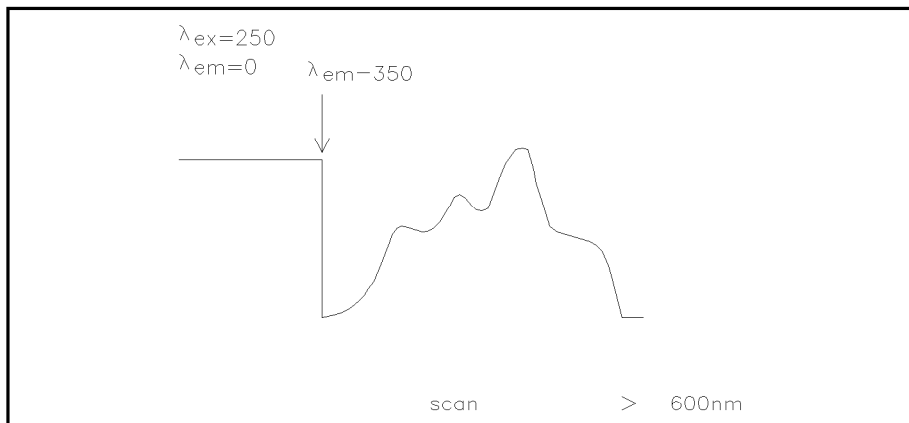


Figure 1-6. Scanning First Peak For Optimum Emission Wavelength

5. Press **4** **0** **0** **Enter** to set the excitation wavelength near to the optimum.
6. Press the **▼** key repeatedly to decrease the emission wavelength in steps of 1 nm.
7. Press the **▲** key repeatedly to increase the emission wavelength in steps of 1 nm. The plot will be similar to Figure 1-7.

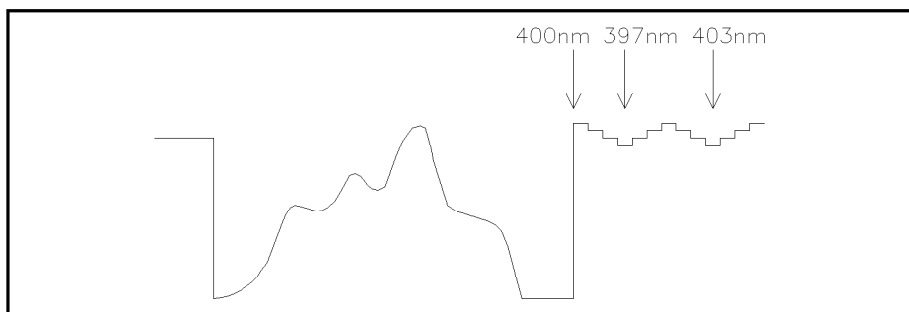


Figure 1-7. Scanning First Peak Near Fluorescence Maximum

Now you can see the optimum emission wavelength is 400 nm.

8. Press **4** **0** **0** **Enter** to set the emission wavelength to 400 nm.

Stage 5: Finding Optimum Wavelengths for Other Peaks

1. Repeat “Stage 3: Finding the Optimum Excitation Wavelength” and “Stage 4: Finding the Optimum Emission Wavelength” for the other two peaks, stopping or diverting flow just before the peak apex.

You may need to amplify the signal, selectively for each peak. Use higher values for PMTGAIN in the same way as you increased the wavelength, and observe the effect on the signal.

Stage 6: Running a Complete Analysis

In this stage you set up the detection wavelengths and signal amplification for the complete analysis, including a timetable for programmed parameter changes.

1. Set the detector parameters to the optimum values for the first peak.

λ Ex 2 5 0 Enter

λ Em 4 0 0 Enter

Pmtgain 8 Enter

Using Fluorescence Detection

2. Set the detector to change the excitation wavelength to 265 nm and the emission wavelength to 500 nm after 0.9 min.

Timetable

AT_

0 . 9

AT0.9!

λ Ex 2 6 5

AT0.9 EX=265!

λ Em 5 0 0

AT0.9 EX=265 EM=500!

Enter

3. Set the detector to change the signal amplification to 10 after 1.5 min.

Timetable

AT_

1 . 5

AT1.5!

Pmtgain 1 0

AT1.5 PMTGAIN=10!

Enter

Using Phosphorescence Detection

In this example you will simulate the data handling in the HP 1046A, because special experimental conditions are required to perform an analysis.

1. Press **Lamp** **1** **Enter** to turn on the xenon lamp.
2. Press **λ Ex** **0** **Enter** to illuminate flow cell with full spectrum of light from xenon lamp.
3. Press **λ Em** **0** **Enter** to measure light intensity at all wavelengths.
4. Press **Delay** **0** **.** **0** **3** **Enter** to delay detector from measuring until 0.03 ms after the xenon lamp has flashed.
5. Press **Monitor** to display the luminescence monitor.

EX= 0 nm EM= 0 nm F= 1.00

6. Press **Gate** **4** **Enter** to increase the time period for measurement to 4 ms.
7. Press **Monitor** to display the luminescence monitor.

EX= 0 nm EM= 0 nm F=10.05

The intensity of the phosphorescence (F) has increased, because the duration of the detector measurement was longer. This applies to all measurements, so you should subtract this value from your signal before you decide on the best measurement duration (GATE setting).

Using Chemiluminescence Detection

In this example you will simulate the technique using the HP 1046A, because special experimental conditions are required to perform an analysis.

1. Press **Lamp** **0** **Enter** to turn off the xenon lamp.

The detector's data-acquisition and data-processing electronics continue to measure, even with the lamp off.

2. Press **λ Ex** **0** **Enter**.

3. Press **λ Em** **0** **Enter** to measure light intensity at all wavelengths.

4. Press **Delay** **0** **Enter** *not* to delay detector from measuring.

5. Press **Gate** **0** **.** **0** **3** **Enter** to increase the time period for measurement to 0.03 ms.

6. Press **Monitor** to display the luminescence monitor.

EX= 0 nm EM= 0 nm C= 1.01

The intensity of the chemiluminescence (C) is caused by background light. Try a longer measurement period and see how this affects the intensity of the background light. The ideal value for chemiluminescence is exactly 4.00 ms.

7. Press **Gate** **4** **Enter** to increase the time period for measurement to 4 ms.

Remember, when you use increased measurement time periods, to subtract the increased value from your measurements, as explained in "Using Phosphorescence Detection".

8. Press **Monitor** to display the luminescence monitor.

EX= 0 nm EM= 0 nm C=28.84

The intensity of the chemiluminescence has become stronger, because the duration of the detector measurement was longer.

Optimizing Detection

Optimizing Detection

Detection with the HP 1046A, either fluorescence, phosphorescence or chemiluminescence, can give you very sensitive and selective results if you understand what you are doing and why. This chapter supplements Chapter 1, explaining in detail how to optimize detection.

Design Features Help Optimization

The HP 1046A has several design features you can use to optimize detection:

<i>Gratings</i>	For selecting excitation and emission wavelengths
<i>Slits</i>	For bandwidth selection, before and after the cell, and before the photomultiplier tube
<i>Cut-off filter</i>	For removing stray light

And, the HP 1046A has several electronic features you can use to optimize detection:

PMTGAIN	Amplification factor
LAMP	Flash frequency
RESPONSETIME	Data reduction interval

Check Performance Before You Start

Before you start you should check that your detector is performing according to the specifications published by Hewlett-Packard, see “Verifying the Performance of Your Detector” in Chapter 7.

Use fluorescence grade solvents only. Your normal HPLC grade solvents may give good results most of the time, but our experience shows that baseline noise can be higher with HPLC grade solvents than with fluorescence grade solvents.

Flush your solvent delivery system for at least 15 minutes before checking sensitivity. If your pump has multiple channels, you should also flush the channels not in use.

Do not use a syringe to fill the flow cell with solvent. The flow cell's low volume makes it nearly impossible to evacuate all the air pockets.

Finding the Best Wavelengths

The most important parameters to be optimized in fluorescence detection are the excitation and emission wavelengths. Generally it is assumed that the best excitation wavelength can be taken from the excitation spectrum acquired on a spectrofluorimeter. It is also assumed that once the optimal excitation wavelength has been found for one particular instrument type, this wavelength can also be applied for other instruments.

Both assumptions are wrong.

The optimum wavelength for the excitation depends on the absorption of the compounds, but also on the instrument characteristics, for example, the lamp type and the gratings. As most organic molecules absorb best in the ultra-violet range, the HP 1046A was designed to give an optimum signal-to-noise ratio in the 210 nm to 280 nm range of the spectrum. To achieve greatest sensitivity, the absorbance wavelength of your sample molecule should match the wavelength range for your instrument. In other words, an excitation wavelength in the ultra-violet range. Your HP 1046A has a broad excitation wavelength range, but for higher sensitivity you should choose a wavelength in the ultra-violet range.

The design elements that contribute to higher efficiency in the ultra-violet range are the xenon flash lamp and the gratings. Flash-type lamps shift the optimum wavelength to lower wavelength ranges—with the HP 1046A to a maximum of 231 nm. The excitation grating is blazed for highest efficiency at 225 nm.

A Real Example

Although an excitation wavelength of 340 nm is quoted in the literature, the HP 1046A scan of orthophthalaldehyde derivative of the amino acid alanine (Figure 2-1) shows a maximum between 220 nm and 240 nm.

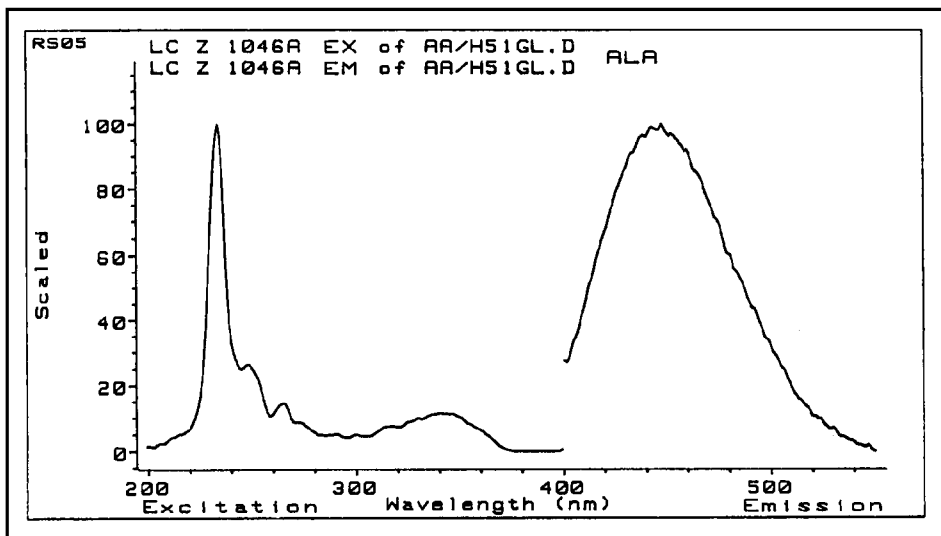


Figure 2-1. Scan Orthophthalaldehyde Derivative of Alanine

When you are looking for the wavelength by scanning as described in “Using Fluorescence Detection” in Chapter 1, scan over the whole range. As this example shows, a maximum may be in a completely different wavelength range.

Choosing the Correct Slits

The slit width is the most important parameter in determining the spectral bandwidth of spectrofluorimeters. Smaller slits sharpen the spectral resolution, bringing increased selectivity. However, decreasing the slit width decreases sensitivity since the intensity of light emerging from the monochromator decreases. The HP 1046A has interchangeable slits: You can choose either to optimize the selectivity or the sensitivity. Table 2-1 lists the combinations and their characteristics.

Table 2-1. Slit Width Combinations

Ex	1	2	2	2	2	1	4	2
Em #1	2	2	4	2	4	4	4	4
Em #2	2	2	2	4	4	4	4	-
Signal (mV)	2.0	4.0	9.0	11	22	10	27	35
Noise (mV)	0.32	0.55	0.95	1.2	2.0	0.9	3.0	4.7
S/N	6.2	7.3	9.5	9.2	11	11	9	7.5

When opening the slits up to 4 mm, the signal increases significantly, but so does the noise. Remember the optimum slit configuration depends on the spectra of the individual compounds.

With the Hewlett-Packard isocratic sample, the best signal-to-noise ratio for the biphenyl peak is obtained with a 1×1 mm or a 2×2 mm slit on the excitation side and two 4×4 mm slits on the emission side.

Finding the Best Signal Amplification

Increasing the PMTGAIN increases the signal and the noise. Up to certain factor, the increase in signal is higher than the increase in noise.

In Figure 2-2 the PMTGAIN was raised stepwise from 11 up to 17 (the peak is from the Hewlett-Packard isocratic sample which was diluted 1000 times). Below 11, the biphenyl could not be measured at all. With increasing PMTGAIN, there was an improvement in signal-to-noise up to 15. Above 15, the noise increased proportionally to the signal, with no improvement in appearance of the peak.

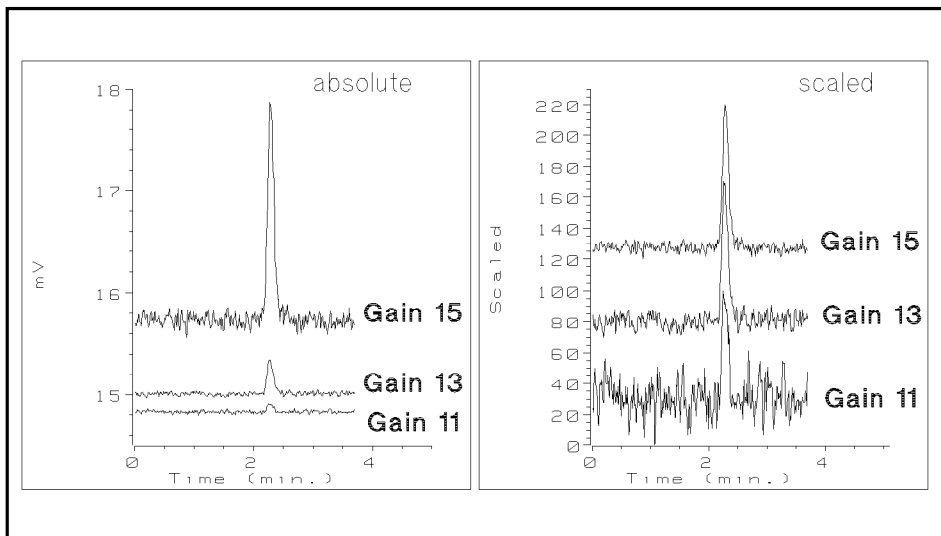


Figure 2-2. Finding Best PMTGAIN for Biphenyl

Finding the Best Flash Frequency

The lamp flash frequency can be regulated continuously or as a function of your run. Positive integers (1, 2, and 3) set the lamp flashing continuously, whereas negative integers (-1, -2, and -3) use the lamp only when an analysis is running. We recommend positive integers during method development, because you can monitor the baseline before the run. Negative integers are useful for routine work, because it increases the lifetime of the lamp.

For example, a flash frequency of 220 Hz has the lowest noise and therefore the highest sensitivity, see Figure 2-3.

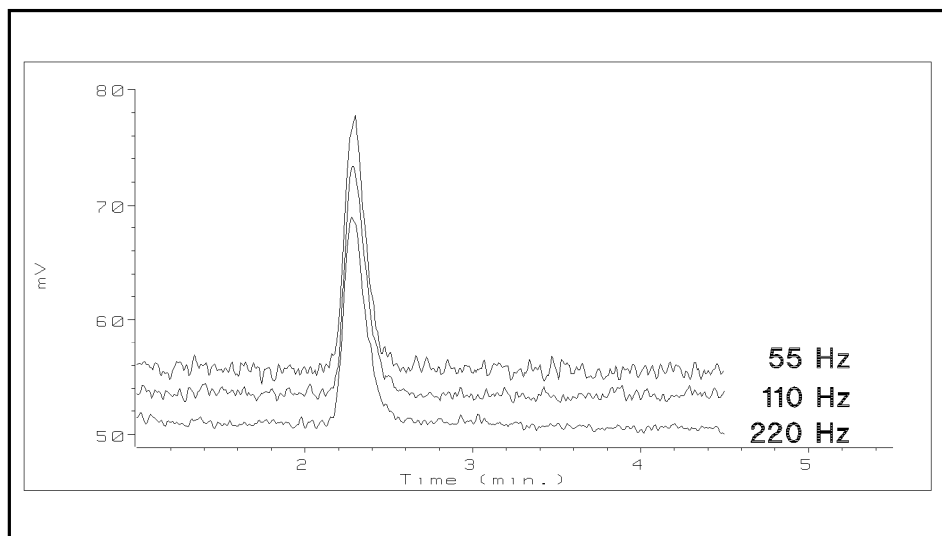


Figure 2-3. Finding Best Flash Frequency

Selecting the Best Response Time

As a rule-of-thumb you will need at least 20 data points spread over your peak to be able to integrate and quantify properly. Data reduction using the `RESPONSETIME` function can smooth your data, increasing your signal-to-noise ratio even further. By increasing the time interval for summing and filtering you decrease the contribution of the interfering baseline noise to your signal.

For example, see Figure 2-4.

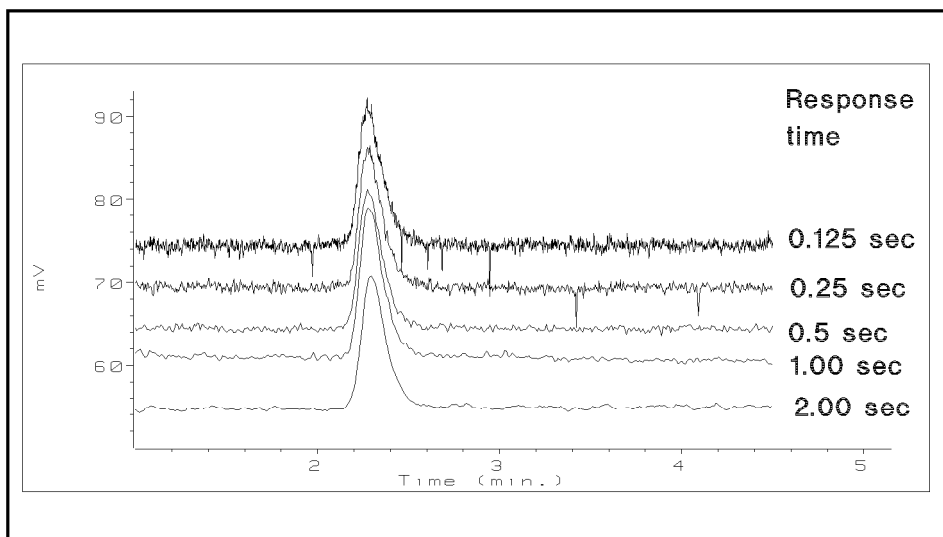


Figure 2-4. Finding Best Response Time

Reducing Stray Light

Cut-off filters are used to remove stray light and 2nd-order or higher reflected light, by allowing complete transmission above the cut-off and little or no transmission below the cut-off point. They are used between excitation and emission gratings, to prevent any stray excitation light from reaching the photomultiplier tube when it is measuring emission.

When the emission and excitation wavelengths are close together, the distortion due to scattering severely limits the sensitivity. When the emission wavelength is twice the excitation wavelength, the 2nd-order light is the limiting factor. To explain the effect of such higher order light, assume the detector is on, but no sample is eluting through the flow cell.

The lamp sends 1 million photons into the flow cell at, for example, 240 nm. Scattering on the surface of the flow cell, and scattering from the molecules of solvent allow 0.1% of this light to leave the cell through the window at right angles to the incident light. Without a cut-off filter, these remaining 1000 photons will reach the emission grating. 90% will be reflected totally without dispersion onto the photomultiplier. The other 10% disperses at 240 nm, (1st order) and at 480 nm (2nd order). To remove this stray light, you need a cut-off filter above 240 nm.

For example, Figure 2-5 shows a comparison of emission scans obtained from a pure solvent made up of 70% methanol and 30% water, with excitation at 240 nm, and cut-off at 335 nm.

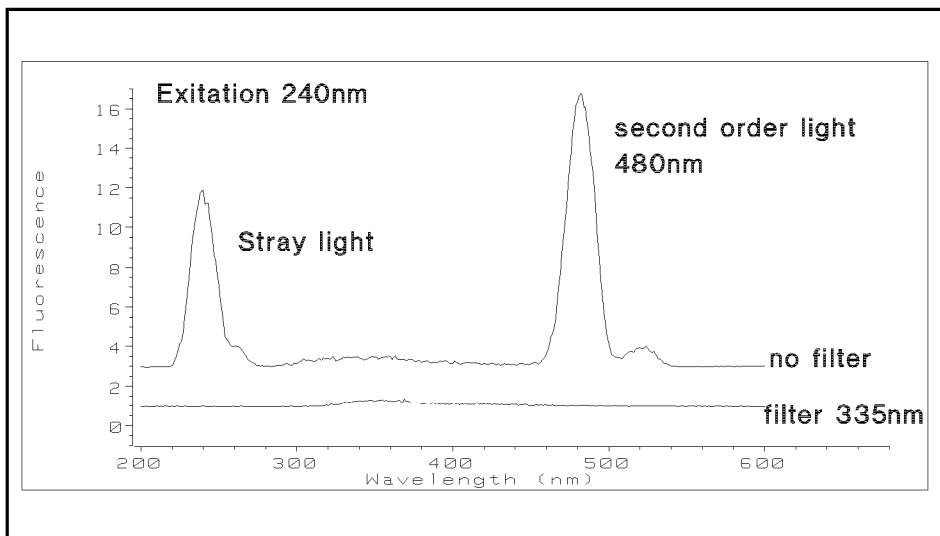


Figure 2-5. Reducing Stray Light

Without filter, the upper trace shows maxima from 1st-order light at around 240 nm and from 2nd-order light at around 480 nm. The second maximum is larger than the first. Normally we would expect the opposite. However the HP 1046A was designed with the optimum emission wavelength in mind, and the efficiency of the emission grating is higher for the 400 nm to 600 nm range of the spectrum than at 240 nm.

The scan with filter does not have any maxima—the stray light at 240 nm does not get through the 335 nm filter, explaining the absence of a maximum at 240 nm. As the stray light never gets to the emission grating, it cannot disperse to give the 2nd-order light, explaining the absence of a maximum at 480 nm.

Choosing the Correct Cut-Off Filter

Select your cut-off filter at a wavelength above excitation, thereby removing that stray light source, but below the emission wavelength to allow that light

Reducing Stray Light

through. For example, if the optimum excitation wavelength (λ_{ex}) is 290 nm and the optimum emission wavelength (λ_{em}) is 360 nm, a 270 nm cut-off filter is totally ineffective, while one at 380 nm will black out your results completely.

If λ_{em} is within 20% of λ_{ex} , or if λ_{em} is around twice λ_{ex} , we recommend you use a cut-off filter. Cut-off filters can increase the signal-to-noise ratio by a factor of 5.

Troubleshooting Your Detector

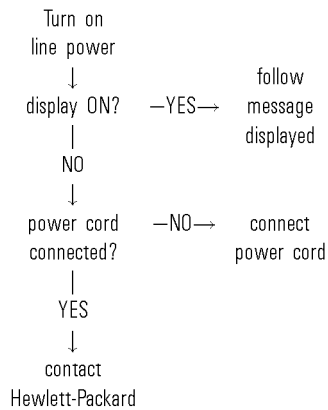
Troubleshooting Your Detector

In this chapter we have organized the error conditions and malfunctions together, starting from the point where the condition can occur. This organization gets you to the source of a problem—fast.

Whenever you must make a repair or replace a part, we will reference the appropriate procedure in Chapter 4.

Troubleshooting During Start-up

Power Lamp Off and Detector Appears Dead



Troubleshooting During Operation

The messages described in this section can occur at any time during operation.

- The *Run* lamp on the front panel will be on during a run.
- The *Not ready* or *Error* lamp (or both) on the front panel will be on, depending on the message.
- You will see a list of the full messages by pressing **Status** and then **▼**.

If a not-ready condition occurs, the detector makes a connection between pins 1 and 7 of the REMOTE connector. The detector makes this connection for as long as the not ready condition exists. When the not ready condition is removed the connection is broken and the detector operates again.

If an error condition occurs, the detector makes a connection between pins 1 and 4 of the REMOTE connector. The detector makes this connection for as long as the error condition exists. When the error condition is removed, the connection is broken and the detector operates again.

If an error remains after you have checked or replaced the parts described, contact Hewlett-Packard.

ADC Overflow

NOTREADY: ADC overflow

The detector cannot measure intensity values which exceed 99 on the luminescence monitor, because the measured analog signal has reached its maximum.

Reduce PMTGAIN.

Another Keyword Expected

another keyword expected

You have made an incorrect entry on the keyboard. This message is displayed for about 2 seconds before redisplaying the line where you made the incorrect entry. The detector waits for you to make a correct entry.

Press correct key.

DAC Overflow

NOTREADY: DAC overflow

The detector cannot output intensity values which exceed 99 on the luminescence monitor, since the analog output signal has reached its maximum.

Reduce ZERO until the intensity value is < 99 .

DAC Underflow

NOTREADY: DAC underflow

The detector cannot output intensity values which are less than 0 in the luminescence monitor.

Increase ZERO until the intensity value is > 0 .

Detector On

HP 1046A detector on (A 2617)

You have turned on line power, the detector has initialized the monochromators, looked for and found the calibration values.

The detector is ready to use.

EM Monochromator

NOTREADY: EM monochromator

The stepper motor is moving to the position you have just entered.

Wait before you press **Start** to begin measuring.

EM Reference Position

ERROR: EM reference position

The detector electronics have tried and failed to find the stepper motor's reference position.

Press **Ctrl** **1** **Enter** to initialize the monochromator. If error remains, turn off at the **~LINE** switch and turn on. If error persists, contact Hewlett-Packard.

Equal Expected

equal expected

You have made an incorrect entry on the keyboard. This message is displayed for about 2 seconds before redisplaying the line where you made the incorrect entry. The detector waits for you to make a correct entry.

Press correct key.

EX Monochromator

NOTREADY: EX monochromator

The stepper motor is moving to the position you have just entered.

Wait before you press **Start** to begin measuring.

EX Reference Position

ERROR: EX reference position

The detector electronics have tried and failed to find the stepper motor's reference position.

Press **Ctrl** **1** **Enter** to initialize the monochromator. If error remains, turn off at the **VL** switch and turn on. If error persists, contact Hewlett-Packard.

Illegal Key Pressed

```
illegal key pressed
```

You have made an incorrect entry on the keyboard. This message is displayed for about 2 seconds before redisplaying the line where you made the incorrect entry. The detector waits for you to make a correct entry.

Press correct key.

Initializing

```
initializing....
```

You have just turned on line power and the detector is initializing the monochromators. The firmware checks any parameters you had before line power was turned off and looks for the calibration values, both stored in memory.

Wait about 1 minute until detector has initialized the monochromators and then follow message displayed.

Invalid Format

```
invalid format
```

You have made an incorrect entry on the keyboard. This message is displayed for about 2 seconds before redisplaying the line where you made the incorrect entry. The detector waits for you to make a correct entry.

Press correct key.

Keyword Not Identified

`keyword not identified`

You have made an incorrect entry on the keyboard. This message is displayed for about 2 seconds before redisplaying the line where you made the incorrect entry. The detector waits for you to make a correct entry.

Press correct key.

Leak Detected

`ERROR: leak detected`

Back pressure has forced flow cell windows to separate, flow cell could be damaged, or detector outlet blocked. The detector generates a shut-down signal at REMOTE connector and turns off the xenon lamp.

Turn off your solvent pump. Check flow cell, and compartment. Check back pressure—look for possible blockages.

Leak Sensor Failed

`ERROR: leaksensor failed`

The detector electronics have tried to test the leak sensor. Either the leak sensor or its connector is faulty. The detector generates a shut-down signal at REMOTE connector and turns off the xenon lamp.

Turn off your solvent pump. Contact Hewlett-Packard.

Not Time Programmable

`not time programmable`

You have tried to enter a parameter that cannot be programmed with the timetable.

You can program the excitation wavelength, emission wavelength and the PMTGAIN function.

Parameters Lost

`HP 1046A parameters lost !!!`

You have turned on line power, the detector has initialized the monochromators, looked for and found the calibration values, but not found your previous setpoints. The detector sets the parameters to their default values.

The detector is ready to use.

Parameter Missing

`parameter missing`

You have made an incorrect entry on the keyboard. This message is displayed for about 2 seconds before redisplaying the line where you made the incorrect entry. The detector waits for you to make a correct entry.

Press correct key.

Parameter Out Of Range

`parameter out of range`

You have made an incorrect entry on the keyboard. This message is displayed for about 2 seconds before redisplaying the line where you made the incorrect entry. The detector waits for you to make a correct entry.

Press correct key.

Parameter Overflow

`parameter overflow`

You have made an incorrect entry on the keyboard. This message is displayed for about 2 seconds before redisplaying the line where you made the incorrect entry. The detector waits for you to make a correct entry.

Press correct key.

Timetable is Empty

`timetable is empty`

You have pressed **Timetable** and there is no timetable, or you have just deleted the timetable.

Enter a timetable or continue.

Timetable Overflow

`timetable overflow`

You have tried to insert another line into the timetable, but the detector memory allocated to the timetable is full.

Either change an existing line or delete a line.

Top of Timetable

`top of timetable`

The first line of the timetable is displayed and you have pressed .

Press correct key.

Wavelength Calibration Lost

`wavelength calibration lost !!!`

You have turned on line power, the detector has initialized the monochromators, looked for, but failed to find the calibration values. The detector sets the calibration values to zero.

The detector is ready to use.

Troubleshooting the Chromatogram

The quality of your chromatographic results depends on the performance of all modules in the chromatographic system (pump, injector, column, detector, etc.). If all the modules in your system perform according to the manufacturer's specification, an application problem might be preventing good chromatographic results. Use the following guide to help identify the source of the fault.

No Signal

Lamp is off	Switch lamp on
Wrong cut-off filter	Change filter
Wrong excitation or emission wavelengths	Reset wavelengths
Wrong time, excitation or emission wavelengths in timetable	Reset time or wavelengths
Wrong GATE or DELAY	Change GATE or DELAY

Low Response

Wrong excitation or emission wavelengths	Reset wavelengths
Low PMTGAIN	Increase PMTGAIN
Small slits	Install wider slits
Wrong GATE or DELAY	Change GATE or DELAY

Troubleshooting the Chromatogram

Air in mobile phase

Degas mobile phase

Noisy Baseline

Air in flow cell

Disconnect column and purge flow cell with high flow rate

Lamp frequency too low

Increase lamp frequency

Response time too short

Increase interval

Poor quality solvent

Use better quality solvent

High Baseline Offset

Poor quality solvent

Use better quality solvent

Maintaining Your Detector

Maintaining Your Detector

In this chapter we describe the maintenance tasks you can do on your detector. Do only the maintenance specified in this guide. Other maintenance or repairs must be done by Hewlett-Packard trained personnel. Unauthorized maintenance can be dangerous. Damage caused by unauthorized maintenance is not covered by warranty.

Before you attempt any maintenance, observe the following warning.

High Voltages: Disconnect the line-power cord before removing any of the HP 1046A panels.

UV Radiation: Continuous exposure to intense ultra violet radiation (the radiation emitted by the xenon flash lamp) may cause permanent damage to the eyes or other parts of the body. Avoid exposure to this radiation. Do not open the detector when the lamp is turned on.

Hochspannung: Bevor ein Gehäuseteil entfernt werden soll, muß das Netzkabel abgezogen werden.

UV Strahlung: Setzen Sie sich keiner direkten UV-Strahlung aus (ausgestrahlt von der Detektorlampe). Die Strahlung kann Ihre Augen oder andere Körperteile gefährden. Vermeiden Sie den Kontakt mit dieser Strahlung. Öffnen Sie nicht das Lampengehäuse, wenn die Lampe eingeschaltet ist.

Alta tensión: Desconecte el instrumento de la línea antes de sacar el panel posterior del instrumento.

Radiación UV: Una exposición continuada a la luz ultravioleta (que es la radiación emitida por la lámpara del detector) puede dañar irreversiblemente los ojos y otros órganos del cuerpo humano. Evite cualquier exposición a esta radiación: no abra nunca el compartimento de la lámpara cuando esté conectado.

ATTENTION

Tensions élevées: Débranchez le cordon secteur avant d'ôter le panneau arrière.

Radiation UV: Une exposition continuelle à une radiation ultra- violette intense (la radiation émise par la source lumineuse du détecteur) peut provoquer des lésions irréversibles aux yeux ou à d'autres parties du corps. Evitez de vous exposer a cette radiation. N'ouvrez pas le logement de la lampe quand celle-ci est allumée.

ATTENZIONE

Alte tensioni: Scollegare il cavo di alimentazione prima di rimuovere il pannello posteriore.

Radiazioni UV: L'esposizione continuata ad una intensa radiazione ultravioletta (radiazione emessa dalla lampada del rivelatore) può causare danni permanenti agli occhi o altre parti del corpo. Evitate l'esposizione a queste radiazioni: non aprite lo scomparto della lampada quando questa è accesa.

Before you replace a printed circuit board, observe the following caution.

CAUTION

The printed circuit boards contain many electronic parts that are sensitive to electronic discharge. Do not attempt to remove the boards, unless you use a portable grounding kit. Do not touch any of the components on the boards. Do not place printed circuit boards on any plastic or plastic-coated surface.

ACHTUNG

Die elektronischen Leiterplatten enthalten viele elektronische Bauteile, die sehr empfindlich gegenüber elektrostatische Entladung sind. Entfernen Sie keine Leiterplatten ohne Verwendung einer antistatischen Ausrüstung (Portable Grounding Kit). Berühren Sie keine Bauteile auf den Leiterplatten. Legen Sie keine Leiterplatten auf Oberflächen mit Kunststoffbeschichtung.

PRECAUCION

Les circuits impresos contienen muchas partes sensibles a las descargas electricas. No intente sacar los circuitos si no dispone del kit portátil de tierra. No toque ninguno de los componentes de las placas. No coloque ningun circuito impreso sobre material o superficies de plástico.

Troubleshooting the Chromatogram

EMENT

Les circuits imprimés possèdent des éléments sensibles aux décharges électrostatiques. N'essayez pas de sortir les cartes sans utiliser l'accessoire portable de mise à la terre. Ne touchez pas aucun des composants des cartes. Ne posez pas les circuits imprimés sur des matières plastiques ou des surfaces recouverts de matières plastiques.

ONE

Le schede a circuito stampato contengono molte parti elettriche. Non tentate di rimuovere le schede se non siete dotati di un kit per la messa a terra dell'operatore. Non toccate nessun componente sulle schede. Non appoggiate le schede su superfici plastiche o plastificate.

IT

De elektronische boards bevatten veel elektronische onderdelen die zeer gevoelig zijn voor elektrostatische ontlading. Verwijder geen boards zonder gebruik te maken van de "Portable Grounding Kit". Raak geen onderdelen op de boards aan. Leg geen boards op oppervlakken uit kunststof of die met een kunststoflaag bedekt zijn.

What You Will Need

In Table 4-1 the items marked with the † symbol are part of the standard accessory kit (part number 01046-68701) shipped with every HP 1046A, see Chapter 7. The items marked with the ‡ symbol are included in the spare parts kit, (order number 1046A option #502).

Table 4-1. What You Will Need to Maintain Your Detector

Description	Hewlett-Packard Part Number	Quantity
Wrench, $\frac{5}{16}$ " and $\frac{1}{4}$ "	8710-0510	1
† Pozidriv screwdriver #1	8710-0899	1
† Hexagonal key, 1.5 mm	8710-0909	1
‡ 5 μ l flow chamber	01046-28102	2
‡ Stepped-window gasket	01046-27101	10
‡ Transparent stepped-window	01046-28101	3
‡ Absorbent stepped-window	01046-28103	1
‡ Cell gasket	01046-27102	10
‡ Guide ring	01046-27103	10
‡ Cell spring	79881-09103	10
‡ Smooth point tweezers	8710-0007	1
‡ Tooth picks	9340-0005	box
Polyester gloves	8650-0030	pair
Xenon lamp	2140-0549	1

Removing the Top Cover

1. Remove the 2 screws in the top-left and top-right corners of the rear panel.
2. Remove the 2 screws at the top-left and top-right sides of the detector.
3. Lift off the top cover.

Replacing the Xenon Lamp

WARNING

Make sure line-power cord is disconnected.

WARNUNG

Stellen Sie sicher, daß das Netzkabel herausgezogen ist.

CUIDADO

Asegúrese que el cable de red está desconectado.

ATTENTION

Assurez-vous que le câble secteur n'est pas connecté.

ATTENZIONE

Assicuratevi che il cavo di alimentazione sia scollegato.

WAARSCHUWING

Zorg dat het netsnoer is losgekoppeld.

Stage 1: Removing the Old Lamp

1. Turn off the line power at the LINE switch on the rear panel.
2. Remove the top cover, as described in “Removing the Top Cover”.
3. Using a 1.5 mm hexagonal key, loosen the front screw holding the trigger pack in the lamp compartment, see Figure 4-1. *Do not* remove this screw.
Never loosen the inner screw.

Replacing the Xenon Lamp

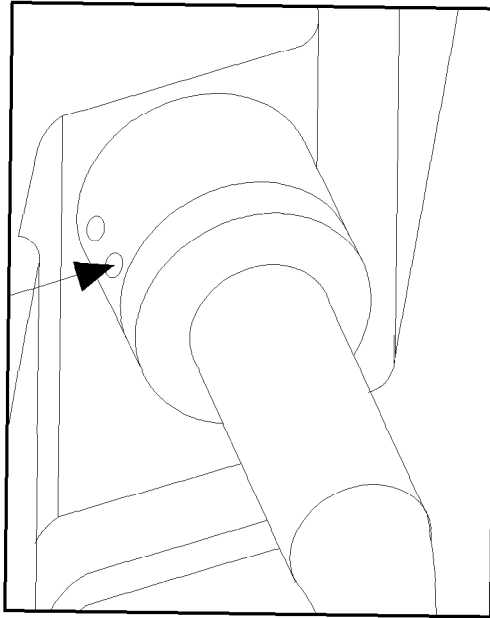


Figure 4-1. Location of Screw Holding Trigger Pack

4. Disconnect the flash power supply cable at the connector on the FPS board, see Figure 4-2.

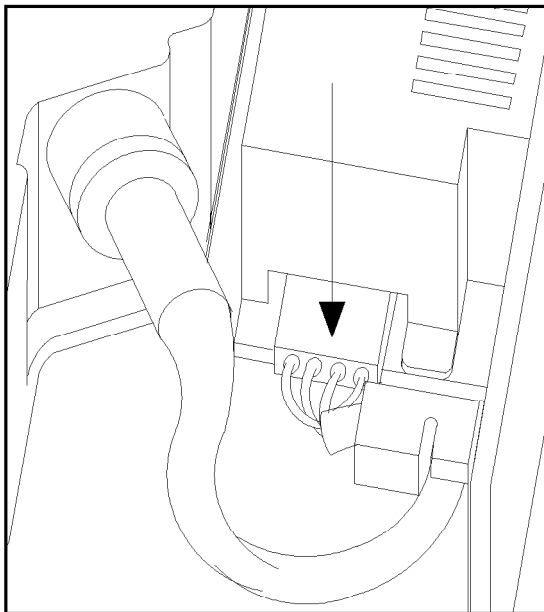


Figure 4-2. Disconnecting Flash Power Supply Cable

5. Pull the lamp and trigger pack assembly out of the lamp compartment.
6. Pull the old lamp out of the trigger pack.

Stage 2: Inserting the New Lamp

1. Put on a pair of polyester gloves.
2. Place a clean optical tissue on your lab bench or desk.
3. Remove the new lamp from its box.

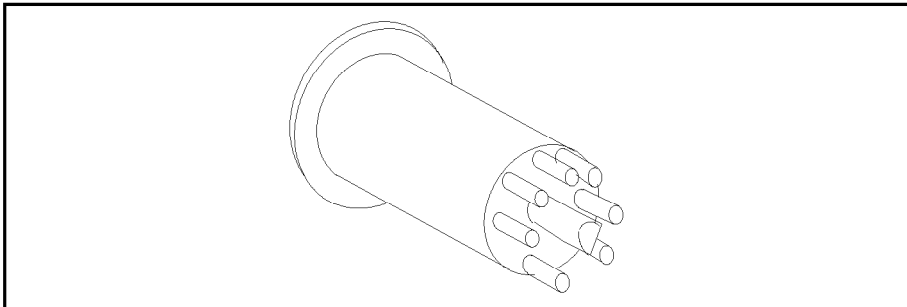


Figure 4-3. Xenon Lamp

Important

Do not touch the quartz envelope with your fingers—fingerprints absorb light in the ultra-violet range.

Wichtig

Berühren Sie das Quarzglas der Lampe nicht mit Ihren Fingern. Fingerabdrücke absorbieren Licht im UV-Bereich.

Importante

No toque la ampolla de cuarzo con los dedos. Las huellas digitales absorben en el UV.

Important

Ne touchez pas à l'enveloppe en quartz avec vos doigts—les traces de doigts absorbent la lumière en UV.

Importante

Non toccate l'involucro di quarzo con le dita—le impronte digitali assorbono energia nella zona dell'ultravioletto.

4. Holding the new lamp with the polyester gloves, insert the lamp into the trigger pack.
5. Place the top surface of the lamp face-down onto the optical tissue. Press down firmly on the trigger pack. *Do not* use excessive force. The seven pins on the lamp should fit into the seven holes in the trigger pack. Note that the eighth position is blank.

Replacing the Xenon Lamp

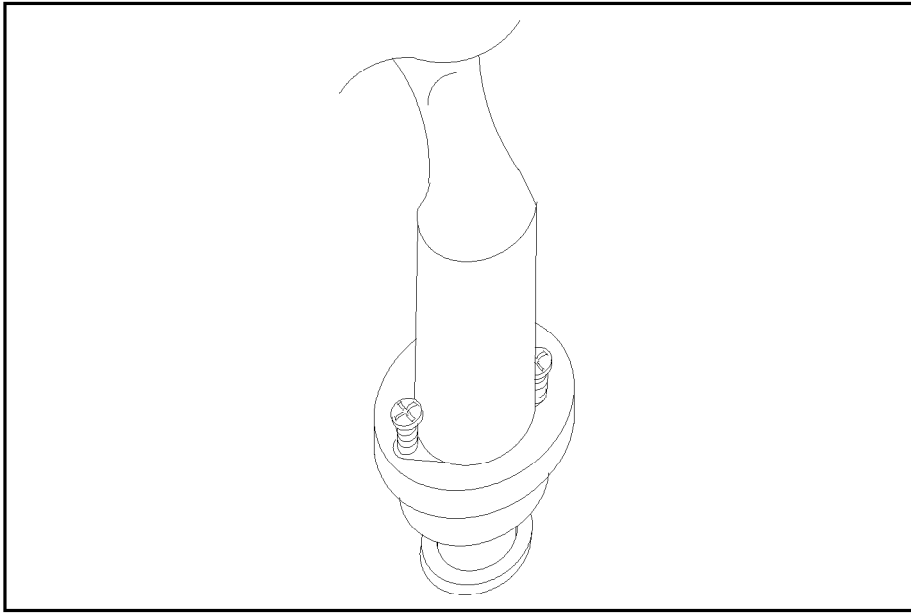


Figure 4-4. Inserting Lamp into Trigger Pack

Stage 3:

Securing the Assembly in Lamp Compartment

1. Place the lamp and trigger pack assembly into the recess in the optical housing. Align the pin with the hole in the collar.
2. Tighten the hexagonal screw.

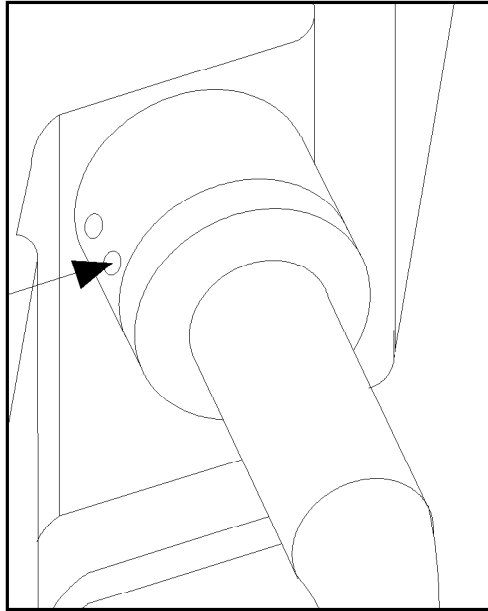


Figure 4-5. Tightening the Hexagonal Screw

3. Plug the trigger pack cable connector into the socket on the flash power supply (FPS) board.

Replacing the Xenon Lamp

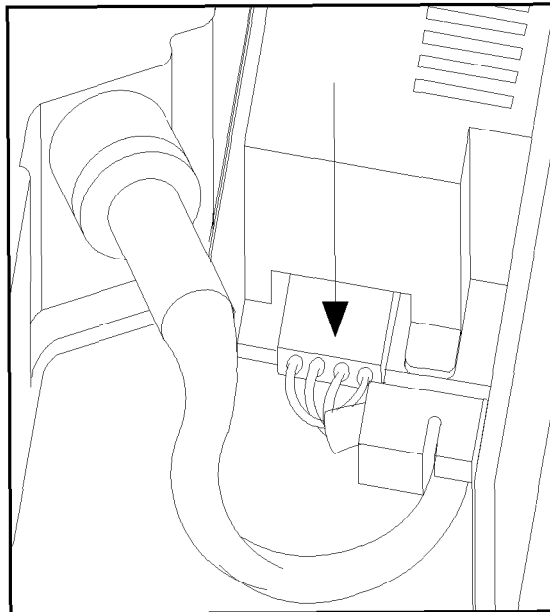


Figure 4-6. Connecting Flash Power Supply Cable

4. Replace the top cover and keyboard.
5. Turn on the Λ LINE switch on the rear panel.
6. Turn on the xenon lamp.

Ultra-violet radiation is harmful to the unprotected eye. Do not operate the lamp outside its compartment.

UV Strahlung ist schädlich für ungeschützte Augen. Deshalb sollte die Lampe nicht außerhalb des Lampengehäuses betrieben werden.

La radiación UV puede dañar la vista. No maneje la lámpara encendida fuera de su compartimento.

Les radiations UV sont dangereuses pour les yeux non protégés. N'allumez pas la lampe en dehors de son logement.

ATTENZIONE

Le radiazioni ultraviolette sono dannose per gli occhi senza protezione.
Non accendete la lampada se questa si trova fuori dalla sua sede.

Cleaning the Flow Cell

Stage 1: Removing the Flow Cell

1. Turn off the xenon lamp.
2. Open the keyboard door.
3. Loosen the screw in the door of the flow-cell compartment and open the door, see Figure 4-7.

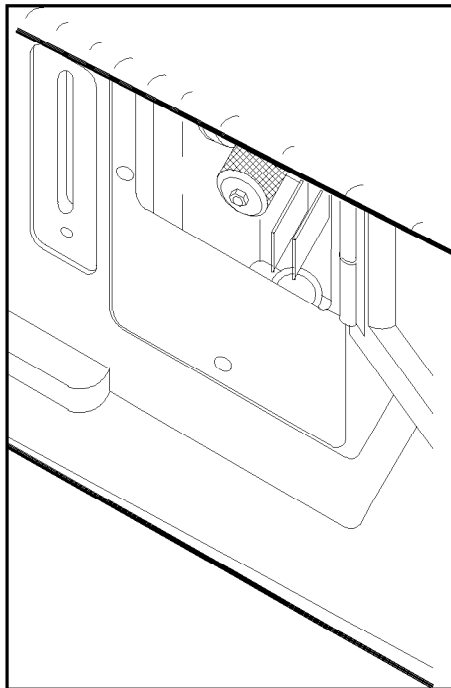


Figure 4-7. Flow Cell Compartment

Pull the spring clip backwards, slide the flow cell out of the recess and lift the flow cell out of the compartment, see Figure 4-8.

Do not remove the flow cell by pulling the capillaries.

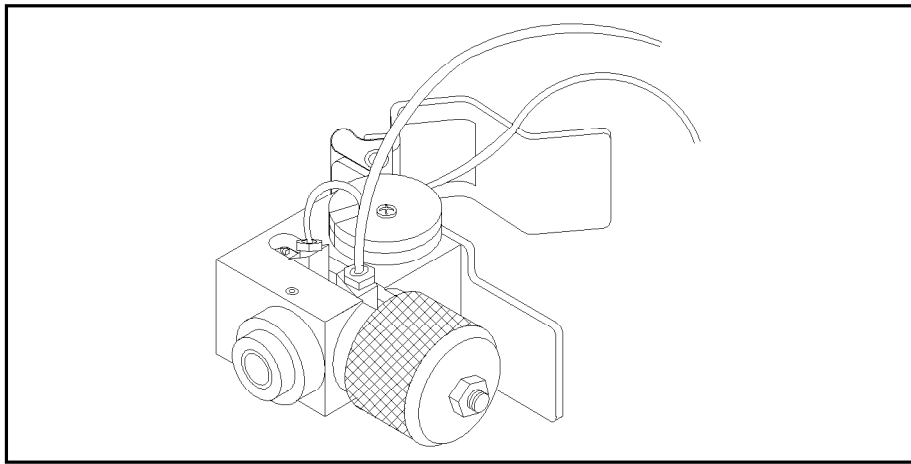


Figure 4-8. Flow Cell

4. Using the Pozidriv screwdriver, remove the screw in the center of the heat exchanger on the flow cell, see Figure 4-8.
5. Using the wrench, disconnect the inlet capillary together with the heat exchanger and the outlet capillary.

The flow cell is assembled from many small parts. Before you begin to disassemble it, make yourself familiar with the appearance and nomenclature of these parts, see Figure 4-9. The part numbers are given in Table 4-1.

Cleaning the Flow Cell

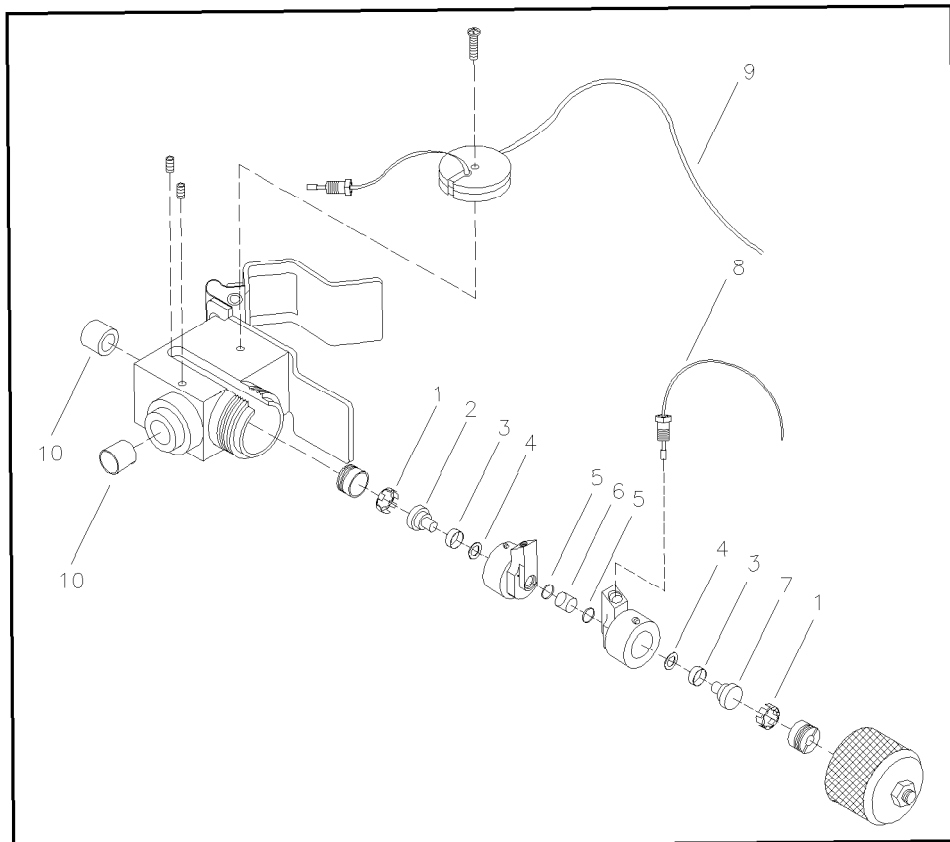


Figure 4-9. Exploded View of Flow Cell

Figure legend:

1. Cell spring
2. Transparent stepped-window
3. Guide ring
4. Stepped-window gasket
5. Cell-piece gasket
6. Flow chamber
7. Absorbant stepped-window
8. Cell outlet capillary
9. Cell inlet capillary
10. Lens rings

Stage 2: Removing the Cell Pieces

1. Place a clean optical tissue on your lab bench or desk.
2. Unscrew the knurled cap.
3. Using the hexagonal key, unscrew the small screw in the top of the flow cell housing and remove the lens ring from the left side of the flow cell. Using the same key, unscrew the small screw in the cell piece and remove the lens ring from the rear of the flow cell.

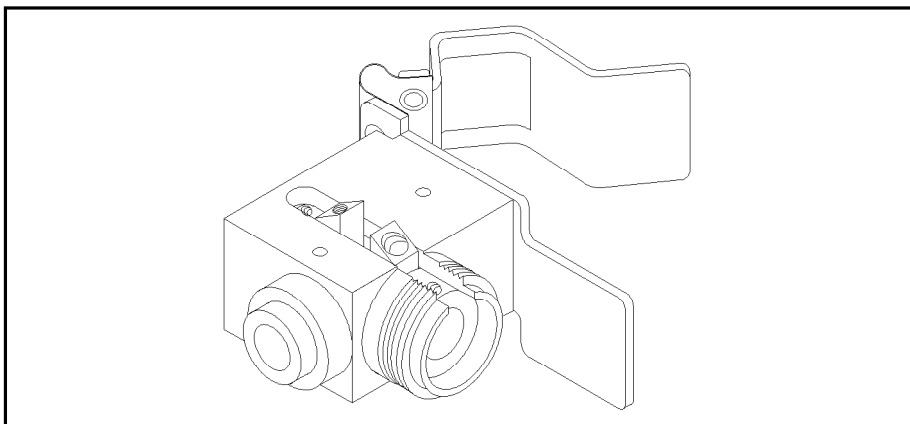


Figure 4-10. Disassembling Flow Cell

4. Tip the flow cell to remove the two cell pieces and the flow chamber.
5. Using the screwdriver, unscrew the cell screw from each cell piece, see Figure 4-11. The cell window and cell ring will come out with the cell screw.

Cleaning the Flow Cell

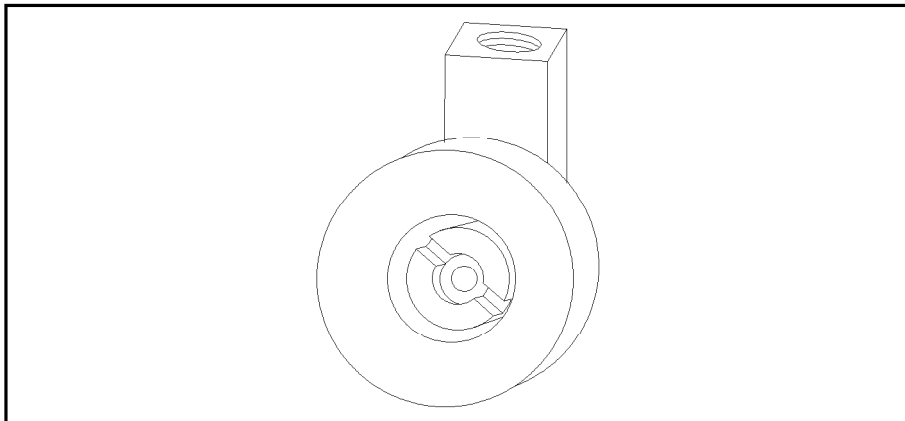


Figure 4-11. Cell Piece

6. The cell gasket will probably remain stuck inside the cell piece. Use a toothpick to prise out the gasket, see Figure 4-12. Discard the used gasket.

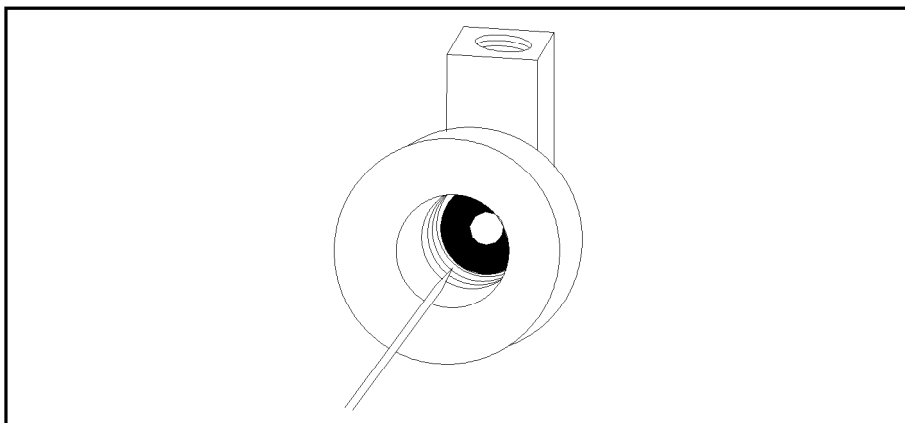


Figure 4-12. Removing the Used Gasket

Stage 3: Cleaning the Flow Cell Parts

1. Gently drop the cell windows with ring attached and lens rings into a beaker containing acetone or a similar solvent. Avoid quartz attacking solvents. Use an ultra-sonic bath, if necessary, to remove any stains. Repeat for the cell housing, cell pieces and cell screws.
2. Remove the pieces from the beaker using the smooth-tipped tweezers included in the spare parts kit. Place on an optical cleaning tissue.
3. Polish the glassware with optical cleaning-tissue. Dry the cell screw, cell piece and cell housing thoroughly.

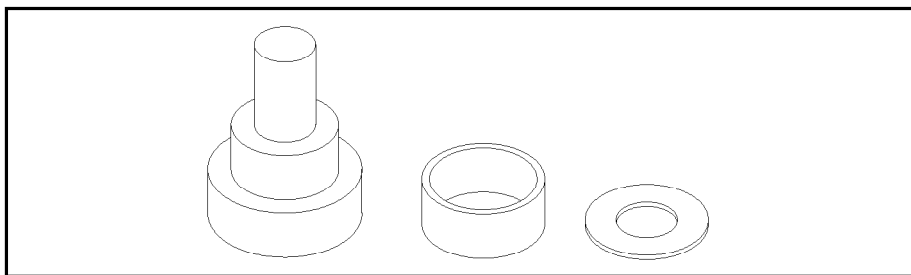


Figure 4-13. Cell Screw, Piece, and Housing after Cleaning

Stage 4: Assembling the Cell Pieces

If the windows or guide ring are badly marked, discard them and use new parts from the spare parts kit. If your flow cell window and guide ring are clean, go to step 2.

1. Place the transparent window, base down on the optical cleaning tissue. Using the smooth-tipped tweezers, carefully drop the guide ring over the

Cleaning the Flow Cell

window. Press the guide ring onto the second tier of the window, see Figure 4-14.

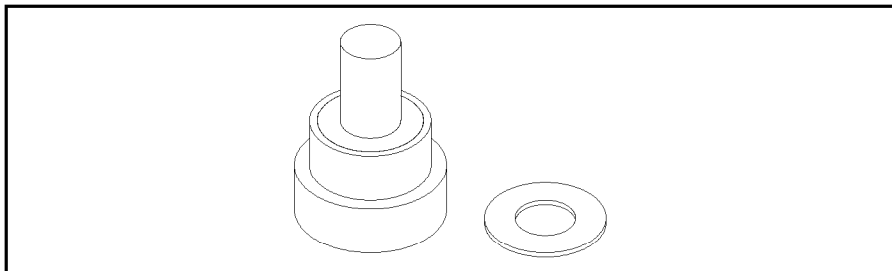


Figure 4-14. Placing Guide Ring on Window

2. Using the tweezers, place the step-window gasket over the guide ring, see Figure 4-15.

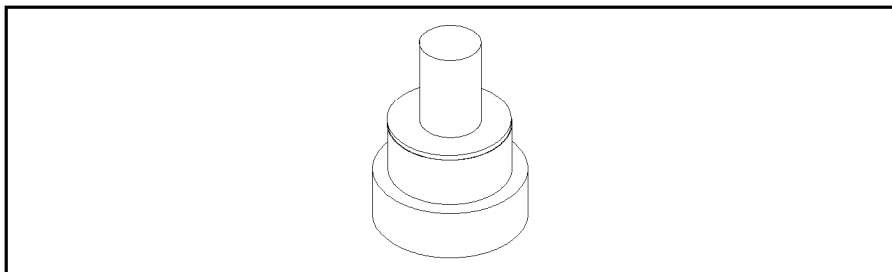


Figure 4-15. Placing Step-Window Gasket over Guide Ring

NOTE

Whenever you re-assemble the flow cell, use *new* springs and gaskets.

3. Place a cell spring on the tissue beside the window, see Figure 4-16.

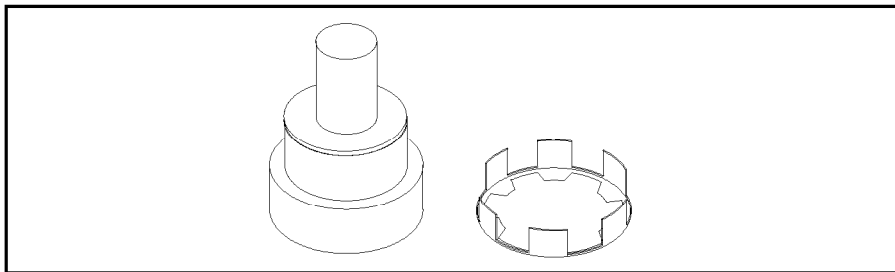


Figure 4-16. Cell Spring and Window

4. Using the tweezers, lift the window, as you assembled it in step 2, and place it on top of the cell spring. Press down gently, so that the window and cell spring hold together, see Figure 4-17.

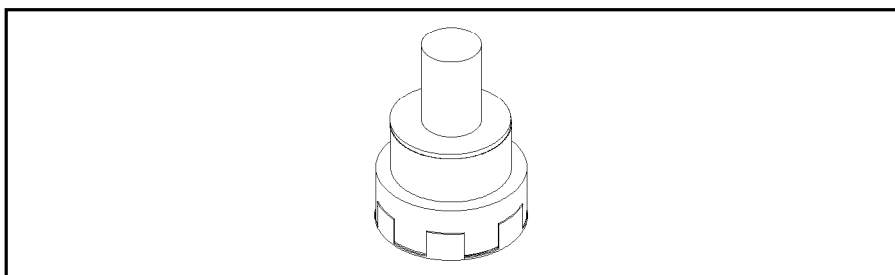


Figure 4-17. Placing Window on Cell Spring

5. Lift the window and place it gently into the cell screw, see Figure 4-18 and Figure 4-19. Press down gently until the window sits tight.

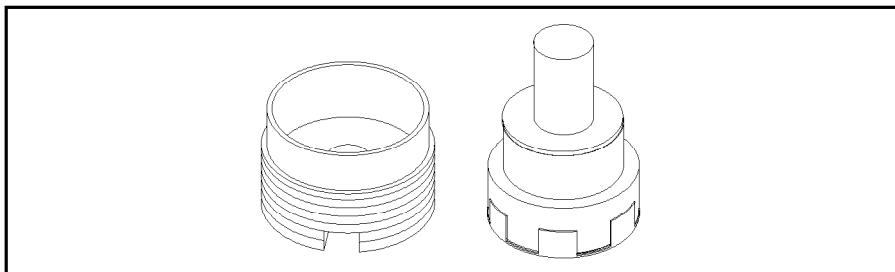


Figure 4-18. Window and Cell Screw

Cleaning the Flow Cell

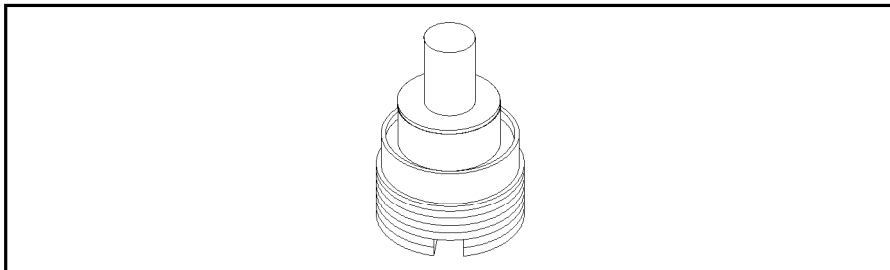


Figure 4-19. Placing Window into Cell Screw

6. Using the slot screwdriver, screw the assembled cell screw into the cell piece, see Figure 4-20.

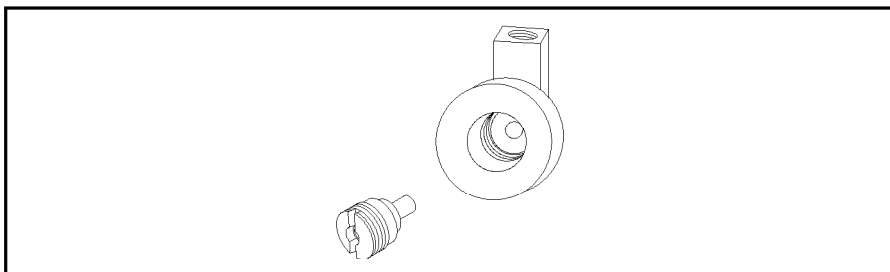


Figure 4-20. Screwing Assembled Cell Screw into Cell Piece

7. Repeat steps 1 through 5 for the absorbant stepped-window in second cell piece.
8. Take the two complete cell pieces, two cell-piece gaskets and the flow chamber, see Figure 4-21.

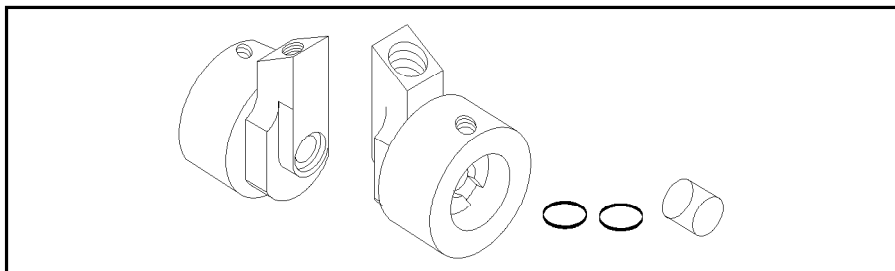


Figure 4-21. Cell Pieces, Cell-Piece Gaskets, and Flow Chamber

9. Wet the cell piece surface and put one gasket in each cell piece, see Figure 4-22.

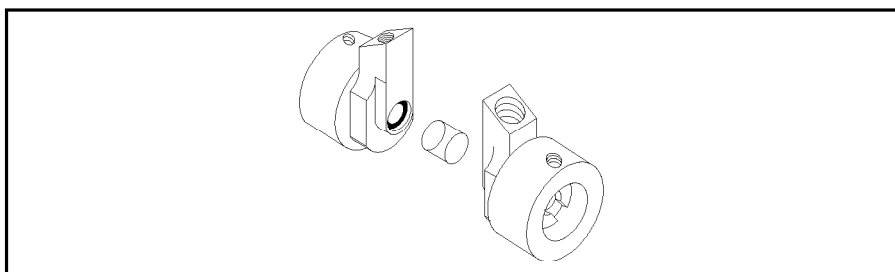


Figure 4-22. Placing Gaskets in Cell Pieces

10. Place the flow chamber between the two gaskets, see Figure 4-23.

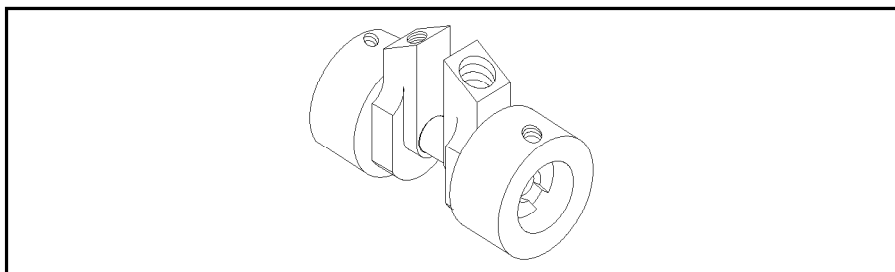


Figure 4-23. Placing Flow Chamber Between Gaskets

Stage 5: Reconstructing the Flow Cell

1. Holding the cell pieces together, slide the flow chamber (as assembled in step 10, of stage 4, shown in Figure 4-23) into the flow-cell housing, see Figure 4-24.

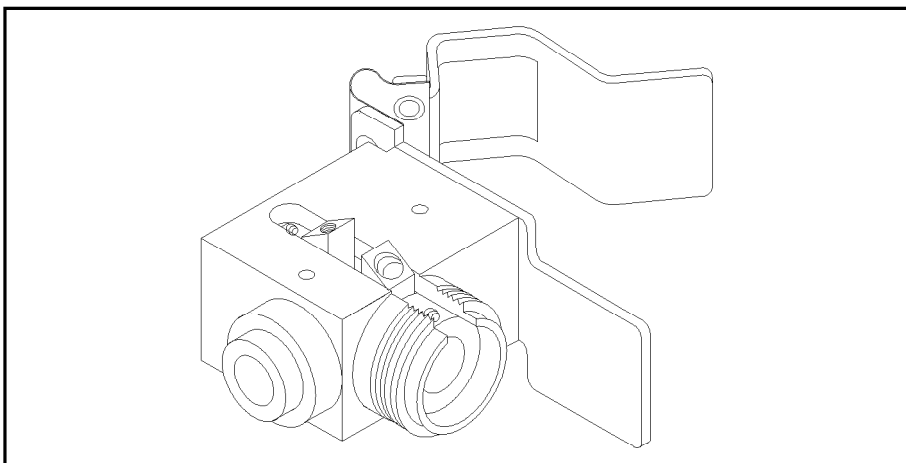


Figure 4-24. Placing Flow Chamber into Flow-Cell Housing

2. Screw the knurled cap over the cell to secure the cell pieces in place.
3. Return the clean lens rings to the recesses at the back and the left side of the flow-cell housing. Using the wrench, secure the lens rings in place with the screws.
4. Reconnect the inlet capillary (the red color-coded capillary with the heat exchanger attached) to the cell piece at the front of the cell, see Figure 4-25.

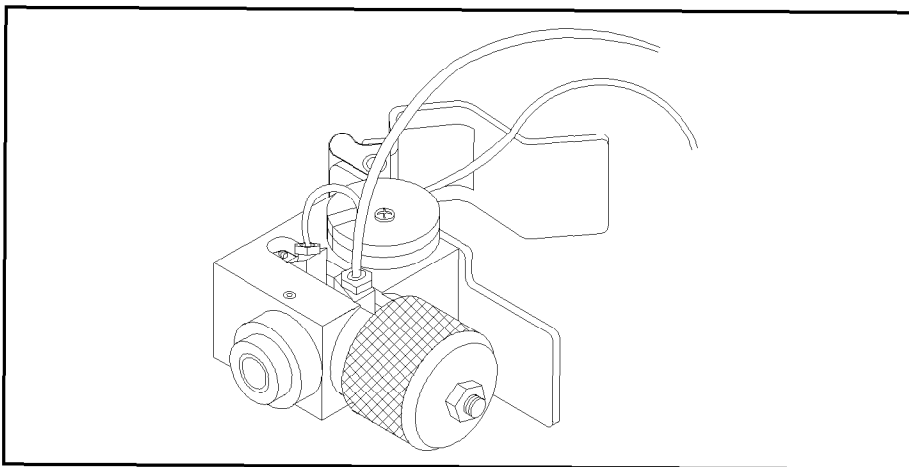


Figure 4-25. Connecting Capillaries to Flow Cell

5. Using the Pozidriv screwdriver, secure the heat exchanger in position on top of the flow cell, with the screw.
6. Reconnect the outlet capillary (the blue color-coded capillary) to the cell piece at the rear of the flow cell, see Figure 4-25.

Changing the Cut-Off Filter

The cut-off filter installed at the factory removes all light below 280 nm. A 370 nm cut-off filter is supplied in the accessory kit. Other cut-off filters are available for the HP 1046A, for example in the following kits:

- HP 1046A option 810, special low fluorescent filters, five filters for 389 nm, 408 nm, 450 nm, 500 nm, and 550 nm.
- HP 1046A option 811, special low fluorescent filters, five filters for 380 nm, 399 nm, 418 nm, 470 nm, and 520 nm.
- HP 1046A option 812, color glass filters, five filters for 280 nm, 295 nm, 305 nm, 335 nm, and 345 nm.

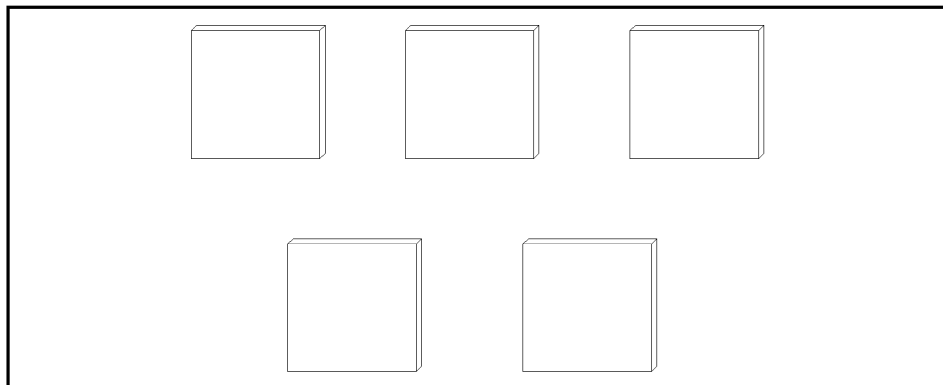


Figure 4-26. Filter Kit for HP 1046A

To exchange a filter:

1. Open the keyboard door.
2. Using the Pozidriv screwdriver included in the standard accessory kit, loosen the screw in the filter holder, shown in Figure 4-27. Pull out the holder.

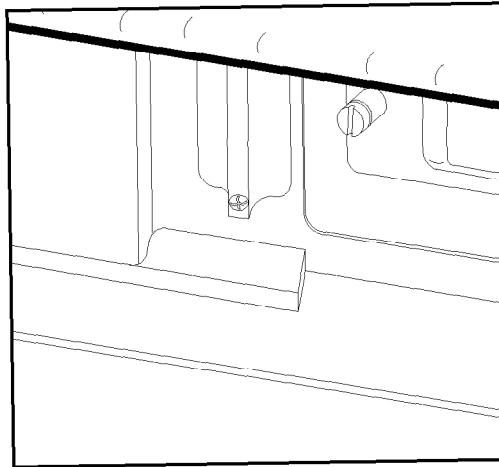


Figure 4-27. Loosening Screw of Filter Holder

3. Lift out the cut-off filter.
4. Place the new filter between the spring and the holder.
5. Push the filter-holder back into the optical unit.
6. Tighten the screw.

Changing the Slits

The bandwidth of light in each part of the optical path is set using slits. At the factory, there is a 2×2 mm slit before the cell, giving a bandwidth of 25 nm, a 4×4 mm after the cell, and another 4×4 mm slit before the photomultiplier, giving a bandwidth of 25 nm.

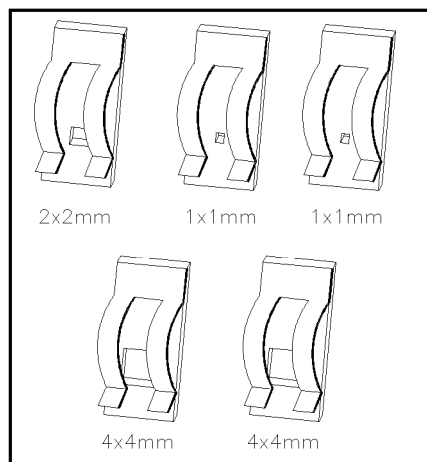


Figure 4-28. Slit Kit for HP 1046A

If you want to exchange the slits for slits from the slit kit, HP 1046A option 805, or for the slits supplied in the accessory kit, proceed as follows:

1. Remove the top cover.
2. The slits are positioned underneath the special slit covers (see Figure 4-29) on the top of the optical unit. Using the slot screwdriver supplied in the standard accessory kit, loosen one or two of the screws and swing the cover to one side.

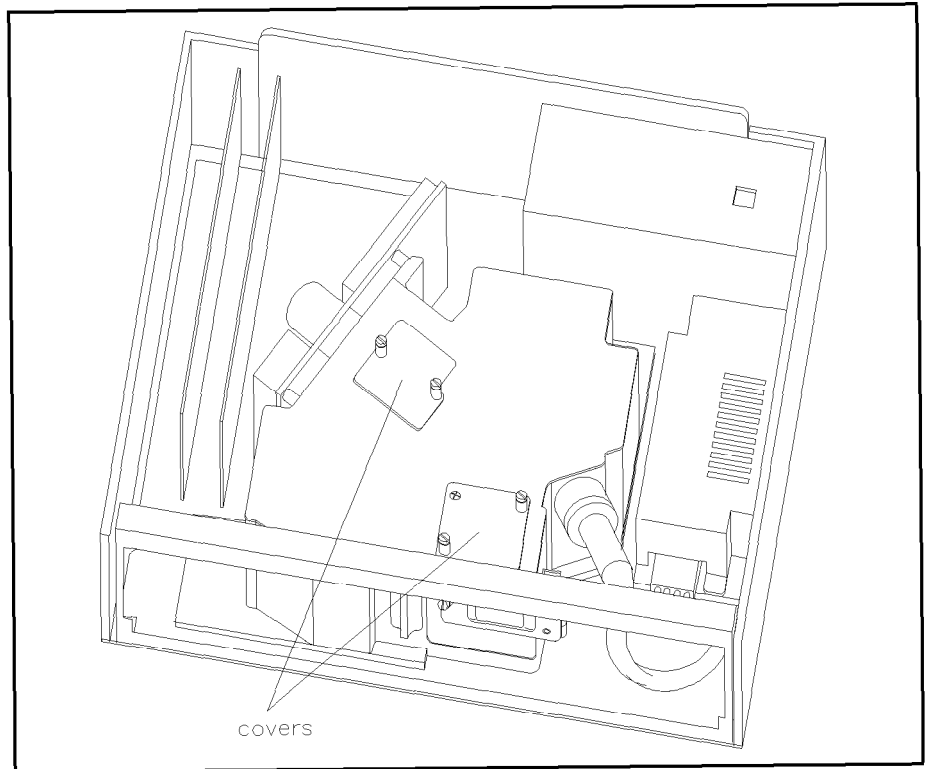


Figure 4-29. Location of Slit Covers

3. Pull the slit out of the recess in the wall of the optical unit.
4. Push the new slit down into the recess. The spring side of the slit must face away from the grating.
5. When the slit is completely in, move it gently to the left, until it is securely in place, to ensure correct alignment in the optical path.

Changing the Slits

Touring The HP 1046A

Touring The HP 1046A

This chapter describes the optical and electrical hardware in the HP 1046A Programmable Fluorescence Detector and gives a more detailed overview of operation than you will find in Chapter 1 or Chapter 2.

Luminescence Detection

Luminescence, the emission of light, happens when molecules change from an excited state to their ground state. Molecules can be excited by different forms of energy, each with its own excitation process. For example, when the excitation energy is light, the process is called *photoluminescence*, and when the excitation energy comes from a chemical reaction (a so-called *non-photon excitation*) the process is called *chemiluminescence*. The HP 1046A can measure both photoluminescence and luminescence from non-photon excitation.

In simple cases, the emission of light is the reverse of absorption, see Figure 5-1. With sodium vapor, for example, the absorption and emission spectra are a single line at the same wavelength. The absorption and emission spectra of organic molecules in solution produce bands instead of lines.

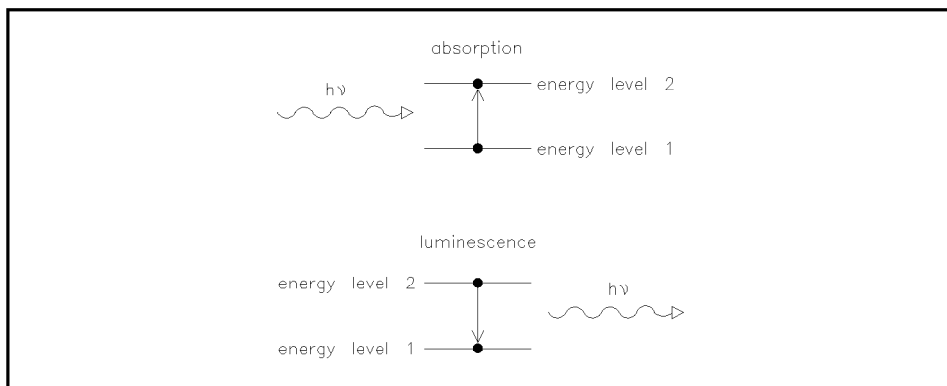


Figure 5-1. Absorption of Light Versus Emission of Light

When a more complex molecule transforms from its ground energy state into an excited state, the absorbed energy is distributed into various vibrational and rotational sub-levels. When this same molecule returns to the ground state, this vibrational and rotational energy is first lost by relaxation without any radiation. Then the molecule transforms from this energy level to one of the vibrational and rotational sub-levels of its ground state, emitting light, see

Figure 5-2. The characteristic maxima of absorption for a substance is its λ_{ex} , and for emission is its λ_{em} .

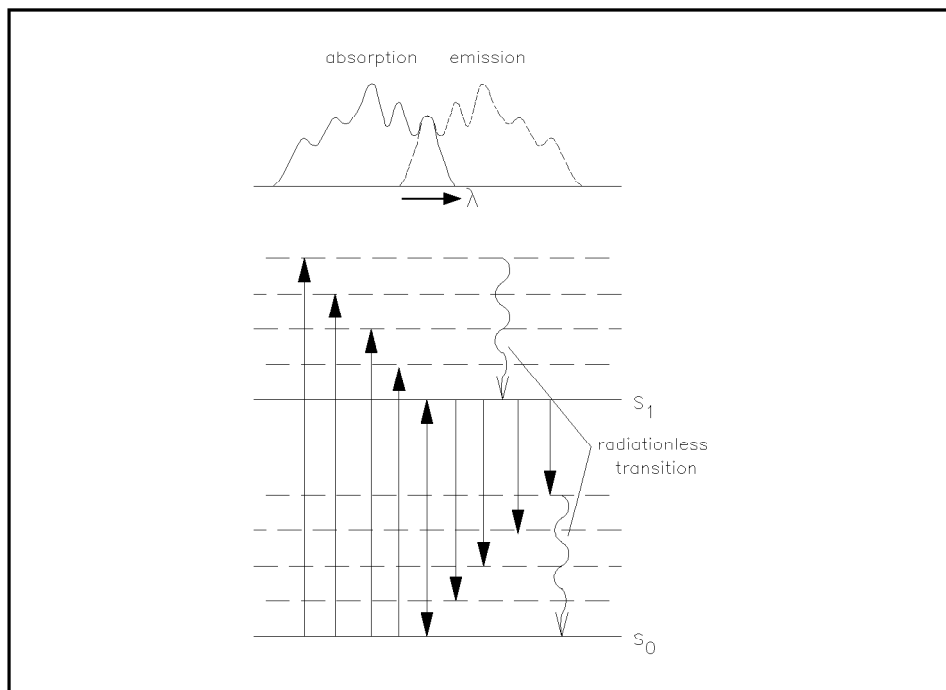


Figure 5-2. Relationship of Excitation and Emission Wavelengths

Photoluminescence is the collective name for two phenomena, *fluorescence* and *phosphorescence*, which differ from each other in one characteristic way—the delay of emission after excitation. If a molecule emits light 10^{-9} to 10^{-5} seconds after it was illuminated then the process was fluorescence. If a molecule emits light longer than 10^{-3} seconds after illumination then the process was phosphorescence.

Phosphorescence is a longer process because one of the electrons involved in the excitation changes its spin, during a collision with a molecule of solvent, for example. The excited molecule is now in a so-called triplet state, T, see Figure 5-3.

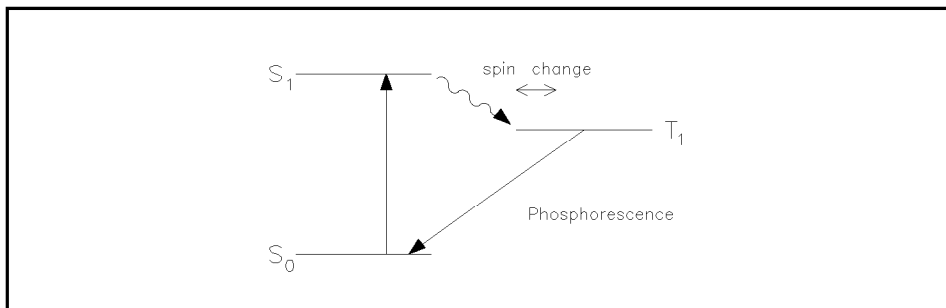


Figure 5-3. Phosphorescence Energy Transitions

The molecule must change its spin back again before it can return to its ground state. Since the chance of colliding with another molecule with the necessary spin for change is slight, the molecule remains in its triplet state for some time. During the second spin change the molecule loses more energy by relaxing without radiation. The light which is emitted during phosphorescence therefore has less energy and is at a longer wavelength than fluorescence.

$$E = h\lambda^{-1}$$

In this equation:

E is energy
h is Planck's constant
 λ is the wavelength

Contemporary applications for room-temperature phosphorescence in liquids divide into three groups: native, sensitized and quenched.

- Native phosphorescence is found in those compounds that emit light naturally.
- In the sensitized mode, the illuminated sample molecule transfers its energy to an acceptor (for example, bi-acetyl) which in turn phosphoresces. You could follow reports in the literature to detect polychlorobiphenyls and polychloronaphthalenes this way.
- In the quenched mode, the bi-acetyl is added to the solvent producing a high background phosphorescence at the baseline. When the sample is added, the energy of the bi-acetyl transfers before phosphorescence occurs: the background phosphorescence is quenched. Literature reports

Luminescence Detection

of detection of aromatic and aliphatic amines, pyrrole-type nitrogen compounds and inorganic ions could help you optimize your method using this technique.

To achieve better detection limits, it can be useful to excite fluorescent compounds by a chemical reaction instead of photons. For example, bis (2, 4, 6-trichlorophenyl) oxalate, or TCPO, and hydrogen peroxide (H_2O_2) form 1, 2,-dioxethanedione which can excite fluorophors in the post-column eluent. The presence of H_2O_2 can be determined in this way.

Chemiluminescence detection methods have been applied to the analysis of many compounds including amino acids, PAHs, H_2O_2 , and various drugs.

Optical Unit

All the elements of the optical system, shown in Figure 5-4, including xenon lamp (1), excitation grating (2), flow cell (3), cut-off filter (4), slits (5), emission grating (6) and photomultiplier tube (7) are housed in the metal casting inside the detector compartment. The HP 1046A has grating/grating optics, enabling the selection of both excitation and emission wavelengths. The flow cell and cut-off filter can be accessed from the front of the HP 1046A by opening the keyboard outwards.

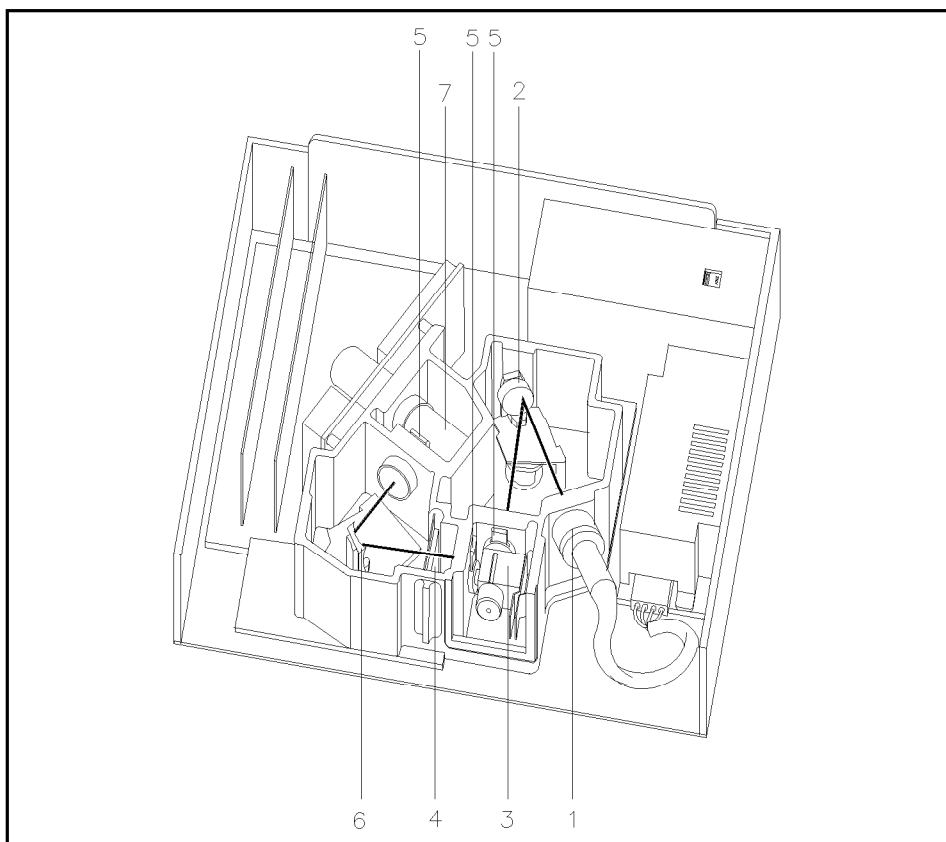


Figure 5-4. Optical Unit

Optical Unit

The radiation source is a xenon-arc flash-lamp. The 30 μs flash produces a continuous spectrum of light from 190 nm to 800 nm. The light output distribution can be expressed as a percentage in 100 nm intervals, see Figure 5-5. The lamp can be used for 1500 hours at high flash frequency, reducing intensity by 50%. You can economize during automatic operation using keyboard setpoints (see “**Lamp**” in Chapter 6), so the lamp flashes during your analysis only. The lamp can be used until it no longer ignites, but the noise level may increase with usage.

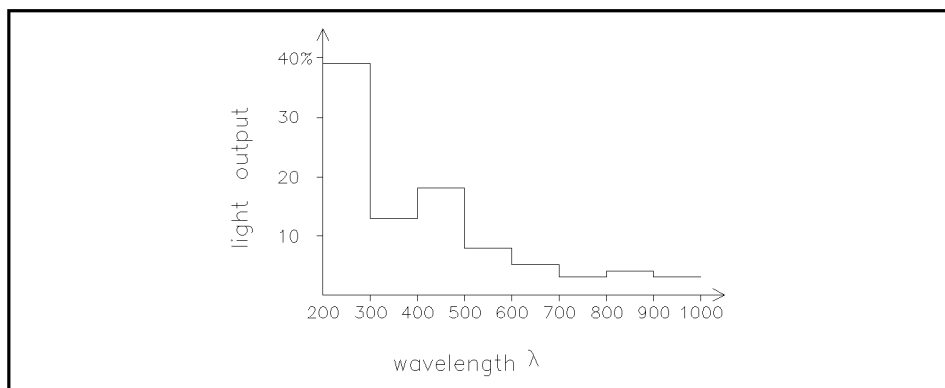


Figure 5-5. Lamp Energy Distribution

The radiation emitted by the lamp is dispersed and reflected by the excitation monochromator grating onto the slit before the aperture of the flow cell. For wider bandwidths of incident light, the slits can be exchanged.

The holographic concave grating is the main part of the monochromator, dispersing and reflecting the incident light. The surface contains many minute grooves, 1200 of them per millimeter.

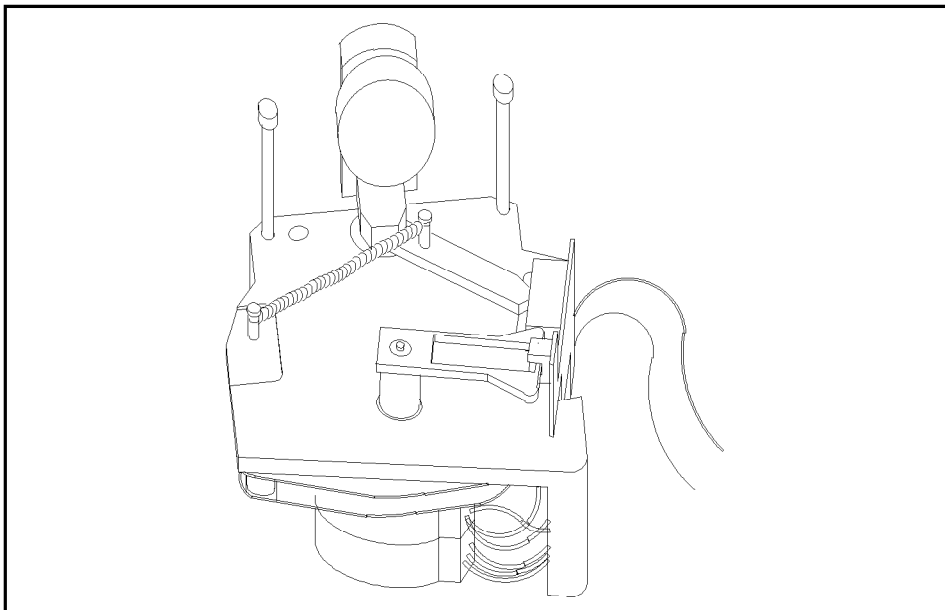
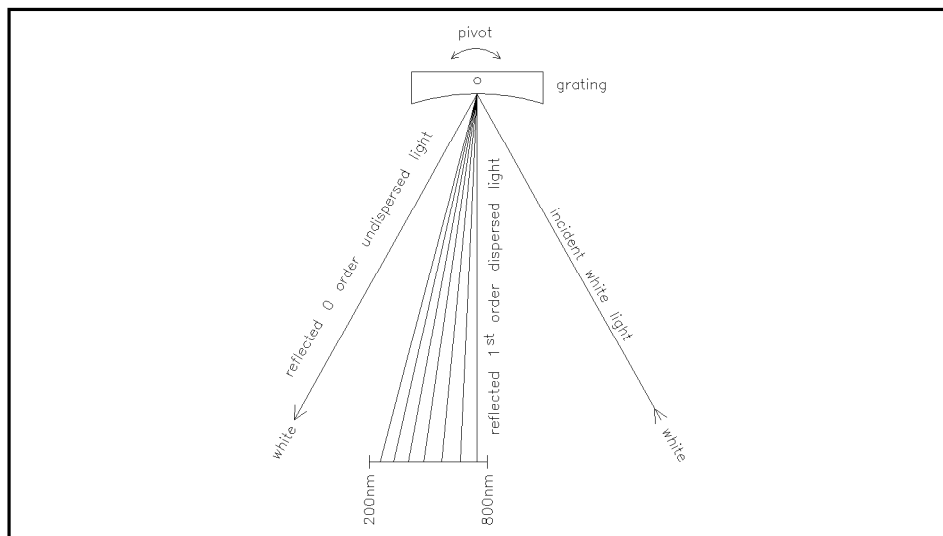


Figure 5-6. Grating Assembly

The geometry of the grooves is optimized to reflect almost all of the incident light, in the 1st order and disperse it with about 70% efficiency in the ultra-violet range. Most of the remaining 30% of the light is reflected at zero order, with no dispersion. Figure 5-7 illustrates the light path at the surface of the grating.

Optical Unit**Figure 5-7. Dispersion of Light by a Grating**

The grating is turned around its pivot by a stepper motor, the position of the grating determining the wavelength or wavelength range of the light falling onto the flow cell. The grating can be programmed to change its position and therefore the wavelength during a run.

The excitation and emission gratings are similar in design, but have different surfaces. The excitation grating reflects most 1st-order light in the ultra-violet range around 225 nm, whereas the emission grating reflects better in the visible range around 450 nm.

The flow cell is assembled from cell pieces for flow inlet and outlet, each with a window; one transparent to light, the other absorbent to light, or black. These two windows are specially shaped to measure front surface luminescence, to reduce scatter and stray light, and to minimize peak dispersion within the flow chamber.

Each window is stepped inwards, see Figure 5-8. Between the two windows is a cylindrical quartz flow chamber sealed with small gaskets. The design of the flow cell allows the windows and flow chamber to separate under excessive back pressure (>80 bar), so that solvent flows out of the cell and is registered underneath by the leak sensor. When pressure returns to

normal (<80 bar), the tension of springs inside the cell pieces ensure that the windows and flow chamber reseal.

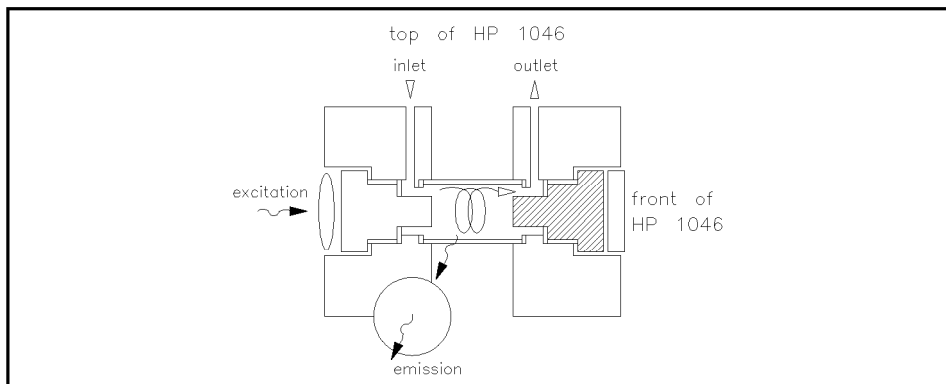


Figure 5-8. Cross-Section of Flow Cell

The luminescence from the sample in the flow cell is collected at right angles to the incident light by a second lens, and passes through a second slit. Before the luminescence reaches the emission monochromator, a cut-off filter removes light below a certain wavelength, to reduce noise from scatter and 2nd-order stray light. There are three cut-off filter kits available from Hewlett-Packard, each containing five filters for you to choose from. A 280 nm cut-off filter is built-in at the factory. The remaining luminescence above 280 nm falls onto the second monochromator.

The selected wavelength of light is reflected onto the slit in the wall of the photomultiplier compartment of the optical unit. The bandwidth of emitted light is a product of the bandwidth after the cell and the slit here. The accessory kit contains one 1 × 1 mm slit, and one 2 × 2 mm slit, see “Changing the Slits” in Chapter 4.

On the photocathode, see Figure 5-9, incident photons generate electrons. These electrons are accelerated by an electrical field between several arc-shaped dynodes. Depending on the voltage difference between any pair of dynodes, an incident electron may spark-off further electrons which accelerate onto the next dynode. An avalanche effect results: finally so many electrons are generated that a current can be measured. The amplification is a function of the voltage at the dynodes and is microprocessor controlled. You can set the amplification using the PMTGAIN function.

Optical Unit

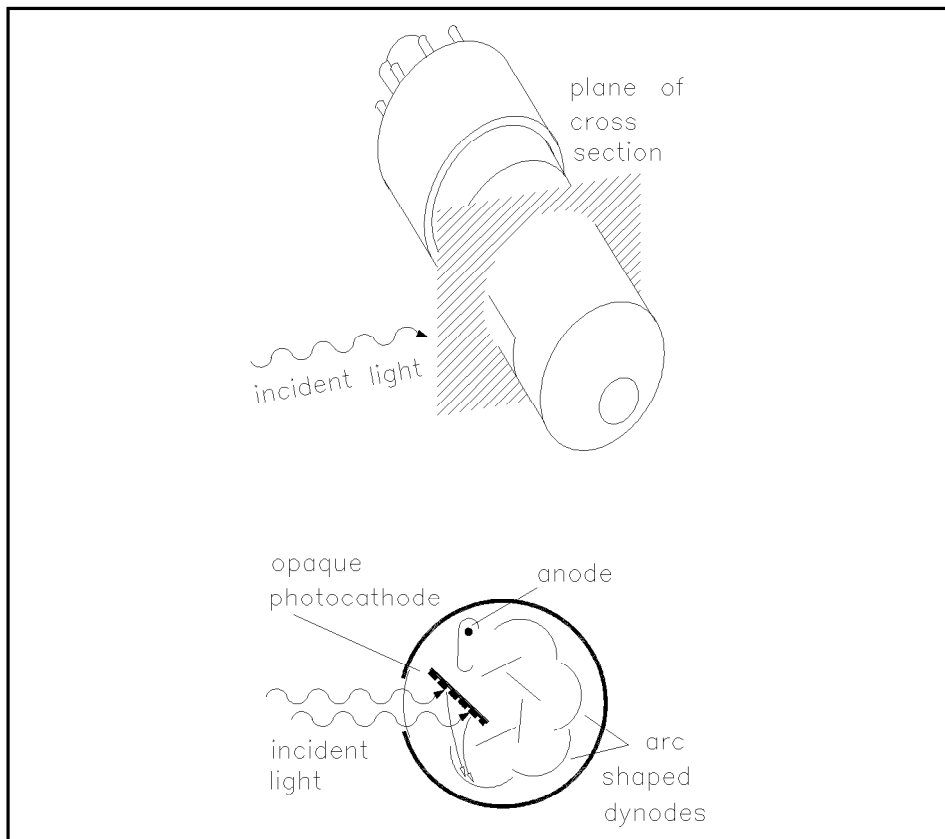


Figure 5-9. Photomultiplier Tube

This type of so-called side-on photomultiplier is compact ensuring fast response, conserving the advantages of the short optical path shown in Figure 5-9.

Analytical Information From Primary Data

We now know how the primary data from your sample is acquired in the optical unit. But how can this data be used as information in analytical chemistry? Depending on the chemistry of your application, the luminescence measured by the HP 1046A will have different characteristics. You must decide, using your knowledge of the sample, what mode of detection you will use.

Fluorescence Detection

When the lamp flashes, the fluorescing compounds in your sample will luminesce almost simultaneously, see Figure 5-10. The luminescence is short-lived, therefore the HP 1046A need only measure over a short period of time after the lamp has flashed, as defined by the GATE function.

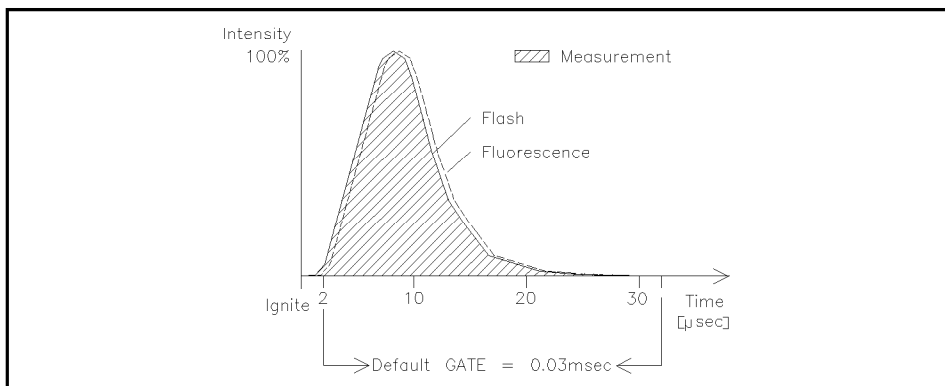


Figure 5-10. Measurement of Fluorescence

When you have set a value for LAMP other than 0 (zero), but GATE and DELAY remain at 0, the fluorescent mode of measurement, F, is displayed in the luminescence monitor, together with the intensity during your analysis.

Phosphorescence Detection

To measure phosphorescence sensitively, we must be able to measure where stray light from the flash is minimum. The **DELAY** function allows you to set a waiting period, during which the lamp flashes, before the HP 1046A starts to measure.

As we saw in “Luminescence Detection”, phosphorescence is a longer process than fluorescence. You will have to set a measurement time period, using the **GATE** function, based on the phosphorescence lifetime of the compound you want to analyze, see Figure 5-11.

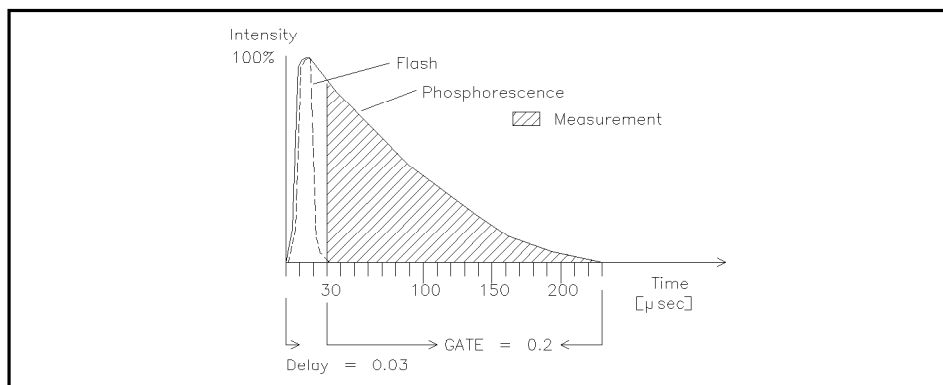


Figure 5-11. Measurement of Phosphorescence

When you have set values other than 0 for **LAMP** and either **GATE** or **DELAY**, the phosphorescence mode of measurement, **P**, is displayed in the luminescence monitor, together with the intensity during your analysis.

Figure 5-11 is only a representation of the dynamics of phosphorescence. In reality, the situation approaches that of chemiluminescence, see “Chemiluminescence Detection”.

Chemiluminescence Detection

When your sample luminesces as a result of a chemical reaction either before the column or after, you must switch off the lamp, because the components do not need to be illuminated. The HP 1046A continues to measure intensity, see Figure 5-12, during the time you define by the GATE function. The HP 1046A requires a short amount of time to convert the data acquired by the photomultiplier into a signal.

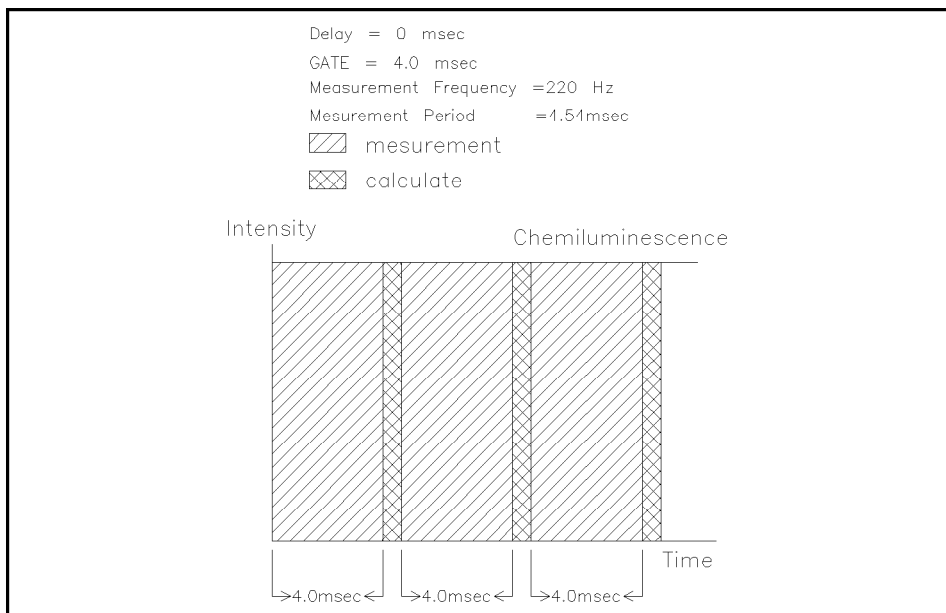


Figure 5-12. Measurement of Chemiluminescence

If LAMP=0, the chemiluminescence mode of measurement, C, is displayed in the luminescence monitor, together with the intensity during your analysis.

Use exactly the setpoints given in Figure 5-12, DELAY=0 and GATE=4, for optimum results.

Processing of Raw Data

The luminescence data is generated at the same rate as the lamp flash frequency, set with the `LAMP` function, see Figure 5-13. If the lamp flashes at 220 Hz, then the fluorescence data rate is also 220 Hz. That means that your sample is illuminated 220 times per second, and any luminescence generated by the components eluted from the column is measured 220 times per second.

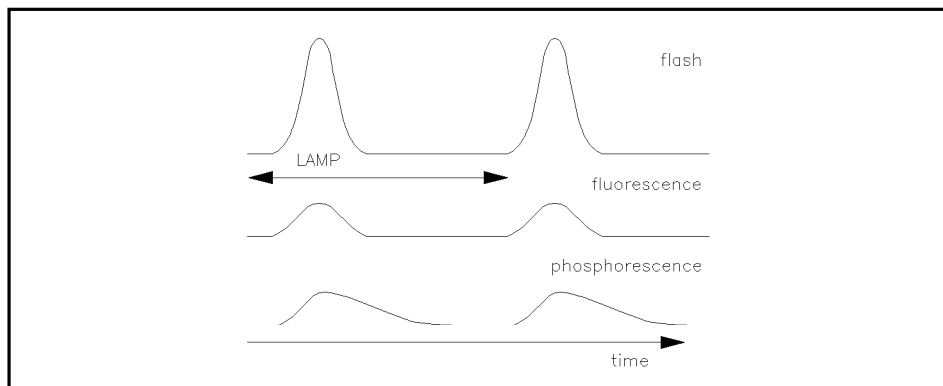


Figure 5-13. LAMP: Frequency of Flash, Fluorescence, and Phosphorescence

By using a high flash frequency, you can improve the signal-to-noise characteristics. The higher the flash frequency, the more signal points generated: so the proportion of noise becomes smaller. However, with high flash frequencies, the total number of flashes the lamp is capable of producing (that is, lamp lifetime) will be reached sooner than with a low frequency.

The analog-to-digital (A-to-D) converter has a full scale resolution of 4000 points. Weak signals may effect errors in quantification because of insufficient resolution. You can amplify the signal using `PMTGAIN`. A multiple of electrons, depending on the `PMTGAIN` you have set, is generated for every photon falling on the photomultiplier. You can quantify large and small peaks in the same chromatogram by programming changes in `PMTGAIN` during the run.

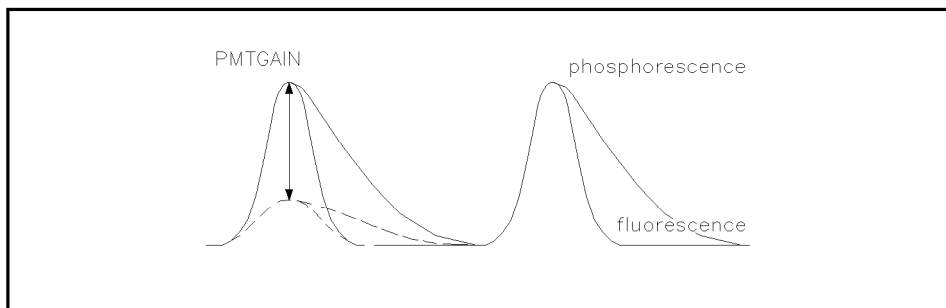


Figure 5-14. PMTGAIN: Amplification of Signal

Do not use the attenuation (or similar function) on your integrator to amplify a weak signal, see Figure 5-15. This can only amplify the already bunched-and-filtered data points. Use PMTGAIN first to amplify the raw data. Bunching and filtering of the amplified raw data produces more correct results.

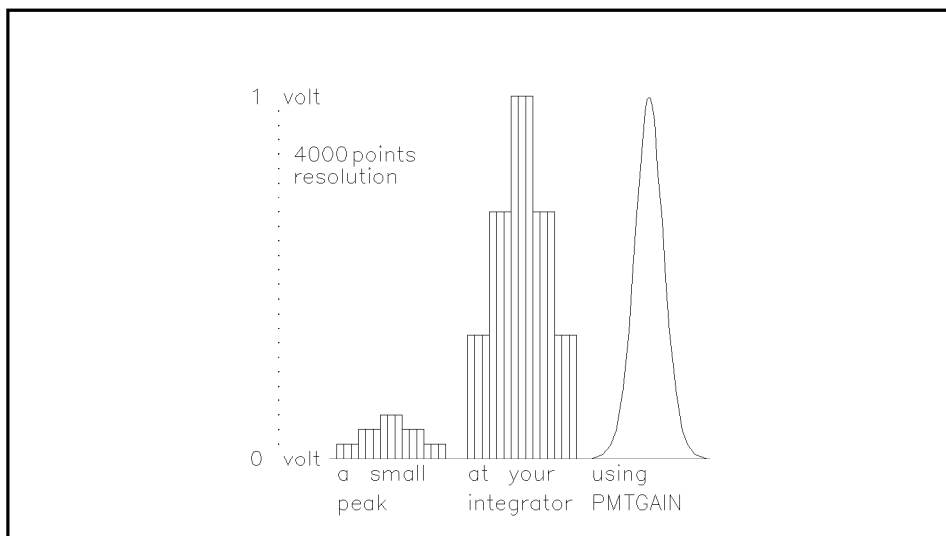


Figure 5-15. Correct Signal Amplification

Each PMTGAIN step is approximately a factor of 2 increase. To optimize your amplification for the peak with the most emission, raise the PMTGAIN setting

Processing of Raw Data

until the luminescence reading in the luminescence monitor is between 50 and 100.

After the photons are converted and multiplied into an electronic signal, the signal (at present analog) is integrated in the electronics behind the photomultiplier. After integration, the signal is converted by an A-to-D converter to give one raw data point (digital), shown as a circle with cross in Figure 5-16. Eleven of these data points are bunched together as the first step of data processing. Bunching improves your signal-to-noise ratio.

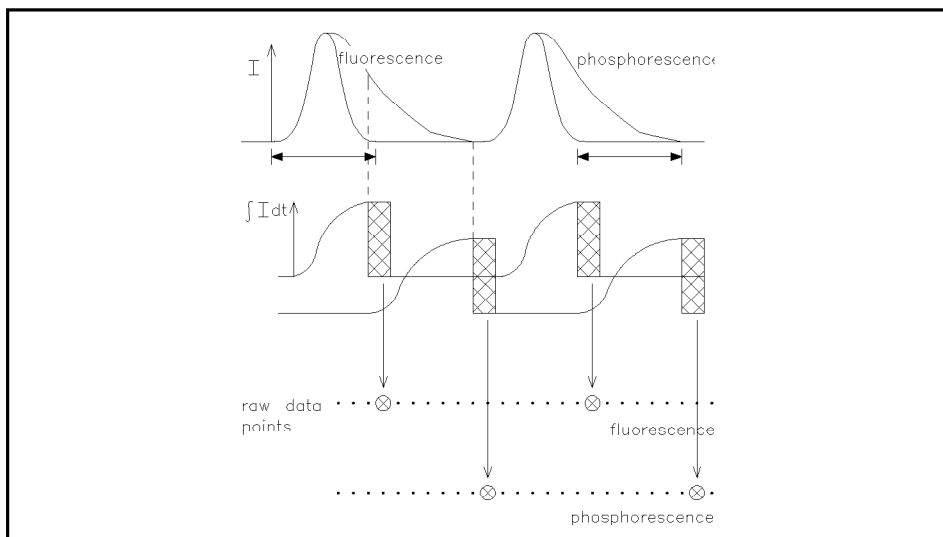


Figure 5-16. Integration of Intensity and Conversion to Signal Data Points

The bunched data, shown as larger black dots in Figure 5-17, is then filtered using a boxcar filter. The data is smoothed, without being reduced, by taking the mean of a number of points. The mean of the same points minus the first plus the next, and so on, is calculated so that there are the same number of bunched- and-filtered points as the original bunched points. You can define the length of the boxcar element using the `RESPONSETIME` function: the longer the `RESPONSETIME`, the greater the number of data points averaged. A four-fold increase in `RESPONSETIME` (for example, 1 ms to 4 ms) doubles the signal-to-noise ratio.

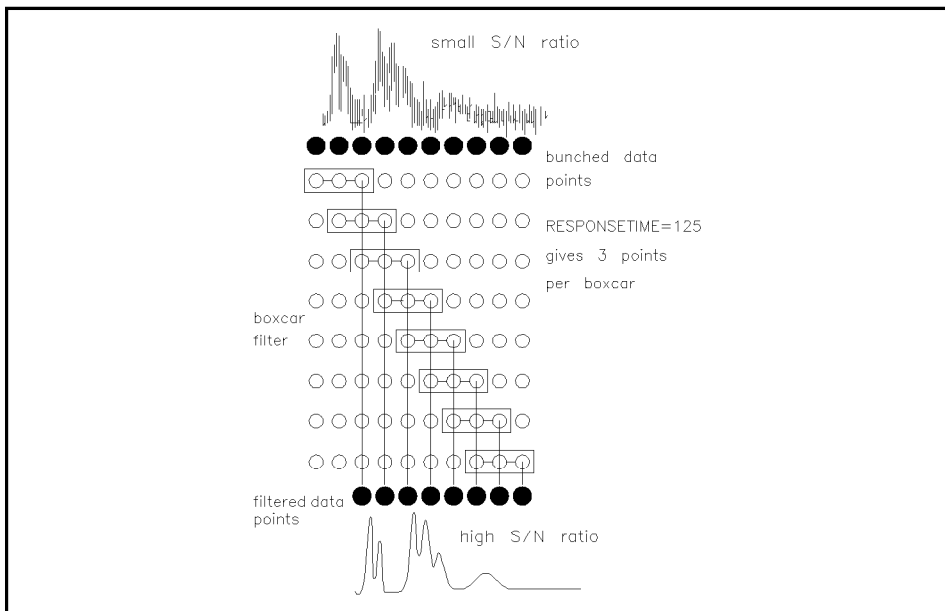


Figure 5-17. RESPONSETIME: Signal-to-Noise Ratio

Literature References

If you are interested in knowing more about the theory of luminescence spectroscopy, the following literature references should help you understand the HP 1046A optimization process even better.

1. A Wiese, H. Elgass and L. Huber, Programmable Fluorescence Detector for HPLC, Hewlett-Packard Technical Note, publication number 12-5954-6280
2. J.N. Miller, Standards in Fluorescence Spectrometry, Chapman and Hall, London—New York—Rheine, 1981, ISBN 0-412-22500-X
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5. E.L. Wehry, Fluorescence Spectroscopy, vols. 1 & 2, Heyden, London—New York—Rheine, 1976
6. K. D. Mielenz, Optical Radiation Measurements, Academic Press, New York—London, 1982
7. R. Weinberger, Drug Determination in Biological Fluids by Liquid Chromatography-Fluorescence, pp 159/189, in Therapeutic Drug Monitoring and Toxicology by Liquid Chromatography, Marcel Dekker Inc., New York and Basel, 1985

Detector Reference

Detector Reference

board

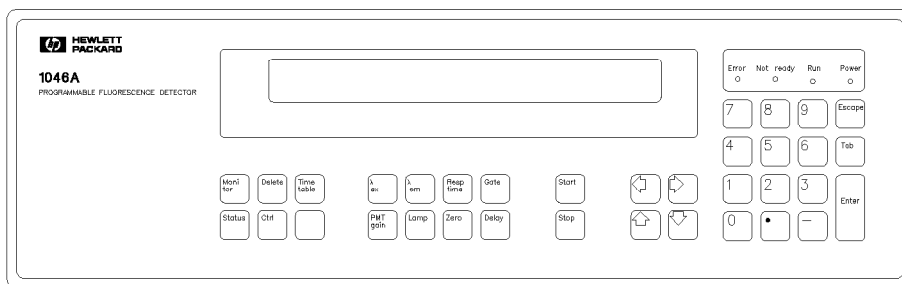


Figure 6-1. The Keyboard

Direction Keys

The direction keys, , ,  and , allow you to select different options and move the cursor in the display.



If cursor is on left of display,  or  displays next or previous line of list.

If cursor is in entry field, enter numeric value, press  or  to display next or previous choice. In the luminescence monitor  and  control the wavelength setting.



Moves cursor to the entry field on right or to the next digit in a numeric entry field.



Moves cursor to previous character or entry field on left or to previous digit in numeric entry field.

Numeric Keys



Display the numbers 0 through 9 in numeric entry fields.



Displays a decimal point (.) in numeric entry fields.



Displays a minus sign (–) in numeric entry fields.

Parameter and Function Entry Keys

Enter

Enters value of parameter or function displayed. When you begin to edit a display the cursor will be an exclamation mark (!). When you press **Enter** the cursor becomes a rectangle.

A exclamation mark (!) cursor means you are editing the display and the displayed value is not necessarily the same as the actual setting of the parameter.

A rectangle cursor means the displayed value is the same as the actual setting.

When you are editing a display, the detector's parameter or function is not changed until you press **Enter**.

Esc

Displays original value of parameter or function.

Tab

Moves cursor to next entry window, like a tabulator.

Analysis Control Keys

You use the analysis control keys, **Start** and **Stop**, to start and stop an analysis.

Start

Starts an analysis. Remember that you can use the REMOTE connectors or the HP-IB interface to start an analysis from another device, for example, your injector. After pressing **Start** (or receiving a start signal) the detector displays the run time in the luminescence monitor.

Stop

Stops an analysis. Remember that you can use the REMOTE connectors or the HP-IB interface to stop an analysis from another device, or you can use the detector's **STOPTIME** function. After pressing **Stop** (or receiving a stop signal) the detector reverses programmed parameters changes made with the timetable, checks the positions of the monochromators, and—if you used negative lamp values—turns off the xenon lamp.

NOTE

The detector does not send out start and stop signals through the REMOTE connectors. The start and stop lines of these connectors are for input of start and stop signals to the detector.

Display and Control Functions

You access the display and control functions using the block of keys on the left of the keyboard.

Monitor

Displays the luminescence monitor—the excitation and emission wavelengths, and the measured intensity as a percentage of the full scale, for example:

```
EX= 300nm EM= 500nm F= 1.09
```

Where:

EX= is the excitation wavelength in nm;
EM= is the emission wavelength in nm;
F= is the measured intensity as a percentage of output (10% means 100 mV at 1 V full scale).

When the cursor is in the EX or EM field you can change the wavelength setting of either monochromator in 1 nm intervals using the  and  keys. Use the  and  keys or the  key to move the cursor between the EX and EM fields.

If you press and hold down the  or  key the monochromator moves continuously, scanning the wavelengths until it reaches the end of the spectral range. The monochromator moves with a default speed of 50 nm/s. You can change the scanning speed using the SCANSPEED function, see “”.

The label of the measured intensity can be F, P, or C:

- F for fluorescence—when

```
LAMP ≠ 0
DELAY = 0
GATE = 0
```
- P for phosphorescence—when

Display and Control Functions

```
LAMP ≠ 0  
DELAY ≠ 0  
GATE ≠ 0
```

- C for chemiluminescence—when

```
LAMP = 0
```

Status

Displays the current operating condition of your detector, for example:

```
PRERUN      HP 1046A ready
```

```
PRERUN      error
```

```
RUN 2.79
```

The detector is in the PRERUN state when line power has been turned on, after the STOPTIME has elapsed, after you have pressed **Stop**, or after a stop signal has been received through REMOTE or HP-IB connector.

During RUN the detector displays the time elapsed since the start of the analysis (**Start** pressed or start signal received through REMOTE or HP-IB connector) and the RUN lamp will be on.

On the right of the display the detector will display an error or not ready message when a corresponding condition is detected. Press the **▼** to display the full error message.

NOTE

For details of what to do when the detector displays an error message, see Chapter 3.

Delete

You use the **Delete** key to delete a line from the timetable or to delete the complete timetable.

Deleting a Line From the Timetable

1. Press **Timetable**, then **▲** or **▼** to select the line you want to delete.

```
AT 5.00 PMTGAIN = 1
```

2. Press **Delete**.

```
DEL 5.00 PMTGAIN = 1
```

3. Press **Enter** to delete the line.

If you do not want to delete the line, press **Escape**.

Deleting the Complete Timetable

Press **Delete** **Timetable** **Enter** to delete the complete timetable.

Ctrl

You use the **Ctrl** key with the numeric keys, **0**, **1**, and **2**, the **λ EX**, **λ EM**, and **Stop** keys to access advanced functions that you do not need in day-to-day use.

SCANSPEED

Press **Ctrl** **0** to access the SCANSPEED function.

```
SCANSPEED = 6 ; 50 nm/sec
```

SCANSPEED sets the speed of the motor beneath the monochromator, for scanning faster or slower using the **▲** or **▼** in the luminescence monitor. SCANSPEED does not change the speed of wavelength switching in the timetable. The scanning speeds you can select are shown in Table 6-1.

Table 6-1. Scanning Speeds

Entry	Scan Speed
1	1.5 nm/sec
2	3 nm/sec
3	6 nm/sec
4	12 nm/sec
5	25 nm/sec
6	50 nm/sec

INIT MONOCHROMATOR

Press **Ctrl** **1** to access the INIT MONOCHROMATOR function.

```
INIT MONOCHROMATOR
```

INIT MONOCHROMATOR initializes the monochromators by moving both monochromators to reference position and then back to your setpoints. Press **Enter** to initialize the monochromators. Use this function to recover from the error messages;

```
EX reference position
```

or
EM reference position
If successful, this will display:

initializing....
HP 1046A detector on (X-2718)

RESET INSTRUMENT

Press **(Ctrl) 2** to access the RESET INSTRUMENT function.

RESET INSTRUMENT

RESET INSTRUMENT restarts the HP 1046A and resets all parameters, including the timetable, to default values. Press **(Enter)** to reset the detector. This will display:

HP 1046A parameters lost !!!

The default values are:

```
EXCITATION =_0  ; zero order
EMISSION   =_0  ; zero order
PMTGAIN    =_1  ;    1
RESPONSETIME =_4  ; 1000 msec
ZERO       =_0  ; % of full scale
LAMP       =_0  ; off
GATE       =_0.00 ; off
DELAY      =_0.00 ; off
SCANSPEED  =_6  ; 50 nm/sec
timetable is empty
```

EXCALIB

Press **(Ctrl)** **(λ Ex)** to access the EXCALIB function.

EXCALIB = 0 ; nm

EXCALIB calibrates the wavelength setting of the **(λ Ex)** key. You can enter a value from -14 to 14 nm in steps of 1. The calibrated wavelength is the value with EXCALIB = 0 less the expected value.

EMCALIB

Press **(Ctrl)** **(λ Em)** to access the EMCALIB function.

EMCALIB = 0 ; nm

EMCALIB calibrates the wavelength setting of the **(λ Em)** key. You can enter a value from -14 to 14 nm in steps of 1. The calibrated wavelength is the value with EMCALIB = 0 less the expected value.

STOPTIME

Press **(Ctrl)** **(Stop)** to access the STOPTIME function.

STOPTIME = 0 ; off

Limits: 0 for off, or 0.01 to 1440.00 min in steps of 0.01 min.

If you cannot connect a remote stop signal from your liquid chromatograph to the HP 1046A, you can use the STOPTIME function. The timetable will stop running as soon as the actual run time overruns the time you set here.

Timetable

You use the **Timetable** key to select the timetable for editing, for example:

```

Timetable
AT 4.00   EX= 220   EM= 400
▼
AT 4.00   PMTGAIN = 2
▼
:

```

The first line of the current timetable is displayed. You can display the other lines using the **▲** and **▼** keys.

If you have not yet entered a timetable, a message is displayed for about 2 seconds followed by a new empty line of the timetable:

```

timetable is empty
AT

```

You can program the excitation wavelength, emission wavelength, and PMTGAIN function.

Entering a Timetable

1. Display a new line of timetable by pressing the **Timetable** key (and **▼** key, if a timetable has already been entered):

```

Timetable
AT

```

2. Type time in entry window after AT. You can enter times between 0 and 1440 min in steps of 0.01 min:

```

3
AT3!

```

Display and Control Functions

3. Select the variable you want to program by pressing the corresponding parameter key:

(λ Ex)

AT3 EX=!

4. Type set value in entry window after variable:

(2) (5) (4)

AT3 EX=254!

5. Select emission wavelength (EM) entry field by pressing the corresponding parameter key:

(λ Em)

AT3 EX=254 EM=!

6. Type set value in entry window after variable:

(4) (0) (5)

AT3 EX=254 EM=405!

7. Press **(Enter)**

AT_

A new empty line is displayed. To display the line you have just entered, press the **(▲)** key:

(▲)

AT_3.00 EX= 254 EM= 405

Notes on Entering a Timetable

- If you overwrite an existing timetable line and then press **(Enter)**, the old line is deleted and replaced by the new line.
- If the first line of the timetable is displayed and you press **(▲)**, the detector will display:

top of timetable

- If you try to enter a variable that cannot be programmed, the detector will display:

`not time programmable`

- A timetable comprises a maximum of about 100 lines. If you try to insert another line, the detector will display:

`timetable overflow`

- You can delete a line from the timetable or delete the complete timetable using the **Delete** key, see “**Delete**”.

Parameter Keys

You access the parameters using the block of keys near the center of the keyboard.

λ Ex

Sets the wavelength on the excitation monochromator.

EXCITATION = 190 ; 190 nm

Limits: 0 (for zero order) or 190 to 800 nm in steps of 1.

When set to 0 the full spectrum of light from the xenon lamp illuminates the flow cell. Each compound can absorb its characteristic wavelength of light and then emit maximum fluorescence. This is not very sensitive because of the corresponding noise, from zero-order stray light at wavelengths above the 280 nm cut-off filter. Ultra-violet light output is also lower at zero order.

When set to a wavelength value the excitation monochromator is selecting light of this wavelength from the xenon lamp to illuminate the flow cell. The excitation wavelength is programmable, see “**Timetable**”. You can also scroll the excitation wavelength in the luminescence monitor, see “**Monitor**”.

Pmtgain

Sets an amplification factor for your signal.

PMTGAIN = 1 ; 1

Limits: 1 to 16 in steps of 1.

The PMTGAIN function defines the voltage across the dynodes inside the photomultiplier. Each step approximately doubles the signal. The PMTGAIN function is programmable, see “**Timetable**”.

λ Em

Sets the wavelength on the emission monochromator.

EMISSION = 190 ; 190 nm

Limits: 0 (for zero order) or 190 to 800 nm in steps of 1.

When set to 0 the photomultiplier is illuminated with the total sample spectrum. The detector measures the light intensity over all wavelengths above 280 nm (the cut-off filter after the flow cell absorbs all light below 280 nm).

When set to a wavelength value the emission monochromator is selecting light of this wavelength from the flow cell to be measured at the photomultiplier. The emission wavelength is programmable, see “**Timetable**”. You can also scroll the emission wavelength in the luminescence monitor, see “**Monitor**”.

The optimum emission wavelength will always be higher than the optimum excitation wavelength so you can begin scanning at a higher wavelength than the optimum excitation wavelength.

Lamp

Turns on and sets the flash frequency of the xenon lamp.

LAMP = 0 ; off

Limits:

0 for lamp off

Parameter Keys

1 for on (1.25 W or 55 Hz)

2 for on (2.5 W or 110 Hz)

3 for on (5 W or 220 Hz)

When LAMP=0, the chemiluminescence mode of detection is annotated C in the luminescence monitor, see “**Monitor**”.

You can economize during routine work, by using the lamp during actual measurements only, that is, when the detector is in RUN status. The lamp function is then controlled by the **Start** or **Stop** keys or the equivalent remote control signals through the REMOTE or HP-IB connectors. Use negative values (–1, –2, or –3) if you want the lamp to be on only during the run. Do not use negative values if your integrator balances at the start of each run, unless you can reset the baseline approximately 0.01 minutes after each start.

NOTE

The flash frequency determines the data acquisition rate for fluorescence or phosphorescence only. For chemiluminescence, use the recommended values for the GATE and DELAY functions, see “**Gate**” and “**Delay**”.

Resptime

Sets the digital filter for the analog output.

RESPONSETIME = 1 ; 125 msec

Limits:

1 for 125 ms

2 for 250 ms

3 for 500 ms

4 for 1000 ms

5 for 2000 ms

6 for 4000 ms

The response time selects the time period during which data points are summed and averaged during data processing. The length of time determines the number of data points in the boxcar filter. Such digital filtering improves the signal-to-noise characteristics of your final chromatogram.

Zero

Adds a constant offset to the analog signal output.

`ZERO = 0 ; % of full scale`

Limits: -99 to 99% of full scale in steps of 1. For example, ZERO = -10, baseline of 0.1 V reset to 0 V and maximum output is 0.9 V.

ZERO does not balance the detector. As ZERO values larger than 0 reduce the available dynamic range, use the zero function on your integrator, if possible.

Gate

Sets a time period during which light is collected and integrated during data acquisition.

`GATE = 0 ; off`

Limits: 0.02 to 10.00 in steps of 0.01 ms. 0 uses default (0.03 ms duration of the flash) to measure the light, see “Notes on Rate of Data Acquisition”.

Use 0 for fluorescence. Optimum is 4.00 ms for chemiluminescence.

Parameter Keys**Delay**

Sets a waiting period, after lamp flash, before signal is measured and integrated during data acquisition.

DELAY = 0 ; off

Limits: 0 to 10.00 in steps of 0.01 ms.

With DELAY>0 and LAMP on, the phosphorescence mode of detection is annotated as P in the luminescence monitor, see “**Monitor**”.

Notes on Rate of Data Acquisition

When the lamp is not being used (LAMP=0), the functions GATE and DELAY determine your rate of data acquisition—the greater the sum of your GATE and DELAY, the slower the rate of data acquisition.

Table 6-2.
Relationship Between GATE and DELAY Settings and Acquisition Rate

GATE + DELAY	Acquisition Rate
≤ 4 ms	220 Hz
≤ 8.5 ms	110 Hz
≤ 17 msec	55 Hz

Similar limits apply to your data acquisition with the lamp on—the more often the lamp flashes (and therefore the faster the rate of data acquisition), the shorter the sum of GATE and DELAY.

Table 6-3. Relationship Between LAMP Setting, GATE and DELAY

LAMP Setting	Limit for Sum GATE + DELAY
0, 1 or -1	≤ 17 ms
2 or -2	≤ 8.5 msc
3 or -3	≤ 4 ms

Parameter Keys

Installing Your Detector

Installing Your Detector

This chapter describes how to install your detector and verify that it is functioning correctly.

Check carefully before you begin that you have all the necessary cables and connection points for the HP 1046A: read “Choosing a Suitable Place” and “Unpacking”. If you wish to install or interface the HP 1046A to instruments or accessories not specified here, you should first contact your local Hewlett-Packard office to check that the safety of the HP 1046A will not be affected by this intended connection.

The HP 1046A panels and cabinet must be grounded to protect yourself during operation. The HP 1046A, and any peripheral instruments connected to it, must be operated from a three-contact power source, with the ground contacts securely connected to ground. Get an electrician to check and verify ground at the source.

High Voltages: Disconnect the line-power cord before removing any of the HP 1046A panels.

UV Radiation: Continuous exposure to intense ultra violet radiation (the radiation emitted by the xenon flash lamp) may cause permanent damage to the eyes or other parts of the body. Avoid exposure to this radiation. Do not open the detector when the lamp is turned on.

Um den Benutzer zu schützen, muß das Gerät geerdet sein. Der HP 1046A und alle Peripherie müssen mit einem 3-adrigen Netzkabel ausgestattet sein, das den HP 1046A mit der Schutzterde verbindet, sobald der Stecker in eine passende Steckdose gesteckt wird. Überprüfen Sie die Schutzterde, bevor der HP 1046A installiert wird.

Hochspannung: Bevor ein Gehäuseteil entfernt werden soll, muß das Netzkabel abgezogen werden.

UV Strahlung: Setzen Sie sich keiner direkten UV-Strahlung aus (ausgestrahlt von der Detektorlampe). Die Strahlung kann Ihre Augen oder andere Körperteile gefährden. Vermeiden Sie den Kontakt mit dieser Strahlung. Öffnen Sie nicht das Lampengehäuse, wenn die Lampe eingeschaltet ist.

CUIDADO

Para proteger al operador, todos los paneles del instrumento deben estar conectados a tierra. Tanto el HP 1046A como los periféricos deben trabajar con todas las tomas de tierra conectadas correctamente. Verifique su toma de tierra antes de instalar el HP 1046A.

Alta tensión: Desconecte el instrumento de la línea antes de sacar el panel posterior del instrumento.

Radiación UV: Una exposición continuada a la luz ultravioleta (que es la radiación emitida por la lámpara del detector) puede dañar irreversiblemente los ojos y otros órganos del cuerpo humano. Evite cualquier exposición a esta radiación: no abra nunca el compartimento de la lámpara cuando esté conectado.

ATTENTION

Pour protéger l'opérateur, les panneaux de l'instrument et le four doivent être raccordés à la terre; le HP 1046A ainsi que tout périphérique associé doivent être branchés à une prise électrique à 3 contacts dont celui de terre est relié correctement à la terre du bâtiment. Vérifiez la prise de terre avant d'installer le HP 1046A.

Tensions élevées: Débranchez le cordon secteur avant d'ôter le panneau arrière.

Radiation UV: Une exposition continuelle à une radiation ultra- violette intense (la radiation émise par la source lumineuse du détecteur) peut provoquer des lésions irréversibles aux yeux ou à d'autres parties du corps. Evitez de vous exposer a cette radiation. N'ouvrez pas le logement de la lampe quand celle-ci est allumée.

ATTENZIONE

Per proteggere l'utilizzatore, i pannelli dello strumento e la struttura esterna devono essere collegati a terra. Sia il vostro HP 1046A che le varie periferiche devono lavorare con un cavo di alimentazione a 3 conduttori con il filo di terra adeguatamente collegato a terra. Verificate la vostra linea di terra prima di installare lo strumento.

Alte tensioni: Scollegare il cavo di alimentazione prima di rimuovere il pannello posteriore.

Radiazioni UV: L'esposizione continuata ad una intensa radiazione ultravioletta (radiazione emessa dalla lampada del rivelatore) può causare danni permanenti agli occhi o altre parti del corpo. Evitate l'esposizione a queste radiazioni: non aprite lo scomparto della lampada quando questa è accesa.

Choosing a Suitable Place

Check carefully before you begin that the place you have chosen to install your detector meets requirements below. Check each item as you read through the list.

Dimensions

430 mm × 370 mm × 150 mm

(17 in × 14.5 in × 6 in)

(width × depth × height)

Leave adequate space to the right (or left, as you prefer) of the HP 1046A for capillary connections, and at least 8 cm (3 in) at the rear for drainage of any leaks to waste, and access to the LINE switch.

Weight

13 Kg (29 lb)

Environment

Temperature 0 to 55°C (50 to 131 F)

Humidity 5 to 95% (40°C)

Line Voltage

100 to 120 V AC, $\pm 10\%$ or 220 to 240 V AC, $\pm 10\%$

Line Frequency

48 to 66 Hz

Power Consumption

50 VA maximum

Unpacking

Inspect the carton. If you find signs of external damage, contact your local Hewlett-Packard office.

After you have removed the plastic straps and staples, open the top of the carton. Lift out the accessory box. Check the accessories against the packing list below.

Table 7-1. Checklist of Accessories

Description	Hewlett-Packard Part Number	Check
Power cord	as ordered	...
Signal cables	as ordered	...
Remote cables	as ordered	...
HP-IB cable	if ordered	...
<i>Quick Reference Guide</i>	01046-90002	...
<i>User's Guide</i>	01046-90004	...
Capillaries	as ordered	...

Table 7-1. Checklist of Accessories (continued)

Description	Hewlett-Packard Part Number	Check
Accessory kit, including:	01046-68701	...
Slot screwdriver	8730-0019	...
Pozidriv screwdriver #1	8710-0899	...
2 fuses, T1 Amp, 250 V	2110-0007	...
2 fuses, T0.5 Amp, 250 V	2110-0202	...
Wrench, 4 mm	8710-1534	...
Hexagonal key, 1.5 mm	8710-0909	...
Hexagonal key, 0.05"	8710-0857	...
Hexagonal key, 2.5 mm	8710-1597	...
Flexible tubing	0890-1486	...
1 × 1 mm slit	01046 60021	...
2 × 2 mm slit	01046-60022	...
Waste tube	01040-67602	...
Capillary column adapter	01048-87302	...

Each HP 1046A is identified by a 10-digit serial number on a label attached to the rear panel.

The serial number of your HP 1046A is:

.....

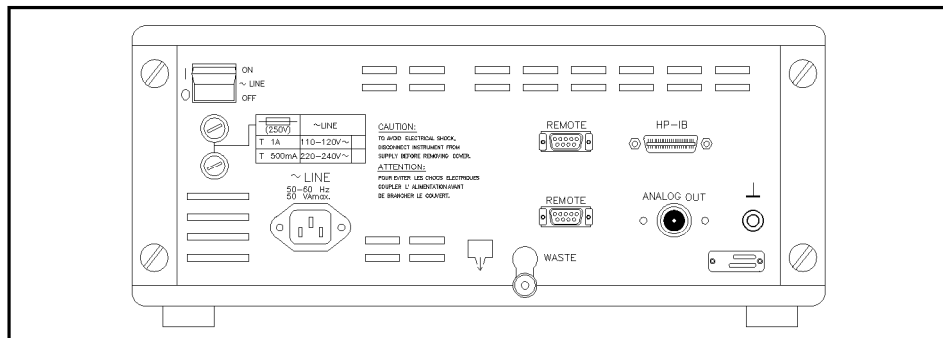


Figure 7-1. Rear Panel of Detector

ANALOG OUT Connectors

The ANALOG OUT connector provides signal output. You can vary the scaling range of the output according to your application, see “Changing the Full-Scale Range”.

Check that you have the correct cable from the detector to your integrator or plotter, see Table 7-2. The part number is in a sheath around the cable.

Screw the metal cap of the signal cable onto the connector. Push the other end of the signal cable into the connector for analog input on your integrator or plotter.

Table 7-2. Signal Cables

From HP 1046A to:	Requires signal cable:
HP 3390A Integrator	01040-60101
HP 3392A Integrator	01040-60101
HP 3393A Integrator	01040-60101
HP 3394A Integrator	35900-60750
HP 3396A Integrator	35900-60750
HP 18652A Interface	01046-60103
HP 35900A Dual Channel Interface	35900-60750
HP 1082B Liquid Chromatograph	8120-1840
HP 1084B Liquid Chromatograph	8120-1840
General purpose	01046-60105

If you are using a non-Hewlett-Packard device, connect the spade lugs on the end of the general purpose signal cable 01046-60105 to the contacts of your instrument.

Changing the Full-Scale Range

The full-scale range of the ANALOG OUT output is set at 0 to 1 V. You can change this setting to 0 to 100 mV by moving a slide-switch on the detector's DAC board.

1. Remove the 2 screws at each side of the rear panel.
2. Remove the 2 screws at both the left and right side of the detector.
3. Lift off the top cover.
4. Find the full-scale definition switch—it is located on the DAC board (see Figure 7-2) near to the ANALOG OUT connector.

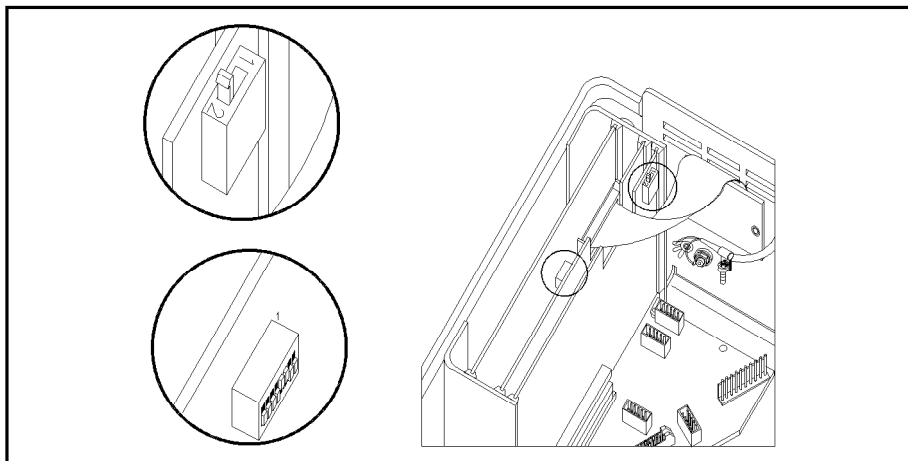
Cables

Figure 7-2. Location of Full-Scale Range Switch

5. Slide switch to desired position (position 1 is 0 to 100 mV and position 2 is 0 to 1 V).
6. Put the top cover back into place.
7. Secure the side panels and top cover with the 2 screws on each side and the 2 screws on the rear panel.

REMOTE Connectors

These connectors communicate start, stop, prepare, error and not-ready signal inputs and outputs, in a chain arrangement between your liquid chromatograph and integrator. The connectors are identical: you can connect your liquid chromatograph or integrator to either one. The HP 1046A simply passes on the signals it receives, in either direction. If the flow cell leaks, a shut-down signal is generated, which you can use to stop your solvent pump.

Check that you have the correct cable from the HP 1046A to your instrument, and from your instruments to the HP 1046A if necessary, see Table 7-3 and Table 7-4. The part number is in a sheath around the cable.

Table 7-3. Remote Control Cables From HP 1046A

From HP 1046A to:	Requires remote control cable:
HP 3390A Integrator	01046-60203
HP 3392A Integrator	01046-60206
HP 3393A Integrator	01046-60206
HP 3394A Integrator	01046-60210
HP 3396A Integrator	03394-60600
HP 18652A Interface	01046-60204
HP 35900A Dual Channel Interface	5061-3378
General purpose	01046-60201

Table 7-4. Remote Control Cables To HP 1046A

To HP 1046A from:	Requires remote control cable:
HP 1050 Series of HPLC modules	5061-3378
HP 1081B Liquid Chromatograph	01046-60200
HP 1082B Liquid Chromatograph	01046-60201
HP 1084B Liquid Chromatograph	01046-60201
HP 1090 Liquid Chromatographs	01046-60202

Push the metal plug of the remote control cable onto one of the REMOTE connectors. There is only one way to position the plug. Tighten the two screws to secure the connector. Connect the other end of the cable to the contacts for remote start, stop or prepare on your instrument.

If you are using a non-Hewlett-Packard device, connect the crimp contacts on the end of the general purpose remote cable 01046-60201 to the contacts of your instrument. To help you make the correct connections, the signals carried on each pin are listed below, the colors refer to the wires of remote cable part number 01046-60201.

Cables**Table 7-5. Signals Carried by Remote Cable**

Pin	Signal	Wire color	Activity
1	Digital ground	white	
2	Prepare run	brown	low
3	Start	gray	low
4	Shut down	blue	low
5	Not used	pink	
6	Not used	yellow	
7	Ready	red	high
8	Stop	green	low
9	†Start request	black	low

† The HP 1046A does not use the start request signal.

HP-IB Connector

Check that the device address switch is set to 12 (the switch is preset at the factory—you should not need to change it). The switch is on the HP-IB board. Remove the HP 1046A top cover. The HP-IB board and the position of the switch is shown in Figure 7-3. Replace the top cover.

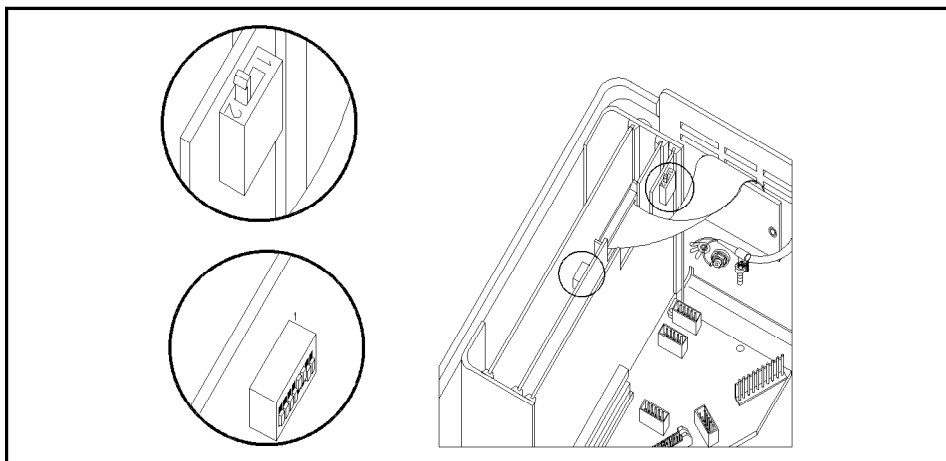


Figure 7-3. Position and Setting of HP-IB Switch

Connect the HP-IB cable between your computer and the HP 1046A.

Chassis Terminal

If you have a measuring instrument that has a safety ground connector, connect grounding cable from your instrument to chassis terminal on rear panel of your HP 1046A.

Connecting the Hydraulic Lines

The inlet and outlet connections are factory installed in the side panel at the left of the HP 1046A. If you want to keep the length of the capillary connections from your liquid chromatograph to a minimum, you can exchange the left side with the right, as follows.

Changing Position of Inlet and Outlet Connections

1. Remove the 4 screws at each side of the rear panel.
2. Remove the 2 screws at both the left and right side of the detector.
3. Lift off the top cover.
4. Lift out the right side panel and put to one side.
5. Lift out the left side panel and turn it over to the right side of the detector.
6. Turn over the other panel and place it on the left side of the detector.
7. Pull off leak drain from inlet and outlet connections, turn leak drain through 180° and replace it beneath the connections.
8. Put the top cover back into place.
9. Secure the side panels and top cover with the 2 screws on each side and the 4 screws on the rear panel.

Connecting the Detector Inlet and Outlet

The detector inlet and outlet are the 2 fittings labeled IN (in *red*) and OUT (in *blue*) on the side panel of your detector.

Select your inlet capillaries narrower than outlet capillaries, see Table 7-6. To avoid high back pressure, never install an inlet capillary on the outlet.

Table 7-6. Inlet and Outlet Capillaries

Internal Diameter	Length	Hewlett-Packard Part Number
0.12 mm (inlet)	75 mm	01090-87611
0.12 mm (inlet)	280 mm	01090-87610
0.25 mm (outlet)	130 mm	01090-87308
0.25 mm (outlet)	320 mm	79835-87638

1. Connect the inlet capillary from your column to the IN fitting on the side of the detector.
2. Connect the outlet capillary from the OUT fitting on the side of the detector to a safe waste container.

CAUTION

It is your responsibility to ensure that a detector-in-series is safely connected to the HP 1046A.

ACHTUNG

Wenn Sie einen externen Detektor anschließen, ist es Ihre Aufgabe, dafür zu sorgen, dass der Detektor sicher in den HP 1046A Flußweg integriert wird.

ATENCION

Cuando conecte un detector externo, recuerde que es su responsabilidad asegurarse de que el detector está correctamente conectado a la salida de la columna del HP 1046A.

AVERTISSEMENT

Lors de la connexion d'un détecteur extérieur, il est de votre responsabilité d'assurer la bonne connexion du détecteur au circuit de solvant du HP 1046A.

PRECAUZIONE

Quando collegate un rivelatore esterno è vostra responsabilità controllare che questo sia correttamente collegato al sistema idraulico del vostro HP 1046A.

Connecting the Waste Outlet

Push a length of corrugated tubing (supplied with your detector) onto fitting at lower center of rear panel, see Figure 7-1. Lead tubing to a safe waste disposal container.

Connecting Line Power

Observe the following safety information.

WARNING

Ensure that line-power cord is disconnected before changing line- voltage setting. Ensure that the line-power cord is disconnected before installing or replacing a fuse.

WARNUNG

Wenn der Netzspannungswahlschalter betätigt wird, darf das Netzkabel nicht angeschlossen sein. Während des Auswechselns einer Sicherung darf das Netzkabel nicht angeschlossen sein.

CUIDADO

Asegúrese que el cable de red está desconectado antes de cambiar el selector de voltaje. Asegúrese de tener desconectado el instrumento cuando necesite cambiar un fusible.

ATTENTION

Assurez vous que le cable secteur n'est pas connecté avant de changer la tension d'alimentation. Vérifiez que le cordon secteur est déconnecté avant de mettre en place ou de remplacer le fusible.

ATTENZIONE

Assicuratevi che il cavo do alimentazione sia scollegato prima di spostare li elettore di voltaggio. Assicuratevi che il cavo di alimentazione sia staccato prima di installare o sostituire il fusibile.

Setting the Line Voltage

Check that the line-voltage selector is set for the correct line voltage for operation in your area. The switch is set at the factory, you should not need to change it. The position of the voltage selector switch is marked on the label on top of the main power supply cover, see Figure 7-4.

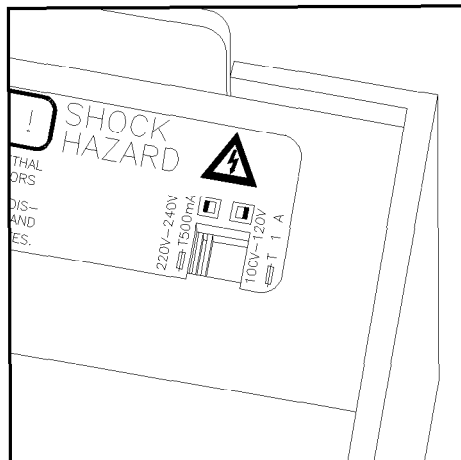


Figure 7-4. Setting the Line Voltage

To change the setting: Turn off the detector at the $\sim$ LINE power switch at the rear. Insert tip of small screwdriver into slot of switch and slide switch to correct position.

Fuses

Check that the correct fuses for the voltage operation you have selected are installed.

Table 7-7. Fuses

Line Voltage Setting	Fuse	Hewlett-Packard part number
110 V	T1 Amp, 250 V	2110-0007
220 V	T0.5 Amp, 250 V	2110-0202

To install a fuse:

1. Use a screwdriver to push the fuse cap inwards and then turn counterclockwise.
2. Place new fuse in fuse cap.
3. Insert fuse and cap into fuse receptacle, pressing and turning clockwise until cap locks in place.

Connecting the Power Cord

Connect the line power cord to the LINE socket at the rear of the detector.

Connect the other end of the line power cord to a suitable power source, as indicated on the rear panel.

NOTE

Connect the power cords of *all* units of your system to the *same* power source. This prevents current loops that can cause systematic baseline interference.

Verifying the Performance of Your Detector

The performance test described here assumes that you have installed your detector correctly, according to the instructions at the beginning of this chapter.

You will verify the sensitivity performance of the detector using a dilute solution of biphenyl in water. You can either make up your own biphenyl solution or use the Hewlett-Packard isocratic standard sample supplied with every Hewlett-Packard liquid chromatograph. You can order the isocratic standard sample from Hewlett-Packard by quoting the order number 01080-68704.

The biphenyl solution you prepare will have a concentration of about 8 $\mu\text{g/l}$, corresponding to about 40 pg of biphenyl in the 5 μl flow cell.

The resulting signal-to-noise ratio at this concentration must be at least 40. Under optimum conditions you may find your detector able to reach signal-to-noise ratios of greater than 200.

Stage 1: Preparing Your System

1. Set the analog output voltage range on the HP 1046A to 1 V, see “Changing the Full-Scale Range”.
2. Install the following slits and filters in the HP 1046A:

Excitation slit	2 × 2 mm
Emission slit #1	4 × 4 mm
Emission slit #2	4 × 4 mm
Cut-off filter	280 nm

To change a slit, see “Changing the Slits” in Chapter 4. To change the cut-off filter, see “Changing the Cut-Off Filter” in Chapter 4.

3. Set the following parameters on the HP 1046A:

```

EXCITATION = 246 ; 246 nm
EMISSION   = 317 ; 317 nm
PMTGAIN    = 14 ; 14
RESPONSETIME = 4 ; 1000 msec
LAMP       = 1 ; 55 Hz
ZERO       = 0 ; % of full scale
GATE       = 0.00 ; off
DELAY     = 0.00 ; off

```

4. Flush *all* channels of your solvent delivery system with bidistilled water at a flow rate of 1 ml/min.
5. Set your solvent delivery system to pump bidistilled water through the detector at a flow rate of 1 ml/min.
6. Press **Monitor** and wait until the fluorescence value (F) is stable.

The fluorescence value must be less than 10 and must not fluctuate more than ± 0.15 .

If the fluorescence value is < 10 and stable, go to “Preparing the Test Solution”.

If the fluorescence value is > 10 and unstable, continue with “Fluorescence Too High or Unstable?”.

Fluorescence Too High or Unstable?

If the fluorescence value is > 10 and unstable, try the following:

1. Check you have set the detector parameters correctly.
2. Check there are no air bubbles or leaks in the flow cell.
3. Check the bidistilled water is not contaminated.
4. Increase the flow rate (for example, 4 ml/min) to flush out air bubbles from the flow cell.

Verifying the Performance of Your Detector

5. Reduce the PMTGAIN to 13 or 12.

If the fluorescence value remains >10 and unstable, go to “Troubleshooting the Chromatogram” in Chapter 3.

Preparing the Test Solution

1. Make up a $8\text{ }\mu\text{g/l}$ solution of biphenyl in water.

If you have the Hewlett-Packard isocratic standard sample, add $100\text{ }\mu\text{l}$ of this sample to 1 l of bidistilled water and mix thoroughly.

Use a freshly prepared test solution. Do not use a test solution that has been stored for longer than a few hours.

2. Set your solvent delivery system to pump the test solution through the detector at a flow rate of 1 ml/min.
3. Press **Monitor** and wait until the fluorescence value (F) is stable.

The fluorescence value should be between 45 and 95.

If the fluorescence value is *not* between 45 and 95, adjust the PMTGAIN setting to bring the fluorescence value within range. If you have to adjust the PMTGAIN setting by more than ± 2 steps, check you have prepared the test solution correctly.

Measuring the Sensitivity Performance

1. Set your solvent delivery system to pump bidistilled water through the detector at a flow rate of 1 ml/min.
2. Press **Monitor** and wait until the fluorescence value is than 10 and does not fluctuate more than ± 0.15 .

3. Set the attenuation of your integrator to 64 mV full scale and record the signal for about 3 min.
4. Set your solvent delivery system to pump the test solution through the detector at a flow rate of 1 ml/min.
5. Set the attenuation of your integrator to 1024 mV full scale and record the signal for about 7 min.
6. Measure the peak-to-peak noise recorded during the first 3 minutes.
7. Measure the baseline offset recorded after changing from bidistilled water to the test solution.
8. Calculate the signal-to-noise ratio from the equation:

$$\text{Signal to Noise} = 16 \frac{\text{Baseline Offset}}{\text{Noise}}$$

Verifying the Performance of Your Detector

Legal, Regulatory and Safety Information

Legal, Regulatory and Safety Information

This chapter contains legal, regulatory and safety information

Warranty Statement

All Analytical Products

Hewlett-Packard (HP) warrants its Analytical Products against defects in materials and workmanship for the period specified in Table 8-1. During the warranty period, HP will, at its option, repair or replace products which prove to be defective. Products that are installed by HP are warranted from the installation date, all others from the ship date.

If Buyer schedules or delays installation more than 30 days after delivery, then warranty period starts on 31st day from date of shipment (60 and 61 days, respectively for products shipped internationally).

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

Limitation of Warranty

On-site warranty services are provided at the initial installation point. Installation and on-site warranty services are available only in HP service travel areas, and only in the country of initial purchase unless Buyer pays HP international prices for the product and services. Warranties requiring return to HP are not limited to the country of purchase.

For installation and warranty services outside of HP's service travel area, HP will provide a quotation for the applicable additional services.

If products eligible for installation and on-site warranty services are moved from the initial installation point, the warranty will remain in effect only if

Warranty Statement

the customer purchases additional inspection or installation services, at the new site.

The foregoing warranty shall not apply to defects resulting from:

1. Improper or inadequate maintenance, adjustment, calibration or operation by Buyer;
2. Buyer-supplied software, hardware, interfacing or consumables;
3. Unauthorized modification or misuse;
4. Operation outside of the environmental and electrical specifications for the product;
5. Improper site preparation and maintenance; or
6. Customer induced contamination or leaks.

THE WARRANTY SET FORTH IS EXCLUSIVE AND NO OTHER WARRANTY, WHETHER WRITTEN OR ORAL, IS EXPRESSED OR IMPLIED. HP SPECIFICALLY DISCLAIMS THE IMPLIED WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE.

Limitation of Remedies and Liability

THE REMEDIES PROVIDED HEREIN ARE BUYER'S SOLE AND EXCLUSIVE REMEDIES. IN NO EVENT SHALL HP BE LIABLE FOR DIRECT, INDIRECT, SPECIAL, INCIDENTAL, OR CONSEQUENTIAL DAMAGES (INCLUDING LOSS OF PROFITS) WHETHER BASED ON CONTRACT, TORT OR ANY OTHER LEGAL THEORY.

Responsibilities of the Customer

The customer shall provide:

1. Access to the products during the specified periods of coverage to perform maintenance.
2. Adequate working space around the products for servicing by Hewlett-Packard personnel.
3. Access to and use of all information and facilities determined necessary by Hewlett-Packard to service and/or maintain the products. (Insofar as these items may contain proprietary or classified information, the customer shall assume full responsibility for safeguarding and protection from wrongful use.)
4. Routine operator maintenance and cleaning as specified in the Hewlett-Packard Operating and Service Manuals.
5. Consumables such as paper, disks, magnetic tapes, ribbons, inks, pens, gases, solvents, columns, syringes, lamps, septa, needles, filters, frits, fuses, seals, detector flow cell windows, etc.

Warranty Statement

Responsibilities of Hewlett-Packard

Hewlett-Packard will provide warranty services as described in Table 8-1.

Table 8-1. Warranty Services

Services During Warranty¹	Warranty Period²	Type
Spectrophotometers, Gas and Liquid Chromatographs, Integrators, Lab Automation Systems, Samplers and Injectors	90 Days	On-site
GC, LC, UV-VIS, and LAS supplies and accessories	90 Days	On-site
Columns and Consumables ³	90 Days	Return to HP
Gas Discharge and Tungsten Lamps	30 Days	Return to HP
Repairs performed on-site by HP ⁴	90 Days	On-site

1 This warranty may be modified in accordance with the law of your country. Please consult your local HP office for the period of the warranty, for shipping instructions and for the applicable wording of the local warranty.

2 Warranty services are included as specified for Analytical products and options purchased concurrently provided customer is located within a HP defined travel area. HP warranty service provides for 8 am to 5 pm on-site coverage Monday through Friday, exclusive of HP holidays.

3 Columns and Consumables are warranted to be free from defects for a period of 90 days after shipment and will be replaced on a return-to-HP basis if unused.

4 HP repair warranty is limited to only the item repaired or replaced.

Safety Information

The following general safety precautions must be observed during all phases of operation, service, and repair of this instrument. Failure to comply with these precautions or with specific warnings elsewhere in this manual violates safety standards of design, manufacture, and intended use of the instrument. Hewlett-Packard Company assumes no liability for the customer's failure to comply with these requirements.

General

This is a Safety Class I instrument (provided with terminal for protective earthing) and has been manufactured and tested according to international safety standards.

Operation

Before applying power, comply with the installation section. Additionally the following must be observed:

Do not remove instrument covers when operating. Before the instrument is switched on, all protective earth terminals, extension cords, auto-transformers, and devices connected to it must be connected to a protective earth via a ground socket. Any interruption of the protective earth grounding will cause a potential shock hazard that could result in serious personal injury. Whenever it is likely that the protection has been impaired, the instrument must be made inoperative and be secured against any intended operation.

Safety Information

Make sure that only fuses with the required rated current and of the specified type (normal blow, time delay, etc.) are used for replacement. The use of repaired fuses and the short-circuiting of fuseholders must be avoided.

Some adjustments described in the manual, are made with power supplied to the instrument, and protective covers removed. Energy available at many points may, if contacted, result in personal injury.

Any adjustment, maintenance, and repair of the opened instrument under voltage should be avoided as much as possible. When inevitable, this should be carried out by a skilled person who is aware of the hazard involved. Do not attempt internal service or adjustment unless another person, capable of rendering first aid and resuscitation, is present. Do not replace components with power cable connected.

Do not operate the instrument in the presence of flammable gases or fumes. Operation of any electrical instrument in such an environment constitutes a definite safety hazard.

Do not install substitute parts or make any unauthorized modification to the instrument.

Capacitors inside the instrument may still be charged, even though the instrument has been disconnected from its source of supply. Dangerous voltages, capable of causing serious personal injury, are present in this instrument. Use extreme caution when handling, testing and adjusting.

Safety Symbols

Below you will find some symbols that are used on the instrument and throughout the documentation.

Symbol



Description

The apparatus is marked with this symbol when the user should refer to the instruction manual in order to protect the apparatus against damage.



Indicates dangerous voltages.



Indicates a protected ground terminal.

WARNING

The "warning sign" denotes a hazard. It calls attention to a procedure, practice or the like, which, if not correctly done or adhered to, could result in injury or loss of life. Do not proceed beyond a "warning sign" until the indicated conditions are fully understood and met.

WARNUNG

Das "Warnung Zeichen" weist auf eine Gefahr für den Menschen hin. Wenn die Anweisungen und Verfahrensweisen oder ähnliches nicht befolgt und korrekt ausgeführt werden, könnte das eine erhebliche Verletzungsgefahr oder Lebensgefahr zur Folge haben. Solange die neben dem "Warnung Zeichen" angegebenen Anweisungen nicht vollständig verstanden und ausgeführt sind, sollten keine weiteren Schritte unternommen werden.

ADVERTENCIA

El "signo de advertencia" denota un riesgo. Remite a un procedimiento o práctica que de no ser llevada a cabo correctamente, podría resultar en un daño o pérdida de vida. No continúe cuando exista un "signo de advertencia" hasta que las condiciones indicadas hayan sido completamente entendidas y satisfechas.

DANGER

Le signe "Danger" dénote un risque. Il appelle une précaution quant à une procédure ou une pratique qui, si elle n'est pas correctement suivie, peut résulter en une blessure ou même en un risque pour la vie. S'assurer d'avoir rempli toutes les conditions indiquées avant de continuer.

Safety Information

NE	La scritta "Attenzione" indica un pericolo. Essa richiama l'attenzione su una situazione che può portare a danni anche gravi per le persone. Non proseguire oltre tale indicazione senza aver ben compreso il rischio ed aver seguito le istruzioni per evitarlo.
UWING	Het "waarschuwingsteken" wijst op gevaar. Indien de aanwijzingen, procedures etc. niet opgevolgd en korrekt uitgevoerd worden, kan dat een aanzienlijk risico en zelfs levensgevaar met zich mee brengen. Zolang de naast het "waarschuwingsteken" aangegeven aanwijzingen niet volledig begrepen en opgevolgd zijn, dienen geen verdere stappen ondernomen te worden.
	The "caution sign" denotes a hazard. It calls attention to an operating procedure, practice or the like, which, if not correctly done or adhered to, could result in damage to or destruction of part or all of the equipment. Do not proceed beyond a "caution sign" until the indicated conditions are fully understood and met.
	Das "Achtung Zeichen" weist auf eine mögliche Beschädigung der Geräte hin. Wenn Anweisungen und Verfahrensweisen oder ähnliches nicht befolgt und korrekt ausgeführt werden, könnte das eine Beschädigung oder Zerstörung eines Teils oder des gesamten Gerätes zur Folge haben. Solange die neben dem "Achtung Zeichen" angegebenen Anweisungen nicht vollständig verstanden und ausgeführt sind, sollten keine weiteren Schritte unternommen werden.
ON	El "signo de precaución" denota un riesgo. Remite a un procedimiento o práctica que de no ser llevada a cabo correctamente podría resultar en un daño o destrucción parcial o total del equipo. No continúe cuando exista un "signo de precaución" hasta que las condiciones indicadas hayan sido completamente entendidas y satisfechas.
N	Le signe "Attention" dénote un risque. Il appelle une précaution quant à une procédure ou une pratique qui, si elle n'est pas correctement suivie, peut résulter en un dommage ou même une destruction du matériel. S'assurer d'avoir rempli toutes les conditions indiquées avant de continuer.

PRECAUZIONE

La scritta "Precauzione" indica un pericolo. Essa richiama l'attenzione su una situazione che può portare a danni anche permanenti dello strumento. Non proseguire oltre tale indicazione senza aver ben compreso il rischio ed aver seguito le istruzioni per evitarlo.

VOORZICHT

Het teken "voorzicht" wijst op gevaar voor een mogelijke beschadiging van de instrumentatie. Het vraagt de aandacht voor een praktijk, werkwijze etc. welke, indien niet opgevolgd en korrekt uitgevoerd, kan leiden tot beschadiging of vernieling van de apparatuur of een deel ervan. Zolang de naast het teken "voorzicht" aangegeven aanwijzingen niet volledig begrepen en opgevolgd zijn, dienen geen verdere stappen ondernomen te worden.

Solvent Information

Observe the following recommendations on the use of solvents.

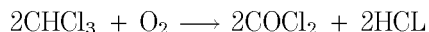
Flow Cell

Avoid the use of alkaline solutions (pH >9.5) which can attack quartz and thus impair the optical properties of the flow cell.

Solvents

Always filter solvents—small particles can permanently block the capillaries. Avoid the use of the following steel-corrosive solvents:

- Solutions of alkali halides and their respective acids (for example, lithium iodide, potassium chloride, and so on).
- High concentrations of inorganic acids like nitric acid, sulfuric acid especially at higher temperatures (replace, if your chromatography method allows, by phosphoric acid or phosphate buffer which are less corrosive against stainless steel).
- Halogenated solvents or mixtures which form radicals and/or acids, for example:



This reaction, in which stainless steel probably acts as a catalyst, occurs quickly with dried chloroform if the drying process removes the stabilizing alcohol.

- Chromatographic grade ethers, which can contain peroxides (for example, THF, dioxane, di-isopropylether) such ethers should be filtered through dry aluminium oxide which adsorbs the peroxides.
- Solutions of organic acids (acetic acid, formic acid, and so on) in organic solvents. For example, a 1 % solution of acetic acid in methanol will attack steel.
- Solutions containing strong complexing agents (for example, EDTA, ethylene diamine tetra-acetic acid);
- Mixtures of carbon tetrachloride with 2-propanol or THF.

Radio Interference

Manufacturer's Declaration

This is to certify that this equipment is in accordance with the Radio Interference Requirements of Directive FTZ 1046/1984. The German Bundespost was notified that this equipment was put into circulation, the right to check the series for compliance with the requirements was granted.

Test and Measurement.

If test and measurement equipment is operated with equipment unscreened cables and/or used for measurements on open set-ups, the user has to assure that under operating conditions the radio interference limits are still met within the premises.

Herstellerbescheinigung

Hiermit wird bescheinigt dass dieses Gerät/System in Übereinstimmung mit den Bestimmungen von Postverfügung 1046/84 funkentstört ist. Der Deutschen Bundespost wurde das Inverkehrbringen dieses Gerätes/Systems angezeigt und die Berechtigung zur Überprüfung der Serie auf Einhaltung der Bestimmungen eingeräumt.

Test- und Messgeräte.

Werden Mess- und Testgeräte mit ungeschirmten Kabeln und/oder in offenen Messaufbauten verwendet, so ist vom Betreiber sicherzustellen, daß die Funk-Entstörbestimmungen unter Betriebsbedingungen an seiner Grundstücksgrenze eingehalten werden.

Sound Emission

Manufacturer's Declaration

This statement is provided to comply with the requirements of the German Sound Emission Directive, from 18 January 1991.

This product has a sound pressure emission (at the operator position) < 70 dB.

- Sound Pressure $L_p < 70$ dB (A).
- At Operator Position.
- Normal Operation.
- According to ISO 7779 (Type Test).

Herstellerbescheinigung

Diese Information steht im Zusammenhang mit den Anforderungen der Maschinenlärminformationsverordnung vom 18 Januar 1991.

- Schalldruckpegel $L_p < 70$ dB (A).
- Am Arbeitsplatz.
- Normaler Betrieb.
- Nach DIN 45635 T. 19 (Typprüfung).

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Are the instructions clear and complete?

Yes No

Are there enough examples and illustrations?

Yes No

Are concepts explained clearly?

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**2 Please rate the following features of the book for their
usefulness. 1=Inadequate. 2=Adequate. 3=Superior.**

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Examples 1 2 3

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HP 1046A Fluorescence Detector

User's Guide

HP Part No. 01046-90004 August 1994 Printed in Germany

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Glossary

This glossary contains abbreviations and terms used in this user's guide.

You can find a glossary of chromatographic terms in the reference section of your *Analytical Supplies Catalog and Chromatography Reference Guide*.

λ_{em}

Characteristic emission maximum of a substance.

λ_{ex}

Characteristic excitation maximum of a substance.

boxcar filter

Method of smoothing data points by successively taking the mean value of a number of points (similar to a moving average) without reducing the total number of data points.

chemiluminescence

Type of **luminescence**. Occurs after energy from a chemical reaction has raised molecules from their ground energy state to an excited energy state.

cut-off filter

Optical device that absorbs light of wavelengths shorter than the specified wavelength (cut-off wavelength) for the filter and transparent to light of wavelengths longer than the cut-off wavelength.

fluorescence

Type of **photoluminescence**. Occurs between 10^{-9} and 10^{-5} seconds after light energy has raised molecules from their ground energy state to an excited energy state.

grating

Optical device that reflects and disperses incident radiation into its constituent wavelengths.

HP-IB

Hewlett-Packard Interface Bus

luminescence

Emission of light. Occurs when molecules relax from an excited energy state to their ground energy state.

monochromator grating

See grating.

non-photon excitation

Process of raising molecules from their ground energy state to an excited energy state by a type of energy other than light. For example, chemical energy, see chemiluminescence.

phosphorescence

Type of photoluminescence. Occurs longer than 10^{-3} seconds after light energy has raised molecules from their ground energy state to an excited energy state.

photoluminescence

Type of luminescence. Occurs after light energy has raised molecules from their ground energy state to an excited energy state.

photomultiplier tube

Device for amplifying the electrical current generated when light falls on a photodiode.

photon excitation

Process of raising molecules from their ground energy state to an excited energy state by light energy, see photoluminescence.

PMT

Photomultiplier tube.

slit

Aperture in the optical path that determines the spectral bandwidth of a spectrofluorimeter.

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