



# Agilent 1100 Series Fluorescence Detector



## Reference Manual



**Agilent Technologies**

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### Software Revision

This guide is valid for A.01.xx revisions of the Agilent 1100 Series Fluorescence Detector software, where xx refers to minor revisions of the software that do not affect the technical accuracy of this guide.

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## In This Guide...

### **1 Installing the Fluorescence Detector**

How to install the fluorescence detector

### **2 First Steps with the Fluorescence Detector**

How to start with the detector

### **3 Optimizing the Fluorescence Detector**

Detection with the Agilent 1100 series fluorescence detector, either fluorescence or phosphorescence, can give you very sensitive and selective results if you understand what you are doing and why. This chapter explains in detail how to optimize detection.

### **4 Troubleshooting and Test Functions**

This chapter describes the detector's built in troubleshooting and test functions.

### **5 Repairing the Fluorescence Detector**

Instructions on how to repair the detector

### **6 Identifying Parts and Materials**

Detailed illustrations and listings for parts and materials identification for the detector

### **7 Introduction to the Fluorescence Detector**

An introduction to the detector

### **8 Theory of Operation**

Instrument overview, theory of operation, external communication and internal connectors

## **9 Introduction to the Control Module**

This chapter is intended to introduce an operator to the screens available for operation of the Agilent 1100 diode array detector with the Agilent 1100 control module.

Please use the manual of Control module for further detailed reference.

## **10 Specifications**

Performance specifications of the detector

# Contents

## 1 Installing the Fluorescence Detector

Site Requirements	14
Physical Specifications	16
Unpacking the Detector	17
Detector Accessory Kit Contents	18
Optimizing the Stack Configuration	20
Installing the Detector	22
Flow Connections to the Detector	24

## 2 First Steps with the Fluorescence Detector

Before You Start	30
Optimization Overview	31
Getting Started and Checkout	33
Starting Your Detector	33
Setting the Chromatographic Conditions	34
Observe the maxima via the isoabsorbance plot	36
Method Development	37
Step 1: Check the LC system for impurities	38
Step 2: Optimize limits of detection and selectivity	39
Step 3: Set up routine methods	48
Example: Optimization for Multiple Compounds	52

## 3 Optimizing the Fluorescence Detector

Optimization Overview	62
-----------------------	----

Design Features Help Optimization	63
Check Performance Before You Start	63
Finding the Best Wavelengths	64
A Real Example	65
Finding the Best Signal Amplification	66
Changing the Xenon Flash Lamp Frequency	67
Lamp life savings	68
Selecting the Best Response Time	69
Reducing Stray Light	71

#### **4 Troubleshooting and Test Functions**

Overview of the Detector's Indicators and Test Functions	74
Status Indicators	75
Power Supply Indicator	75
Detector Status Indicator	76
Error Messages	77
Time-out	78
Shutdown	79
Remote Time-out	80
Synchronization Lost	81
Leak	82
Leak Sensor Open	84
Leak Sensor Short	85
Compensation Sensor Open	86
Compensation Sensor Short	87
Fan Failed	88
Motor Errors	89
Lamp Cover Open	91
FLF Board not found	92
ADC Not Calibrated	93

A/D Overflow	94
Flash Lamp Current Overflow	95
Flash Trigger Lost	96
Wavelength Calibration Failed	97
Wavelength Calibration Lost	98
Flow Cell Removed	99
No Peaks	100
Wavelength Verification and Calibration	101
Check of Raman Band of Water	104
The Wavelength Calibration Procedure	110
Preparation of the Glycogen Calibration Sample	110
Preparation of the Flow Cell	111
Wavelength Calibration	112
Verification	113
Test Functions	114
Lamp Intensity Test	115
Lamp Intensity History	116
Dark-Current Test	117
Excitation and Emission Grating Resistance History	118
Raman ASTM Signal-to-Noise Test	119
Using the Built-in Test Chromatogram	124
Using the Built-in DAC Test	127

## **5 Repairing the Fluorescence Detector**

Introduction into Repairing the Fluorescence Detector	130
Overview of the Repairing of the Fluorescence Detector	133
Simple Repairs	134
Exchanging a Flow Cell	135
How to use the Cuvette	138
Flow Cell Flushing	139
Correcting Leaks	140

Replacing Leak Handling System Parts	141
Exchanging Internal Parts	142
Removing the Covers	144
Removing the Rear Foam	146
Exchanging the Lamp	147
Exchanging the EX-condenser	152
Replacing the Lamp Cover Window	155
Exchanging the Processor Board	159
Exchanging the Fan	163
Exchanging the Leak Sensor or Leak Panel	165
Replacing the EM-Condenser or Cutoff-Filter	168
Replacing the Mirror Assembly	172
Replacing the Slits	176
Removing the Optical Unit	178
Replacing PMT and/or FLF board	180
Replacing FLL board and Trigger Pack	183
Replacing the EM and EX Grating	187
Exchanging the Power Supply	190
Replacing Status Light Pipe	193
Installing the Optical Unit	194
Replacing the Foams and Covers	196
Assembling the Main Cover	198
Replacing the Interface Board	199
Replacing the Detector's Firmware	200

## **6 Identifying Parts and Materials**

Overview of Main Assemblies	202
Optical Unit Assembly	204
Lamp Cover Parts	207
Control Module	208
Sheet Metal Kit	209

Plastic Parts	210
Foam Parts	211
Power and Status Light Pipes	212
Leak Parts	213
Cuvette Kit	214
Spare Parts	215
Accessory Kit	216
Cable Overview	218
Analog Cables	220
Remote Cables	223
BCD Cables	229
Auxiliary Cable	232
CAN Cable	233
External Contact Cable	234
RS-232 Cable Kit	235
LAN Cables	236

## **7 Introduction to the Fluorescence Detector**

Introduction to the Detector	238
How the Detector Operates	239
Raman Effect	242
Optical Unit	243
Reference System	250
Analytical Information From Primary Data	251
Fluorescence Detection	251
Phosphorescence Detection	252
Processing of Raw Data	253
Electrical Connections	256

Instrument Layout	258
Early Maintenance Feedback (EMF)	259
EMF Counters	259
Using the EMF Counters	261

## 8 Theory of Operation

Electronics	264
Detector Main Board (FLM)	265
Detector Frontend Board (FLF)	269
Detector Lamp Supply Board (FLL)	273
Firmware Description	274
Firmware Updates	275
Raw Data Conversion to Fluorescence (LU) Units	276
Data flow for chromatographic output	277
Data flow for spectral output	278
Spectra Acquisition Modes	281
Optional Interface Boards	282
BCD Board	283
LAN Board	284
Interfaces	285
Analog Signal Output	286
GPIB Interface	286
CAN Interface	286
Remote Interface	287
RS-232C	288
Setting the 8-bit Configuration Switch	290
GPIB Default Addresses	291
Communication Settings for RS-232C Communication	292
Forced Cold Start Settings	293

Stay-Resident Settings 294

The Main Power Supply Assembly 295

## **9 Introduction to the Control Module**

Major keys on the Agilent 1100 Control Module 298

Screens available from the Analysis screen 299

Screens available from the System screen 315

Screens available from the Records screen 318

Diagnostics and Tests 323

## **10 Specifications**

Performance Specifications 328

### **A Safety Information**

General Safety Information 332

Lithium Batteries Information 335

Radio Interference 336

Sound Emission 337

UV-Radiation 338

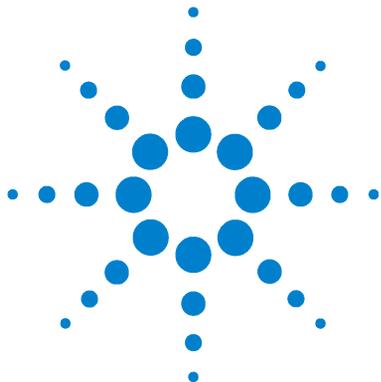
Solvent Information 339

Declaration of Conformity for HOX2 Filter 340

Agilent Technologies on Internet 341

## **Index**





# 1 Installing the Fluorescence Detector

Site Requirements	14
Physical Specifications	16
Unpacking the Detector	17
Optimizing the Stack Configuration	20
Installing the Detector	22
Flow Connections to the Detector	24



### Site Requirements

A suitable environment is important to ensure optimal performance of the detector.

#### Power Consideration

The detector power supply has wide ranging capabilities and accepts any line voltage in the range mentioned in [Table 1](#) on page 16. Consequently, there is no voltage selector in the rear of the detector. There are also no externally accessible fuses, because automatic electronic fuses are implemented in the power supply.

#### **WARNING**

**To disconnect the detector from line, unplug the power cord. The power supply still uses some power, even if the power switch on the front panel is turned off.**

---

#### **WARNING**

**Shock hazard or damage of your instrumentation can result, if the devices are connected to a line voltage higher than specified.**

---

#### Power Cords

Different power cords are offered as options with the detector. The female end of all power cords is identical. It plugs into the power-input socket at the rear of the detector. The male end of each power cord is different and designed to match the wall socket of a particular country or region.

#### **WARNING**

**Never operate your instrumentation from a power outlet that has no ground connection. Never use a power cord other than the Agilent Technologies power cord designed for your region.**

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#### **WARNING**

**Never use cables other than the ones supplied by Agilent Technologies to ensure proper functionality and compliance with safety or EMC regulations.**

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### Bench Space

The detector dimensions and weight (see [Table 1](#) on page 16) allows you to place the detector on almost any desk or laboratory bench. It needs an additional 2.5 cm (1.0 inches) of space on either side and approximately 8 cm (3.1 inches) in the rear for air circulation and electric connections.

If the bench should carry a Agilent 1100 Series system, make sure that the bench is designed to bear the weight of all modules.

The detector should be operated in a horizontal position.

### Environment

Your detector will work within the specifications at ambient temperatures and relative humidity described in [Table 1](#) on page 16.

ASTM drift tests require a temperature change below 2 °C/hour (3.6 °F/hour) over one hour period. Our published drift specification (refer also to "[Performance Specifications](#)" on page 328) is based on these conditions. Larger ambient temperature changes will result in larger drift.

Better drift performance depends on better control of the temperature fluctuations. To realize the highest performance, minimize the frequency and the amplitude of the temperature changes to below 1 °C/hour (1.8 °F/hour). Turbulences around one minute or less can be ignored.

#### CAUTION

Do not store, ship or use your detector under conditions where temperature fluctuations could cause condensation within the detector. Condensation will damage the system electronics. If your detector was shipped in cold weather, leave it in its box and allow it to warm up slowly to room temperature to avoid condensation.

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## Physical Specifications

**Table 1** Physical Specifications

Type	Specification	Comments
Weight	11.5 kg (26 lbs)	
Dimensions (width × depth × height)	345 × 435 × 140 mm (13.5 × 17 × 5.5 inches)	
Line voltage	100 – 120 or 220 – 240 VAC, ± 10 %	Wide-ranging capability
Line frequency	50 or 60 Hz ± 5 %	
Power consumption	180 VA / 70 W / 239 BTU	Maximum
Ambient operating temperature	0 – 40 °C (32 – 104 °F)	
Ambient non-operating temperature	-40 – 70 °C (-4 – 158 °F)	
Humidity	< 95%, at 25 – 40 °C (77 – 104 °F)	Non-condensing
Operating altitude	Up to 2000 m (6500 ft)	
Non-operating altitude	Up to 4600 m (14950 ft)	For storing the detector
Safety standards: IEC, CSA, UL, EN	Installation category II, pollution degree 2	

## Unpacking the Detector

### Damaged Packaging

If the delivery packaging shows signs of external damage, please call your Agilent Technologies sales and service office immediately. Inform your service representative that the detector may have been damaged during shipment.

### CAUTION

If there are signs of damage, please do not attempt to install the detector.

### Delivery Checklist

Ensure all parts and materials have been delivered with the detector. The delivery checklist is shown below. Please report missing or damaged parts to your local Agilent Technologies sales and service office.

**Table 2** Detector Checklist

Description	Quantity
Detector	1
Power cable	1
CAN cable (Part number 5181-1519)	1
Flow cell	1 (built-in)
Optional flow cell/cuvette	as ordered
<i>Reference Manual</i>	1
Accessory kit (see <a href="#">Table 3</a> on page 18)	1

## Detector Accessory Kit Contents

**Table 3** Accessory Kit Contents (Part Number G1321-68705)

Description	Part Number	Quantity
Teflon Tubing flexible i.d. 0.8 mm (flow cell to waste), re-order 5 m	5062-2462	2 m
Corrugated tubing (to waste), re-order 5 m	5062-2463	1.2 m
Fitting male PEEK	0100-1516	2
Capillary column-detector, one side preinstalled 380 mm lg, 0.17 mm i.d. includes:	G1315-87311	1
Ferrule front SST	0100-0043	1
Ferrule back SST	0100-0044	1
Fitting SST	79814-22406	1
Hex key set 1 – 5 mm	8710-0641	1
Screwdriver hexagonal 4 mm, 100 mm long	5965-0027	1
Screwdriver hexagonal 2.5 mm, 100 mm long	5965-0028	1
Needle-Syringe	9301-0407	
Glass-Syringe	9301-1446	
Calibration Sample, Glycogen	5063-6597	
Sample filter, diameter=3 mm, pore size 0.45 µm	5061-3367 (pack of 100)	5
Wrench open end 1/4 – 5/16 inch	8710-0510	1
ESD wrist strap	9300-1408	1

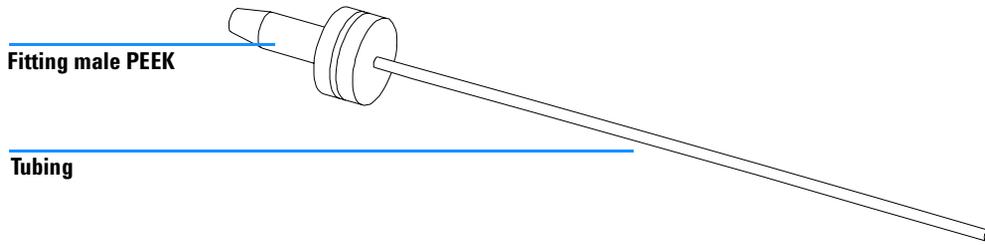


Figure 1 Waste Tubing Parts

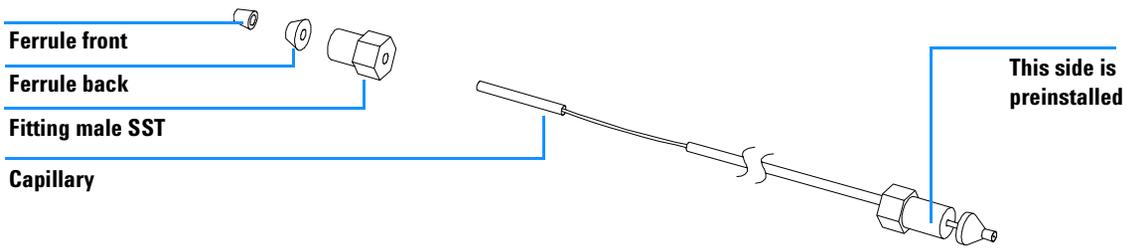
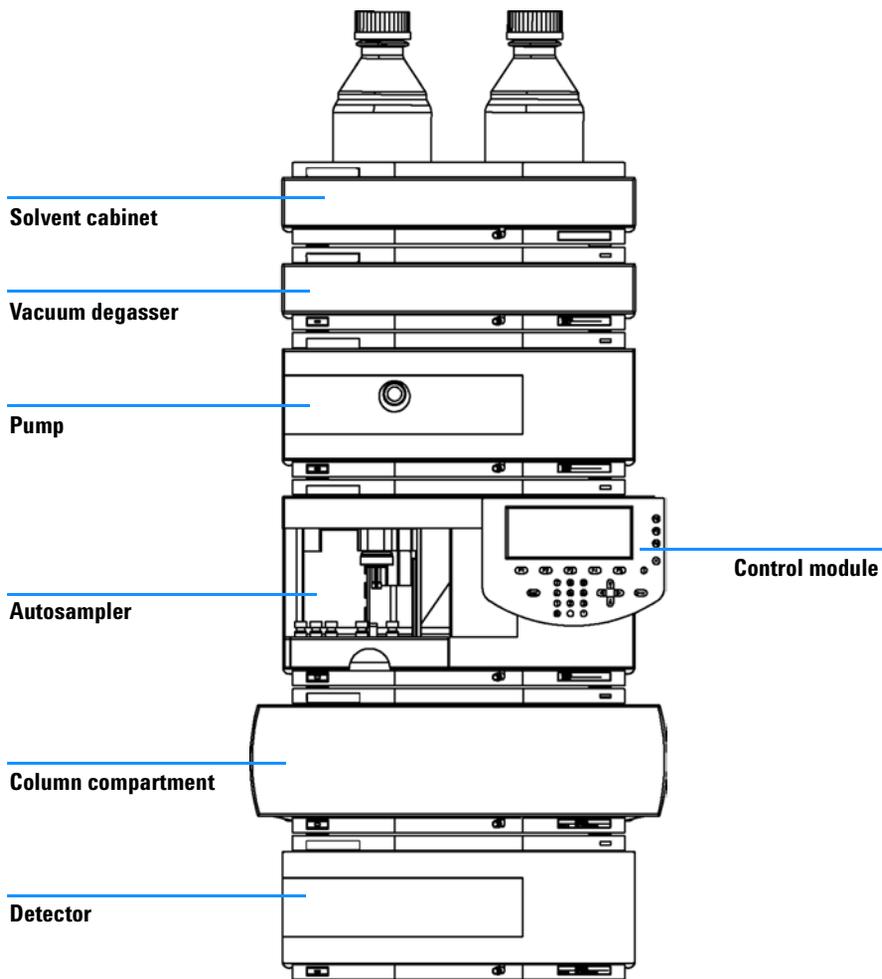


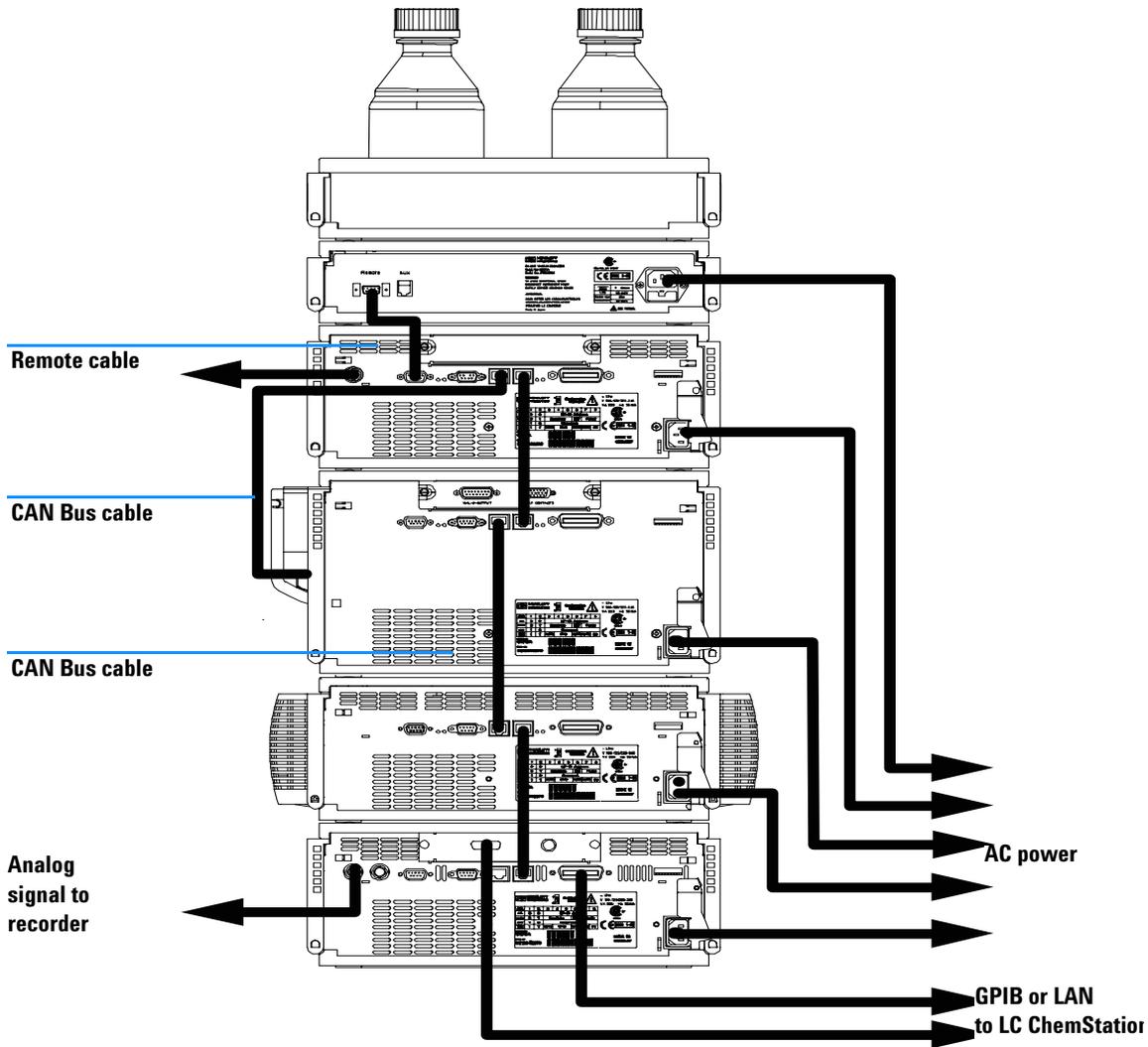
Figure 2 Inlet Capillary (Column-Detector) Parts

## Optimizing the Stack Configuration

If your detector is part of a complete Agilent 1100 Series system, you can ensure optimum performance by installing the following configuration. This configuration optimizes the system flow path, ensuring minimum delay volume.



**Figure 3** Recommended Stack Configuration (Front View)



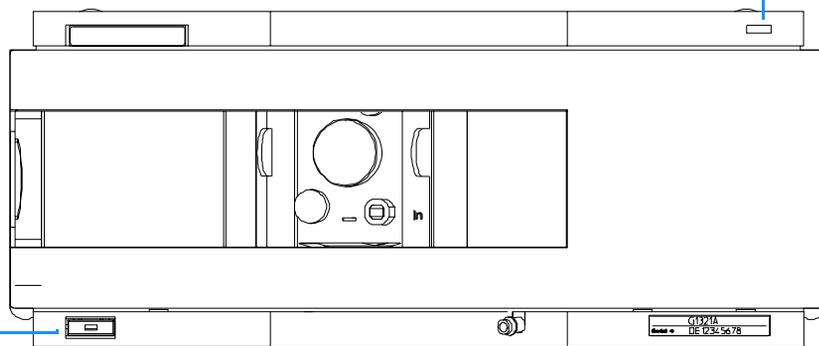
**Figure 4** Recommended Stack Configuration (Rear View)

### Installing the Detector

<b>Preparations</b>	Locate bench space Provide power connections
<b>Parts required</b>	Unpack the detector Detector Power cord, for other cables see below and <a href="#">"Cable Overview"</a> on page 218, ChemStation and/or Control Module G1323A/B.

- 1 Install the LAN interface board in the detector (if required), see ["Replacing the Interface Board"](#) on page 199.
- 2 Place the detector in the stack or on the bench in a horizontal position.
- 3 Ensure the line power switch at the front of the detector is OFF.

Status indicator  
green/yellow/red

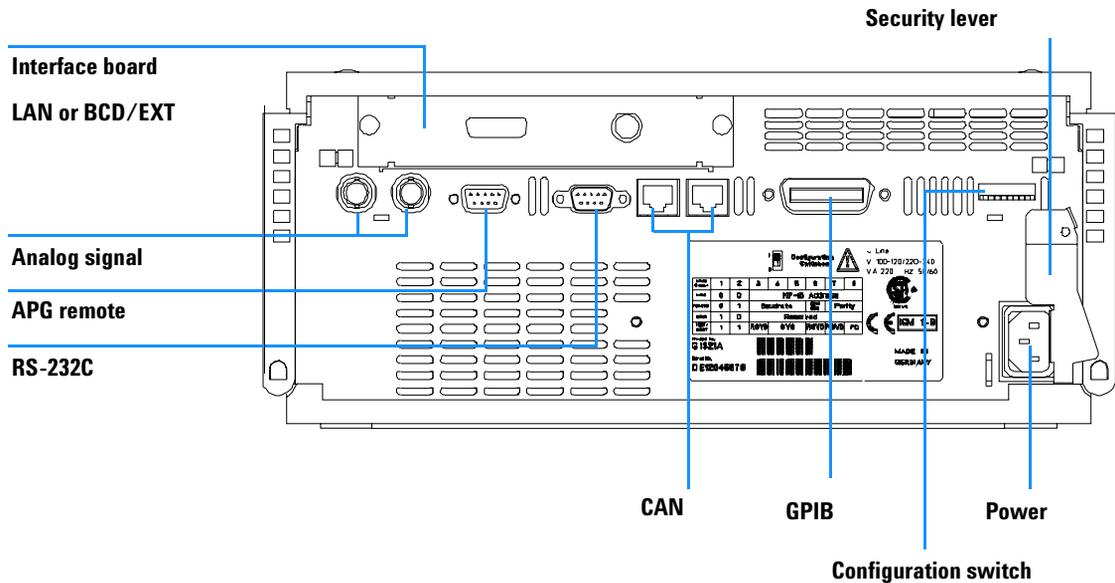


Line power switch  
with green light

**Figure 5** Front View of Detector

- 4 Connect the power cable to the power connector at the rear of the detector.
- 5 Connect the CAN cable to other Agilent 1100 Series modules.
- 6 If a Agilent ChemStation is the controller, connect either
  - the GPIB cable to the detector or
  - the LAN connection to the LAN interface board in the detector.
- 7 Connect the analog cable(s) (optional).

- 8 Connect the APG remote cable (optional) for non-Agilent 1100 Series instruments.
- 9 Turn on power by pushing the button at the lower left hand side of the detector. The status LED should be green.



**Figure 6** Rear View of Detector

#### NOTE

The detector is turned on when the line power switch is pressed and the green indicator lamp is illuminated. The detector is turned off when the line power switch is protruding and the green light is off.

#### WARNING

To disconnect the detector from line, unplug the power cord. The power supply still uses some power, even if the power switch at the front panel is turned off.

#### NOTE

The detector was shipped with default configuration settings. To change these settings see "[Setting the 8-bit Configuration Switch](#)" on page 290.

## 1 Installing the Fluorescence Detector

### Flow Connections to the Detector

**Preparations**  
**Parts required**

Detector is installed in the LC system.  
Other modules  
Parts from accessory kit, see "[Detector Accessory Kit Contents](#)" on page 18.  
Two wrenches 1/4 – 5/16 inch for capillary connections

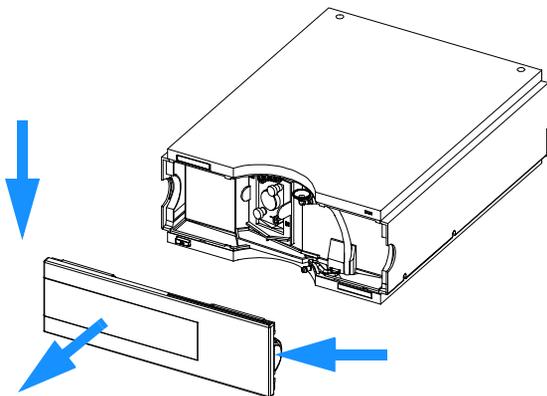
**WARNING**

**When working with solvents please observe appropriate safety procedures (for example, goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet supplied by the solvent vendor, especially when toxic or hazardous solvents are used.**

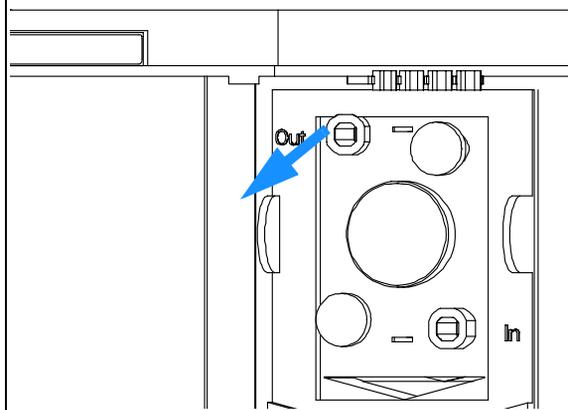
**NOTE**

The flow cell is shipped with a filling of isopropanol (also recommended when the instrument and/or flow cell is shipped to another location). This is to avoid breakage due to subambient conditions.

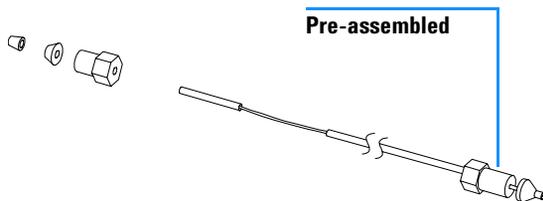
**1** Press the release buttons and remove the front cover to gain access to the flow cell area.



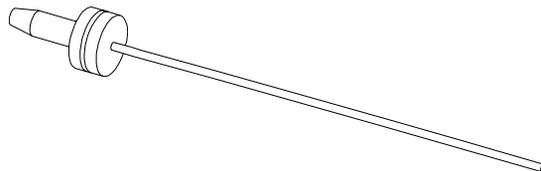
**2** Locate the flow cell.



**3** Assemble the column detector capillary from the accessory kit. One side is already factory-assembled.



**4** Assemble the waste tubing from the accessory kit.



**Note:**

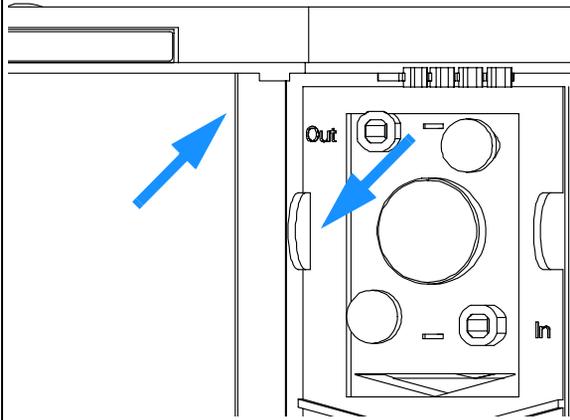
**The fluorescence detector should be the last module in the flow system. An additional detector should be installed before the fluorescence detector to prevent any overpressure to the quartz cell (maximum 20 bar) .**

**When working with detector behind the FLD (on own risk) determine the backpressure of this detector first by**

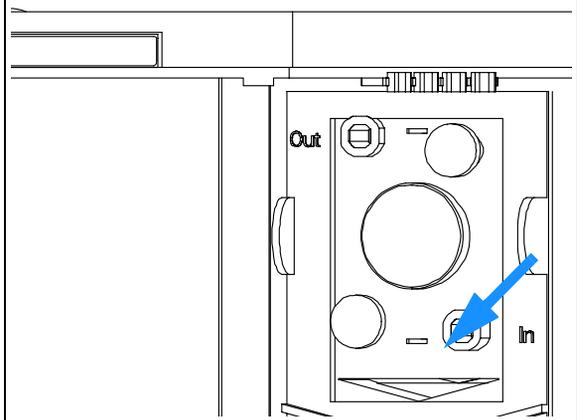
- removing the column and the last detect and measuring system pressure at the application flow rate.
- connecting the last detector (without column and FLD) and measuring the system pressure with flow.
- the difference in measured pressure is due to the back pressure generated by the last detector and is the pressure seen by the FLD.

## 1 Installing the Fluorescence Detector

**5** Insert the flow cell and install the capillaries to the flow cell (top is outlet, bottom is inlet).

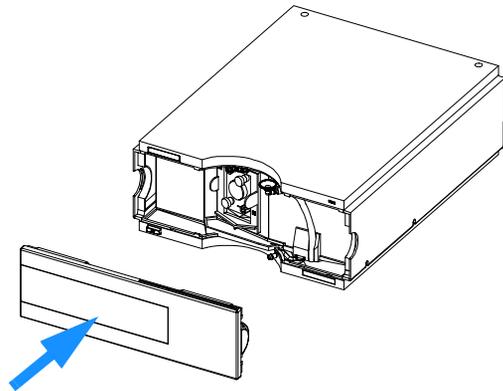


**6** Connect the waste tubing to the bottom waste fitting.

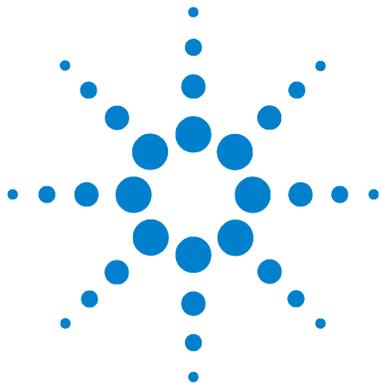


**7** Establish flow and observe if leaks occur.

**8** Replace the front cover.



**The installation of the detector is now complete.**



## 2 First Steps with the Fluorescence Detector

Before You Start	30
Optimization Overview	31
Getting Started and Checkout	33
Method Development	37
Example: Optimization for Multiple Compounds	52



### Before You Start

Your normal LC grade solvents usually give good results most of the time. But experience shows that baseline noise can be higher (lower signal-to-noise ratio) when impurities are in the 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.

## Optimization Overview

### 1 Setting the right PMT value

For most applications a setting of 10 is adequate. The G1321A A/D converter exhibits a large linear range making PMT switching unnecessary for most applications. For example, if at high concentrations a peak is cut off; decrease the PMT setting. Remember that low PMT settings decrease the signal to noise ratio.

The built-in PMT gain test uses the parameters in the detector. When using the PMT gain test, the wavelength setting and lamp energy mode (depending on Multiwavelength-Mode and Lamp-Economy) will affect the pmt gain calculation.

#### NOTE

If you have changed one or more parameter(s), you have to press 'OK' to write down the new settings into the FLD. Then re-enter 'FLD-Signals' and start the PMT gain test.

### 2 Using an appropriate response time

For most applications a setting of 4 seconds is adequate. Only for high speed analyses (short columns at high flow rates) a lower setting is recommended. Bear in mind that even if the response time is too high fast peaks will appear a little smaller and broader but retention time and peak areas are still correct and reproducible.

### 3 Finding the optimum wavelength

Most fluorescent active molecules absorb at 230 nm. Set the excitation wavelength to 230 nm and on-line scan the emission spectra (multi-emission mode). Then set the determined emission wavelength and perform a multi-excitation scan (multi-excitation mode) to find the best excitation wavelength.

## 2 First Steps with the Fluorescence Detector

### 4 Evaluating fluorescence spectra

In contrast to diode array based UV detectors where UV spectra are evaluated by taking a spectrum at the peak maximum and selecting a reference spectrum at the baseline, correct fluorescence spectra are obtained by selecting a peak maximum spectrum and a reference around the inflection points. Selecting reference spectra at the baseline is not useful because the spectrum on the baseline is very noisy (no light!).

### 5 Switching lamp on only for analysis

Unless maximum sensitivity is needed, the lamp lifetime can significantly be increased by switching it on just for analysis. In contrast to other LC detectors the G1321A fluorescence detector equilibrates within seconds after the lamp is switched on.

#### NOTE

For highest reproducibility and linearity change the lamp setting to always ON (default is on only during run).  
One hour of initial warm-up of the instrument is recommended.

---

### 6 Do not overpressurize the detector quartz flow cell

Be aware to not exceed a 20 bar pressure drop after the flow cell when hooking up additional devices like other detectors or a fraction collector. It's better to place a UV detector before the G1321A fluorescence detector.

#### NOTE

When comparing fluorescence excitation spectra directly with DAD spectra or literature based absorbance spectra, you should consider large differences in the used optical bandwidth (FLD=20 nm) which cause a systematic wavelength maximum shift depending on the absorbance spectrum of the compound under evaluation.

---

## Getting Started and Checkout

This chapter describes the check out of the Agilent 1100 series fluorescence detector using the Agilent isocratic checkout sample.

<b>When required</b>	If you want to checkout the detector
<b>Hardware required</b>	LC system with G1321A FLD
<b>Parts required</b>	Start-up Kit 5063-6528, includes Column Hypersil ODS, 125 mm x 4 mm i.d. with 5 µm particles (Part number 7982618-564) Cartridge Holder for column Agilent isocratic checkout sample (Part number 01080-68704) Fittings, Qty=2 (Part number 0100-1516) Capillary, 150 mm long, 0.17 mm i.d. (Part number 5021-1817)

### Starting Your Detector

- 1 Turn on the detector.
- 2 Turn on the lamp.

When the lamp is turned on the first time the instrument performs some internal checks and a calibration check which takes about 5 minutes.

- 3 You are now ready to change the settings of your detector.

### Setting the Chromatographic Conditions

- 1 Set up the system with the following chromatographic conditions and wait until the baseline gets stable.

**Table 4** Chromatographic Conditions

Mobile phases	A = water = 35% B = Acetonitrile = 65%
Column	OSD-Hypersil column, 125 mm x 4 mm i.d. with 5 $\mu$ m particles
Sample	Isocratic standard sample, 1:10 diluted in methanol
Flow rate	1.5 ml/min
Compressibility A (water)	46
Compressibility B (Acetonitrile)	115
Stroke A and B	auto
Stop time	4 minutes
Injection volume	5 $\mu$ l
Oven temperature (1100)	30°C
FLD Excitations/Emission Wavelength	EX = 246 nm, EM = 317 nm
FLD PMT Gain	PMT = 10
FLD Response time	4 seconds

- Set the FLD setpoints according to Figure 7 (on the local control module this information is split across separate screens).

In this example additional excitation wavelengths (B, C, D) are used. This will increase the scan time and may lower the performance.

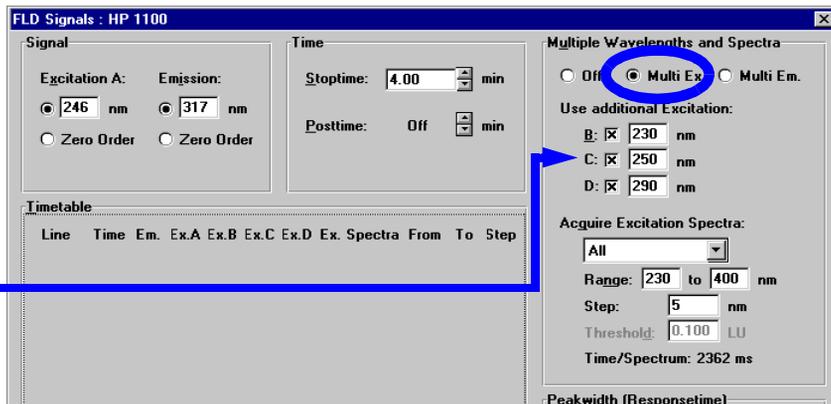


Figure 7 FLD Parameters

- Start the run.
- The resulting chromatograms are shown in Figure 8:

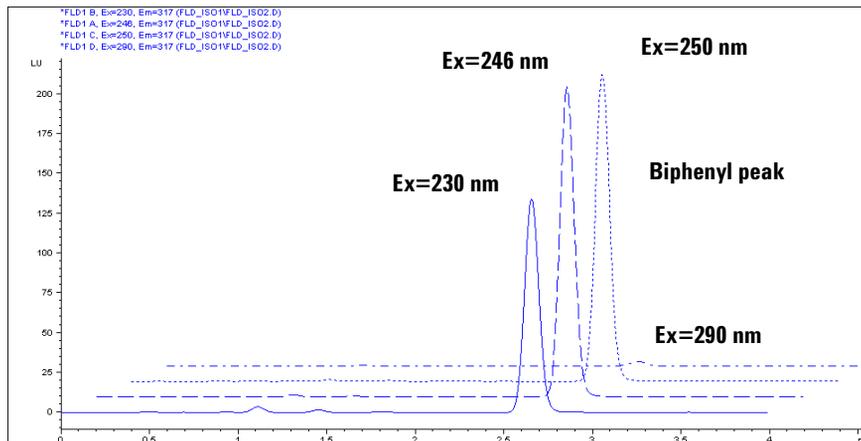


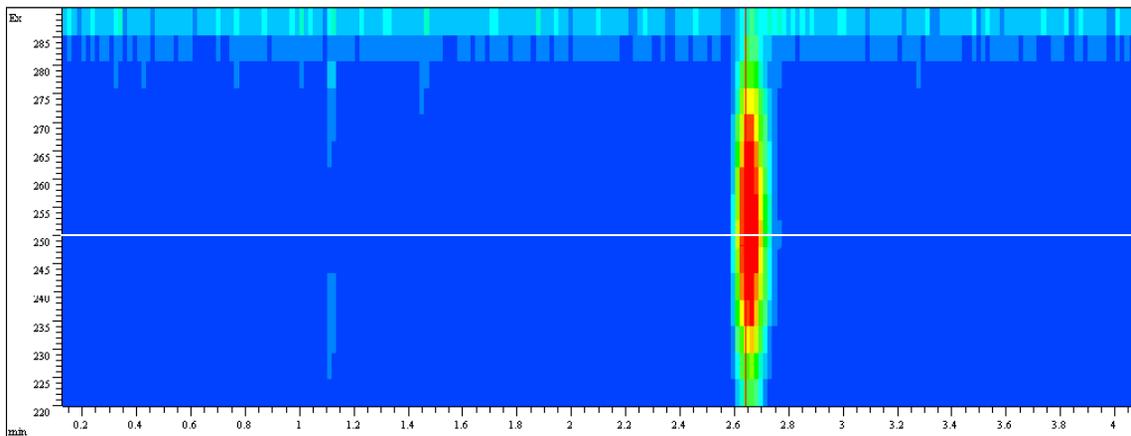
Figure 8 Biphenyl peak with different excitation wavelengths

- The excitation maxima is around 250 nm.

## 2 First Steps with the Fluorescence Detector

### Observe the maxima via the isoabsorbance plot

- 1 Load the data file ( $\lambda_{EX}=246$  nm,  $\lambda_{EM}=317$  nm) and open the isoabsorbance plot.
- 2 The maximum  $\lambda_{EX}$  will be found around 250 nm.



**Figure 9** Isoabsorbance Plot

## Method Development

Fluorescence detectors are used in liquid chromatography when superior limits of detection and selectivity are required. Thorough method development, including spectra acquisition, is fundamental to achieve good results. This chapter describes three different steps that can be taken with the Agilent 1100 Series fluorescence detector. Table 5 gives an overview of how to benefit from the operation modes during these steps.

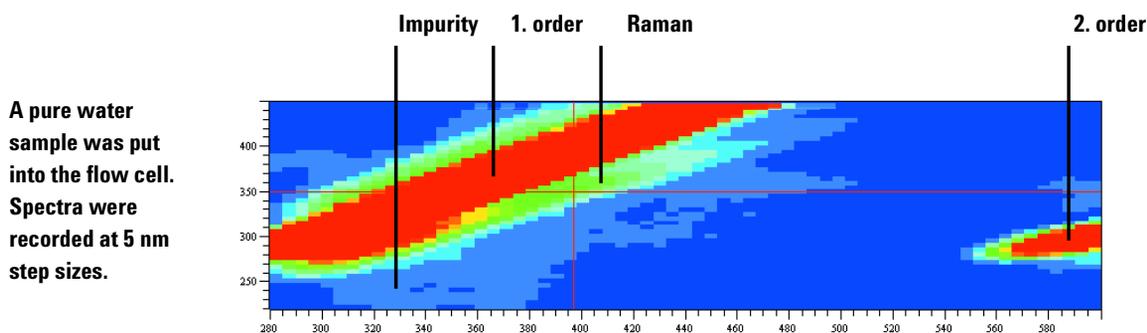
**Table 5** Steps for thorough method development

	<b>Step 1: Check system</b>	<b>Step 2: Optimize limits of detection and selectivity</b>	<b>Step 3: Set up routine methods</b>
Fluorescence scan	Find impurities (for example, in solvents and reagents)	Determine simultaneously the excitation and emission spectra of a pure compound	
Signal mode		Perform wavelength switching	Use for lowest limits of detection
Spectral mode/multi-wavelength detection		Determine Ex/Em spectra for all separated compounds in a single run	Collect online spectra, perform library search, determine peak purity
		Activate up to four wavelength simultaneously	Deactivate wavelength switching

### Step 1: Check the LC system for impurities

A critical issue in trace level fluorescence detection is to have an LC system free of fluorescent contamination. Most contaminants derive from impure solvents. Taking a fluorescence scan is a convenient way to check the quality of the solvent in a few minutes. This can be done, for example, by filling the FLD cuvette directly with the solvent for an offline measurement even before the start of a chromatographic run. The result can be displayed as an isofluorescence plot or a three-dimensional plot. Different colors reflect different intensities.

Figure 10 shows a sample of slightly impure water which was planned for use as mobile phase. The area where fluorescence of the contaminated water sample can be seen is between the stray light areas: the first- and second-order Raleigh stray light and Raman stray light.



**Figure 10** Isofluorescence plot of a mobile phase

Since "excitation" and "emission" wavelength are the same for Raleigh stray light, the area of first-order Raleigh stray light is visible in the left upper area of the diagram. The Raman bands of water are seen below the first-order Raleigh stray light. Since the cut-off filter cuts off light below 280 nm, the second-order Raleigh stray light starts above 560 nm.

Stray light acts in the same way as impurities in that it simulates background noise. In both cases, a higher noise level and therefore a higher limit of detection are obtained. This indicates that high sensitivity measurements should be done away from wavelength settings that have a high stray light background.

## Step 2: Optimize limits of detection and selectivity

To achieve optimum limits of detection and selectivity, analysts must find out about the fluorescent properties of the compounds of interest. Excitation and emission wavelengths can be selected for optimum limits of detection and best selectivity. In general, fluorescence spectra obtained with different instruments may show significant differences depending on the hardware and software used.

The traditional approach is to extract an appropriate excitation wavelength from the UV spectrum that is similar to the fluorescence excitation spectrum (see Figure 11) and to record the emission spectrum. Then with an optimum emission wavelength determined, the excitation spectrum is acquired.

Excitation spectrum with emission at 440 nm, emission spectrum with excitation at 250 nm of 1 ug/ml quinidine.

Detector settings: step size 5 nm, PMT 12, Response time 4 s.

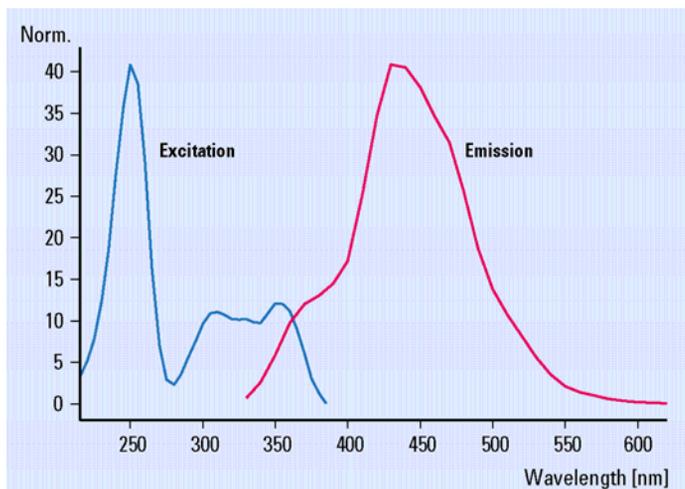


Figure 11 Excitation and emission spectra of quinidine

These tasks have to be repeated for each compound using either a fluorescence spectrophotometer or stop-flow conditions in LC. Usually each compound requires a separate run. As a result, a set of excitation and emission spectrum is obtained (Figure 10 on page 38) for each compound. Since this is a tedious procedure, it is applicable only when there is a limited number of compounds of interest.

The Agilent 1100 Series LC offers three different ways to obtain complete information on a compound's fluorescence:

**Procedure I** - Take a fluorescence scan offline for a single compound as described above for the mobile phase. This is done preferably with a manual FLD cuvette when pure compounds are available.

**Procedure II** - Use two LC runs with the Agilent 1100 Series FLD to separate the compound mix under known conditions and acquire emission and excitation spectra separately.

**Procedure III** - Use an Agilent 1100 Series FLD/DAD combination and acquire UV/Visible spectra (equivalent to excitation spectra) with the DAD and emission spectra with the FLD-both in a single run.

### **Procedure I - Take a fluorescence scan**

Because fluorescence spectra traditionally have not been easily available with previous LC fluorescence detectors, standard fluorescence spectrophotometers have been used in the past to acquire spectral information for unknown compounds. Unfortunately this approach limits optimization, as there are differences expected in optical design between an LC detector and a dedicated fluorescence spectrophotometer, or even between detectors. These differences can lead to variations for the optimum excitation and emission wavelengths.

The Agilent 1100 Series fluorescence detector offers a fluorescence scan that delivers all spectral information previously obtained with a standard fluorescence spectrophotometer, independent of the LC fluorescence detector. Figure 12 on page 42 shows the complete information for quinidine as obtained with the Agilent 1100 Series FLD and a manual cuvette in a single offline measurement. The optima for excitation and emission wavelengths can be extracted as coordinates of the maxima in the three dimensional plot. One of the three maxima in the center of the plot can be chosen to define the excitation wavelength. The selection depends on the additional compounds

that are going to be analyzed in the chromatographic run and the background noise that may be different upon excitation at 250 nm, 315 nm or 350 nm. The maximum of emission is observed at 440 nm.

Details for Figure 12 on page 42:

All excitation and emission spectra of Quinidine (1  $\mu\text{g}/\text{ml}$ ) are shown in graphic. Fluorescence intensity is plotted vs excitation and emission wavelengths.

Detector settings: step size 5 nm, PMT 12, Response time 4 s

## 2 First Steps with the Fluorescence Detector

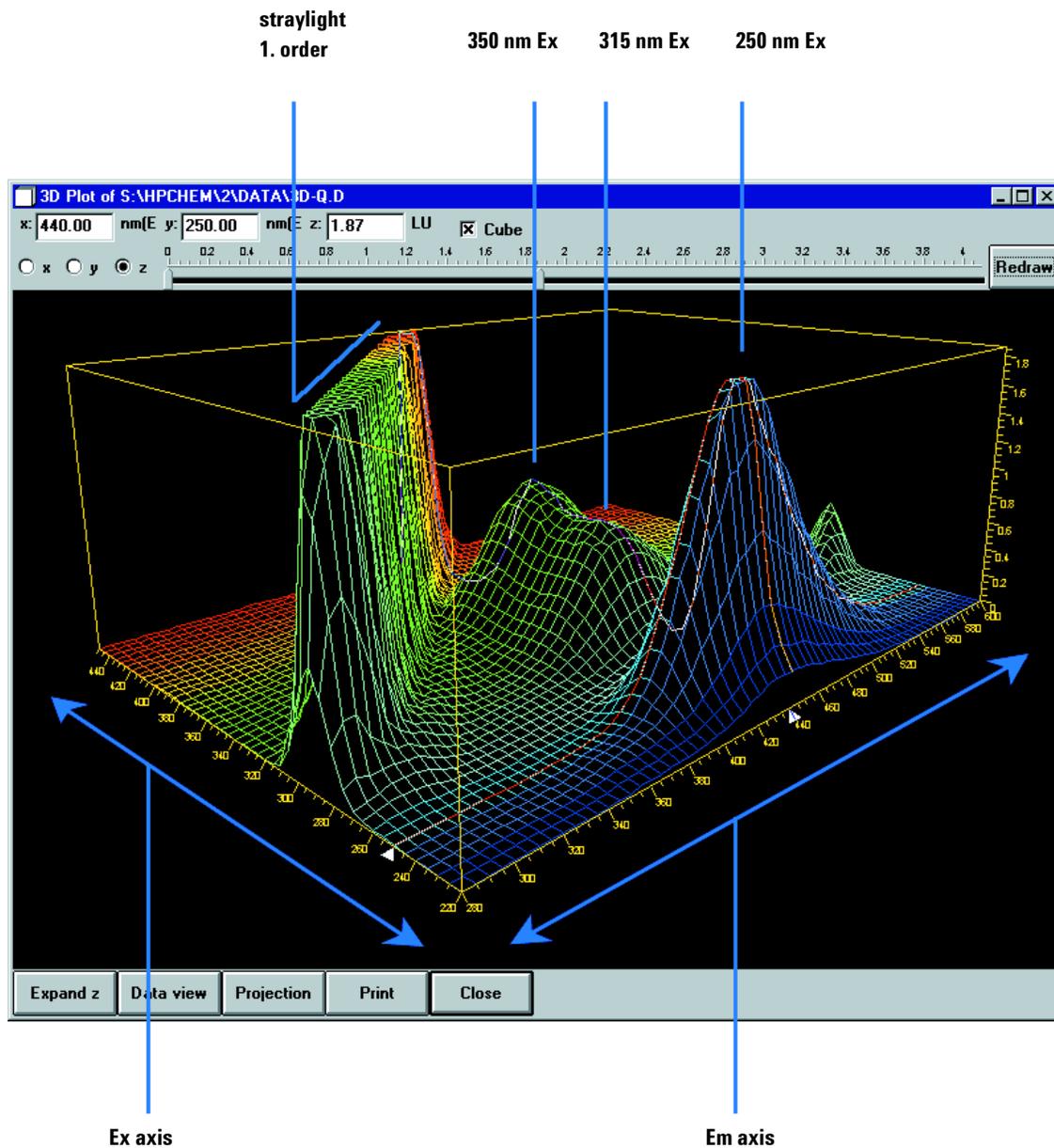


Figure 12 Characterization of a pure compound from a fluorescence scan

### Procedure II - Take two LC runs with the FLD

The conditions for the separation of organic compounds such as polyaromatic nuclear hydrocarbons (PNAs) are well described in various standard methods, including commonly used EPA and DIN methods. Achieving the best detection levels requires checking for the optimum excitation and emission wavelengths for all compounds. Yet taking fluorescence scans individually makes this a tedious process. A better approach is to acquire spectra online for all compounds during a run. This speeds up method development tremendously. Two runs are sufficient for optimization.

During the *first run*, one wavelength is chosen in the low UV range for the excitation wavelength and one emission wavelength in the spectral range for the emission wavelength. Most fluorophores show strong absorption at these wavelengths and the quantum yield is high. Excitation is sufficient for collecting emission spectra.

Figure 13 on page 44 contains all emission spectra obtained in a single run from a mix of 15 PNAs. This set of spectra is used to set up a timetable for optimum emission wavelengths for all compounds.

The individual compound spectra in the isofluorescence plot show that at least three emission wavelengths are needed to detect all 15 PNAs properly:

**Table 6** Timetable for PNA analysis

0 min:	350 nm	for naphthalene to phenanthrene
8.2 min:	420 nm	for anthracene to benzo(g,h,l)perylene
19.0 min:	500 nm	for indeno(1,2,3-cd)pyrene

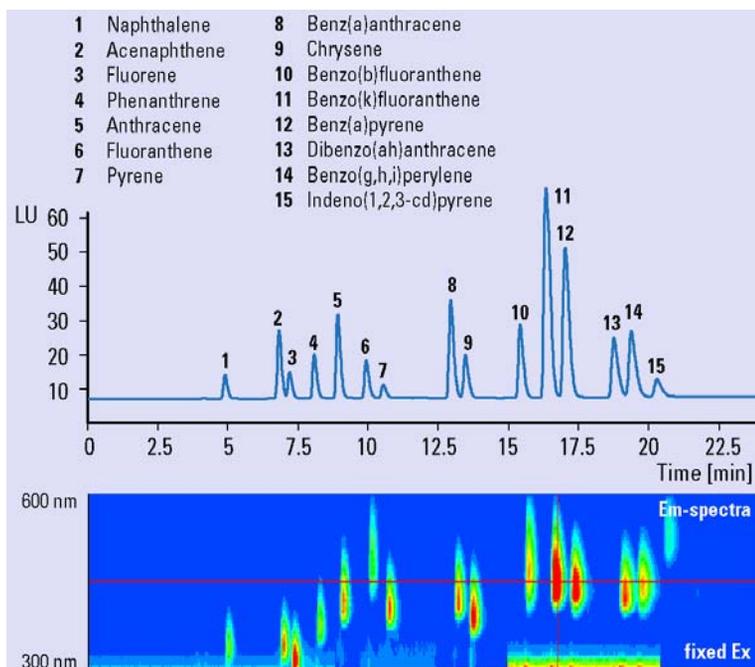
In the second run, three setpoints for emission wavelengths are entered into the time-program and excitation spectra are recorded, as shown in figure 8. The area of high intensity (red) is caused by stray light when emission spectra overlap with the excitation wavelength. This can be avoided by fitting the spectral range automatically. Excitation at 260 nm is most appropriate for all PNAs.

## 2 First Steps with the Fluorescence Detector

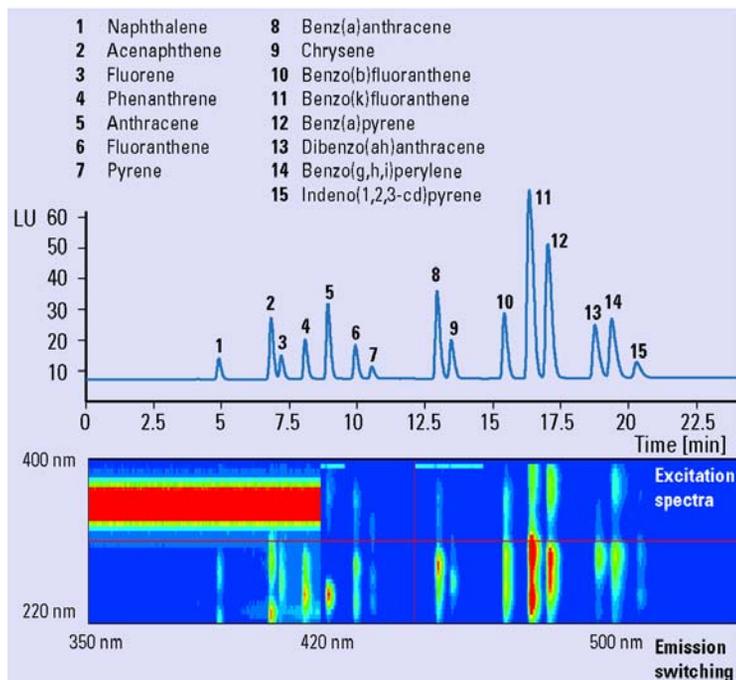
**Table 7** Conditions for Figure 13 and Figure 14 on page 45

Column	Vydac, 2.1 x 200 mm, PNA, 5 µm
Mobile phase	A = water; B = acetonitrile (50:50)
Gradient	3 minutes, 60% 14 minutes, 90% 22 minutes, 100%
Flow rate	0.4 ml/min
Column temperature	18 °C
Injection volume	5 µl
FLD settings	PMT 12, response time 4 s, step size 5 nm

This shows the isofluorescence plot of emission spectra for 15 PNAs (5 µg/ml) with a fixed excitation wavelengths (260 nm).



**Figure 13** Optimization of the time-program for the emission wavelength



**Figure 14** Optimization of the time-program for the excitation wavelength

The obtained data are combined to setup the time-table for the excitation wavelength for best limit of detection and selectivity. The optimized switching events for this example are summarized in Table 8.

**Table 8** Timetable for the analysis of 15 polynuclear aromatic hydrocarbons

Time [min]	Excitation Wavelength [nm]	Emission Wavelength [nm]
0	260	350
8.2	260	420
19.0	260	500

This timetable gives the conditions for optimum detection based on the results of two chromatographic runs.

### **Procedure III - Make a single run with the Agilent 1100 Series DAD/FLD combination**

For most organic compounds, UV-spectra from diode array detectors are nearly identical to fluorescence excitation spectra. Spectral differences are caused by specific detector characteristics such as spectral resolution or light sources.

In practice, combining a diode array detector with a fluorescence detector in series gives the full data set needed to achieve the optimum fluorescence excitation and emission wavelengths for a series of compounds in a single run. With the UV/Visible/excitation spectra available from the diode array detector, the fluorescence detector is set to acquire emission spectra with a fixed excitation wavelength in the low UV range.

The example is taken from the quality control of carbamates. Samples are analyzed for the impurities 2,3-diamino-phenazine (DAP) and 2-amino-3-hydroxyphenazine (AHP). Reference samples of DAP and AHP were analyzed with diode array and fluorescence detection. Figure 9 shows the spectra obtained from both detectors for DAP. The excitation spectrum of DAP is very similar to the UV absorption spectrum from the diode array detector. Figure 16 on page 47 shows the successful application of the method to a carbamate sample and a pure mixture of DAP and AHP for reference. The column was overloaded with the non-fluorescent carbamate (2-benzimidazole carbamic acid methylester/MBC) to see the known impurities, AHP and DAP.

This is an impurity of carbamates. The excitation spectrum in a second run shows the equivalence of UV-spectra and fluorescence excitation spectra. An excitation wavelength at 265 nm was used for taking the emission spectrum and an emission wavelength at 540 nm was used for taking the excitation spectrum.

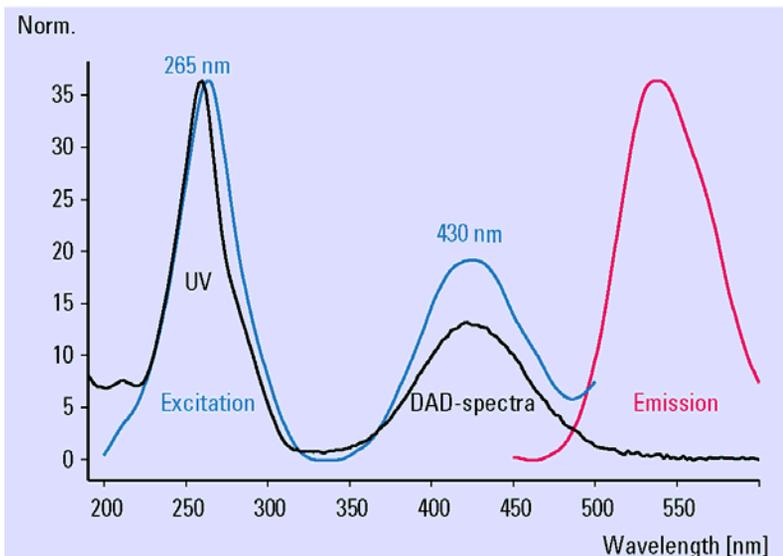


Figure 15 UV-spectrum and fluorescence spectra for 2,3-diaminophenazine (DAP)

The two upper traces are obtained using two different excitation wavelengths. The lower trace is a pure standard of the known impurities.

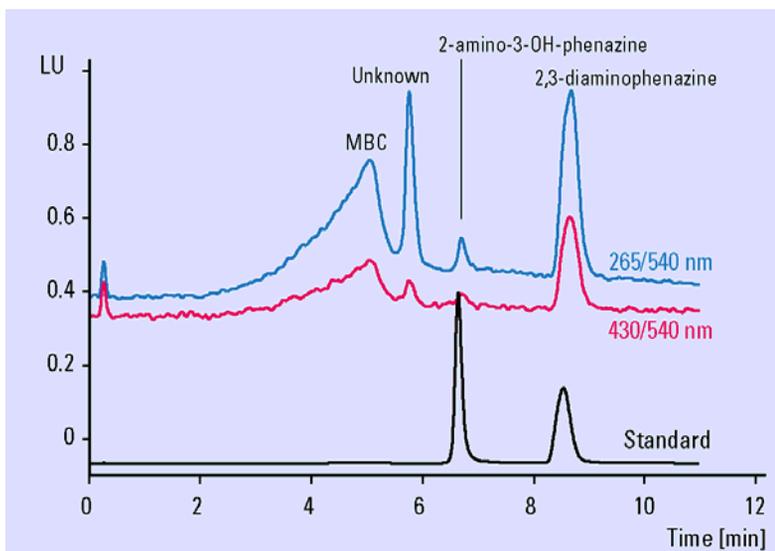


Figure 16 Qualitative analysis of MBC (2-benzimidazole carbamic acid methylester) and impurities

**Table 9** Conditions for Figure 15 and Figure 16 on page 47

Column	Zorbax SB, 2 x 50 mm, PNA, 5 µm
Mobile phase	A = water; B = acetonitrile
Gradient	0 minutes, 5% 10 minutes, 15%
Flow rate	0.4 ml/min
Column temperature	35 °C
Injection volume	5 µl
FLD settings	PMT 12, response time 4 s, step size 5 nm Ex 265 nm and 430 nm Em 540 nm

### Step 3: Set up routine methods

In routine analysis, sample matrices can have a significant influence on retention times. For reliable results, sample preparation must be thorough to avoid interferences or LC methods must be rugged enough. With difficult matrices, simultaneous multi-wavelength detection offers more reliability than timetable-controlled wavelength switching. The Agilent 1100 Series FLD can, in addition, acquire fluorescence spectra while it records the detector signals for quantitative analysis. Therefore qualitative data are available for peak confirmation and purity checks in routine analysis.

### Multi wavelength detection

Time-programmed wavelength switching traditionally is used to achieve low limits of detection and high selectivity in routine quantitative analysis. Such switching is difficult

if compounds elute closely and require a change in excitation or emission wavelength. Peaks can be distorted and quantitation made impossible if wavelength switching occurs during the elution of a compound. Very often this happens with complex matrices, influencing the retention of compounds.

In spectral mode, the Agilent 1100 Series FLD can acquire up to four different signals simultaneously. All of them can be used for quantitative analysis. Apart from complex matrices, this is advantageous when watching for impurities at additional wavelengths. It is also advantageous for reaching low limits of detection or increasing selectivity through optimum wavelength settings at any time. The number of data points acquired per signal is reduced and thus limits of detection may be higher, depending on the detector settings compared to the signal mode.

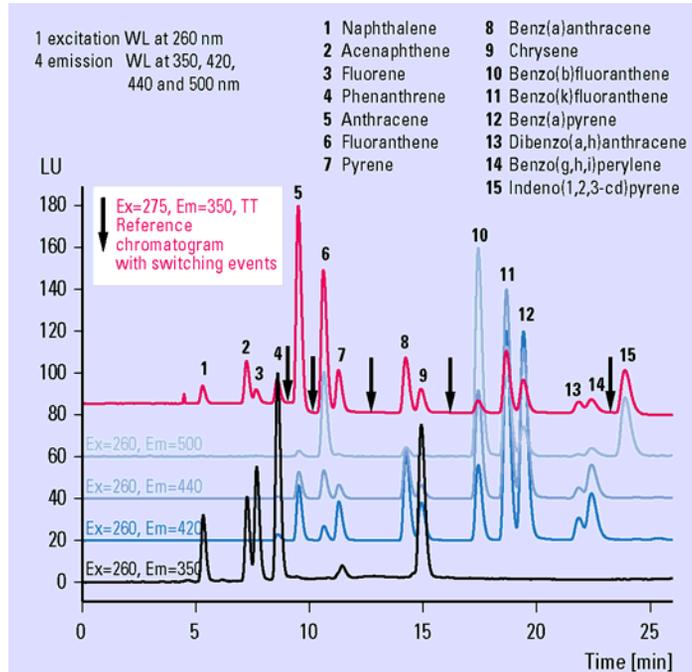
PNA analysis, for example, can be performed with simultaneous multi wavelength detection instead of wavelength-switching. With four different wavelengths for emission, all 15 PNAs can be monitored (Figure 17 on page 50).

**Table 10** Conditions for Figure 17 on page 50

Column	Vydac, 2.1 x 250 mm, PNA, 5 µm
Mobile phase	A = water; B = acetonitrile (50:50)
Gradient	3 minutes, 60% 14.5 minutes, 90% 22.5 minutes, 95%
Flow rate	0.4 ml/min
Column temperature	22 °C
Injection volume	2 µl
FLD settings	PMT 12, response time 4 s,

## 2 First Steps with the Fluorescence Detector

The upper trace was received with traditional wavelength switching.



**Figure 17** Simultaneous multi wavelength detection for PNA-analysis

Previously, only diode array detectors and mass spectrometric detectors could deliver spectral information on-line to confirm peak identity as assigned by retention time.

Now, fluorescence detectors provide an additional tool for automated peak confirmation and purity control. No additional run is necessary after the quantitative analysis.

During method development, fluorescence excitation and emission spectra are collected from reference standards and entered into a library-at the choice of the method developer. All spectral data from unknown samples can then be compared automatically with library data. Table 3 illustrates this principle using a PNA analysis. The match factor given in the report for each peak indicates the degree of similarity between the reference spectrum and the spectra from a peak. A match factor of 1,000 means identical spectra.

In addition, the purity of a peak can be investigated by comparing spectra obtained within a single peak. When a peak is calculated to be within the user-defined purity limits, the purity factor is the mean purity value of all spectra that are within the purity limits.

The reliability of the purity and the match factor depends on the quality of spectra recorded. Because of the lower number of data points available with the fluorescence detector in general, the match factors and purity data obtained show stronger deviations compared to data from the diode array detector, even if the compounds are identical.

Table 11 shows an automated library search based on the emission spectra from a PNA reference sample.

**Table 11** Peak confirmation using a fluorescence spectral library

Meas. Library CalTbI		Signal	Amount	Purity	#	Match	Library Name	
RetTime								
[min]	[min]	[min]	[ng]	Factor				
4.859	4.800	5.178	1	1.47986e-1	-	1	993	Naphthalene@em
6.764	7.000	7.162	1	2.16156e-1	-	1	998	Acenaphthene@em
7.137	7.100	7.544	1	1.14864e-1	-	1	995	Fluorene@em
8.005	8.000	8.453	1	2.56635e-1	-	1	969	Phenanthrene@em
8.841	8.800	9.328	1	1.76064e-1	-	1	993	Anthracene@em
9.838	10.000	10.353	1	2.15360e-1	-	1	997	Fluoranthene@em
10.439	10.400	10.988	1	8.00754e-2	-	1	1000	Pyrene@em
12.826	12.800	13.469	1	1.40764e-1	-	1	998	Benz(a)anthracene@em
13.340	13.300	14.022	1	1.14082e-1	-	1	999	Chrysene@em
15.274	15.200	16.052	1	6.90434e-1	-	1	999	Benzo(b)fluoranthene@em
16.187	16.200	17.052	1	5.61791e-1	-	1	998	Benzo(k)fluoranthene@em
16.865	16.900	17.804	1	5.58070e-1	-	1	999	Benz(a)pyrene@em
18.586	18.600	19.645	1	5.17430e-1	-	1	999	Dibenz(a,h)anthracene@em
19.200	19.100	20.329	1	6.03334e-1	-	1	995	Benzo(g,h,i)perylene@em
20.106	20.000	21.291	1	9.13648e-2	-	1	991	Indeno(1,2,3-cd)pyrene@em

## Example: Optimization for Multiple Compounds

Using PNAs as a sample, this example uses the described scanning functions.

### Setting the Chromatographic Conditions

- 1 This example uses the following chromatographic conditions (the detector settings are shown in [Figure 18](#) on page 53).

**Table 12** Chromatographic Conditions

Mobile phases	A = water = 50% B = Acetonitrile = 50%
Column	Vydac-C18-PNA, 250 mm x 2.1 mm i.d. with 5 µm particles
Sample	PAH 0.5 ng
Flow rate	0.4 ml/min
Compressibility A (water)	46
Compressibility B (Acetonitrile)	115
Stroke A and B	auto
Time Table	at 0 minutes %B=50 at 3 minutes %B=60 at 14.5 minutes %B=90 at 22.5 minutes %B=95
Stop time	26 minutes
Post time	8 minutes
Injection volume	1 µl
Oven temperature (1100)	30°C
FLD PMT Gain	PMT = 15
FLD Response time	4 seconds

Select a Excitation wavelength in the low UV (230...260 nm). This will cover nearly all fluorescence in your sample.

DO NOT select additional emission wavelengths (B, C, D). Doing so will increase the scan time and will lower the performance.

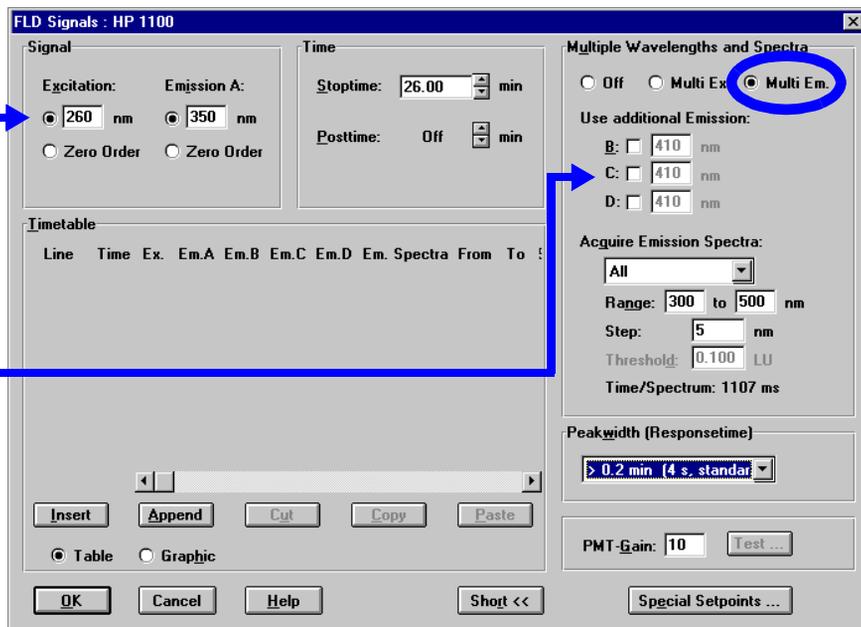
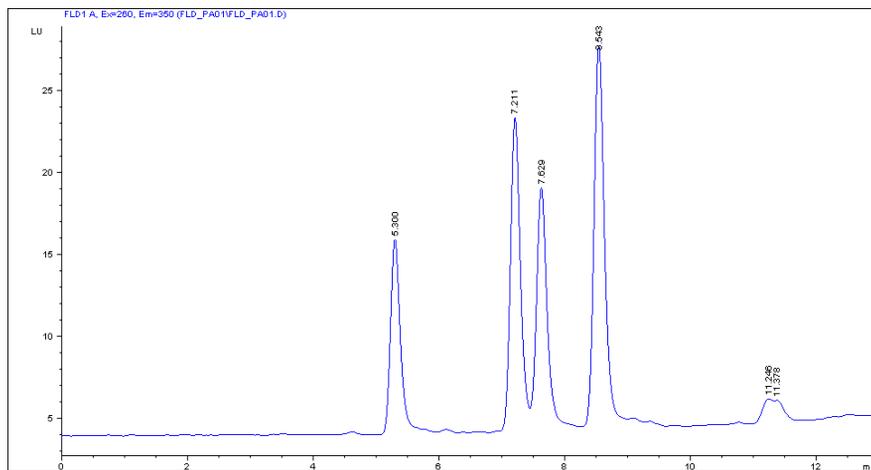


Figure 18 Detector Settings for Emission Scan

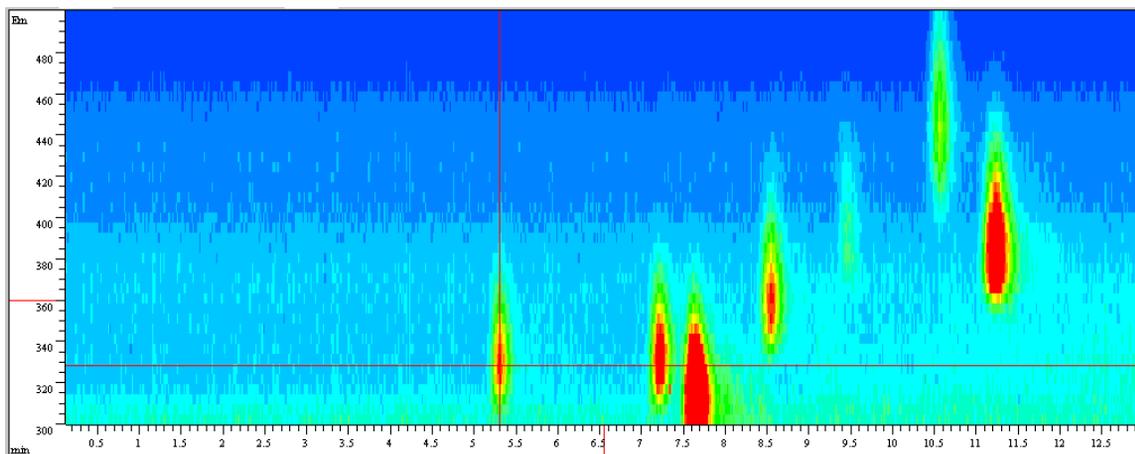
- 2 Wait until the baseline stabilizes. Complete the run.
- 3 Load the signal. (In this example just the time range of 13 minutes is displayed).

## 2 First Steps with the Fluorescence Detector



**Figure 19** Chromatogram from Emissions Scan

- 4 Use the isoabsorbance plot and evaluate the optimal emission wavelengths, shown in the table below.



**Figure 20** Isoabsorbance Plot from Emission Scan

Table 13

Peak #	Time	Emission Wavelength
1	5.3 min	330 nm
2	7.2 min	330 nm
3	7.6 min	310 nm
4	8.6 min	360 nm
5	10.6 min	445 nm
6	<b>11.23 min</b>	385 nm

- Using the settings and the timetable (from previous page), do a second run for the evaluation of the optimal excitation wavelength. See Figure 21.

**DO NOT select additional excitation wavelengths (B, C, D). Doing so will increase the scan time and will lower the performance**

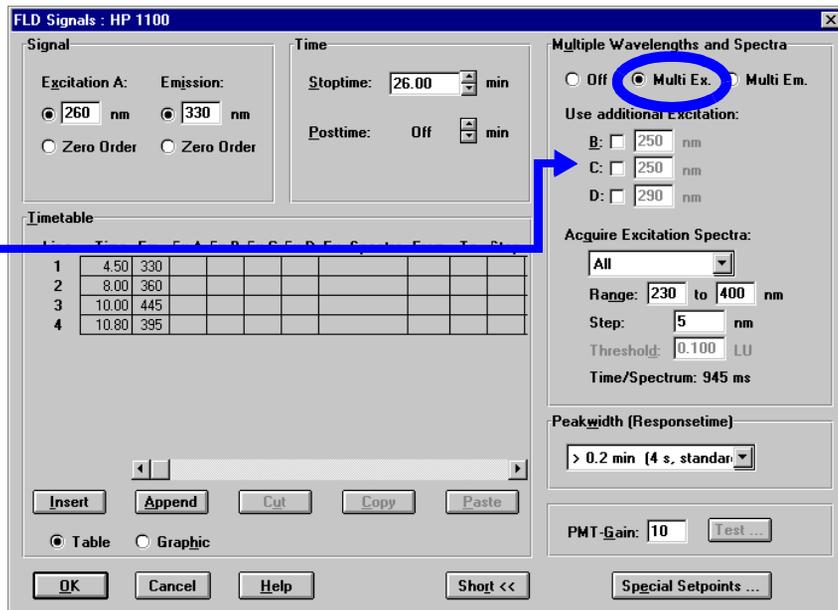
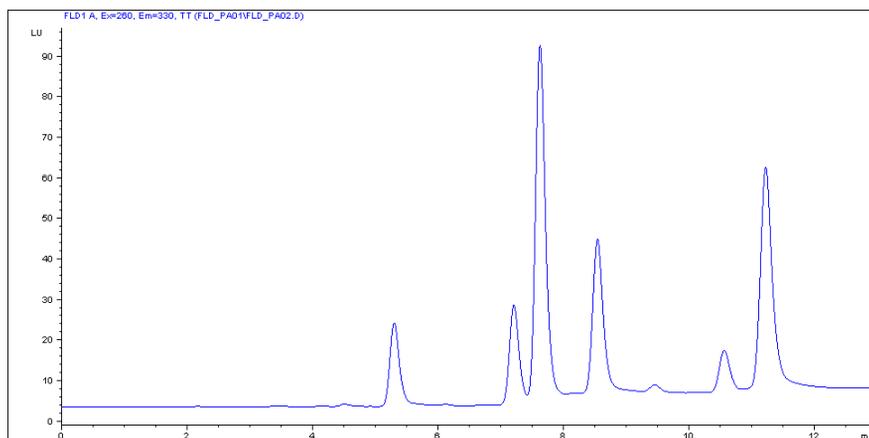


Figure 21 Detector Settings for Excitation Scan

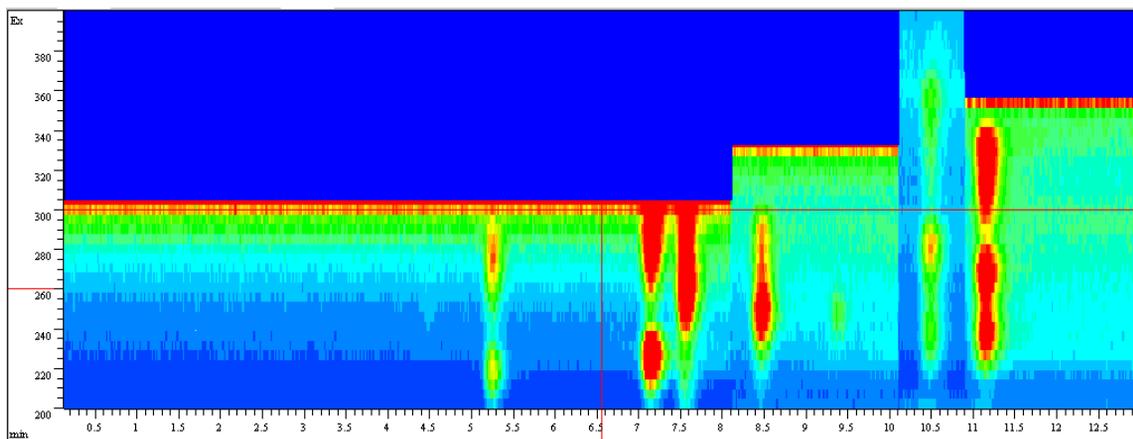
## 2 First Steps with the Fluorescence Detector

- 6 Wait until the baseline stabilizes. Start the run.
- 7 Load the signal.



**Figure 22** Chromatogram - excitation scan at reference wavelength 260/330 nm

- 8 Use the isoabsorbance plot and evaluate the optimal excitation wavelengths (in this example just in the time range of 13 minutes).



**Figure 23** Isoabsorbance Plot - Excitation

The table below shows the complete information about emission (from [Figure 20](#) on page 54) and excitation maxima.

**Table 14**

Peak #	Time	Emission Wavelength	Excitation Wavelength
1	5.3 min	330 nm	220 / 280 nm
2	7.3 min	330 nm	225 / 285 nm
3	7.7 min	310 nm	265 nm
4	8.5 min	360 nm	245 nm
5	10.7 min	445 nm	280 nm
6	<b>11.3 min</b>	385 nm	270 / 330 nm

### Evaluating The System Background

The example below uses water.

- 1 Pump solvent through your system.
- 2 Set the fluorescence scan range under FLD special setpoints according to your needs.

#### NOTE

The scan time will increase when the range is enlarged. With the default values, the scan takes about 2 minutes.

- 3 Set PMT gain to 16.

The wavelength range and step number defines the duration. Using the maximum range, the scan would take approximately 10 minutes

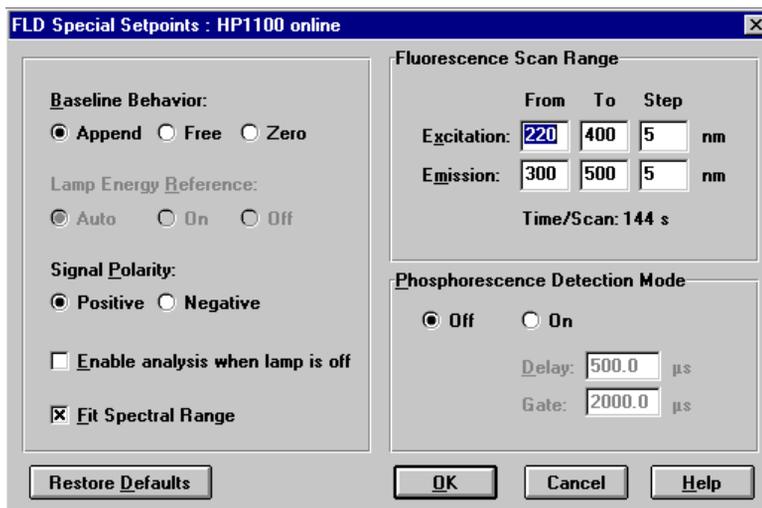
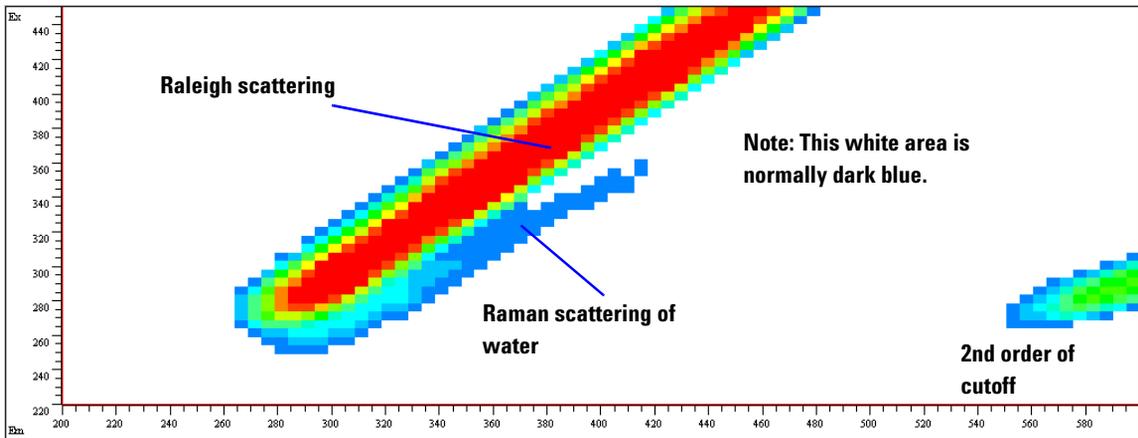


Figure 24 FLD special settings

- 4 Define a data file name and take a fluorescence scan. After the scan is completed, the isoabsorbance scan results appear, see [Figure 25](#) on page 59.

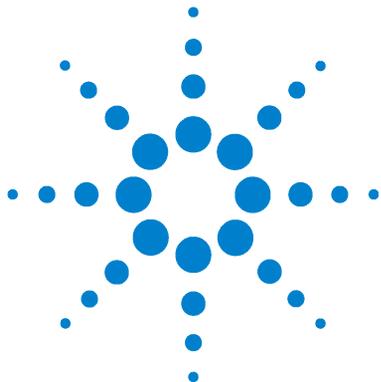
**NOTE**

A low background will improve the signal-to-noise, see also "Reducing Stray Light" on page 71.



**Figure 25** Fluorescence Scan of Water

## **2 First Steps with the Fluorescence Detector**



## 3 Optimizing the Fluorescence Detector

- Optimization Overview 62
- Design Features Help Optimization 63
- Finding the Best Wavelengths 64
- Finding the Best Signal Amplification 66
- Changing the Xenon Flash Lamp Frequency 67
- Selecting the Best Response Time 69
- Reducing Stray Light 71



### **3 Optimizing the Fluorescence Detector**

## **Optimization Overview**

Refer to [“Optimization Overview”](#) on page 31 for details.

## Design Features Help Optimization

The Agilent 1100 fluorescence detector has several features you can use to optimize detection:

**Table 15**

---

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 Agilent Technologies.

Your normal LC grade solvents may give good results most of the time but our experience shows that baseline noise can be higher with LC 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.

## 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 to other instruments.

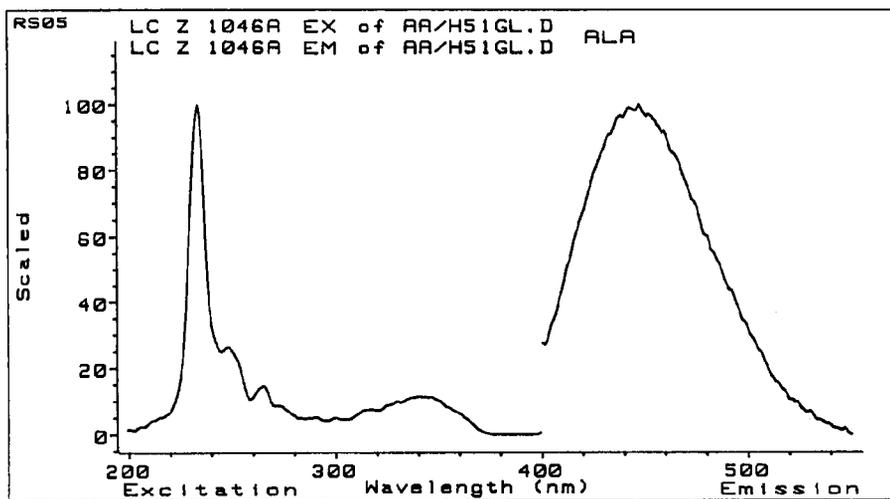
Both assumptions are wrong.

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 Agilent 1100 fluorescence detector was designed to give an optimum signal-to-noise ratio in the 210 nm to 360 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 Agilent 1100 fluorescence detector has a broad excitation wavelength range, but for higher sensitivity you should choose a wavelength in the ultra-violet range (near 250 nm).

The design elements that contribute to lower efficiency in the lower ultra-violet range are the xenon flash lamp and the gratings. Flash-type lamps shift the optimum wavelength to lower wavelength ranges with the Agilent 1100 fluorescence detector to a maximum of 250 nm. The excitation grating is blazed for highest efficiency at 300 nm.

## A Real Example

Although an excitation wavelength of 340 nm is quoted in the literature the Agilent 1100 fluorescence detector scan of orthophthalaldehyde, a derivative of the amino acid alanine, (Figure 26 on page 65) shows a maximum between 220 nm and 240 nm.



**Figure 26** Scan Orthophthalaldehyde Derivative of Alanine

When you are looking for the wavelength by scanning, scan over the whole range. As this example shows a maximum may be found in a completely different wavelength range.

### NOTE

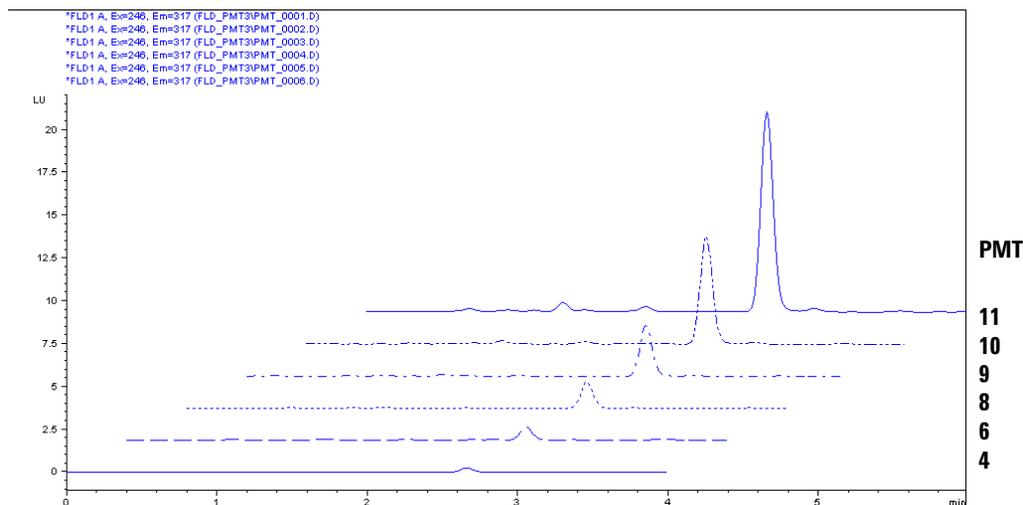
When comparing fluorescence excitation spectra directly with DAD spectra or literature based absorbance spectra, you should consider large differences in the used optical bandwidth (FLD=20 nm) which cause a systematic wavelength maximum shift depending on the absorbance spectrum of the compound under evaluation.

## Finding the Best Signal Amplification

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

The step from gain to gain is equal to a factor of 2 (which is the same as on the HP 1046A FLD).

In [Figure 27](#) the PMTGAIN was gradually raised from 4 up to 11 (the peak is from the Agilent Technologies isocratic sample which was diluted 1000 times). With increasing PMTGAIN there was an improvement in signal-to-noise up to 10. Above 10 the noise increased proportionately to the signal with no improvement in signal-to-noise.



**Figure 27** Finding Best PMTGAIN for Biphenyl

The reason for this is the fact, that quantification of baselines (especially at low background levels) is not sufficient for statistically working filter methods. For the best gain, check your solvent under flow conditions with the auto-gain function. Do not use higher values than proposed by the system, if not necessary, because of excessive high fluorescence signals.

Use the PMT test to automatically determine the setting.

## Changing the Xenon Flash Lamp Frequency

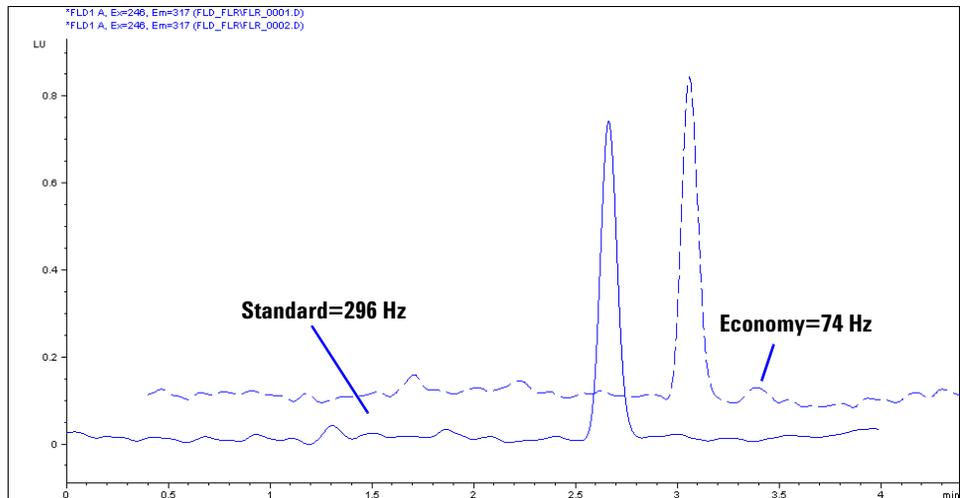
### Modes

The lamp flash frequency can be changed into the following modes:

**Table 16** Flash Lamp Modes

Positioning	296 Hz (Standard), 560 V	63 mJoule (18.8 W)
	74 Hz (Economy), 560 V	63 mJoule (4.7 W)
Rotation (Multi Ex/Em)	74 Hz (Standard), 950 V	180 mJoule (13.3 W)
	74 Hz (Economy), 560 V	63 mJoule (4.7 W)

Best sensitivity can be expected with “no economy”, see [Figure 28](#).



**Figure 28** Xenon Flash Lamp Frequency

#### Lamp life savings

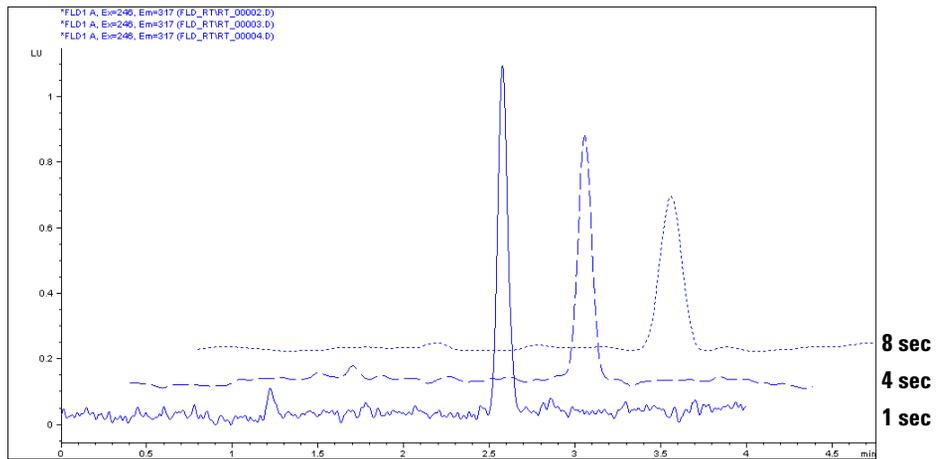
There are three ways to save lamp life:

- switch to “lamp on during run” without loss of sensitivity.
- switch to “economy” mode with a certain loss of sensitivity.
- a combination of the above.

## Selecting the Best Response Time

Data reduction using the RESPONSETIME function will increase your signal-to-noise ratio.

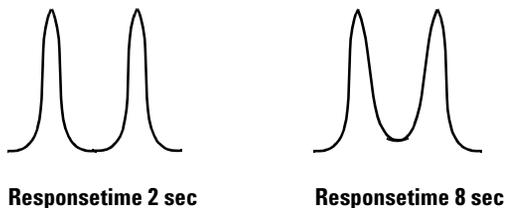
For example, see [Figure 29](#).



**Figure 29** Finding Best Response Time

### 3 Optimizing the Fluorescence Detector

LC fluorescence detectors typically work with response times of 2 or 4 seconds. The default of the Agilent 1100 fluorescence detector is 4 seconds. It is important to know that comparing sensitivity requires using the same response time. A response time of 4 seconds (default) is equivalent to a time constant of 1.8 seconds and appropriate for standard chromatographic conditions.



**Figure 30** Separation of Peaks using Responsetime

## Reducing Stray Light

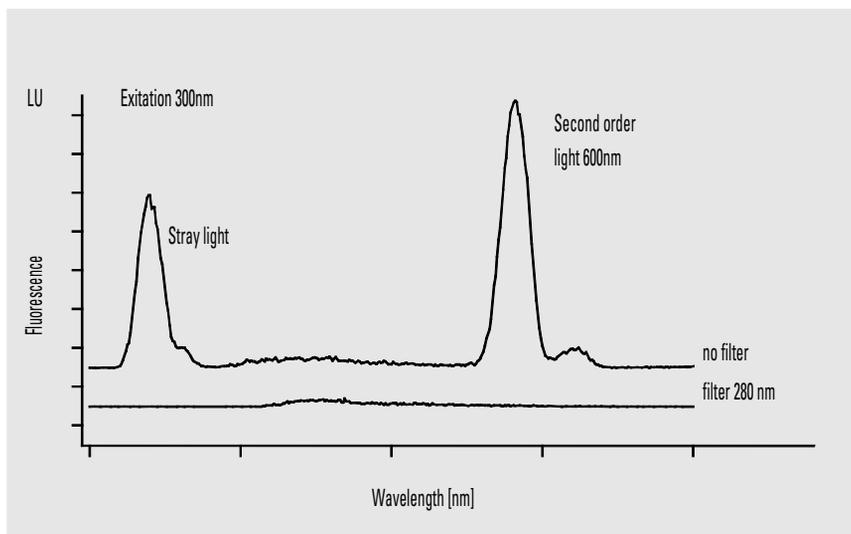
Cut-off filters are used to remove stray light and 2<sup>nd</sup> order or higher stray light by allowing complete transmission above the cut-off and little or no transmission below the cut-off point. They are used between excitation and emission gratings, to prevent any stray excitation light from reaching 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 2<sup>nd</sup> 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.

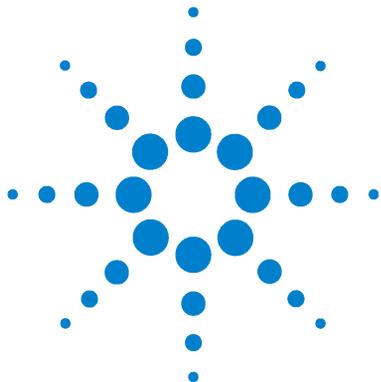
The lamp sends 1 million photons into the flow cell at, for example 280 nm. Scattering on the surface of the flow cell and scattering from the molecules of solvent allow 0.1% of this light to leave the cell through the window at right angles to the incident light. Without a cut-off filter, these remaining 1000 photons will reach the emission grating. 90% will be reflected totally without dispersion onto the photomultiplier. The other 10% disperses at 280 nm (1<sup>st</sup> order) and at 560 nm (2<sup>nd</sup> order). To remove this stray light, you need a cut-off filter around 280 nm.

### 3 Optimizing the Fluorescence Detector

Because of a known set of applications a 295 nm cut-off filter is built-in for undisturbed application up to 560 nm without compromises (see [Figure 31](#) on page 72).



**Figure 31** Reducing Stray Light



## 4 Troubleshooting and Test Functions

Overview of the Detector's Indicators and Test Functions	74
Status Indicators	75
Error Messages	77
Wavelength Verification and Calibration	101
The Wavelength Calibration Procedure	110
Test Functions	114



# Overview of the Detector's Indicators and Test Functions

### Status Indicators

The detector is provided with two status indicators which indicate the operational state (prerun, run, and error states) of the detector. The status indicators provide a quick visual check of the operation of the detector (see [page 75](#)).

### Error Messages

In the event of an electronic, mechanical or hydraulic failure, the detector generates an error message in the user interface. For each message, a short description of the failure, a list of probable causes of the problem, and a list of suggested actions to fix the problem are provided (see ["Error Messages"](#) on [page 77](#)).

### Wavelength Recalibration

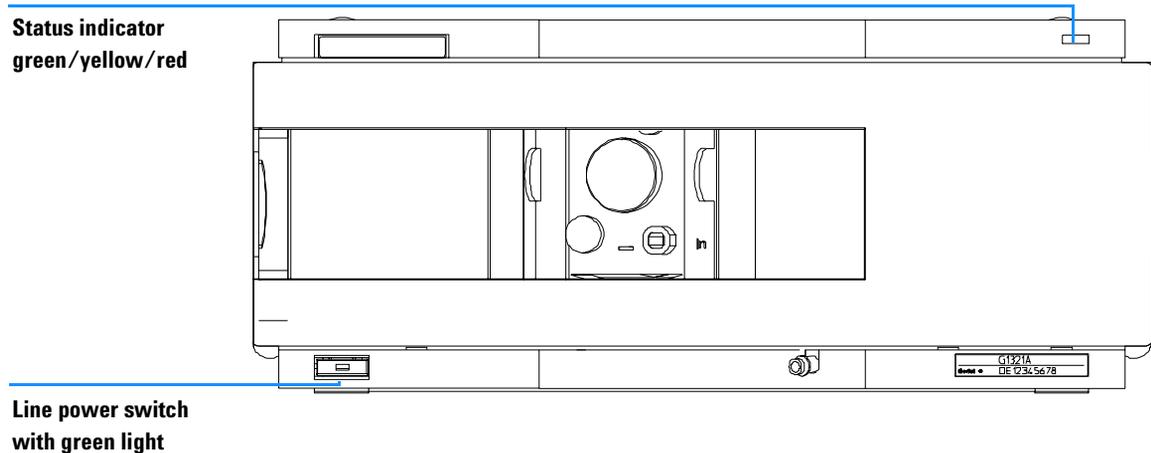
Wavelength recalibration is recommended after repair of internal components to ensure correct operation of the detector. The detector uses specific properties of the excitation and emission light characteristics, see ["Wavelength Verification and Calibration"](#) on [page 101](#)).

### Test Functions

A series of test functions are available for troubleshooting and operational verification after exchanging internal components (see ["Test Functions"](#) on [page 114](#)).

## Status Indicators

Two status indicators are located on the front of the detector. The lower left indicates the power supply status, the upper right indicates the detector status.



**Figure 32** Location of Status Indicators

## Power Supply Indicator

The power supply indicator is integrated into the main power switch. When the indicator is illuminated (*green*) the power is *ON*.

### Detector Status Indicator

The detector status indicator indicates one of four possible detector conditions:

- When the status indicator is *OFF* (and power switch light is on), the detector is in a *prerun* condition, and is ready to begin an analysis.
- A *green* status indicator, indicates the detector is performing an analysis (*run* mode).
- A *yellow* indicator indicates a *not-ready* condition. The detector is in a not-ready state when it is waiting for a specific condition to be reached or completed (for example, immediately after changing a setpoint), or while a self-test procedure is running.
- An *error* condition is indicated when the status indicator is *red*. An error condition indicates the detector has detected an internal problem which affects correct operation of the detector. Usually, an error condition requires attention (e.g. leak, defective internal components). An error condition always interrupts the analysis.

## Error Messages

Error messages are displayed in the user interface when an electronic, mechanical, or hydraulic (flow path) failure occurs which requires attention before the analysis can be continued (for example, repair, or exchange of consumables is necessary). In the event of such a failure, the red status indicator at the front of the detector is switched on, and an entry is written into the detector logbook.

This section describes the meaning of detector error messages, and provides information on probable causes and suggested actions how to correct error conditions.

### Time-out

The time-out threshold was exceeded (set in sequence parameter on the Agilent ChemStation or Configure-LC system).

#### Probable Causes

- The analysis was completed successfully, and the time-out function switched off the pump as requested.
- A not-ready state was present during a sequence or multiple-injection run for a period longer than the time-out threshold.

#### Suggested Actions

- ✓ Check the logbook for the occurrence and source of a not-ready condition. Restart the analysis where required.

## Shutdown

An external instrument has generated a shut-down signal (through CAN or REMOTE lines).

The detector continually monitors the remote input connectors for status signals. A LOW signal input on pin 4 of the remote connector generates the error message.

### Probable Causes

- Leak detected in an external instrument with a remote connection to the system.
- Shut-down in an external instrument with a remote connection to the system.
- The degasser failed to generate sufficient vacuum for solvent degassing.

### Suggested Actions

- ✓ Fix the leak in the external instrument before restarting the pump.
- ✓ Check external instruments for a shut-down condition.
- ✓ Check the degasser for an error condition. Refer to the *Reference Manual* for the Agilent 1100 Series vacuum degasser.

### Remote Time-out

A not-ready condition is still present on the remote input.

When an analysis is started, the system expects all not-ready conditions (e.g. a not-ready condition during detector balance) to switch to run conditions within one minute of starting the analysis. If a not-ready condition is still present on the remote line after one minute the error message is generated.

#### Probable Causes

- Not-ready condition in one of the instruments connected to the remote line.
- Defective remote cable.
- Defective components in the instrument showing the not-ready condition.

#### Suggested Actions

- ✓ Ensure the instrument showing the not-ready condition is installed correctly, and is set up correctly for analysis.
- ✓ Exchange the remote cable.
- ✓ Check the instrument for defects (refer to the instrument's reference documentation).

## Synchronization Lost

During an analysis, the internal synchronization or communication between one or more of the modules in the system has failed.

The system processors continually monitor the system configuration. If one or more of the modules is no longer recognized as being connected to the system, the error message is generated.

### Probable Causes

- CAN cable disconnected.
- Defective CAN cable.
- Defective main board in another module.

### Suggested Actions

- ✓ Ensure all the CAN cables are connected correctly.
- ✓ Switch off the system. Restart the system, and determine which module or modules are not recognized by the system.
- ✓ Ensure all CAN cables are installed correctly.

### Leak

A leak was detected in the detector.

The signals from the two temperature sensors (leak sensor and board-mounted temperature-compensation sensor) are used by the leak algorithm to determine whether a leak is present. When a leak occurs, the leak sensor is cooled by the solvent. This changes the resistance of the leak sensor which is sensed by the leak-sensor circuit on the FLM board.

#### Probable Causes

- Loose fittings.
- Broken capillary.
- Leaking flow cell.

#### Suggested Actions

- ✓ Ensure all fittings are tight.
- ✓ Exchange defective capillaries.
- ✓ Exchange flow cell.

#### formation

Detector leak error may be caused by the Agilent 1100 sampler. In each sampler injection sequence, step# 2 ejects the mobile phase stored in the metering head during the previous injection. This mobile phase is ejected through the short plastic tube connected to port# 4 of the sampler switching valve. The output of port# 4 is integrated into the sampler's contingency leak drain system, which eventually terminates in the leak pan of the bottom module of the stack - the detector. With normal injection volumes and run times, the output of port# 4 is small, and evaporates right in the sampler leak pan. However, the output of port# 4 is significant, and a substantial volume of ejected mobile phase reaches the detector leak pan.

There are two possible fixes. Select the one which is most convenient.

- 1** The waste drain plumbing orientation, shown in [step 6](#) on [page 26](#), eliminates the possibility of any leak drainage from above reaching the detector leak pan. The leak drain for the detector can be connected to the detector's leak drain fitting, and taken to waste separately.
- 2** If it is desired that the system has only one leak drain tube, then it's possible to increase the length of the small plastic tube which is connected to port# 4 of the sampler switching valve. This tube can then be taken to waste separately. The tube which normally serves as the detector cell outlet tube can be used for this purpose.

### Leak Sensor Open

The leak sensor in the detector has failed (open circuit).

The current through the leak sensor is dependent on temperature. A leak is detected when solvent cools the leak sensor, causing the leak sensor current to change within defined limits. If the current falls outside the lower limit, the error message is generated.

#### Probable Causes

- Leak sensor not connected to the FLM board.
- Defective leak sensor.

#### Suggested Actions

- ✓ Ensure the leak sensor is connected correctly.
- ✓ Exchange the leak sensor.

## Leak Sensor Short

The leak sensor in the detector has failed (short circuit).

The current through the leak sensor is dependent on temperature. A leak is detected when solvent cools the leak sensor, causing the leak sensor current to change within defined limits. If the current increases above the upper limit, the error message is generated.

### Probable Causes

- Defective leak sensor.

### Suggested Actions

- ✓ Exchange the leak sensor.

### Compensation Sensor Open

The ambient-compensation sensor (NTC) on the FLM board in the detector has failed (open circuit).

The resistance across the temperature compensation sensor (NTC) on the FLM board is dependent on ambient temperature. The change in resistance is used by the leak circuit to compensate for ambient temperature changes. If the resistance across the sensor increases above the upper limit, the error message is generated.

#### Probable Causes

- Defective FLM board.

#### Suggested Actions

- ✓ Exchange the FLM board.

## Compensation Sensor Short

The ambient-compensation sensor (NTC) on the FLM board in the detector has failed (short circuit).

The resistance across the temperature compensation sensor (NTC) on the FLM board is dependent on ambient temperature. The change in resistance is used by the leak circuit to compensate for ambient temperature changes. If the resistance across the sensor falls below the lower limit, the error message is generated.

### Probable Causes

- Defective FLF board.

### Suggested Actions

- ✓ Exchange the FLF board.

### Fan Failed

The cooling fan in the detector has failed.

The hall sensor on the fan shaft is used by the FLM board to monitor the fan speed. If the fan speed falls below two revolutions/second for more than five seconds, the error message is generated.

#### Probable Causes

- Fan cable disconnected.
- Defective fan.
- Defective FLM board.

#### Suggested Actions

- ✓ Ensure the fan is connected correctly.
- ✓ Exchange fan.
- ✓ Exchange the FLM board.

## Motor Errors

**NOTE**

Monochromator motor errors may show up during the **initialization** or during **operation** of the detector.

There are individual messages for either the excitation or the emission side.

If an error occurs, do a lamp ignition. This will clear the error and a re-initialization of the motors is performed.

**Table 17** Motor Errors

Message	Description	Probable Causes	Suggested Actions
Motor Or Encoder Not Found EX 6705 EM 6706	During initialization of the detector, the excitation and emission monochromator are activated.	encoder cables mixed on FLM board monochromator assembly not connected  monochromator or encoder defective monochromator motor power driver defective	check encoder connections to FLM check motor connections to FLF (Ex) and FLM (Em) and encoders to FLM replace monochromator assembly defective FLF board (Ex) or FLM board (Em)
Encoder Index Not Found EX 6707 EM 6708	During initialization of the detector, the excitation- and emission monochromator are activated and the encoder should generate an index.	encoder defective encoder electric defective monochromator defective or missing one phase  monochromator motor power driver defective	replace monochromator assembly replace FLM board check for connection to FLF (Ex) and FLM (Em) or replace monochromator assembly defective FLF board (Ex) or FLM board (Em)

## 4 Troubleshooting and Test Functions

**Table 17** Motor Errors (continued)

Message	Description	Probable Causes	Suggested Actions
Motor Friction Too High EX 6709 EM 6710	During initialization of the detector, the excitation and emission grating resistance test provides the resistance history of the excitation and the emission grating drives. The number of revolutions after switching off the drives is a measure of friction. The history may show an increasing friction of the drive(s) over a length of time.	friction too high  defective monochromator assembly	replace monochromator assembly  replace monochromator assembly
Motor Position Not Found EX 6711 EM 6712	When the wavelength is changed the monochromator should move to the new position. The position could not be found.	defective monochromator assembly	reset monochromator settings replace monochromator assembly
Motor Position Lost EX 6713 EM 6714	A mechanical shock to the instrument during operation may cause a movement of the monochromator. The position is lost and the lamp will turn off.	short mechanical shock message appears intermittently without mechanical shock	re-ignite the lamp replace monochromator assembly
Motor Speed Too Low EX 6715 EM 6716	For proper operation the monochromator gratings must run at a certain constant revolution.	revolution too low	replace monochromator assembly
Motor Speed Unstable EX 6717 EM 6718	For proper operation the monochromator gratings must run at a certain constant revolution.	defective monochromator assembly	re-ignite the lamp replace monochromator assembly.
Motor Encoder Index Wrong EX 6717 EM 6718	The actual encoder pattern is checked against a known pattern	encoder was replaced and has a different pattern or no reset of pattern was made encoder lost position completely	reset pattern via user interface and recalibrate  replace monochromator assembly

## Lamp Cover Open

The lamp cover in the optical compartment has been removed. The lamp cannot be turned on while this message is on.

### Probable Causes

- lamp cover removed.

### Suggested Actions

- ✓ close lamp cover and tighten the screws.

### FLF Board not found

The FLF board could not be found by the main board (FLM). This message comes together with some other message generated on the FLF board (e.g. Leak, ...).

#### Probable Causes

- FLF board not connected to the FLM board.
- defective FLF board.
- defective FLM board.

#### Suggested Actions

- ✓ Check the connection between the FLF board and the FLM board.
- ✓ Exchange the FLF board.
- ✓ Exchange the FLM board.

## ADC Not Calibrated

The analog-to-digital converter located on the FLF board cannot calibrate.

### Probable Causes

- defective ADC or other FLF electronics.

### Suggested Actions

- ✓ Exchange the FLF board.

### A/D Overflow

This message is not implemented in firmware revision A.03.66 and below.

It indicates an overload situation of the A/D converter (sample signal). The user-interface will show a not-ready condition for the FLD and an info event is written into the logbook. If the message comes up during a run, it includes the time of occurrence and when it disappears.

```
1100 FLD 1 A/D overflow (RT is 0.32 min)16:33:24 02/11/99
1100 FLD 1 A/D overflow finished (RT is 0.67 min)16:33:46 02/11/99
```

#### NOTE

If this condition is present prior to a run, the not-ready will prevent the system to start the run/sequence.

#### Probable Causes

- PMT setting to high
- wavelength setting wrong.

#### Suggested Actions

- ✓ reduce PMT gain
- ✓ change wavelength setting.

## Flash Lamp Current Overflow

The lamp current of the xenon flash lamp is monitored constantly. If the current gets too high, an error is generated and the lamp is turned off.

### Probable Causes

- shortage of flash lamp assembly
- shortage of trigger pack assembly.
- defective FLL board.

### Suggested Actions

- ✓ Exchange the flash lamp assembly.
- ✓ Exchange the FLL board (includes the trigger pack).

### Flash Trigger Lost

This message is displayed when the flash trigger is no longer generated.

#### Probable Causes

- firmware problem
- defective encoder

#### Suggested Actions

- ✓ Reboot the detector (power cycle).
- ✓ Multi Mode Off: exchange the firmware or replace the main board.
- ✓ Multi Mode Ex: replace EX monochromator assembly.
- ✓ Multi Mode Em: replace EM monochromator assembly.

## Wavelength Calibration Failed

This message may show up during a wavelength calibration.

If the expected deviation is larger than the specified wavelength accuracy, the message “*Wavelength Calibration Failed*” is displayed and the instrument stays in a *Not Ready* condition.

### Probable Causes

- Flash lamp not ignited or position not correct.
- Cell position not correct.
- Solvent in the cell not clean or air bubble in the cell.
- monochromator assembly position not correct (after replacement).

### Suggested Actions

- ✓ Check the flash lamp image and position. See also “[No Peaks](#)” on page 100.
- ✓ Check the cell position.
- ✓ Flush the flow cell.
- ✓ Reset monochromator settings and re-run the wavelength calibration.

### Wavelength Calibration Lost

After exchanging the monochromator assemblies, the calibration factors should be reset to default values (a new FLM board comes with default values). In this case “*Wavelength Calibration Lost*” is displayed and the instrument stays in a *Not Ready* condition.

#### Probable Causes

- Reset of monochromator settings after exchange.
- Replacement of FLM board.

#### Suggested Actions

- ✓ Perform a wavelength calibration.

## Flow Cell Removed

The detector has an automatic cell recognition system. When the flow cell is removed, the lamp is turned off and a NOT READY condition exists. If the flow cell is removed during an analysis, a SHUT DOWN is generated.

### Probable Causes

- Flow cell has been removed during analysis.

### Suggested Actions

- ✓ Insert flow cell and turn on the lamp.

### No Peaks

If no peaks are shown in the chromatogram, the user-interface shows the module still in “Ready”. There is no feedback mechanism that checks whether the lamp is on.

#### Probable Causes

- defective FLL board / Trigger pack
- defective Xenon flash lamp
- wrong position of monochromator
- FLF board defective

#### Suggested Actions

- ✓ Perform a “[Lamp Intensity Test](#)” on page 115. If no profile available (very low counts),
- ✓ replace FLL board / Trigger pack
- ✓ replace Xenon flash lamp
- ✓ perform a “[Check of Raman Band of Water](#)” on page 104 to check the wavelength calibration.
- ✓ replace FLF board

## Wavelength Verification and Calibration

The wavelength calibration is based on a Glycogen solution, which acts as a strong elastic light scatterer (refer to ASTM Test Method E388-72-1993 “*Spectral Bandwidth and Wavelength Accuracy of Fluorescence Spectrometers*”). The Glycogen solution is introduced into the flow cell and then the built-in wavelength calibration functionality is used.

The algorithm is based on evaluating different grating orders and calculating the wavelength scales of both, excitation and emission monochromator, by applying the fundamental grating equation.

**NOTE**

A complete wavelength calibration is not always required. In most cases a quick wavelength accuracy verification is sufficient enough, see the table below.

**Table 18** Reasons for doing a Verification or Calibration

	Verification	WL calibration
interest	X	
GLP compliance	X	
cell change	X	( X )
lamp change	X	( X )
monochromator change		X
main board change		X
optical unit change		X

( X ) only required, if deviation is too large.

**NOTE**

Prior to a wavelength calibration, a wavelength accuracy verification should be performed, see “[Check of Raman Band of Water](#)” on page 104. If the deviation is more than  $\pm 3$  nm, the wavelength calibration should be done as described in “[The Wavelength Calibration Procedure](#)” on page 110.

## 4 Troubleshooting and Test Functions

### NOTE

The duration of the wavelength calibration is about 15 minutes plus setup time for the calibration sample and system. Depending on the maximum intensity found during this scan, the PMT gain will be changed automatically and requires an additional 1 minute per scan.

Table 19 on page 103 shows the steps performed during the wavelength calibration.

The excitation grating and the emission grating are calibrated using Rayleigh stray light from the flow cell or cuvette measured with the photomultiplier tube.

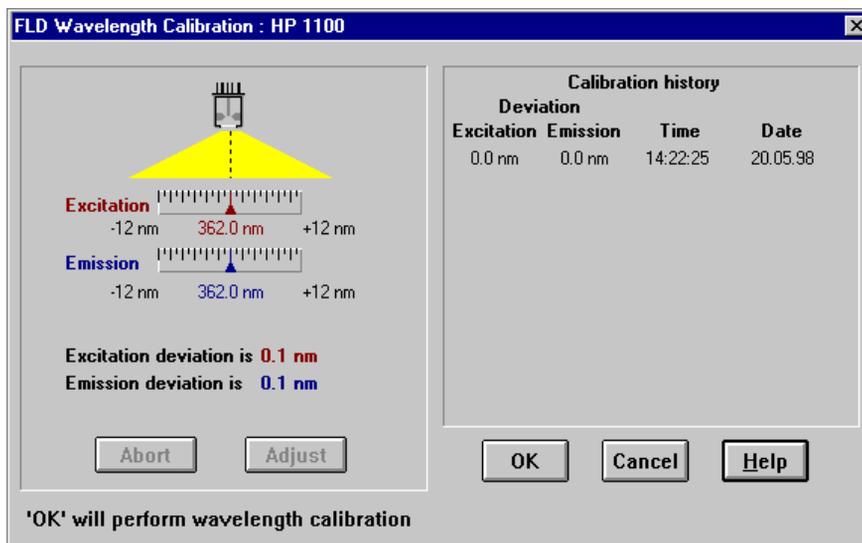


Figure 33 Wavelength Calibration - User Interface

**Table 19** Wavelength Calibration Steps

Step	Description	Duration
1	Preparation	max 30 s
2	Excitation rotation scan, full circle	60 s
3	Excitation rotation scan, high resolution	44 s
4	Excitation position scan, low resolution	55 s variable
5	Excitation position scan, high resolution	260 s variable
6.n	Emission rotation scan, full circle (# of scans depends on the required PMT gain, 1 minute per scan)	61 s variable
6.n	Em rotation scan, full circle" (instrument profile)	9 s
6.n	Em rotation scan, full circle" (instrument profile)	9 s
6.n	Em rotation scan, full circle" (instrument profile)	9 s
6.n	Em rotation scan, full circle" (instrument profile)	9 s
7	Emission rotation scan, high resolution, part I	44 s
8	Emission rotation scan, high resolution, part II	44 s
9	Emission position scan, low resolution	50 s variable
10	Emission position scan, high resolution	250 s variable

Notes:

- 1 Variable times means that they could be a little bit longer.
- 2 Steps “6.n Em rotation scan, full circle" (instrument profile)” have been added with firmware revisions A.03.64 .

**NOTE**

When the lamp is of, the calibration process will stop within the first two steps with “ Wavelength Calibration Failed” , see “Wavelength Calibration Failed" on page 97.

### Check of Raman Band of Water

This test is a verification of the wavelength calibration, described in section “[Wavelength Verification and Calibration](#)” on page 101, using the Raman band of water.

#### NOTE

This test requires the Agilent ChemStation Spectra module or the Control Module.

---

### Using the Agilent ChemStation

#### NOTE

The methods WLEMTEST.M and WLEXTEST.M are part of the A.06.xx ChemStation software CD (\1100FLD\VERIFYWL\).

---

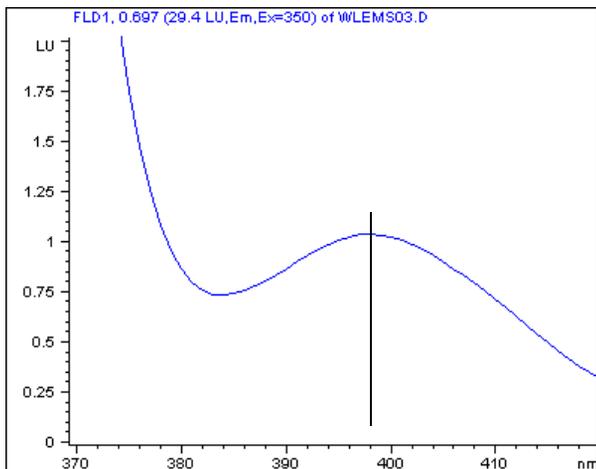
- 1 Copy the method WLEMTEST.M and WLEXTEST.M to \HPCHEM\1\METHODS.
- 2 Load the method WLEXTEST.M. The FLD will change into the multi-emission mode and scan in the range of the expected maximum of 397 nm  $\pm$ 20 nm.
- 3 Turn the pump on and flush with water for a few minutes to assure a clean flow cell. Flow rate should be 0.5 to 1 ml/min and the baseline stable.

#### NOTE

You may remove the flow cell and check for air bubbles. After re-inserting the cell, turn the lamp on.

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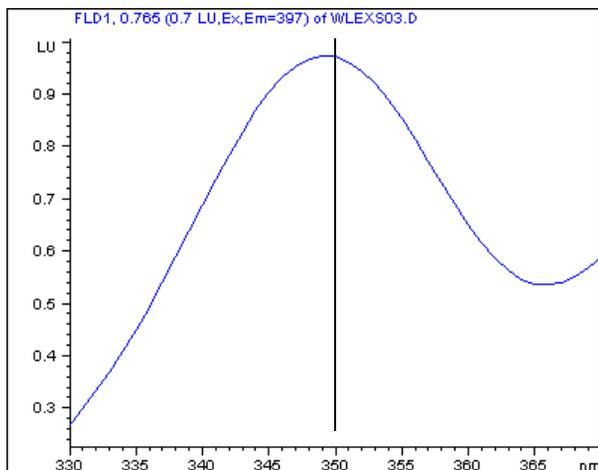
- 4 Define in *Sample Information* the file name and description.
- 5 Start a run. After the 1 minute run has completed, enter the Data Analysis and evaluate the spectrum. The maximum should be found at 397 nm  $\pm$ 3 nm, see [Figure 34](#) on page 105.



**Figure 34** Emission Spectrum

- 6** Load the method WLEMTEST.M. The FLD will change into the multi-excitation mode and scan in the range of the expected maximum of  $350 \text{ nm} \pm 20 \text{ nm}$ .
- 7** Define in *Sample Information* the file name and description.
- 8** Start a run. After the 1 minute run has completed, enter the Data Analysis and evaluate the spectrum. The maximum should be found at  $350 \text{ nm} \pm 3 \text{ nm}$ .

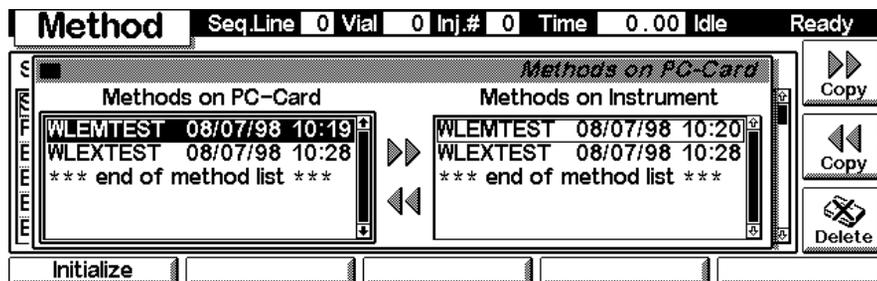
## 4 Troubleshooting and Test Functions



**Figure 35** Excitation Spectrum

### Using the Control Module

- 1 Create the methods WLEMTEST and WLEXTEST as listed [Table 20](#) on page 109.
- 2 Copy the methods to your PC card and re-boot the control module.
- 3 On the *Analysis* screen press the *Method* button and copy the methods into the module.



**Figure 36** Copying Methods into the FLD

- 4 Load the method WLEXTEST. The FLD will change into the multi-emission mode and scan in the range of the expected maximum of 397 nm  $\pm$ 20 nm.

- Turn the pump on and flush with water for a few minutes to assure a clean flow cell. Flow rate should be 0.5 to 1 ml/min and the baseline stable.

**NOTE**

You may remove the flow cell and check for air bubbles. After re-inserting the cell, turn the lamp on.

- From the *Analysis* screen select *Settings - FL Detector*. Press then the *Spectrum* button will open the window below.

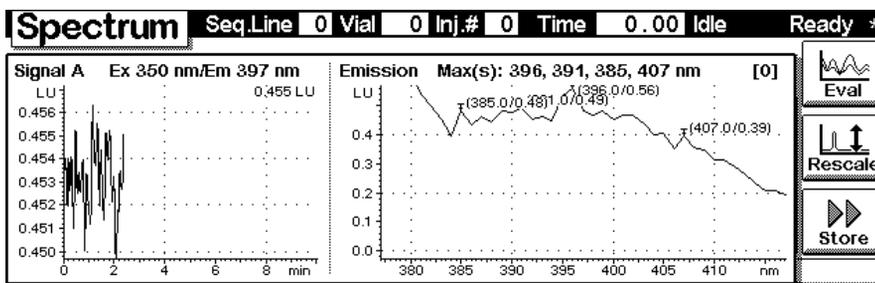


Figure 37 Online Emission Spectra

- Press the *Store* button a few times to store a few spectra in a temporary buffer.
- Press the *Eval* button to change to the following screen.

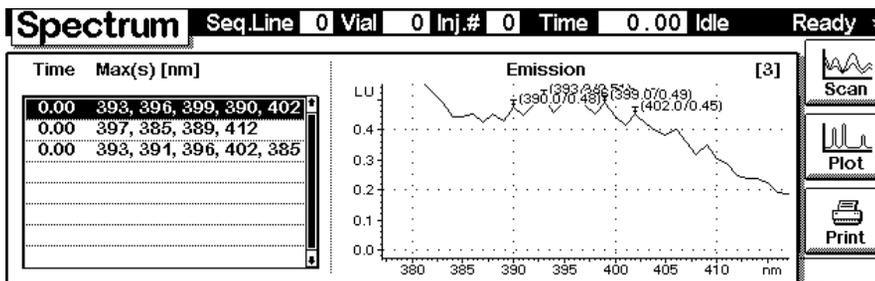
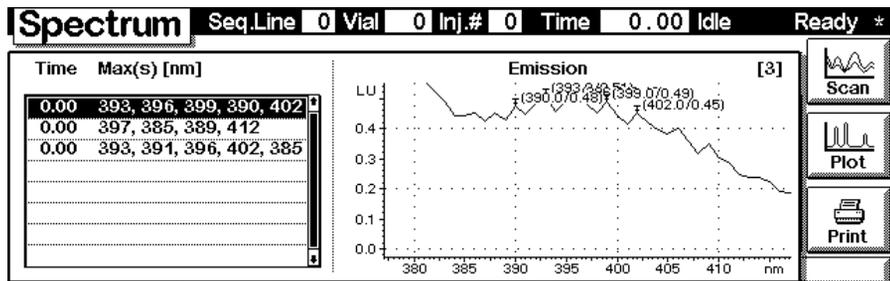


Figure 38 Evaluating Online Emission Spectra

## 4 Troubleshooting and Test Functions

- 9 You can now select a stored spectra and evaluate/print them.  
The maximum should be found at 397 nm  $\pm$ 3 nm.
- 10 Load the method WLEMTEST. The FLD will change into the multi-excitation mode and scan in the range of the expected maximum of 350 nm  $\pm$ 20 nm.
- 11 Repeat steps [step 6](#) to [step 8](#).



**Figure 39** Evaluating Online Excitation Spectra

- 12 You can now select a stored spectra and evaluate/print them.  
The maximum should be found at 350 nm  $\pm$ 3 nm.

### NOTE

The spectra on the control module is a real raw data spectrum. Therefore it looks not that smooth as on the Agilent ChemStation (uses the spline algorithm).

## Method Settings

**Table 20** Method Settings

<b>Setting</b>	<b>Value in WLEXTEST</b>	<b>Value in WLEMTEST</b>
Peak Width	>0.2 min (4 s, standard)	>0.2 min (4 s, standard)
Fit Spectral Range	OFF	OFF
PMT Gain	12	12
Flash Lamp	ON	ON
Spectrum Range	EX 377 - 417 nm step 1 nm	EM 330 - 370 nm step 1 nm
Store Spectra	All w/o signal	All w/o signal
EX Wavelength	350 nm, ON	350 nm, OFF
EM Wavelength	397 nm, OFF	397 nm, ON
Multi WL Settings	Multi EX	Multi EM

### The Wavelength Calibration Procedure

<b>When required</b>	If application requires, or see <a href="#">Table 18</a> on page 101.
<b>Tools required</b>	Laboratory balance
<b>Parts required</b>	Glycogen Calibration Sample, Syringe, needle, sample filter and PEEK fitting from the Accessory Kit

#### Steps

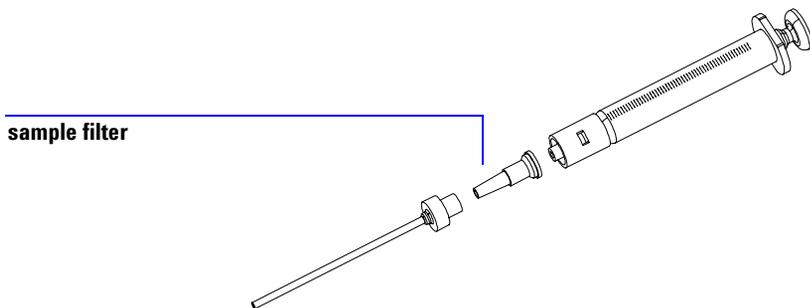
- 1 Preparation of the Glycogen Calibration Sample.
- 2 Preparation of the Flow Cell.
- 3 Wavelength Calibration.
- 4 Verification using "[Check of Raman Band of Water](#)" on page 104.

#### Preparation of the Glycogen Calibration Sample

- 1 To prepare 10 ml of the calibration solution you have to use 10 mg of the Glycogen sample (a tolerance of  $\pm 20\%$  is not critical).
- 2 Fill the prepared amount into a suitable bottle/vial.
- 3 Fill 10 ml of distilled water into the vial and shake.
- 4 Wait 5 minutes and shake again. After 10 minutes the solution is ready.

## Preparation of the Flow Cell

- 1 Flush the flow cell with water.
- 2 Remove the inlet capillary from the flow cell.
- 3 Take the syringe and fix the needle to the syringe adapter.
- 4 Suck about 1.0 ml of the calibration sample into the syringe.
- 5 Keep the syringe in a horizontal position.
- 6 Remove the needle.
- 7 Add the filter to the syringe and fit the needle to filter.



**Figure 40** Syringe with Sample Filter

- 8 Lift the needle tip and carefully eject approximately 0.5 ml to remove air out of the syringe and to flush the needle.
- 9 Add the PEEK fitting to the needle tip and fix both at the flow cell inlet.

**NOTE**

Do not inject the calibration sample without the sample filter.

- 10 Slowly inject about 0.2 ml and wait for about 10 seconds to inject another 0.1 ml. This will assure that the cell is filled properly.

### Wavelength Calibration

- 1 From the user interface start the FLD Wavelength Calibration (see [Figure 41](#)).  
Agilent ChemStation: *Diagnosis - Maintenance - FLD Calibration*  
Control Module: *System - Tests - FLD - Calibrate*.

#### NOTE

If the wavelength calibration process fails, refer to ["Wavelength Calibration Failed"](#) on page 97.

---

- 2 If a deviation is displayed, press Adjust and OK. The history table will be updated.

#### NOTE

To look at the history table (ChemStation) start a wavelength calibration and abort immediately. No changes are made to the calibration at this time.

---

#### NOTE

Rinse the flow cell with pure water at a minimum of 1.5 ml/min to get rid of the Glycogen from the cell and the capillaries. When organic solvent is sequentially applied (without rinsing), a blockage of capillaries may occur.

---

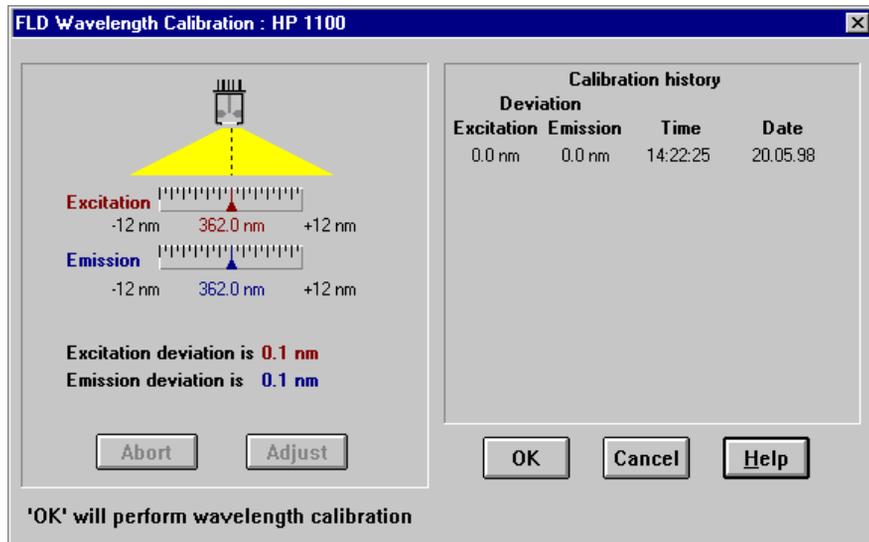


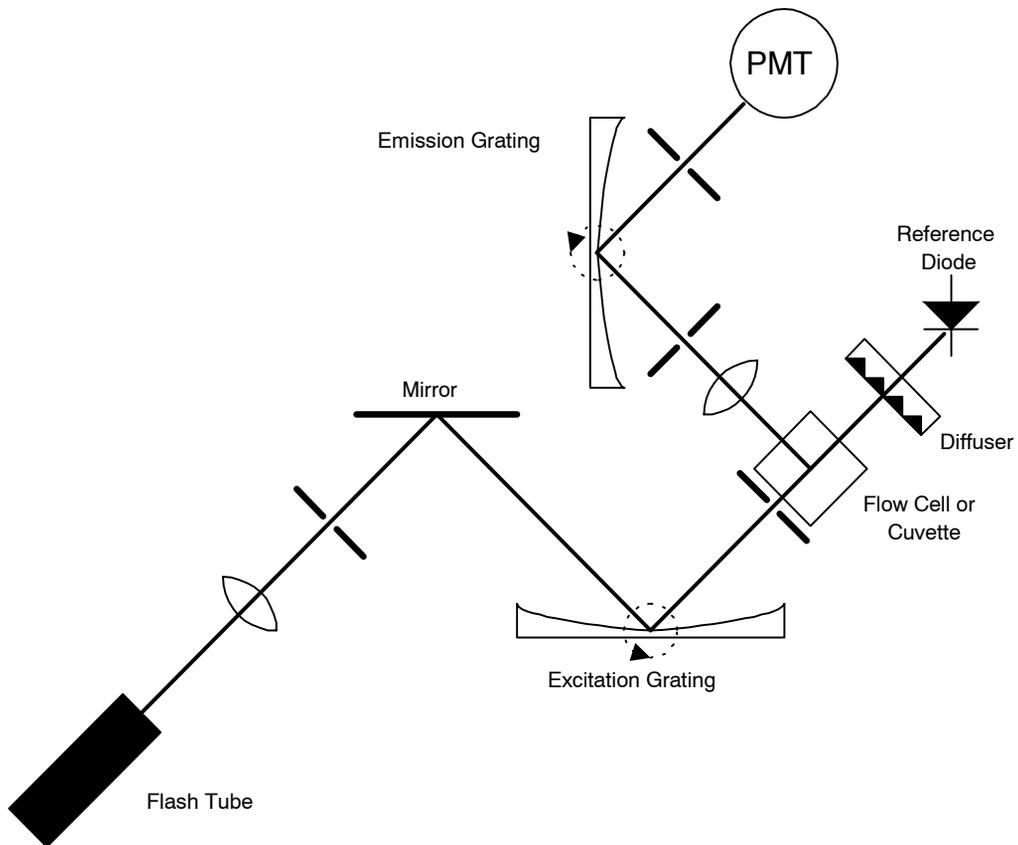
Figure 41 Wavelength Calibration - User Interface

## Verification

- 1 Refit the capillary to the flow cell.
- 2 Follow the procedure “Check of Raman Band of Water” on page 104.

## Test Functions

The light path is shown in [Figure 42](#).

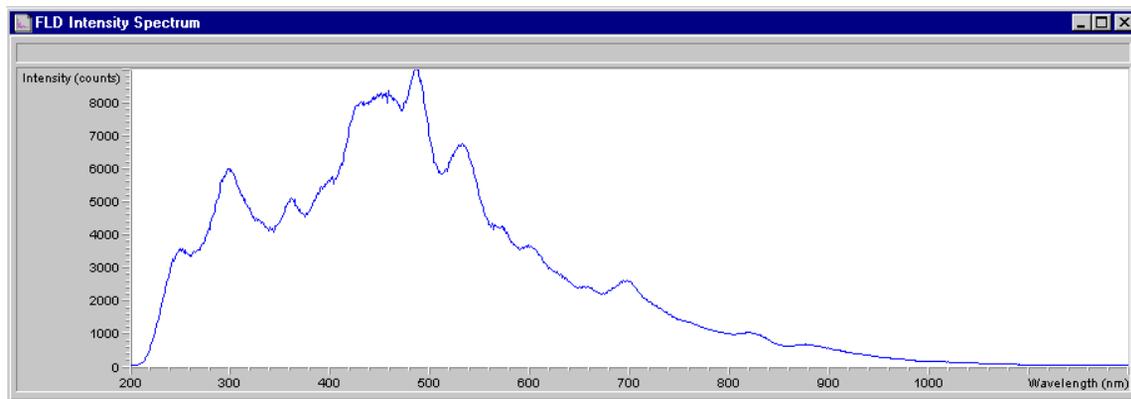


**Figure 42** Schematic of the Light Path

## Lamp Intensity Test

The intensity test scans an intensity spectrum via the reference diode (200 - 1200 nm in 1 nm steps) and stores it in a diagnosis buffer. The scan is displayed in a graphic window. There is no further evaluation of the test.

Results of this test are stored as lamp history (date code, intensity).



**Figure 43** Lamp Intensity Test (typical)

**NOTE**

The profile can vary from instrument to instrument. It is dependig on the age of the lamp and the content of the flow cell (use fresh water).

UV degradation, especially below 250 nm is significantly higher compared to visible wavelength range. Generally the "LAMP ON during run" setting or using "economy mode" will increase lamp life by a magnitude.

## Lamp Intensity History

Results of the lamp intensity test (if the last one is older than one week) are stored as lamp history (date code, intensity of four different wavelengths, 250 nm, 350 nm, 450 and 600 nm) in a buffer. The data/plot can be retrieved via the diagnostics and provides intensity data over a length of time.

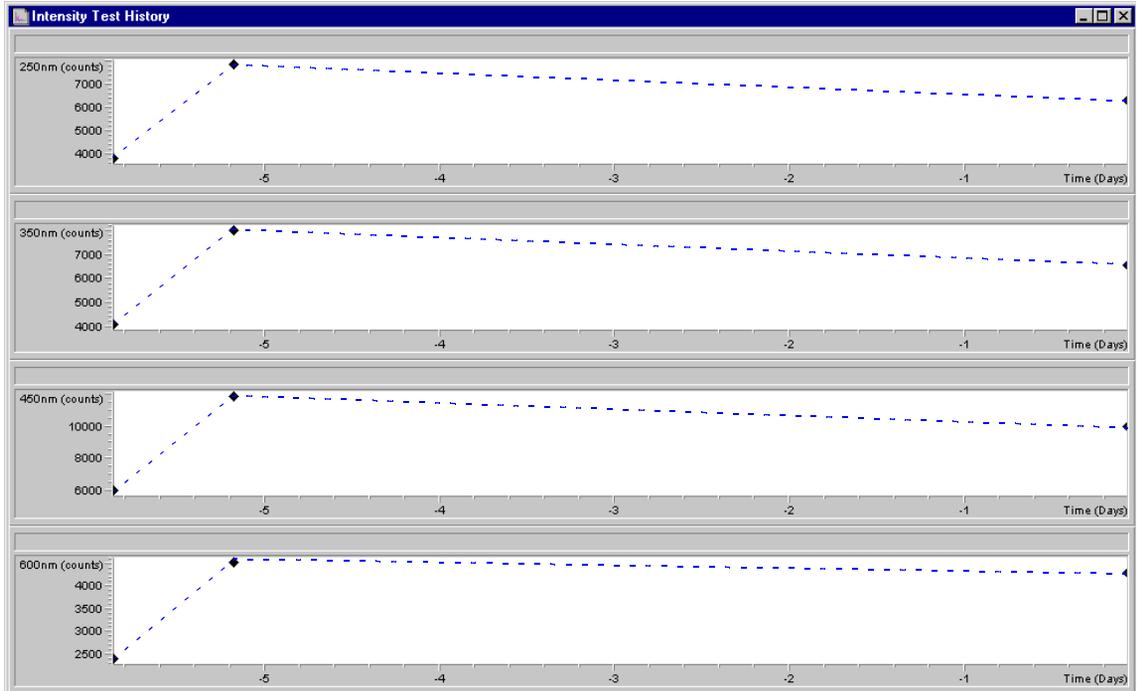


Figure 44 Lamp Intensity History

## Dark-Current Test

The dark-current test measures the PMT signal with maximum and minimum gain while the lamp is off. It also reads the signal of the reference diode. The resulting values (two via reference diode and two from PMT) are shown in a table and checked against reasonable limits (see below).

Date: 21.04.98; Time: 11:50:23	Limits	Measured	Result
PMT dark current	< 10000 cts	1070 cts	Passed
Reference diode dark current	< 5000 cts	1048 cts	Passed

Figure 45 Dark-Current Test

### Dark-Current Test Evaluation

#### Test Failed

#### Probable Causes

- Defective PMT.
- Defective reference diode or A/D converter

#### Suggested Actions

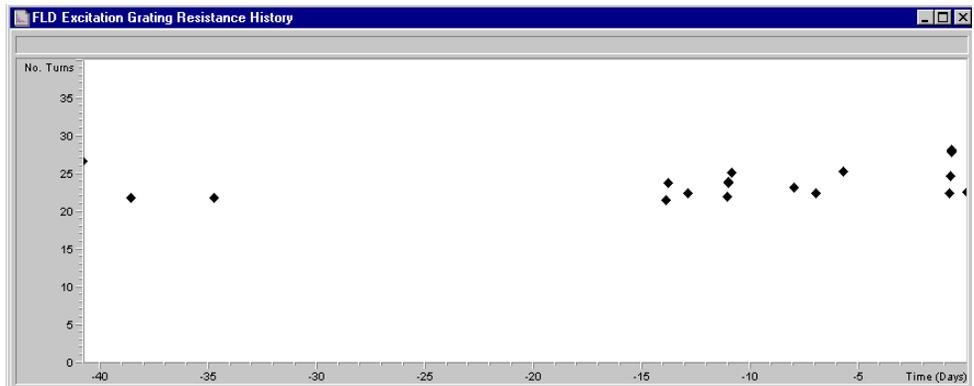
- ✓ Exchange the PMT.
- ✓ Exchange the FLF board

### Excitation and Emission Grating Resistance History

This test runs automatically when the instrument is turned on (not accessible as an external test).

It provides the resistance history of the excitation and the emission grating drives. The number of revolutions after switching off the drives is a measure of friction. The history may show an increasing friction of the drive(s) over a length of time.

The history data contains the data/time information and the number of turns. The data/plot can be retrieved via the diagnostics.



**Figure 46** Resistance History

### Friction Evaluation

#### Motor Error

#### Probable Causes

- Friction too high (< 12 turns)
- Defective monochromator assembly.

#### Suggested Actions

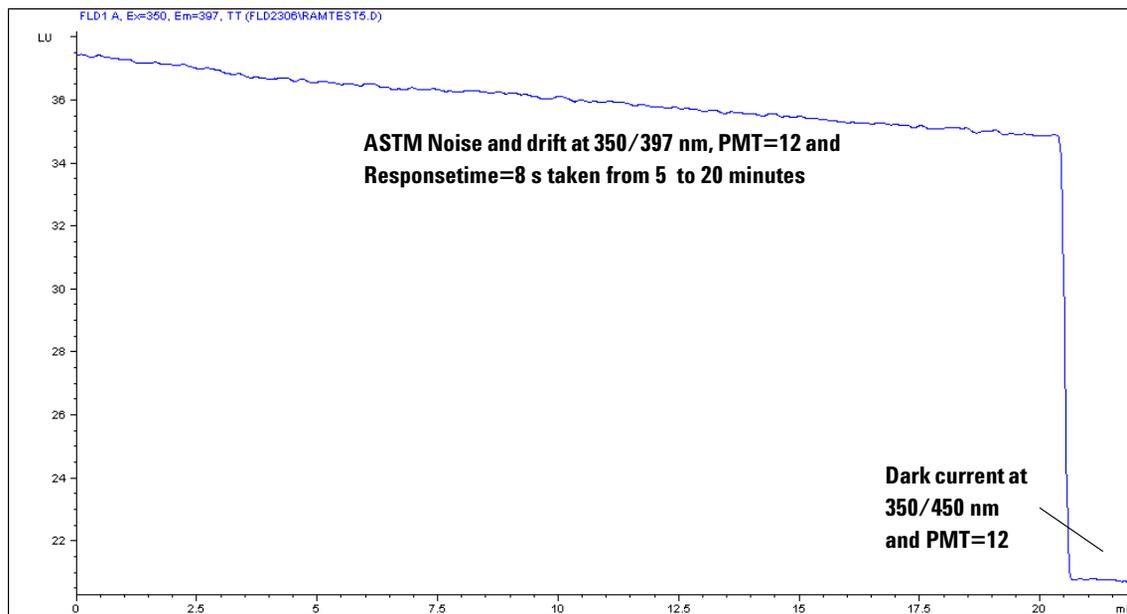
- ✓ Exchange the monochromator assembly.

## Raman ASTM Signal-to-Noise Test

This test verifies the Raman ASTM signal-to-noise.

**Table 21** Raman Signal-to-Noise Test Conditions

Duration	approximately 23 minutes
Report Style (Agilent ChemStation)	Performance+Noise
Noise Determination	5 to 20 minutes
Solvent	LC grade water, degassed
Flow rate	0.5 - 1 ml/min



**Figure 47** Raman ASTM Signal-to-Noise Test

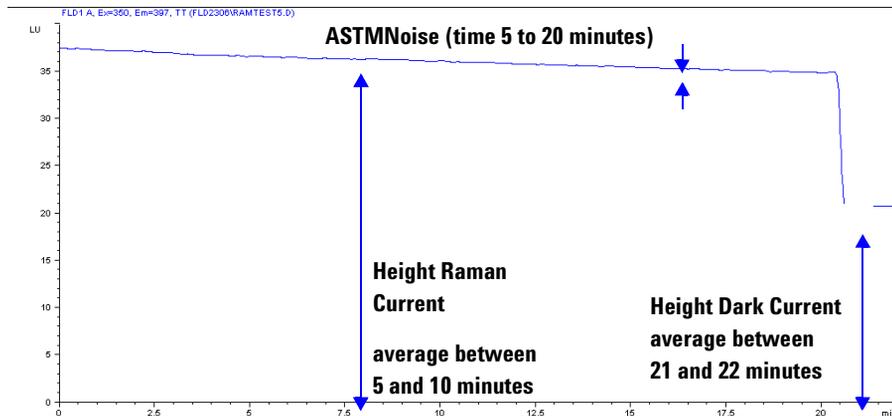
## 4 Troubleshooting and Test Functions

**Formula for the Raman ASTM S/N value** (see [Figure 48](#) for details):

$$RamanASTM = \frac{Height(RamanCurrent) \div Height(DarkCurrent)}{ASTMNoise}$$

The result should be > 200 with FLF board revision A.

The result should be > 400 with FLF board revision B and above.



**Figure 48** Raman ASTM signal/noise calculation

### Procedure using an Agilent ChemStation

#### NOTE

The method FLRAMTST.M and the macro FLRAMTST.MAC are part of the A.06.xx ChemStation software CD (\1100FLD\RAMAN\).

- 1 Assure that the FLD signal is signal 1 (in case you use an additional detector in the system). Otherwise wrong calculations are done.
- 2 Copy the method FLRAMTST.M to \HPCHEM\1\DATA\METHODS and the macro FLRAMTST.MAC to \HPCHEM\CORE.
- 3 Load the method (normally no changes are required).

#### NOTE

**A.06.01 ChemStation CD only: Change the STOP TIME to 23 minutes. The 22-minutes stop time in the method will cause wrong Raman signal/noise ratio values!**

- 4 Define in the *Sample Information* the file name and description.
- 5 Turn the pump on and flush with water for a few minutes to assure a clean flow cell.

#### NOTE

You may remove the flow cell and check for air bubbles. After re-inserting the cell, turn the lamp on.

- 6 Start a blank run with a flow of 0.5 to 1 ml/min.
- 7 When the run is completed a report is displayed and the *status line* shows the "Raman signal/noise ratio = " value (see [Figure 49](#)) which should be >200 (with FLF board revision A) or >400 (with FLF board revision B and above).
- 8 To re-display the number, type `LEO_FLD_EVALUATE_NOISE_TEST` and press Enter.

#### NOTE

If the pre-run macro is no longer loaded, the Raman value shows different values. In this case re-load the pre-run macro.

## 4 Troubleshooting and Test Functions

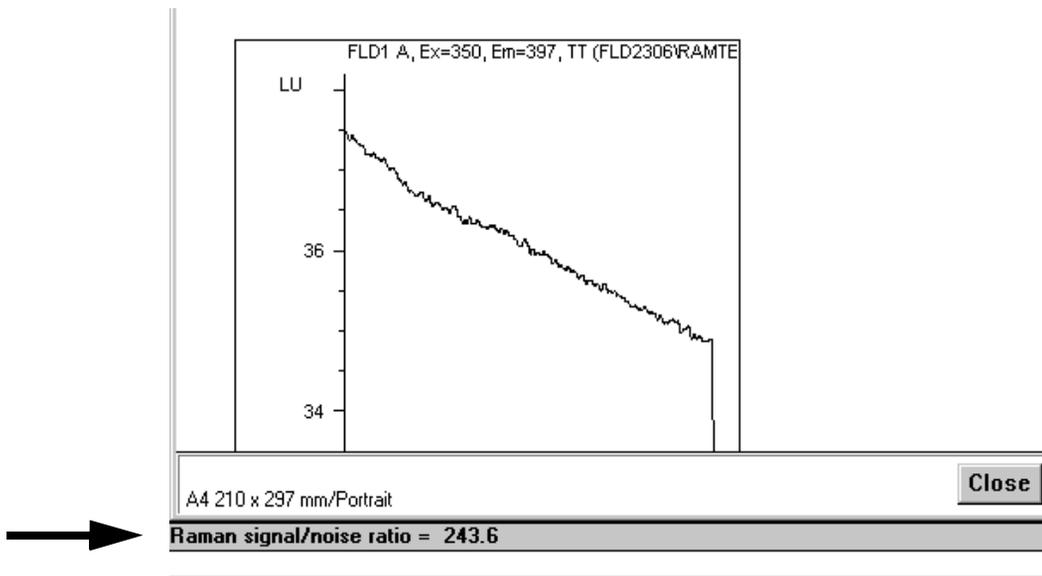
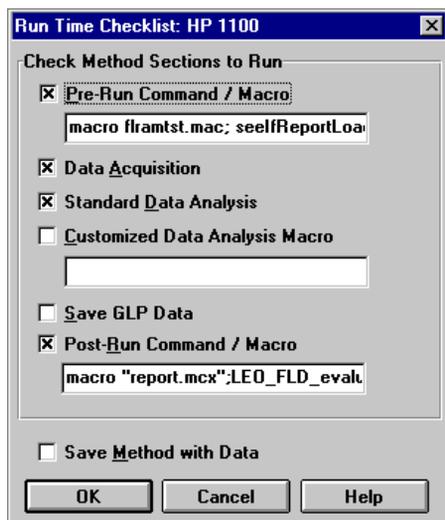


Figure 49 RAMAN value



**macro flramtst.mac; seelfReportLoaded;initNoiseDetermination**

This line loads the raman macro, starts loading the noise evaluation generator and defines ASTM noise performance parameters based on ASTM interval definition of 0.5 minutes.

**macro "report.mcx";LEO\_FLD\_evaluate\_Noise\_Test**

This line starts the FLD ASTM calculation and restores original ASTM noise calculation based on 1 minute intervals

Figure 50 Raman Test - Run Time Checklist (part of method)

Set the Stoptime = 23 minutes, see on page 121.

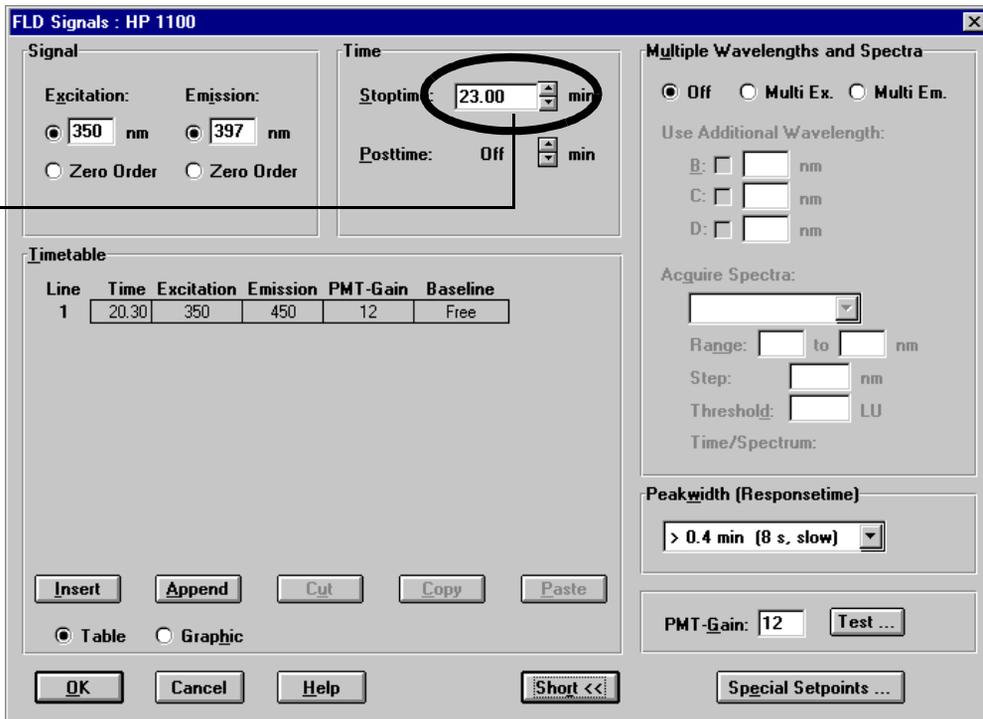


Figure 51 Raman Test - FLD Settings (part of method)

### Interpretation of the Results

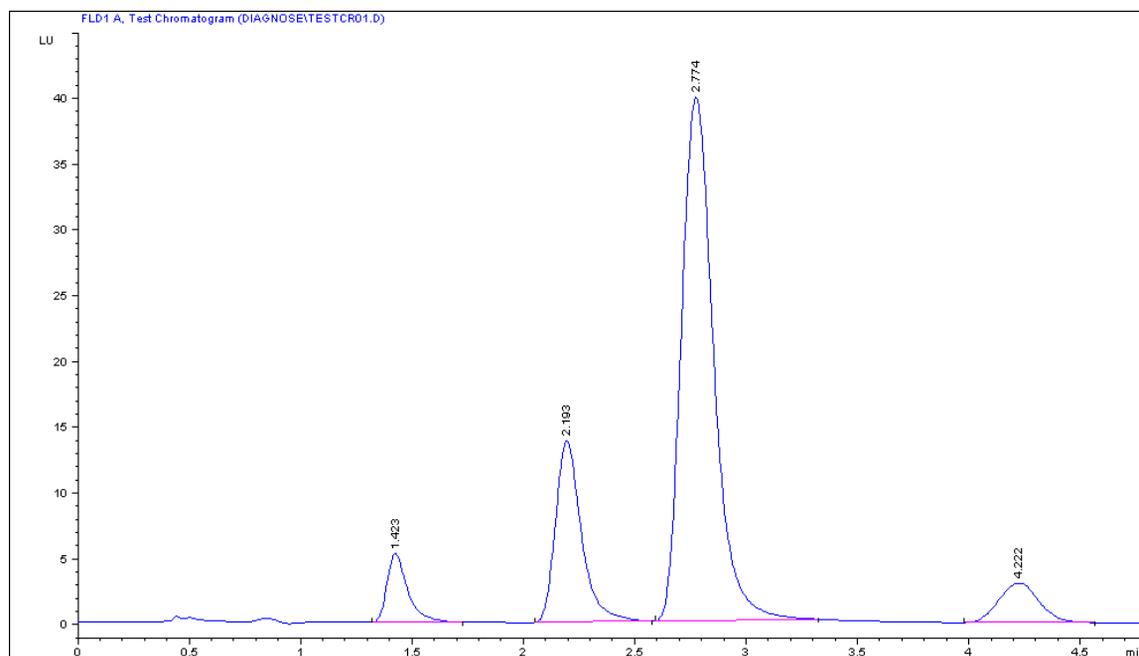
If the test shows low Raman values, check for:

- ✓ correctly positioned flow cell,
- ✓ clean flow cell (flush),
- ✓ no air bubble(s) (check via fluorescence scan or visual check of cell/cuvette),
- ✓ solvent inlet filter (may create air bubbles in flow cell),
- ✓ clean water.

## Using the Built-in Test Chromatogram

This function is available from the Agilent ChemStation and the Control Module.

The built-in Test Chromatogram can be used to check the signal path from the detector to the ChemStation and the data analysis or via the analog output to the integrator or data system. An example is shown in [Figure 52](#). The chromatogram is continuously repeated until a stop is executed either by means of a stop time or manually.



**Figure 52** Built-in Test Chromatogram (default settings)

### NOTE

The peak height is always the same but the area and the retention time depend on the set peakwidth, see examples below.

**Table 22**

Peakwidth = >0.05 min (response time = 1 s fast)	Stop time = 1.2 minutes
Peakwidth = >0.20 min (response time = 4 s STD)	Stop time = 4.8 minutes

**Procedure using the Agilent ChemStation**

- 1 Load the FLD default parameter
  - peakwidth to >0.2 minutes (standard),
  - set stop time to 4.8 minutes.
- 2 Complete the Sample Information (no injection).
- 3 Type into the command line (or as pre-run command):  

```
PRINT SENDMODULE$(LFLD,"SIMU:DFLT").
```
- 4 The logbook shows "Preparing for a simulation".
- 5 Start the run.

The results are processed like a regular chromatogram.

**NOTE**

The test chromatogram is switched off automatically at the end of the run.

### Procedure using the Control Module

- 1 Load the FLD default parameter
  - peakwidth to >0.2 minutes (standard),
  - set stop time to 4.8 minutes.
- 2 Enter System - *Tests - FLD*.
- 3 Select the function *Enable test chromatogram* and press *Execute*.  
The logbook shows “Simulated chromatogram enabled”.
- 4 Enter the *Plot* function and select *Signal A* and/or *Analog x*, set a *time range* of 10 minutes and the *FLD signal* from -1 to 50 LU.

#### NOTE

If an Agilent ChemStation is connected, the next step will start the Agilent ChemStation simultaneously and will overwrite the previous used Data File!

---

- 5 Start the run.

After the run has finished, the plot can be printed to a connected printer by pressing the m-key and selecting *Print Plot*.

#### NOTE

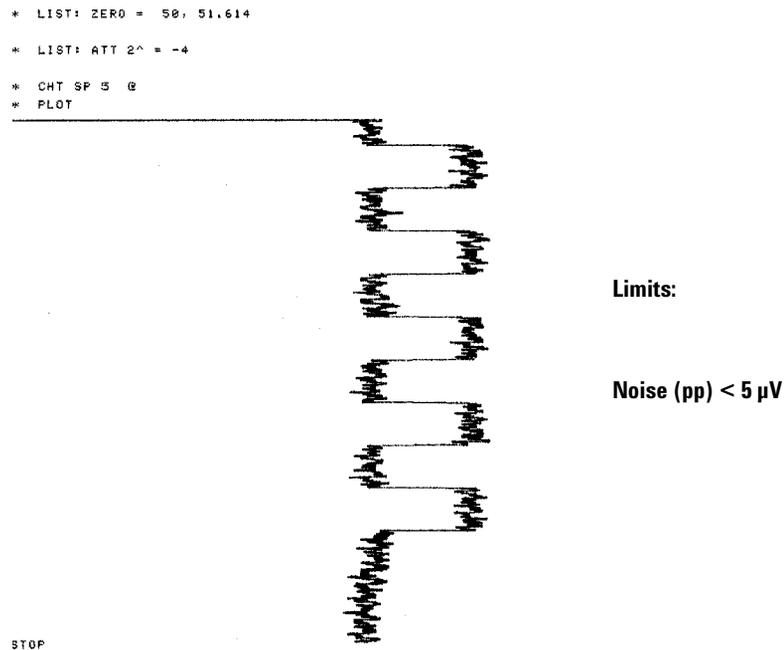
The test chromatogram is switched off automatically at the end of the run.

---

## Using the Built-in DAC Test

This function is available from the Control Module ONLY.

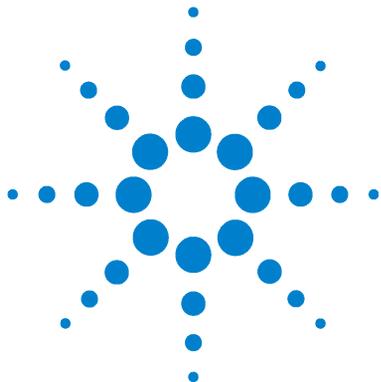
The DAC test provides a test pattern as an analog output. The output voltages (analog 1 and analog 2) should show a constant value corresponding to the ZERO OFFSET value. In addition to the constant voltage a switched voltage with a duration of 12 seconds and a height of 10  $\mu\text{V}$ , see [Figure 53](#).



**Figure 53** DAC Test (Example)

- 1 Enable the function (*System - Tests - FLD - Enable DAC Test Pattern*).
- 2 Start the plot mode (e.g. Agilent 3396 with ATTN -4, ZERO=50, CS=5).
- 3 Stop the plot and disable the TAC Test (*System - Tests - FLD - Disable DAC Test Pattern*).
- 4 Evaluate the noise (should be < 5  $\mu\text{V}$ ).

## **4 Troubleshooting and Test Functions**



## 5 Repairing the Fluorescence Detector

Introduction into Repairing the Fluorescence Detector	130
Overview of the Repairing of the Fluorescence Detector	133
Simple Repairs	134
Exchanging Internal Parts	142



# Introduction into Repairing the Fluorescence Detector

### Simple Repairs

The detector is designed for easy repair. The most frequent repairs such as flow cell change can be done from the front of the detector with the detector in place in the system stack. These repairs are described in “[Simple Repairs](#)” on page 134.

### Exchanging Internal Parts

Some repairs may require exchange of defective internal parts. Exchange of these parts (including flash lamp) requires removing the detector from the stack, removing the covers, and disassembling the detector. The security lever at the power input socket prevents the detector cover from being removed when line power is still connected. These repairs are described in “[Exchanging Internal Parts](#)” on page 142.

#### WARNING

**To prevent personal injury, the power cable must be removed from the instrument before opening the detector cover. Do not connect the power cable to the detector while the covers are removed.**

**When working with solvents please observe appropriate safety procedures (for example, goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet supplied by the solvent vendor, especially when toxic or hazardous solvents are used.**

#### CAUTION

Electronic boards and components are sensitive to electronic discharge (ESD). In order to prevent damage always use an ESD protection (for example, the ESD wrist strap from the accessory kit) when handling electronic boards and components (see “[Using the ESD Strap](#)” on page 132).

#### CAUTION

There is a risk of damaging hardware due to overheating when operating the instrument without covers.

**WARNING**

Eye damage may result from directly viewing the light produced by the Xenon flash lamp used in this product. Always turn the xenon flash lamp off before removing it.



---

**Cleaning the Detector**

The detector case should be kept clean. Cleaning should be done with a soft cloth slightly dampened with water or a solution of water and mild detergent. Do not use an excessively damp cloth allowing liquid to drip into the detector.

**WARNING**

Do not let liquid drip into the detector. It could cause shock hazard and it could damage the detector.

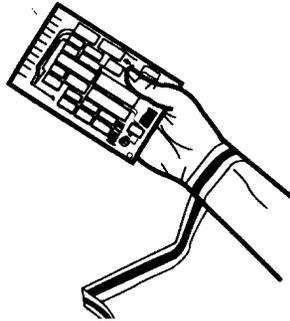
---

## 5 Repairing the Fluorescence Detector

### Using the ESD Strap

Electronic boards are sensitive to electronic discharge (ESD). In order to prevent damage, always use an ESD strap supplied in the standard accessory kit (see “[Accessory Kit](#)” on page 216) when handling electronic boards and components.

- 1 Unwrap the first two folds of the band and wrap the exposed adhesive side firmly around your wrist.
- 2 Unroll the rest of the band and peel the liner from the copper foil at the opposite end.
- 3 Attach the copper foil to a convenient and exposed electrical ground.



**Figure 54** Using the ESD Strap

## Overview of the Repairing of the Fluorescence Detector

Figure 55 shows the main assemblies and their locations.

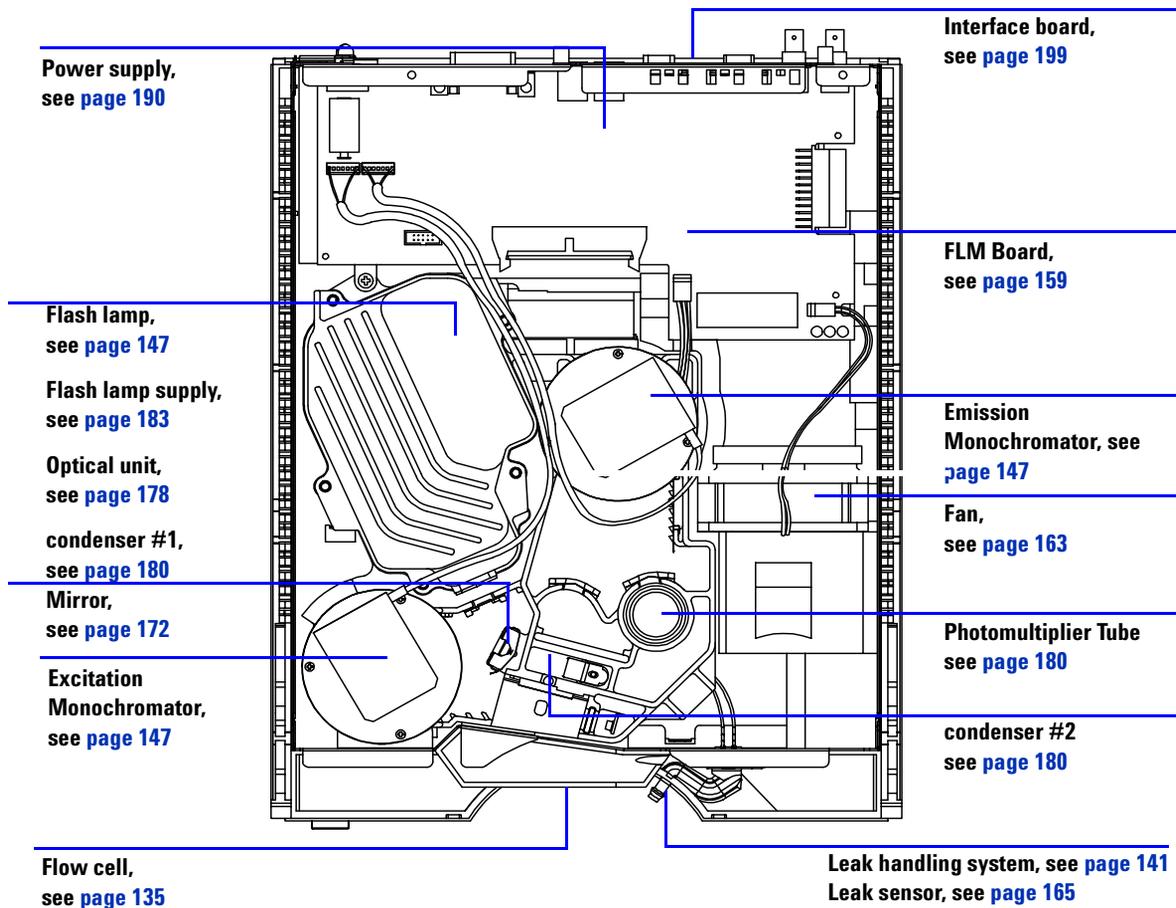


Figure 55 Main Assemblies

## Simple Repairs

On the following pages repairs are described that can be carried out without opening the main cover.

**Table 23** Simple Repairs

Procedure	Typical Frequency	Notes
Flow cell exchange	If application requires a different flow cell type or if defective.	Complete Assembly A wavelength calibration check should be performed after replacement.  If the flow cell is removed and inserted, then a quick calibration check is performed. If this fails, you must do a wavelength recalibration, see <a href="#">"Wavelength Verification and Calibration"</a> on page 101.
Flow cell flushing	If flow cell is contaminated	
Leak sensor drying	If leak has occurred.	Check for leaks.
Leak handling System replacement	If broken or corroded.	Check for leaks.

## Exchanging a Flow Cell

<b>When required</b>	If an application needs a different type of flow cell or the flow cell is defective (leaky).
<b>Tools required</b>	Two 1/4 inch wrenches for capillary connections
<b>Parts required</b>	Standard flow cell, 8 $\mu$ l, 20 bar, G1321-60005 Cuvette for off-line measurements, 8 $\mu$ l, 20 bar, G1321-60007, refer to <a href="#">"How to use the Cuvette"</a> on page 138 for more information on usage.

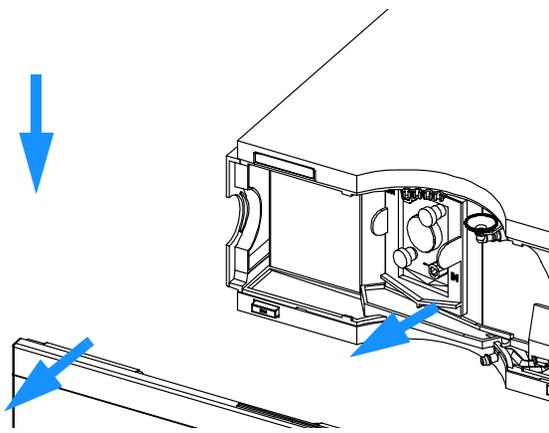
**NOTE**

DO NOT install the inlet capillary to the outlet connection of the flow cell. This will result in poor performance.

**Preparations for this procedure:**

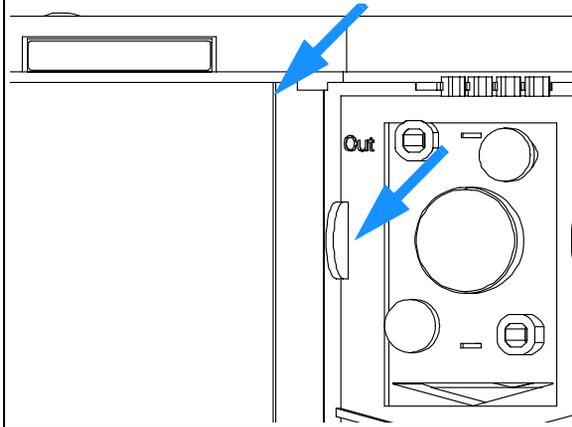
- Turn off the flow.

**1** Press the release buttons and remove the front cover for access to the flow cell area.

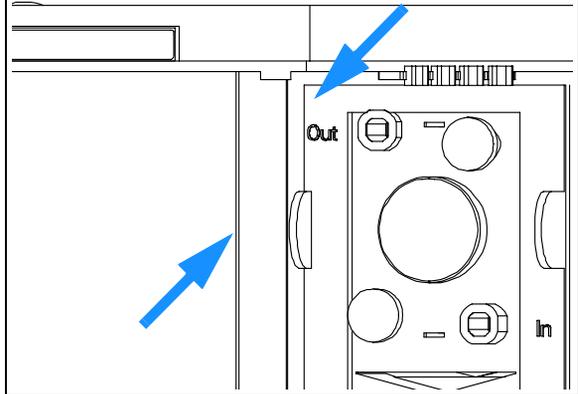


## 5 Repairing the Fluorescence Detector

**2** Disconnect the capillaries from the flow cell.



**3** Unscrew the thumb screws and pull the flow cell out of the compartment.

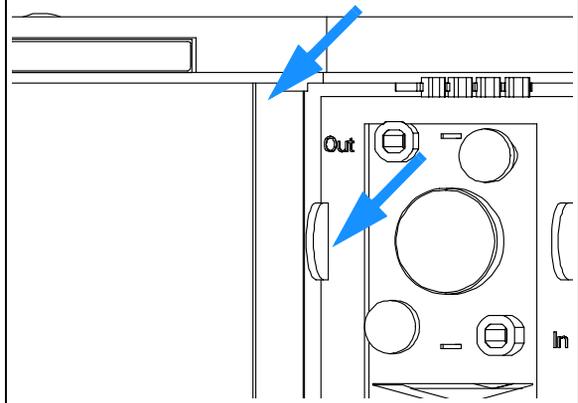


**Note:**

The label attached to the flow cell provides information on part number, cell volume and maximum pressure. The cell type will be automatically detected.

There are no parts that can be replaced on the flow cell. If defective (leaky) the flow cell has to be replaced completely.

**4** Insert the flow cell and tighten the thumb screws. Reconnect the capillaries to the flow cell. **DO NOT** install the inlet capillary to the outlet connection of the flow cell. This will result in poor performance or damage.



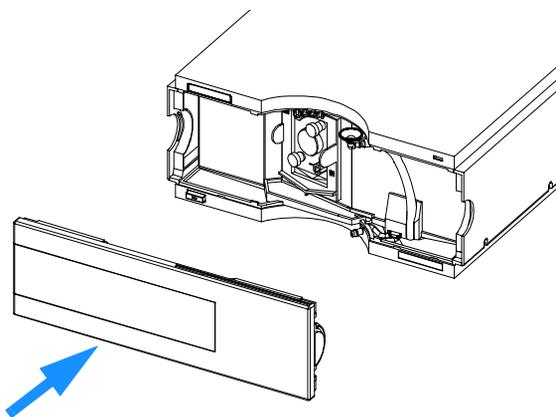
**Note:**

If an additional detector is added to the system, the fluorescence detector should be the last detector in the flow path except for evaporative detectors, like LC-MSD. Otherwise the back pressure generated by the other detector may overload the quartz flow cell and will lead to a defective cell (maximum pressure is 20 bar (2 MPa)).

Always use the outlet capillary set supplied with the accessory kit.

**Note:**

To check for leaks, establish a flow and observe the flow cell (outside of the cell compartment) and all capillary connections.

**5** Replace the front cover.**Note:**

Perform a wavelength verification to check the correct positioning of the flow cell, as described in chapter "[Wavelength Verification and Calibration](#)" on page 101.

### How to use the Cuvette

The cuvette is used for off-line measurements (no flow system required) and is basically a standard flow cell with a few changes:

- wide bore capillary connections for easier injections with a syringe
- identification lever for cell auto-recognition system.

- 1 Install the cuvette instead of the standard flow cell.
- 2 Connect the waste tubing to the outlet of the cuvette.
- 3 Use the syringe (see [“Cuvette Kit”](#) on page 214) to inject the compound.
- 4 Setup the parameters for the Fluorescence Scan (under Special Setpoints).
- 5 Select “Take Fluorescence Scan” on the user-interface to start the off-line measurement.

## Flow Cell Flushing

<b>When required</b>	If flow cell is contaminated
<b>Tools required</b>	Glass syringe, adapter
<b>Parts required</b>	Bidistilled water, nitric acid (65%), tubings to waste

### NOTE

Aqueous solvents in the flow cell can built up algae. Algae do fluoresce. Therefore do not leave aqueous solvents in the flow cell for longer periods. Add a small percentage of organic solvents (e.g. Acetonitrile or Methanol ~5%).

---

In case the cell is contaminated, follow the procedure below.

### Flushing Procedure

- 1 Flush with bidistilled water.
- 2 Flush with nitric acid (65%) using a glass syringe.
- 3 Leave this solution in the cell for about one hour.
- 4 Flush with bidistilled water.

### CAUTION

This concentration of nitric acid is dangerous and proper attention to safety should be given. Also the nitric acid flushing procedure is not an infallible remedy for a dirty cell. It is to be used as a last attempt to salvage the cell before cell replacement. Note that the cell is a consumable item.

---

### NOTE

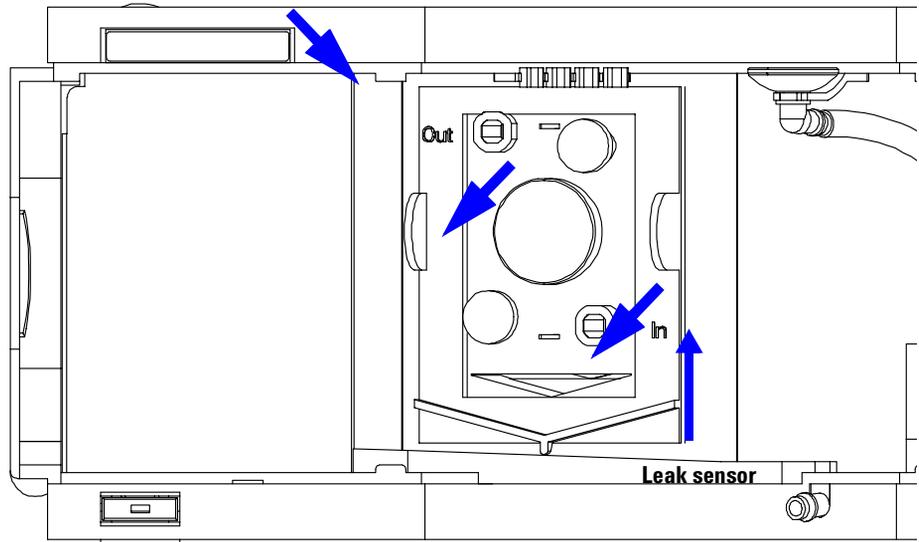
Do not exceed the pressure limit of 20 bar (0.2 MPa).

---

## Correcting Leaks

<b>When required</b>	If a leakage has occurred in the flow cell area or at the capillary connections
<b>Tools required</b>	Tissue Two 1/4 inch wrenches for capillary connections
<b>Parts required</b>	None

- 1 Remove the front cover.
- 2 Use tissue to dry the leak sensor area and the leak pan.
- 3 Observe the capillary connections and the flow cell area for leaks and correct, if required.
- 4 Replace the front cover.

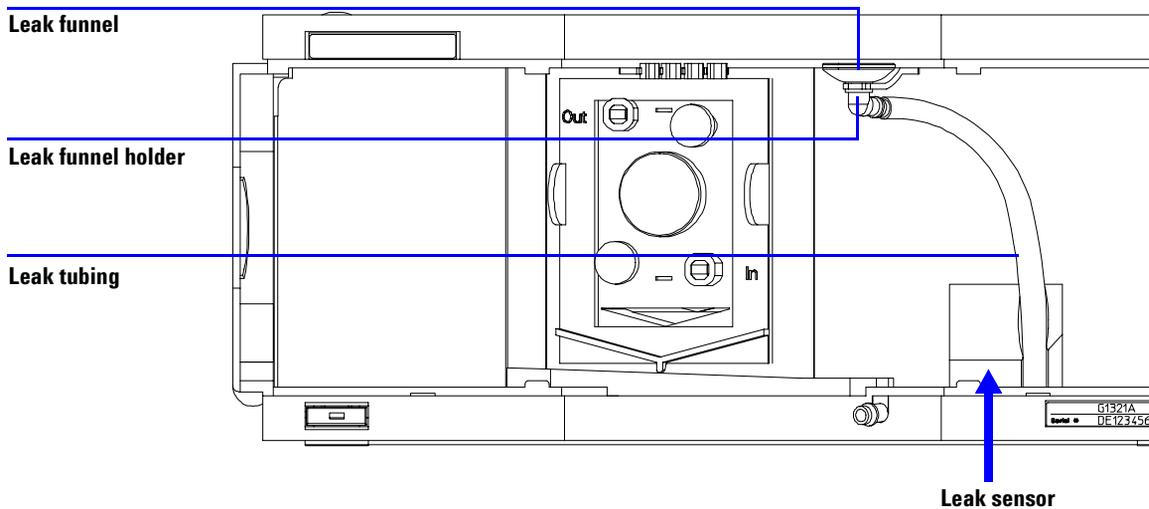


**Figure 56** Observing for Leaks

## Replacing Leak Handling System Parts

<b>When required</b>	If the parts are corroded or broken
<b>Tools required</b>	None
<b>Parts required</b>	Leak funnel 5061-3356
	Leak funnel holder 5041-8389
	Leak tubing (120 mm) 0890-1711

- 1 Remove the front cover.
- 2 Pull the leak funnel out of the leak funnel holder.
- 3 Pull out the leak funnel with the tubing.
- 4 Insert the leak funnel with the tubing in its position.
- 5 Insert the leak funnel into the leak funnel holder.
- 6 Replace the front cover.



**Figure 57** Replacing Leak Handling System Parts

## Exchanging Internal Parts

### WARNING

The following procedures require opening the main cover of the detector. Always ensure the detector is disconnected from the line power when the main cover is removed. The security lever at the power input socket prevents the detector cover from being removed when line power is still connected.

---

### WARNING

To disconnect the detector from line, unplug the power cord. The power supply still uses some power, even if the switch on the front panel is turned off.

When working with solvents please observe appropriate safety procedures (for example, goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet supplied by the solvent vendor, especially when toxic or hazardous solvents are used.

---

### NOTE

Always operate the detector with the top covers in place.

---

### CAUTION

There will be a risk of damaging hardware due to overheating when operating the instrument without covers.

---

### CAUTION

Electronic boards and components are sensitive to electronic discharge (ESD). In order to prevent damage always use an ESD protection (for example, the ESD wrist strap from the accessory kit) when handling electronic boards and components, see ["Using the ESD Strap"](#) on page 132.

---

**WARNING**

Eye damage may result from directly viewing the light produced by the xenon flash lamp used in this product.



## 5 Repairing the Fluorescence Detector

### Removing the Covers

**When required**  
**Tools required**

For all repairs inside the detector  
Screwdriver POZI 1 PT3

**Parts required**

Depends on the work inside and the following procedures

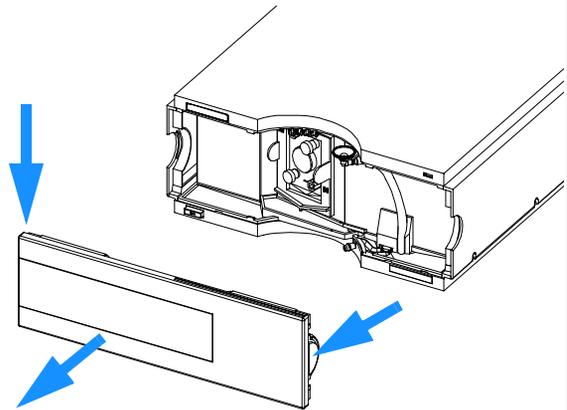
#### CAUTION

There will be a risk of damaging hardware due to overheating when operating the instrument without covers.

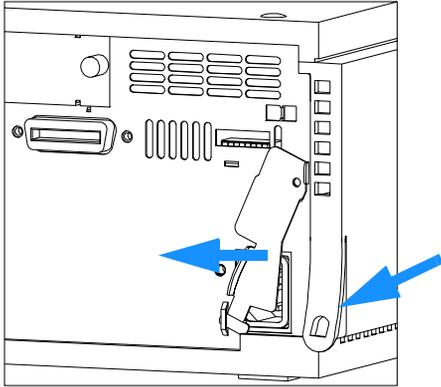
#### Preparations for this procedure:

- Turn off the detector.
- Disconnect the power cable.
- Remove the flow cell.
- Remove detector from stack and place it on the workbench.

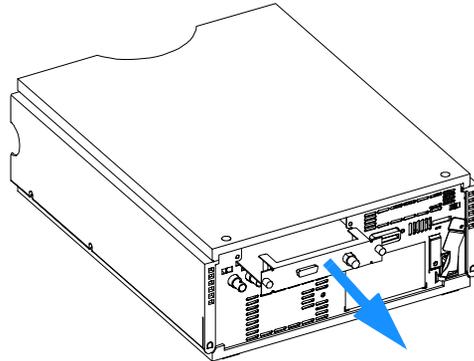
- 1 Press the release buttons and remove the front cover. Unclip and remove the waste funnel assembly.



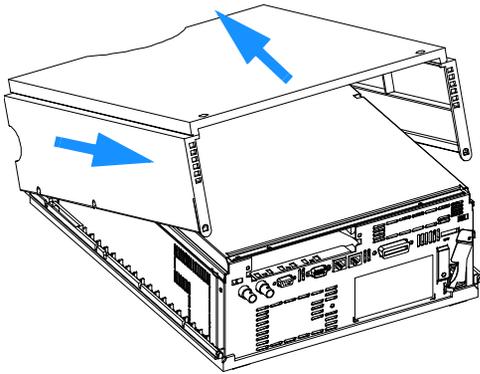
**2** Install the ESD strap. Move the power lock across the power inlet and lift the clips on the rear of the cover.



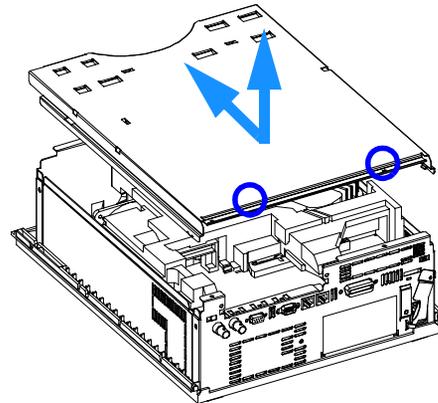
**3** If installed, unscrew and remove the interface board. Place the board on the ESD kit.



**4** Lift the cover up and slide it towards the rear.



**5** Unscrew the screws at the rear of the top plate. Slide the plate towards the front and remove it.



**Warning:**

**Do not connect a power plug to the detector module after removing the top covers.**

## 5 Repairing the Fluorescence Detector

### Removing the Rear Foam

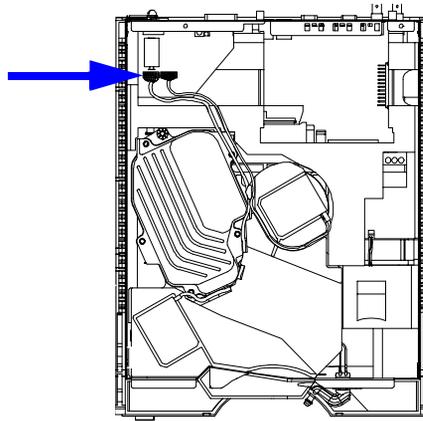
**When required**  
**Parts required**

For all repairs inside the detector  
Depends on the work inside and the following procedures

#### Preparations for this procedure:

- Start with "Removing the Covers" on page 144.

**1** Using a pliers disconnect the cables from the main board.



**2** Carefully remove the rear foam.

## Exchanging the Lamp

**When required  
Tools required**

Lamp: if noise or intensity exceeds limits or lamp does not ignite  
Screwdriver POZI 1 PT3  
Hexagonal screwdriver 2.5 mm, 100 mm long  
Hexagonal screwdriver 4mm, 100 mm long

**Parts required**

Flash lamp 2140-0600

**WARNING**

**Wait until the lamp has cooled down before touching the lamp and/or triggerpack assembly.**

**The instrument should not be operated without Xenon flash lamp. Otherwise the Triggerpack electronics will be damaged.**

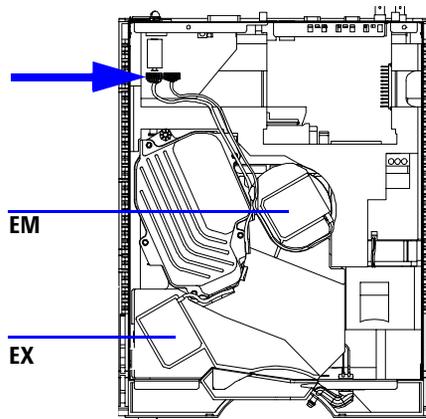
**NOTE**

When loosening or removing screws take care that they do not fall into the instrument. This may result in a complete removal of other assemblies.

**Preparations for this procedure:**

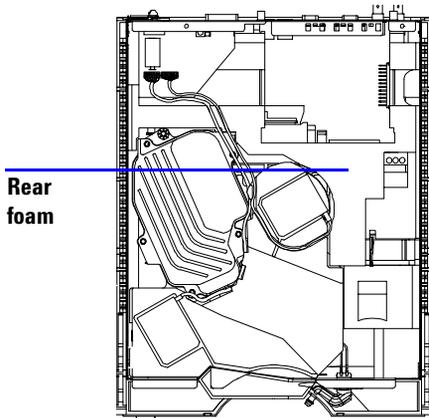
- Turn off the detector.
- Disconnect the power cable.
- Remove the flow cell or disconnect the tubings from the flow cell.
- Remove detector from stack and place it on the workbench.
- Remove the covers as described in "Removing the Covers" on page 144.

**1** Disconnect the emission and the excitation cables and from the detector main board.



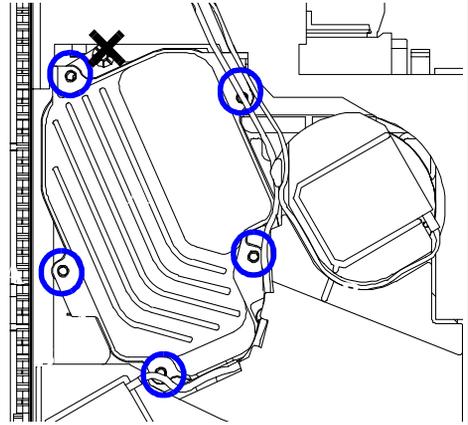
## 5 Repairing the Fluorescence Detector

2 Carefully remove the rear foam.



Rear  
foam

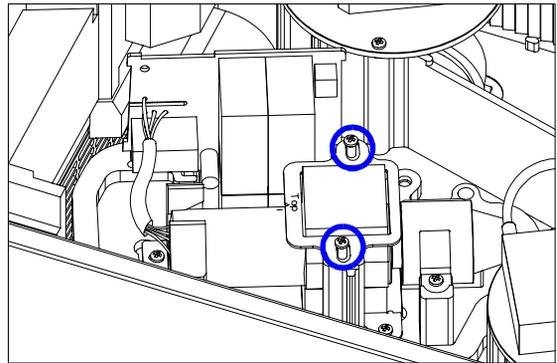
3 Using the 4 mm hexagonal key, unscrew the five screws, remove the cover and place it on the bench without turning it around.



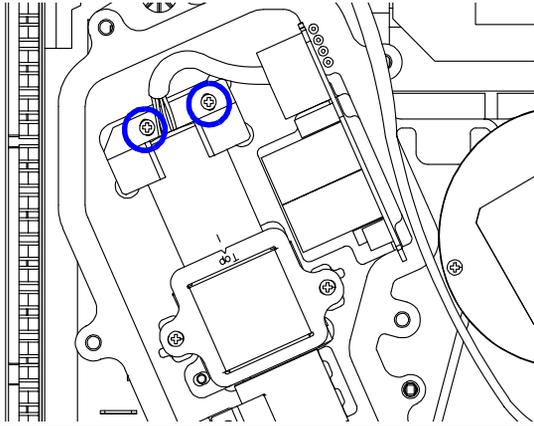
**Note:**

**DO NOT** unscrew the grounding screw (marked with X)

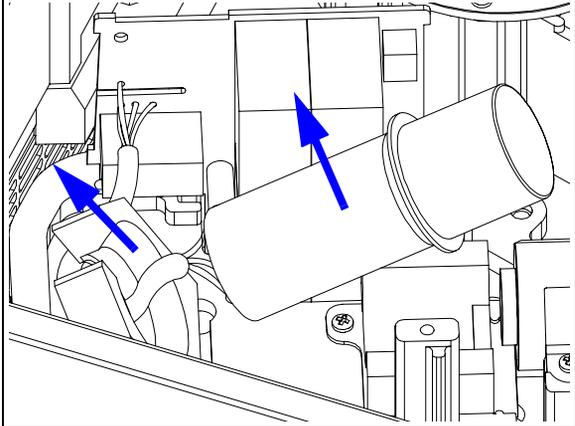
4 Using the 2.5 mm hexagonal key, unscrew the two screws and remove the brass pad together with its holder.



**5** Loosen the screws of the trigger pack holder a few turns.



**6** Tip the holder to release the lamp and trigger pack. Slide the cables of the trigger pack through the holder.



**WARNING:**

**While pulling off the lamp be careful that you do not scrape your skin on the metal plates.**

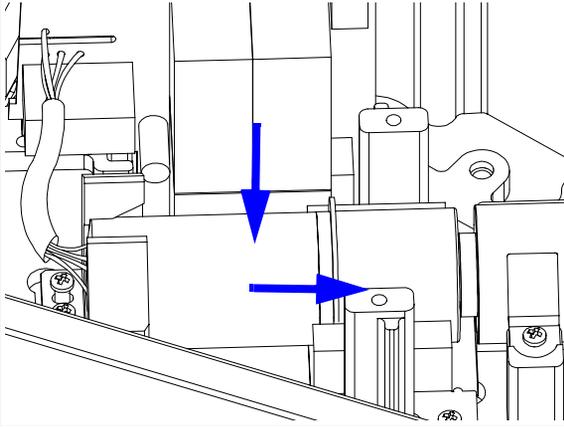
**Do not remove the bottom pad. Otherwise the lamp will lose its correct position during reinstallation.**

## 5 Repairing the Fluorescence Detector

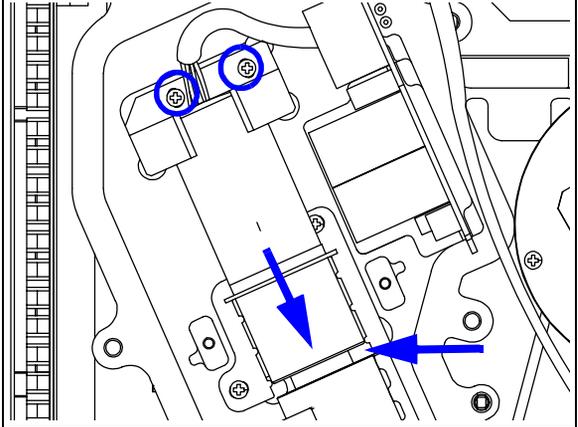
**7** Remove the lamp carefully from the trigger pack..

**8** Carefully insert the new lamp completely into the trigger pack and slide the cables through the holder..

**9** Place the lamp on the bottom pad.



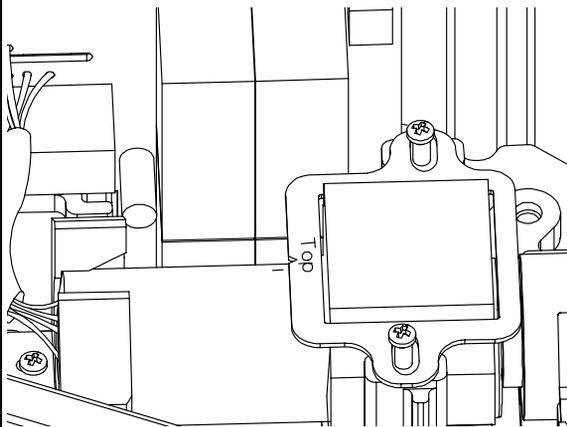
**10** Refit the trigger pack holder by moving the lamp towards the condenser. Leave a distance of about 0.5 mm between each other. Refit the screws.



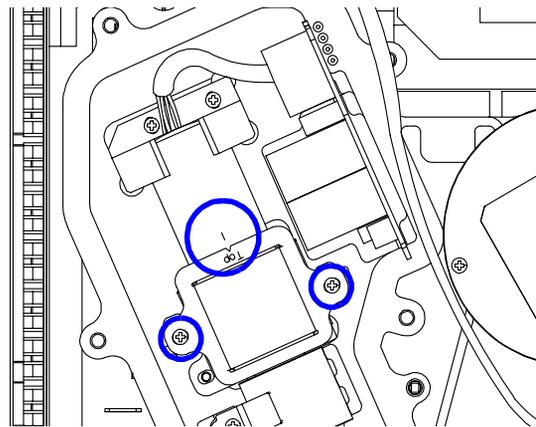
**11** Place the pad with the holder on the stand-offs. The "TOP" sign should be directed towards the trigger pack. Refit the screws.

**Note:**

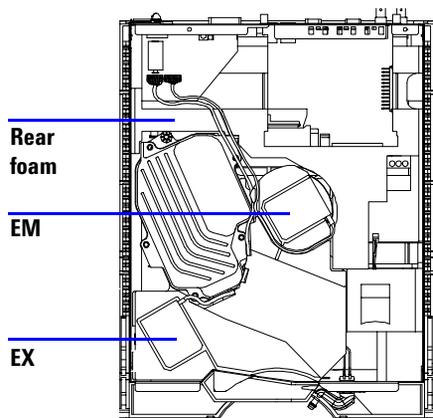
**During the next step no horizontal movement should be made to the pad.**



**12** While pressing the top pad down onto the stand-offs, tighten the screws. Turn the trigger pack such as its marker stays in line with the "TOP" sign of the brass holder.



**13** Replace the lamp housing cover and fix the screws. Carefully insert the rear foam. Reconnect the monochromator cables to the main board. Left is excitation, right is emission.



**Next Steps:**

- Replace the covers as described in "Replacing the Foam and the Top Cover" on page 149.
- Re-install the detector in the stack.
- Enter the replacement of the lamp in the maintenance logbook (this will reset the lamp counter).
- Turn the lamp on.
- Perform a wavelength verification to check the correct positioning of the replaced assemblies, as described in chapter "Wavelength Verification and Calibration" on page 101.

## 5 Repairing the Fluorescence Detector

### Exchanging the EX-condenser

<b>When required</b>	Condenser: if contaminated
<b>Tools required</b>	Screwdriver POZI 1 PT3 Hexagonal screwdriver 2.5 mm, 100 mm long Hexagonal screwdriver 4 mm, 100 mm long
<b>Parts required</b>	EX condenser Assembly 1000-1136

#### WARNING

Wait until the lamp has cooled down before touching the lamp and/or triggerpack assembly.

The instrument should not be operated without Xenon flash lamp. Otherwise the Triggerpack electronics will be damaged.

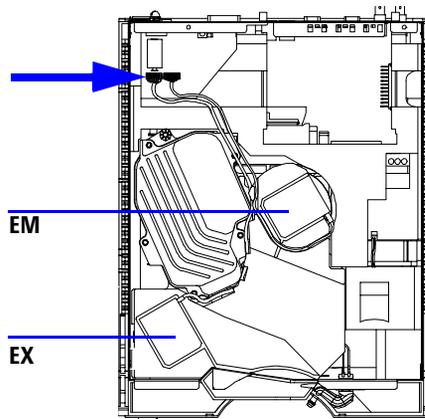
#### NOTE

When loosening or removing screws take care that they do not fall into the instrument. This may result in a complete removal of other assemblies.

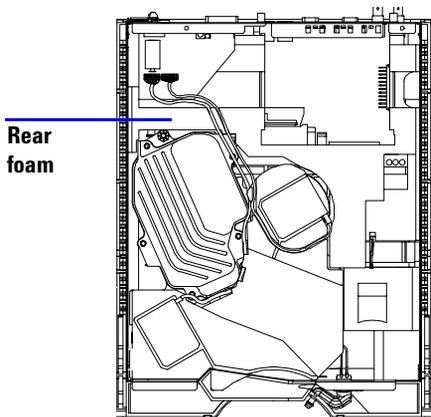
#### Preparations for this procedure:

- Turn off the detector.
- Disconnect the power cable.
- Remove the flow cell or disconnect the tubings from the flow cell.
- Remove detector from stack and place it on the workbench.
- Remove the covers as described in "Removing the Covers" on page 144.

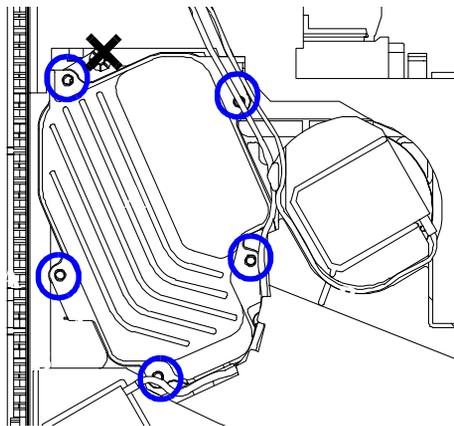
- 1 Disconnect the emission and the excitation cables and from the detector main board.



2 Carefully remove the rear foam.



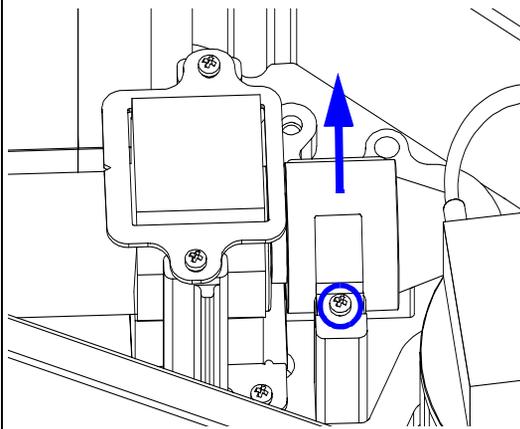
3 Using the 4 mm hexagonal key, unscrew the five screws, remove the cover and place it on the bench without turning it around.



**Note:**

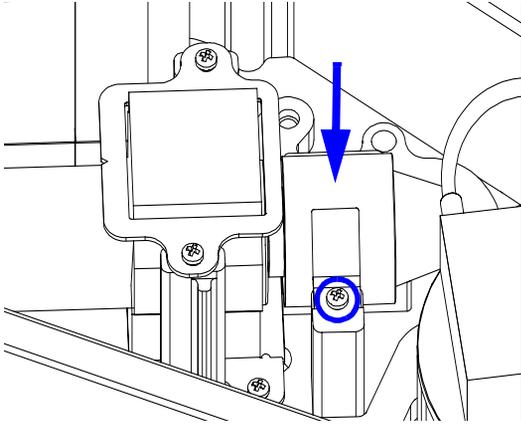
**DO NOT** unscrew the grounding screw (marked with X)

4 Using a 2.5 mm hexagonal key, loosen the screw and remove the condenser. There is a mechanical stop for the condenser.

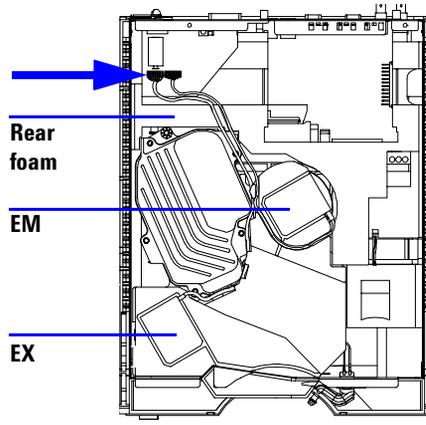


## 5 Repairing the Fluorescence Detector

**5** Insert the new or cleaned condenser, shift it completely to its mechanical stop and fit the screw.



**6** Replace the lamp housing cover and fix the screws. Carefully insert the rear foam. Reconnect the monochromator cables to the main board. Left is excitation, right is emission.



### Next Steps:

- Replace the covers as described in "Replacing the Foam and the Top Cover" on page 149.
- Re-install the detector in the stack.
- Enter the replacement of the lamp in the maintenance logbook (this will reset the lamp counter).
- Turn the lamp on.
- Perform a wavelength verification to check the correct positioning of the replaced assemblies, as described in chapter "[Wavelength Verification and Calibration](#)" on page 101.

## Replacing the Lamp Cover Window

<b>When required</b>	If window is blocking the light throughput due to contamination
<b>Tools required</b>	Screwdriver POZI 1 PT3 Hexagonal screwdriver 2.5 mm, 100 mm long Hexagonal screwdriver 4 mm, 100 mm long
<b>Parts required</b>	Quartz Window 1000-0999

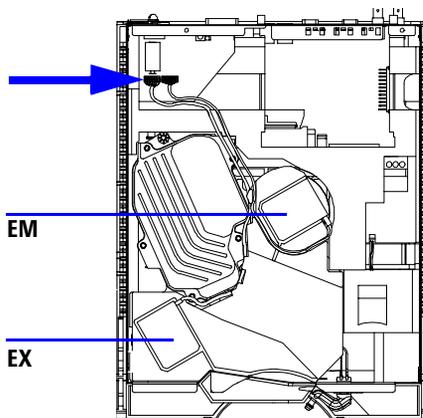
### NOTE

When loosening or removing screws take care that they do not fall into the instrument. This may result in a complete removal of other assemblies.

#### Preparations for this procedure:

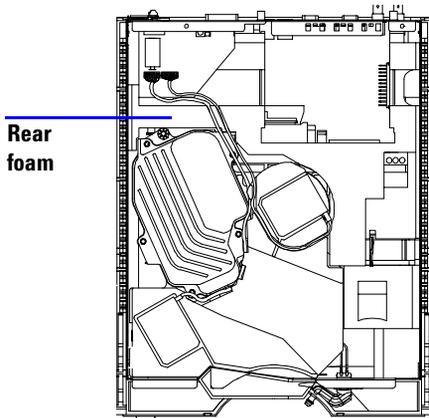
- Turn off the detector.
- Disconnect the power cable.
- Remove the flow cell or disconnect the tubings from the flow cell.
- Remove detector from stack and place it on the workbench.
- Remove the covers as described in "Removing the Covers" on page 144.

- 1 Disconnect the emission and the excitation cables and from the detector main board.

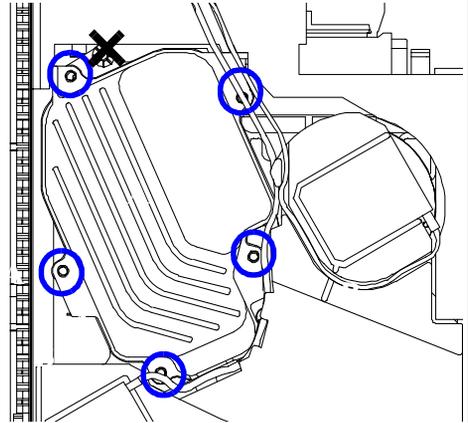


## 5 Repairing the Fluorescence Detector

2 Carefully remove the rear foam.



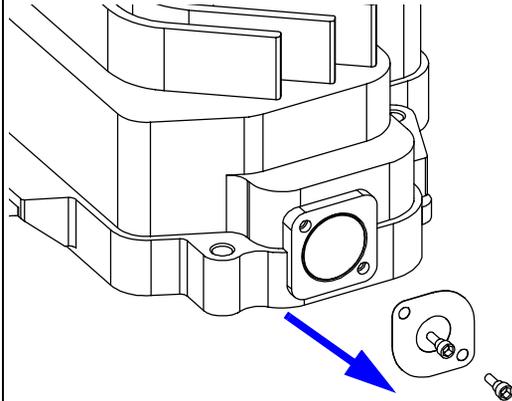
3 Using the 4 mm hexagonal key, unscrew the five screws, remove the cover and place it on the bench without turning it around.



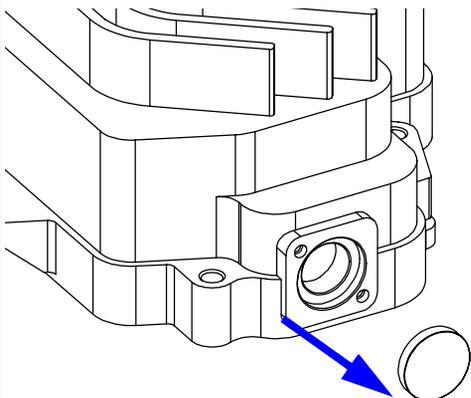
**Note:**

**DO NOT** unscrew the grounding screw (marked with X).

4 Using the 2.5 mm hexagonal key, unscrew one screw and loosen the other one. Slide the window holder out of the window area.



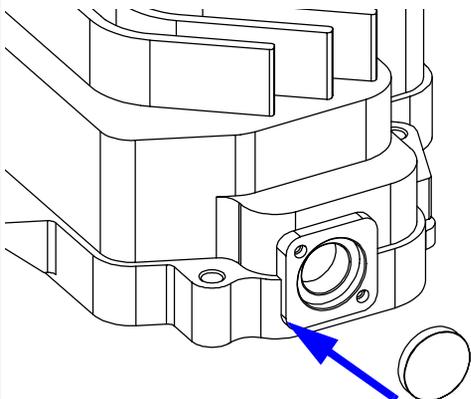
**5** Use a toothpick to remove the window.



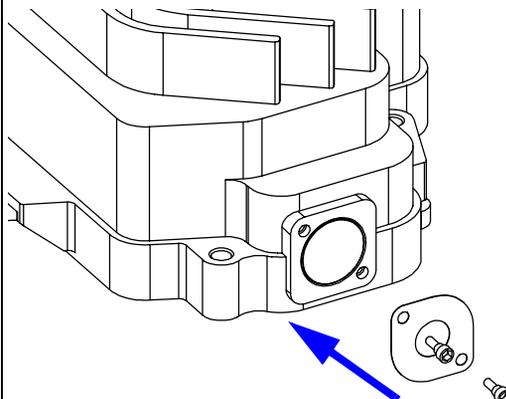
**Note:**

The quartz window may be cleaned with alcohol (e.g. ethanol).

**6** Insert the cleaned or new window.

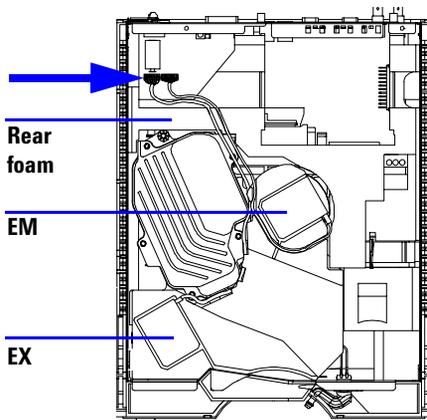


**7** Slide the window holder across the window and fix the screws.



## 5 Repairing the Fluorescence Detector

**8** Replace the lamp housing cover and fix the screws. Carefully insert the rear foam. Reconnect the monochromator cables to the main board. Left is excitation, right is emission.



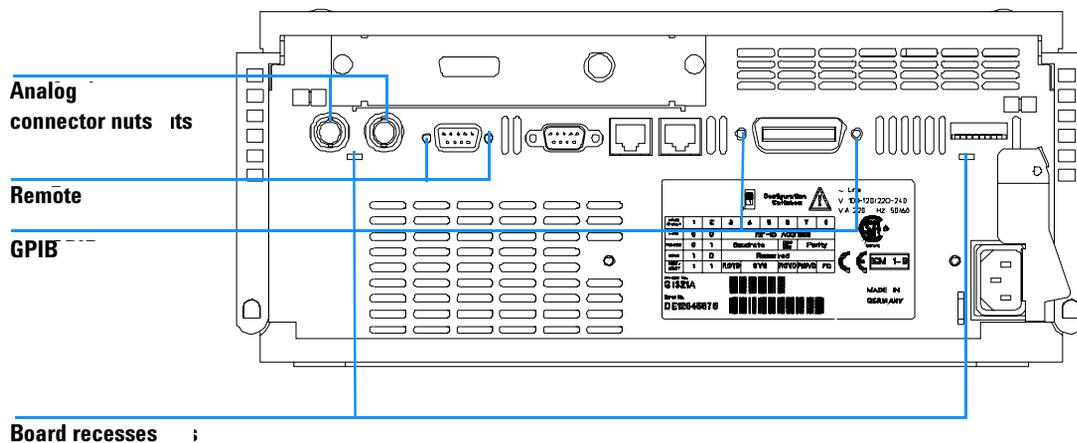
### Next Steps:

- Replace the covers as described in “Replacing the Foam and the Top Cover” on page 149.
- Re-install the detector in the stack.
- Turn the lamp on.

## Exchanging the Processor Board

<b>When required</b>	If detector main board is defective or for repair on other assemblies
<b>Tools required</b>	Screwdriver POZI 1 PT3 Flat screw driver Hexagonal wrenches 5 mm, 7 mm and 15 mm
<b>Parts required</b>	Detector main board (FLM) G1321-69500 (exchange assembly)

- 1 Turn off the lamp.
- 2 Switch off the module, and disconnect the cables.
- 3 Remove module from stack and place it on the workbench.



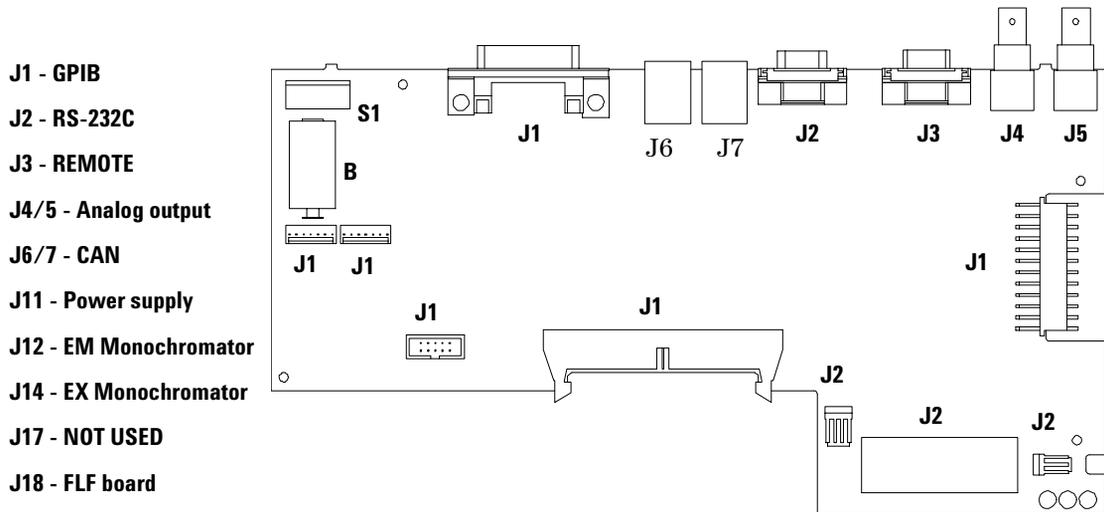
**Figure 58** Unscrew Connectors from Board

- 4 Remove the front cover, top cover and top foam section, see [“Removing the Covers”](#) on page 144.
- 5 Use a 5-mm and 7-mm wrench to unscrew the REMOTE and the GPIB connector and a 15-mm wrench to unscrew the nuts of the ANALOG connectors.
- 6 Disconnect all connectors from the processor board.

## 5 Repairing the Fluorescence Detector

### NOTE

When removing connectors, counter-hold with one hand on connector J12.



**Figure 59** Location of Connectors on FLM Board

- 7 Remove the processor board. Place the board on the ESD kit.
- 8 On the new board check the switch setting of address switch S1, see ["Setting the 8-bit Configuration Switch"](#) on page 290.

### NOTE

An incorrect switch setting (for example, TEST/BOOT) may cause the module to revert to a basic mode (yellow or red flashing status light). In this case turn off the module, reset the address switches, and turn on the module again.

- 9 Install the new processor board and reconnect the connectors. Assure that the board is fitted correctly into the board recess in the rear panel.
- 10 Refit the screws at the REMOTE and GPIB connectors and the nuts of the ANALOG connectors.

- 11 Reinstall the top foam section, top cover and front cover, see [“Replacing the Foams and Covers”](#) on page 196.
- 12 Replace detector into the stack and reconnect the cables.

**NOTE**

If a new FLM board is installed, update the serial number information of the detector in the user interface, see procedure below.

---

- 13 Check the firmware revision of the module. If it is older than the current firmware revision, update the firmware using the standard firmware update procedure, see [“Replacing the Detector’s Firmware”](#) on page 200.

**Entering the Serial Number using the Control Module**

- 1 Connect the control module to the detector. Turn on the detector.
- 2 In the control module, press System (F5), then Records (F4). Using the up/down arrows, make sure that the detector is highlighted.
- 3 Press FW Update (F5). Now, press the m key. This will display a box which says Update Enter Serial#.
- 4 Press Enter. This will display the box labeled Serial#.
- 5 Alphabetic characters and numbers are created using the up and down arrows. In the box labeled Serial#, enter the 10-character serial number for the detector. When the 10-character serial number is entered, press Enter to highlight the complete serial number. Then, press Done (F6).
- 6 Turn the detector off, then on again. The Records screen should display the correct serial number for this module.
- 7 If a Agilent ChemStation is also connected, restart the Agilent ChemStation now as well.

### Entering the Serial Number using the Agilent ChemStation

Module serial numbers are entered by typing specific commands in the command line at the bottom of the main user interface screen.

- 1 To enter a module serial number, type the following command in the command line:

```
print sendmodule$(lfld, "ser YYYYYYYYYY")
```

Where: YYYYYYYYYY is the 10-character serial number of the module in question.

#### NOTE

The first two characters are letters, which should be capitalized.

---

The reply line will respond with RA 0000 SER followed by the module serial number you just entered.

- 2 Turn off the detector, then on again and restart the Agilent ChemStation. If the serial number you have just entered is different than the original module serial number, you will be given the opportunity to edit the configure 1100 access screen during the restart of the Agilent ChemStation.
- 3 After start up, the serial number you have just entered can be seen under the Instrument menu of the main user interface screen. The serial number of the detector can also be seen by typing the following command into the command line:

```
print sendmodule$ (lfld, "ser?")
```

The reply line will give the module serial number.

## Exchanging the Fan

**When required** If the fan is defective or noisy  
**Tools required** Screwdriver POZI 1 PT3

**Parts required** Fan assembly 3160-1017

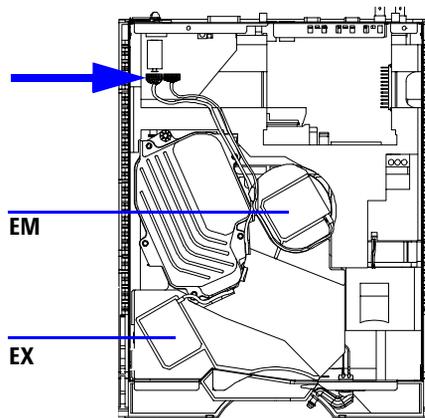
### CAUTION

The fan must be installed in the correct orientation to ensure optimum cooling and operation of the detector.

#### Preparations for this procedure:

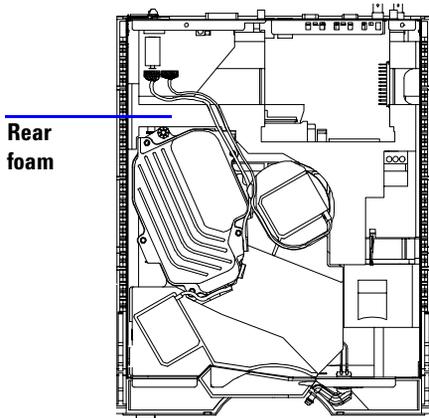
- Turn off the detector.
- Disconnect the power cable.
- Remove the flow cell.
- Remove detector from stack and place it on the workbench.
- Remove the covers as described in ["Removing the Covers"](#) on page 144.

- 1 Disconnect the emission and the excitation cables and from the detector main board.

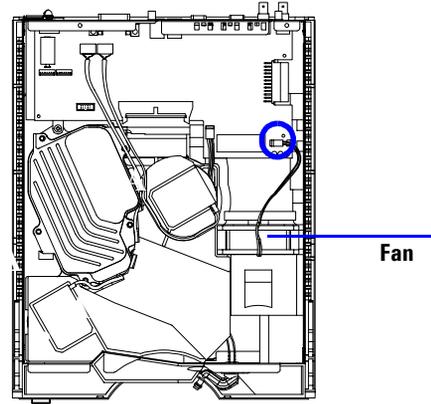


## 5 Repairing the Fluorescence Detector

2 Carefully remove the rear foam.



3 Lift the main board slightly. Bring the fan cable up and disconnect the fan cable. Pull out the fan.

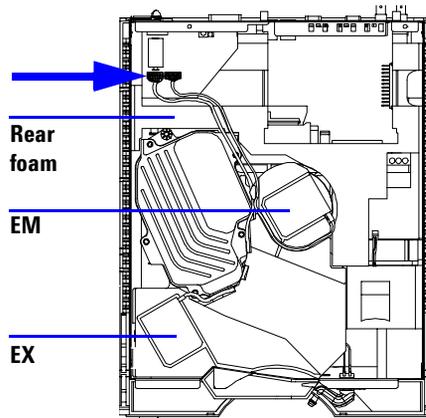


4 When the fan is inserted assure that the air flow is from the rear towards the front of the detector.

Insert the fan.

Lift the main board slightly. Reconnect the fan cable and route it underneath the board.

5 Carefully insert the rear foam. Reconnect the monochromator cables to the main board. Left is excitation, right is emission.



### Next Steps:

- Replace the covers as described in "Replacing the Foam and the Top Cover" on page149.
- Re-install the detector in the stack.

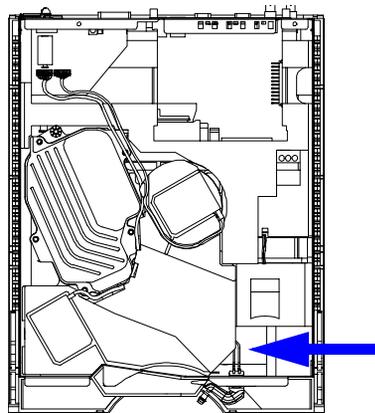
## Exchanging the Leak Sensor or Leak Panel

<b>When required</b>	If defective
<b>Tools required</b>	Screwdriver POZI 1 PT3 Flat screwdriver
<b>Parts required</b>	Leak sensor assembly 5061-3356 Leak Pan G1321-45501

### Preparations for this procedure:

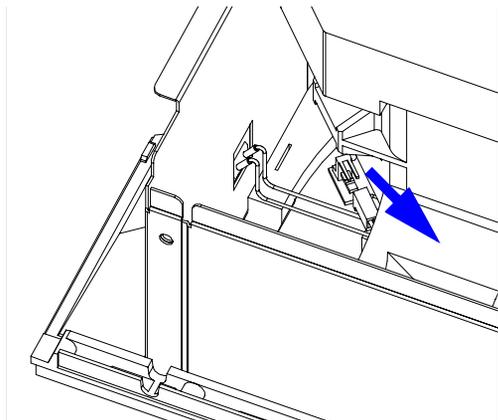
- Turn off the detector.
- Disconnect the power cable.
- Remove the flow cell.
- Remove detector from stack and place it on the workbench.
- Remove the covers as described in ["Removing the Covers"](#) on page 144.
- Remove the flow cell.

### 1 Locate the leak sensor cable.

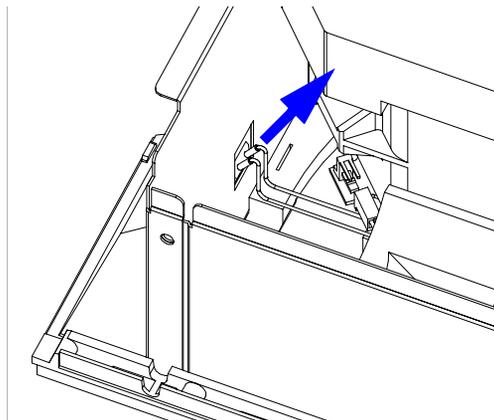


## 5 Repairing the Fluorescence Detector

**2** Disconnect the leak sensor cable using your left hand.



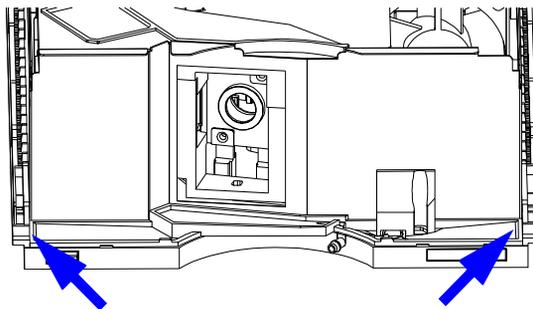
**3** Pull out the leak sensor.



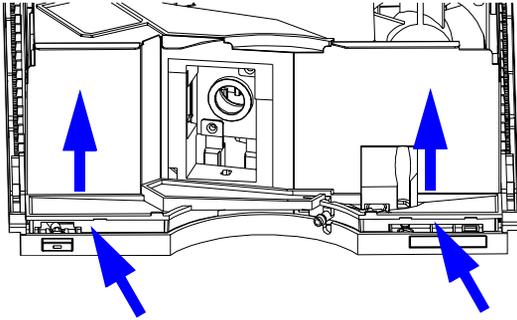
**Note:**

If you want to replace just the leak sensor, continue with [step 8](#).

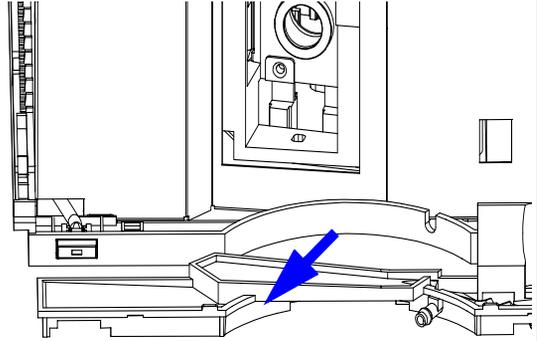
**4** Using flat screw driver, release the leak base (two clasps).



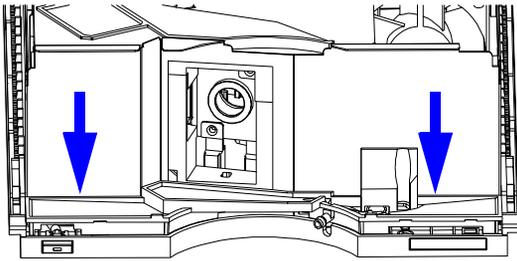
**5** Use a flat screw driver to lift the leak base from the base.



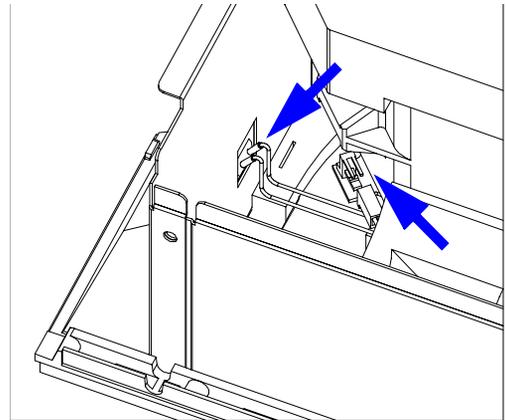
**6** Remove the leak base completely.



**7** Replace the leak pan into the cabinet bottom. Press the leak pan completely down into its recess.



**8** Replace the leak sensor using your left hand and reconnect the connector.



**Next Steps:**

- Replace the covers as described in "Replacing the Foams and Covers" on page 196.
- Re-install the flow cell and the front panel
- Re-install the detector in the stack.

## 5 Repairing the Fluorescence Detector

### Replacing the EM-Condenser or Cutoff-Filter

**When required**  
**Tools required**

If light throughput is too low  
Screwdriver POZI 1 PT3  
Hexagonal key 2.5 mm

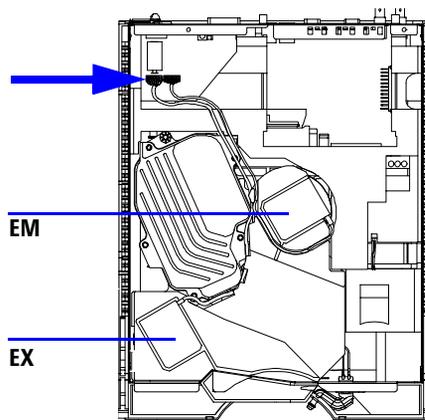
**Parts required**

Condenser assembly EM 1000-1124  
Cutoff filter 295 nm 5062-8530

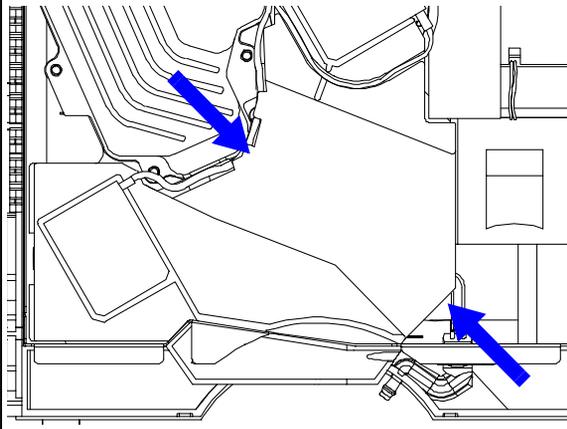
**Preparations for this procedure:**

- Turn off the detector.
- Disconnect the power cable.
- Remove the flow cell.
- Remove detector from stack and place it on the workbench.
- Remove the covers as described in ["Removing the Covers"](#) on page 144.
- Remove the flow cell.

- 1** Disconnect the emission and the excitation cables and from the detector main board.

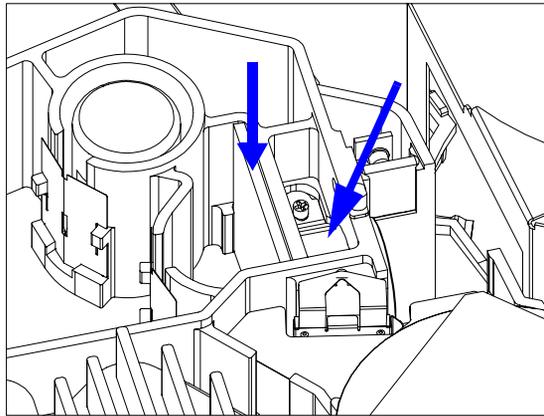


**2** Carefully remove the front foam holding the foam as shown.



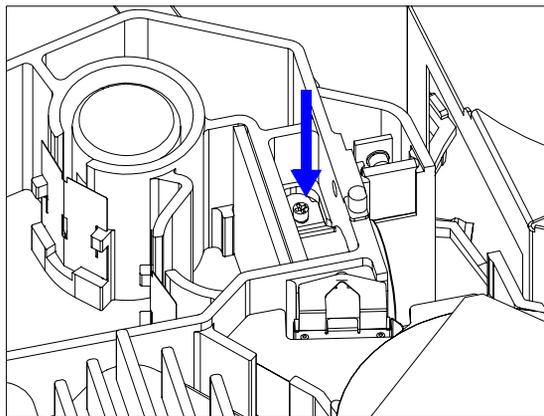
**Note:**

**Locate the condenser assembly and the cutoff filter.**



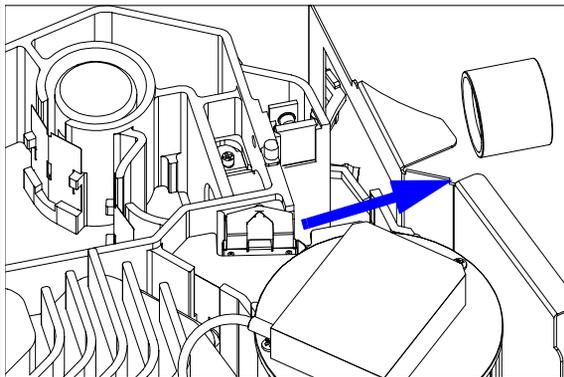
**3** The cutoff filter can be replaced just by sliding it out and back in.

**4** Use a 2.5 mm hexagonal key to loosen the screw.

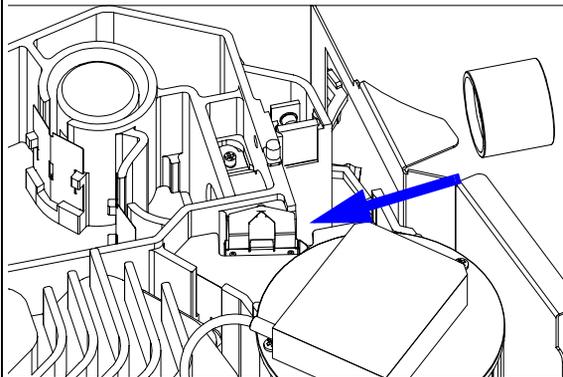


## 5 Repairing the Fluorescence Detector

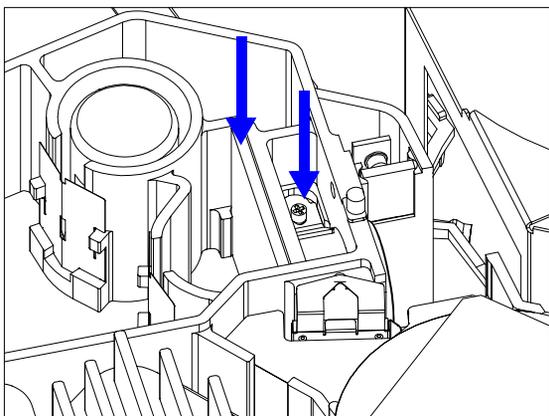
**5** Slide out the condenser assembly and place it in a safe location.



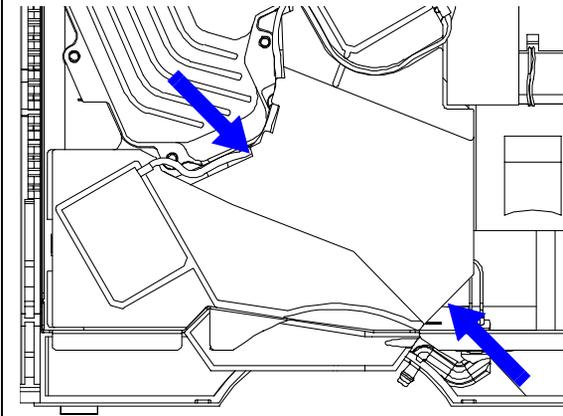
**6** Slide in the condenser assembly completely with the larger lens diameter towards the cutoff filter.



**7** Use a 2.5 mm hexagonal key to fix the screw. Replace the cutoff filter.



**8** Carefully replace the front foam.



**Next Steps:**

- Replace the covers as described in ["Replacing the Foams and Covers"](#) on page 196.
- Re-install the flow cell and the front panel.
- Re-install the detector in the stack.
- Turn the lamp on.
- Perform a wavelength verification to check the correct positioning of the replaced assemblies, as described in chapter ["Wavelength Verification and Calibration"](#) on page 101.

## 5 Repairing the Fluorescence Detector

### Replacing the Mirror Assembly

<b>When required</b>	If reflection is too low (contaminated)
<b>Tools required</b>	Screwdriver POZI 1 PT3 A pair of tweezers
<b>Parts required</b>	Mirror assembly 1000-1000

#### NOTE

DO NOT touch the surface of any optical component during this procedure. This will reduce the reflection efficiency.

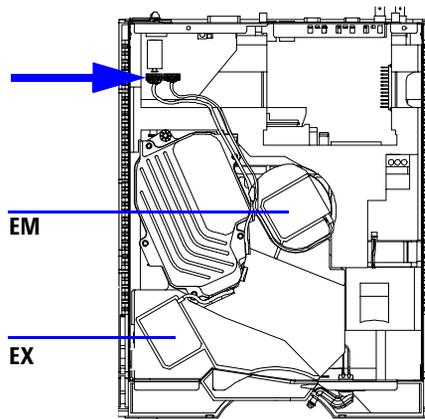
#### NOTE

One side of the mirror is marked "R" representing the rear side.

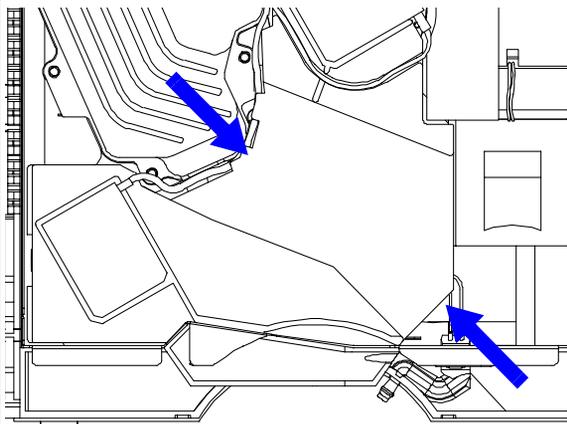
#### Preparations for this procedure:

- Turn off the detector.
- Disconnect the power cable.
- Remove the flow cell.
- Remove detector from stack and place it on the workbench.
- Remove the covers as described in "Removing the Covers" on page 144.

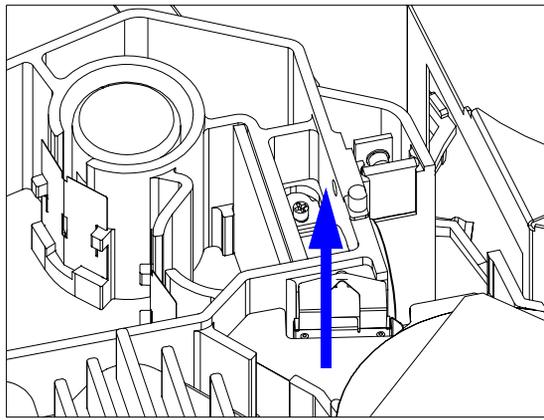
- 1 Disconnect the emission and the excitation cables and from the detector main board.



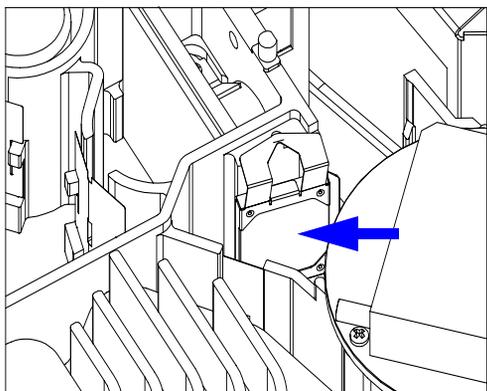
**2** Carefully remove the front foam holding the foam as shown.



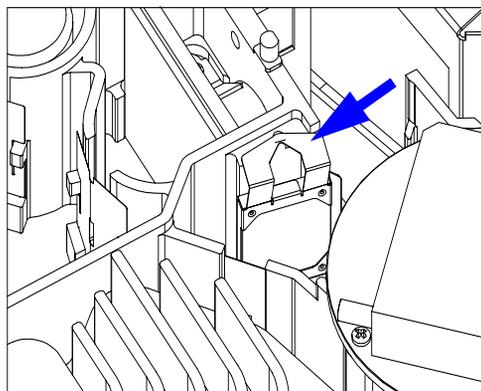
**3** Remove the UV protector.



**4** Locate the mirror assembly.

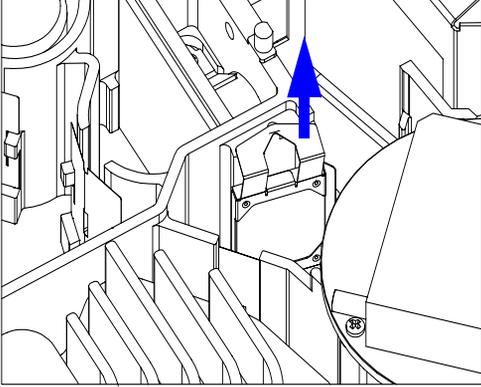


**5** Use a pair of tweezers to lift off the top of the mirror holder.

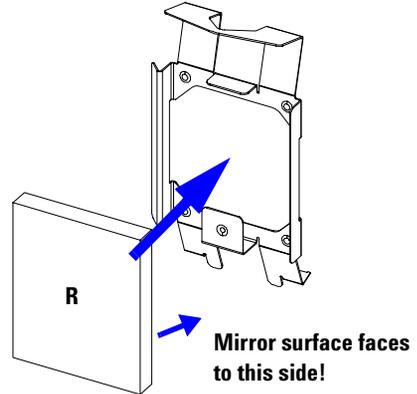


## 5 Repairing the Fluorescence Detector

**6** Remove the mirror assembly.



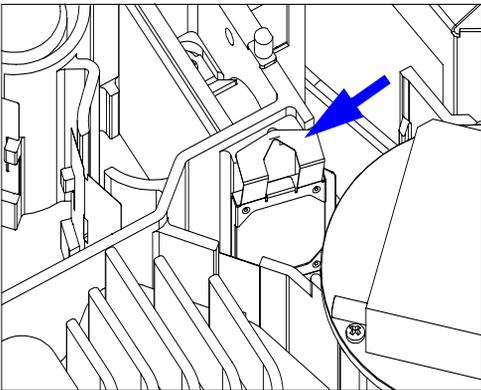
**7** Insert the new mirror into the holder. The mirror surface faces towards the light. The back side is marked as shown.



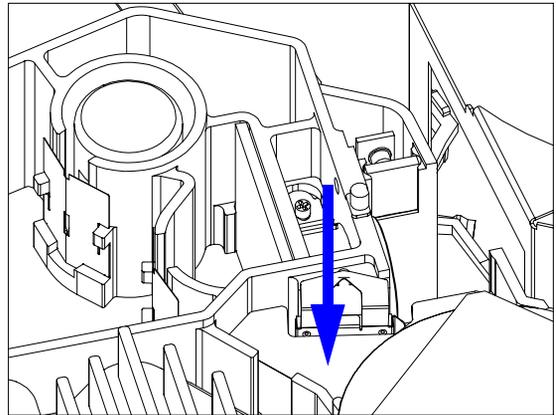
### Note:

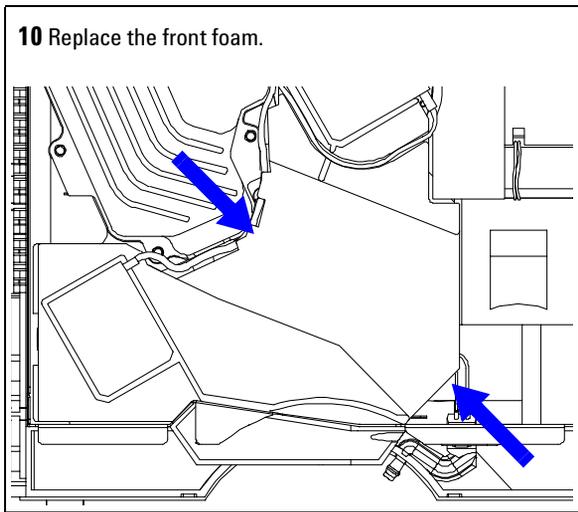
**If the mirror is inserted in the opposite direction, the detector cannot be aligned correctly.**

**8** Replace the mirror assembly and lock the top over the pin.



**9** Replace the UV protector.





- Next Steps:**
- Replace the covers as described in ["Replacing the Foams and Covers"](#) on page 196.
  - Re-install the flow cell and the front panel
  - Re-install the detector in the stack.
  - Turn the lamp on.
  - Perform a wavelength verification to check the correct positioning of the replaced assemblies, as described in chapter ["Wavelength Verification and Calibration"](#) on page 101.

## 5 Repairing the Fluorescence Detector

### Replacing the Slits

**When required**  
**Tools required**

If required.  
Screwdriver POZI 1 PT3

**Parts required**

A pair of tweezers  
Slit EX G1321-08103  
Slit EM G1321-08102 (also used for PMT)

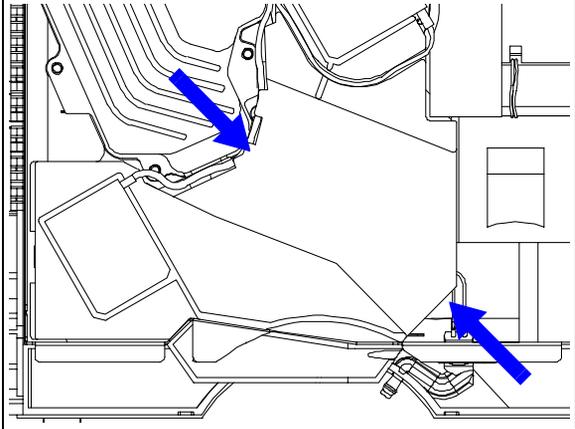
**NOTE**

DO NOT touch the surface of any optical component during this procedure. This will deteriorate the reflection process.

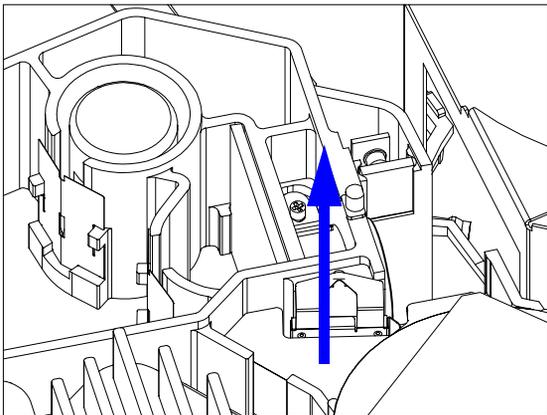
**Preparations for this procedure:**

- Turn off the detector.
- Disconnect the power cable.
- Remove the flow cell.
- Remove detector from stack and place it on the workbench.
- Remove the covers as described in "Removing the Covers" on page 144.

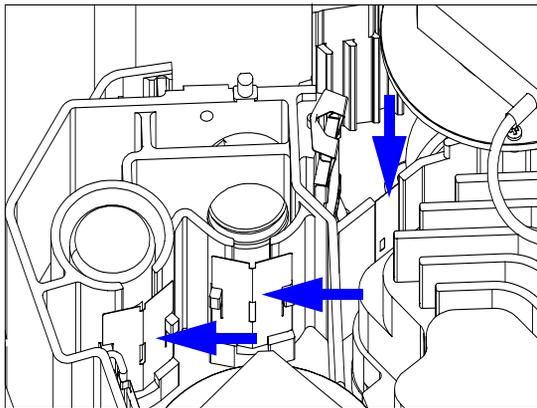
**1** Carefully remove the front foam holding the foam as shown.



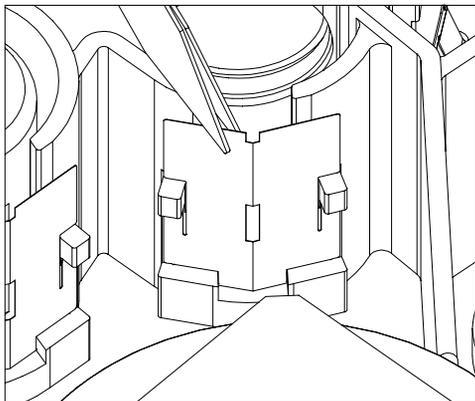
2 Remove the UV protector.



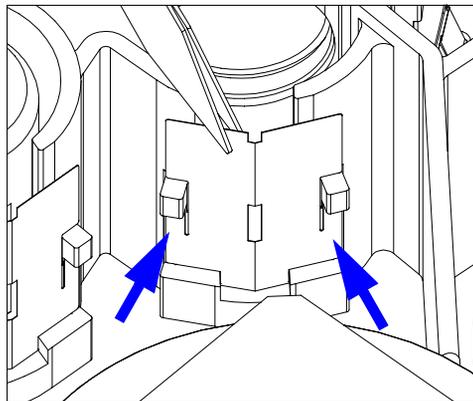
3 Locate the three slits (from left to right: PMT, EM, EX).



4 Use a pair of tweezers to replace the slit.



5 Put slight pressure on to the spring part of the slit.



**Next Steps:**

- Replace the UV protector.
- Replace the front foam.
- Replace the covers as described in ["Replacing the Foams and Covers"](#) on page 196.
- Re-install the flow cell and the front panel
- Re-install the detector in the stack.
- Turn the lamp on.
- Perform a wavelength verification to check the correct positioning of the replaced assemblies, as described in chapter ["Wavelength Verification and Calibration"](#) on page 101.

## 5 Repairing the Fluorescence Detector

### Removing the Optical Unit

<b>When required</b>	For following repairs: power supply, FLL board, Trigger Pack/FLF board and PMT.
<b>Tools required</b>	Screwdriver POZI 1 PT3 Hexagonal key, 4 mm
<b>Parts required</b>	Optical unit G1315-69002 (exchange assembly, comes with foam) or individual parts depending on the following procedures

#### CAUTION

When working on the optical unit, a clean workbench with ESD protection mat must be available. Otherwise optical components or electronic boards may be damaged.

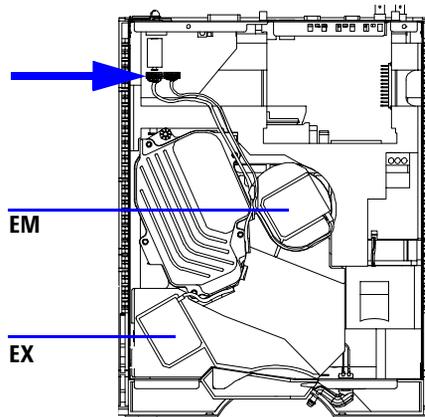
#### NOTE

DO NOT remove the foam from the optical unit unless it is required during a procedure. Otherwise parts can fall out.

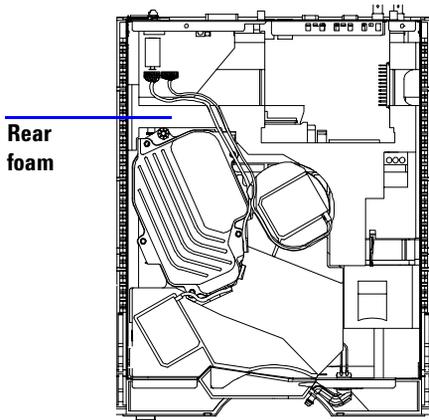
#### Preparations for this procedure:

- Turn off the detector.
- Disconnect the power cable.
- Disconnect capillaries.
- Remove detector from stack and place it on the workbench.
- Remove the covers as described in "Removing the Covers" on page 144.
- Remove the flow cell.

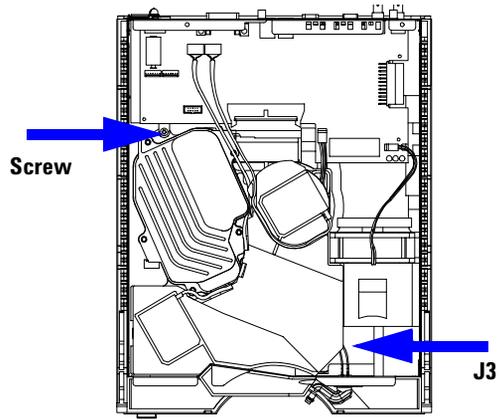
- 1 Disconnect cables (EM monochromator) and (EX monochromator) from the detector main board (FLM).



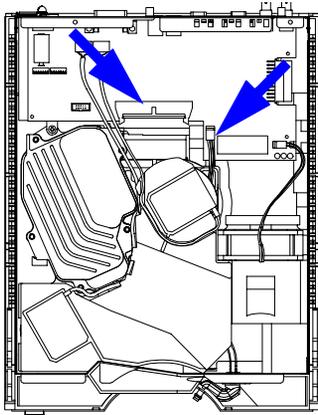
2 Carefully remove the rear foam (A). Leave the other foam on the assembly.



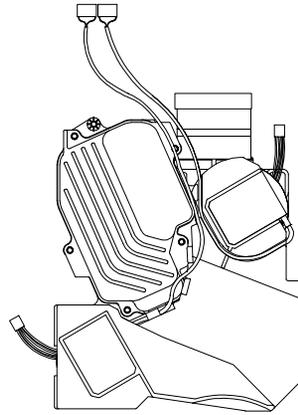
3 Disconnect the leak sensor cable (J3) and unscrew the grounding screw.



4 Disconnect the optical unit cable (J18) and the EM motor cable (J20).



5 Remove the optical unit and place it on the bench (ESD protection required).



**Next Steps:**

- Depending on the replacement you want to perform, proceed to the following procedures.

### Replacing PMT and/or FLF board

<b>When required</b>	If defective or if application specific PMT is required.
<b>Tools required</b>	Screwdriver POZI 1 PT3 Hexagonal key, 4 mm and 2.5 mm Tweezers
<b>Parts required</b>	Photo-multiplier Tube (PMT) 1970-0201 Photo-multiplier Tube (PMT) for other application ranges, please contact Hamamatsu dealers. Alternative PMTs are either R928HA (185 to 900 nm) or R3788HA (185 to 750 nm). No other PMTs are recommended. FLF Board G1321-69531 (requires firmware revision A.04.06 or above), FLR Reference Diode board G1321-66533

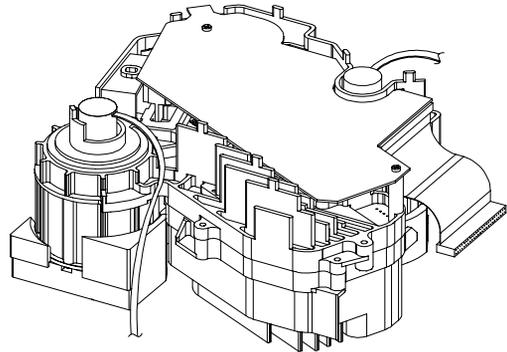
#### CAUTION

When working on the optical unit, a clean workbench with ESD protection mat must be available. Otherwise optical components or electronic boards may be damaged.

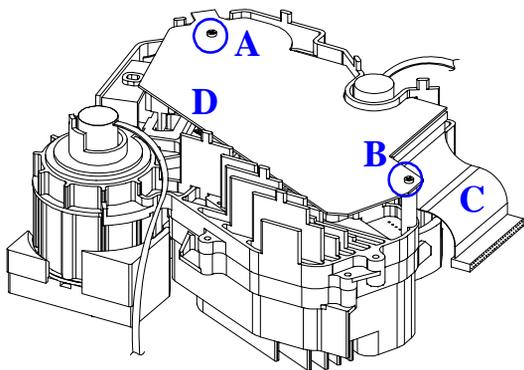
#### Preparations for this procedure:

- Optical unit has been removed as described in "Removing the Optical Unit" on page 178.
- The foam must be in place. Otherwise cutoff filter and diffuser will fall out.

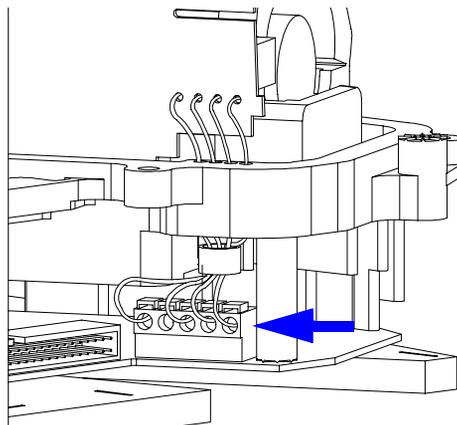
- 1 Place the optical unit upside down on the ESD protection mat.



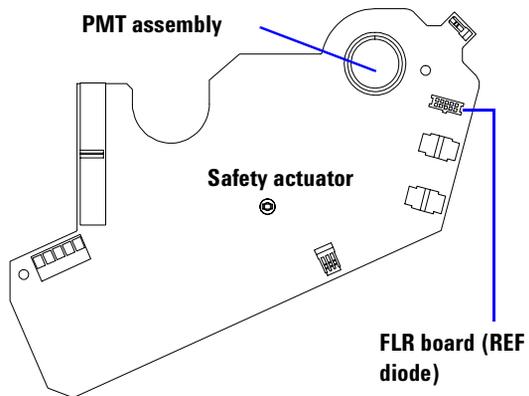
**2** Unscrew screws A and B and disconnect connectors C and D.



**3** Disconnect the wires from the connector. Use a flat screw driver to release the wires.



**4** Remove the FLF board carefully in an upwards motion and place it on an ESD protected mat.



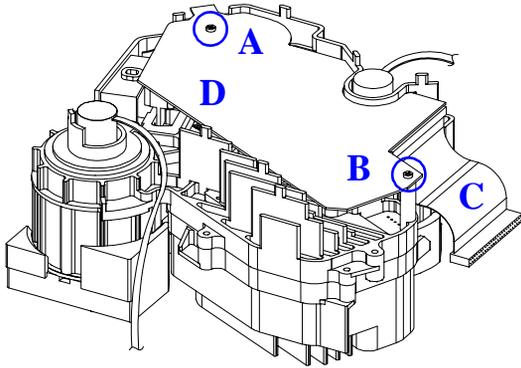
**5** If you need to replace either the board or the PMT assembly, remove the PMT assembly from its socket.

**6** Replace the PMT assembly or the FLF board.

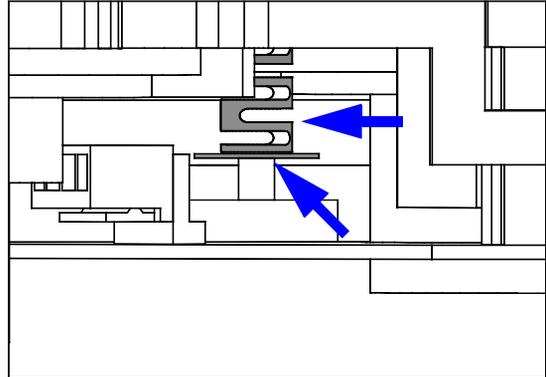
**7** Re-install the PMT assembly in the socket.

## 5 Repairing the Fluorescence Detector

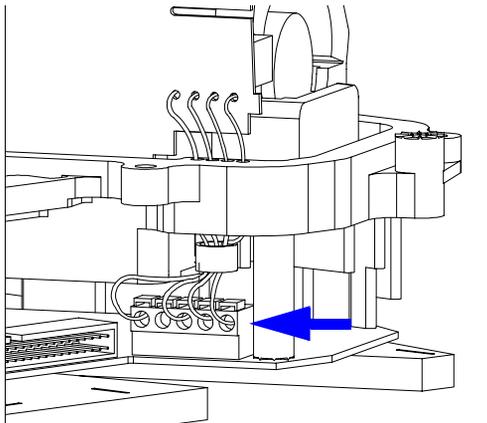
**8** Replace the FLF board and fix the screws (A and B) and connectors (C and D).



**9** Check that the safety switch is positioned correctly. Look through board and casting.



**10** Reconnect the wires to the connector. Use a flat screw driver to release the wire socket.



**11** Put the wires into the released contacts using the tweezers.

Order of wires (left to right):

green  
empty  
brown  
yellow  
white

### Next Steps:

- Reinstall the optical unit as described in "Installing the Optical Unit" on page 194.
- If you replaced a G1321-66501 FLF board by a G1321-66511 FLF board, update the FLD firmware to revision A.03.7x or above (to make it compatible).

## Replacing FLL board and Trigger Pack

<b>When required</b>	If defective
<b>Tools required</b>	Screwdriver POZI 1 PT3 Hexagonal key, 4 mm and 2.5 mm
<b>Parts required</b>	FLL Board G1321-66512 ( <b>includes</b> trigger pack)

### CAUTION

When working on the optical unit, a clean workbench with ESD protection mat must be available. Otherwise optical components or electronic boards may be damaged.

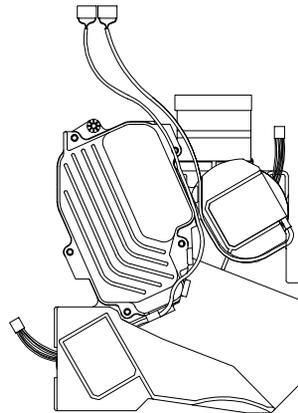
### WARNING

The instrument should not be operated without Xenon flash lamp. Otherwise the Triggerpack electronics will be damaged.

#### Preparations for this procedure:

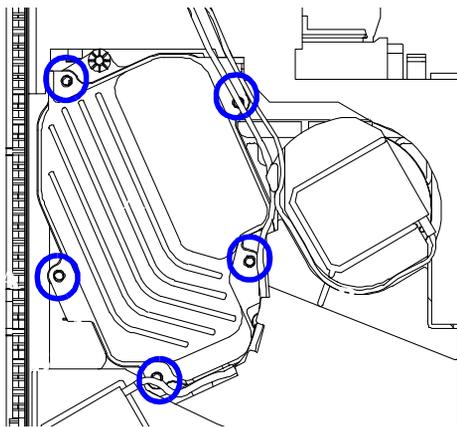
- Optical unit has been removed as described in "Removing the Optical Unit" on page 178.
- The foam must be in place. Otherwise cutoff filter and diffuser will fall out.

1 Place the optical unit on the ESD protection mat.

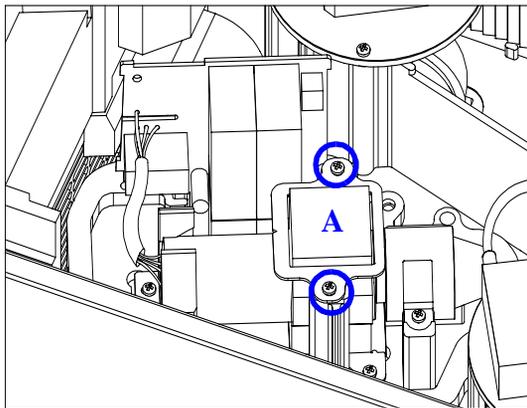


## 5 Repairing the Fluorescence Detector

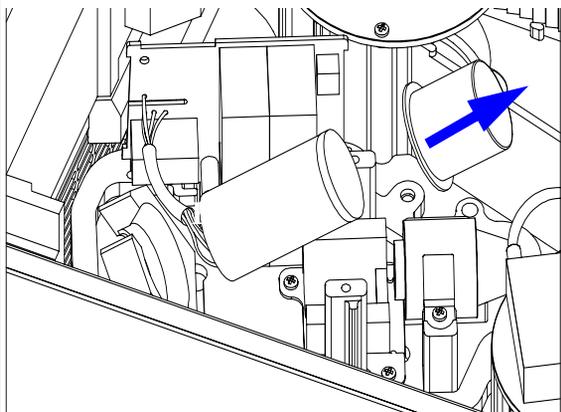
**2** Using the 4 mm hexagonal key, unscrew the five screws and lift the cover.



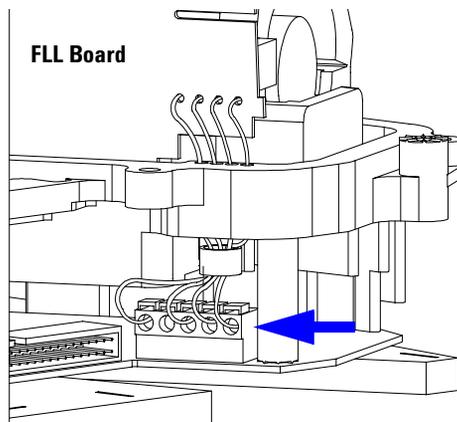
**3** Using the 2.5 mm hexagonal key, unscrew the two screws and remove the pad (A).



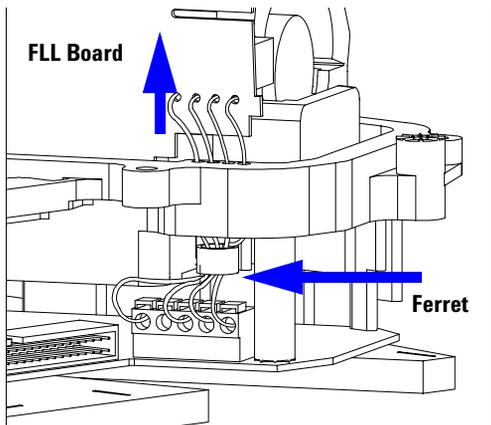
**4** Remove the lamp as described in "Exchanging the Lamp" on page 147.



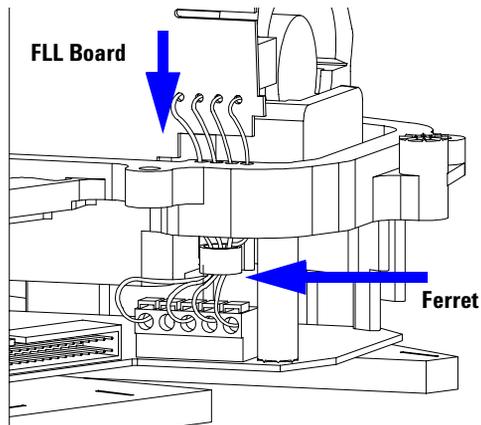
**5** Disconnect the wires from the connector. Use a flat screw driver to release the wires.



**6** Carefully pull off the ferret from the wires and keep it in a safe place. Then pull out the FLL board.



**7** Insert the new FLL board. Fit the wires through the holes and the Ferret.

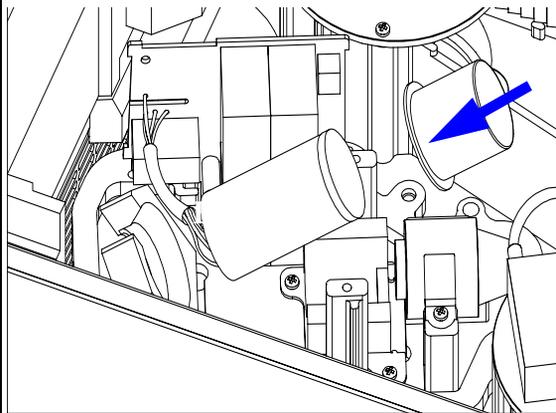


**8** Put the wires into the released contacts using the tweezers.

Order of wires (left to right):

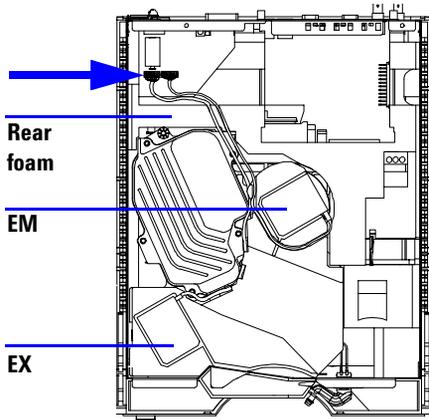
- green
- empty
- brown
- yellow
- white

**9** Reinstall the lamp as described in "Exchanging the Lamp" on page 147.



## 5 Repairing the Fluorescence Detector

**10** Replace the lamp housing cover. Insert the optical unit into its location and carefully insert the rear foam (A). Then reconnect the cables.



### Next Steps:

- Replace the covers as described in “Replacing the Foam and the Top Cover” on page 149.
- Re-install the detector in the stack.
- Reset the lamp counter as described in the user interface documentation.
- Turn the lamp on.
- Perform a wavelength verification to check the correct positioning of the replaced assemblies, as described in chapter “Wavelength Verification and Calibration” on page 101.

## Replacing the EM and EX Grating

<b>When required</b>	If defective
<b>Tools required</b>	Screwdriver POZI 1 PT3 Hexagonal key, 4 mm and 2.5 mm
<b>Parts required</b>	EM Grating assembly G1321-60004 ( <b>includes</b> protection ring) EX Grating assembly G1321-60003 ( <b>includes</b> protection ring)

### CAUTION

When working on the optical unit, a clean workbench with ESD protection mat must be available. Otherwise optical components or electronic boards may be damaged.

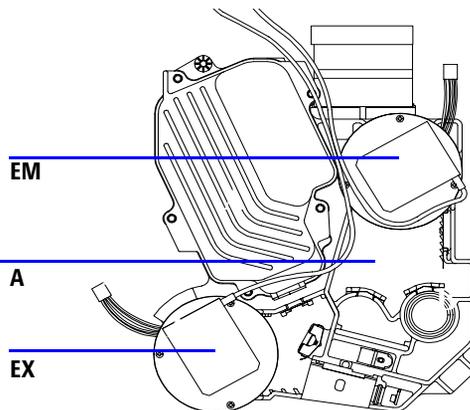
### NOTE

The grating assembly is shipped with a special transport packaging and includes a protection ring. **DO NOT** touch the grating surface and **DO NOT** bend the protection ring.

#### Preparations for this procedure:

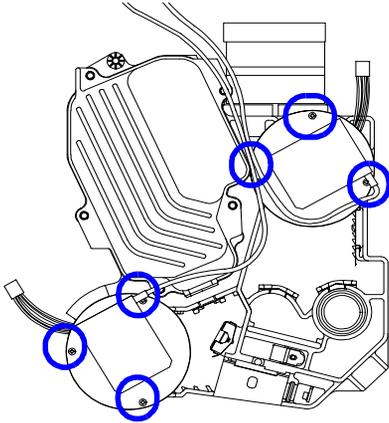
- Optical unit has been removed as described in "Removing the Optical Unit" on page 178.
- The cables from the position encoders (at the rear of the EM or EX grating assembly) should be disconnected.
- Disconnect the encoder cable of the EX-monochromator from FLF board.

**1** Place the optical on the ESD protection mat and remove the front foam (A). Locate the EM and EX grating assembly.

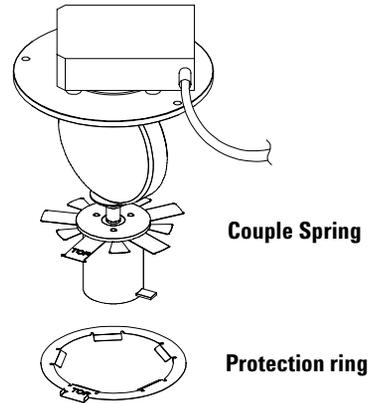


## 5 Repairing the Fluorescence Detector

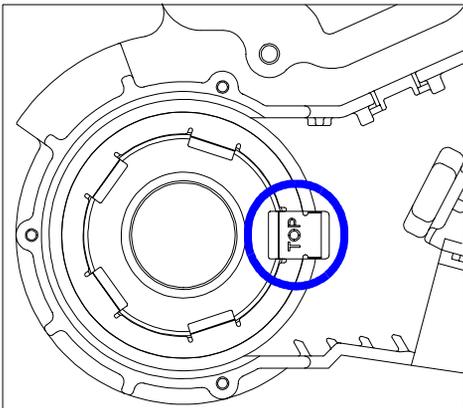
**2** Use a 2.5 mm hex key to unscrew the 3 screws on the assembly you want to remove.



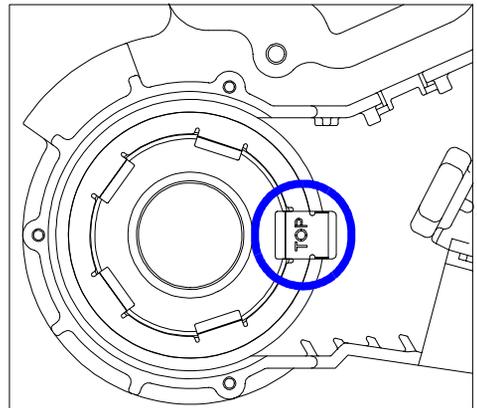
**3** Carefully remove the grating assembly and the protection ring from its location.



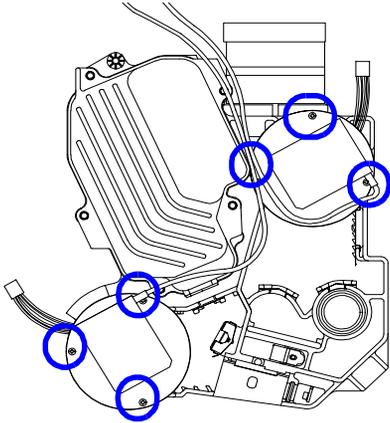
**4** Insert the new protection ring into its position. Do not bend the protection ring.



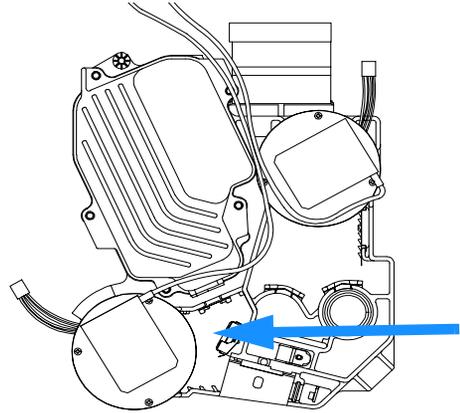
**5** For reinstallation route the cable first through the hole. Carefully insert the grating into its position while pulling the cable slightly from below. The couple spring has to be clipped into the protection ring (TOP mark)!



**6** Bring the top of the assembly into the position shown below and fix the 3 screws.



**7** Assure the UV protector cover is installed. Replace the foam on the optical unit.



**Next Steps:**

- Reconnect the encoder cable of the EX-monochromator to FLF board.
- Reinstall the optical unit as described in "Installing the Optical Unit" on page 194.
- Enter the replacement in the maintenance logbook.
- Reset the monochromator settings to default using the diagnose functions.
- In this case "Wavelength Calibration Lost" is displayed and the instrument stays in *Not Ready* condition until you perform a wavelength calibration.
- Perform a wavelength verification to check the correct positioning of the replaced assemblies, as described in chapter "Wavelength Verification and Calibration" on page 101.

### Exchanging the Power Supply

<b>When required</b>	If defective
<b>Tools required</b>	Screwdriver POZI 1 PT3 Wrench 1/4 inch Wrench 5 mm Wrench 7 mm
<b>Parts required</b>	Power supply 0950-2528

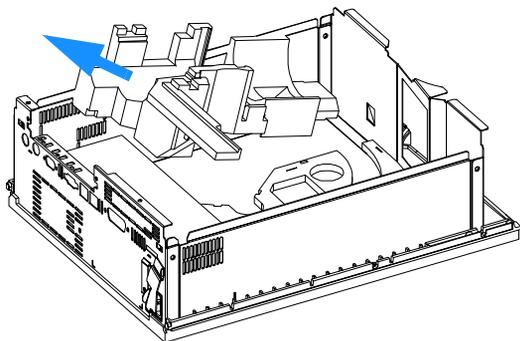
#### NOTE

The repair of the power supply assembly comprises exchanging the complete assembly. No serviceable parts are inside.

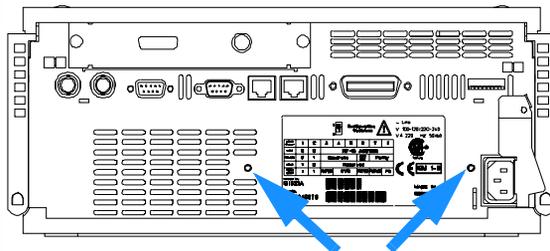
#### Preparations for this procedure:

- Turn off the lamp.
- Switch off the detector, and disconnect the cables.
- Remove the detector from the stack and place it on the workbench.
- Remove the front cover and top cover, see ["Removing the Covers"](#) on page 144.
- Remove the processor board, see ["Exchanging the Processor Board"](#) on page 159.
- Remove the fan assembly, see ["Exchanging the Fan"](#) on page 163.
- Remove the optical unit, see ["Removing the Optical Unit"](#) on page 178.

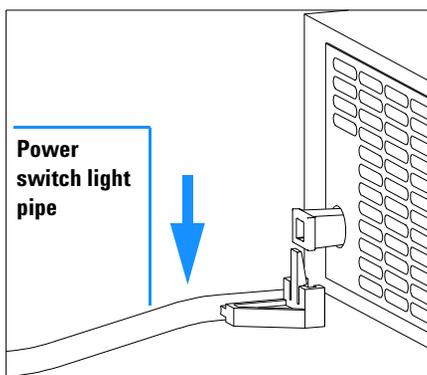
1 Carefully remove the bottom foam piece by sliding it out towards the rear.



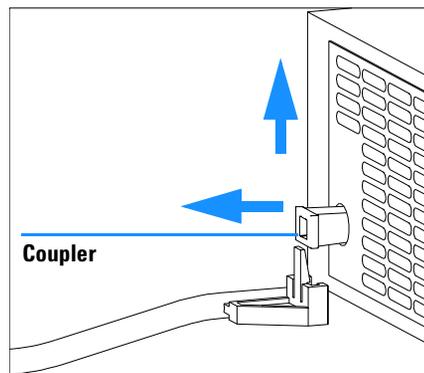
2 Unscrew the power supply at the rear of the module.



3 Press down the power switch light pipe to remove it from the coupler.



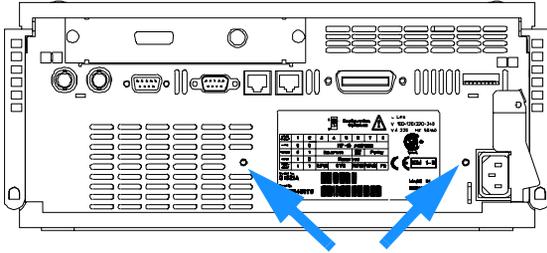
4 Remove the power supply completely. Re-use the coupler on the new power supply.



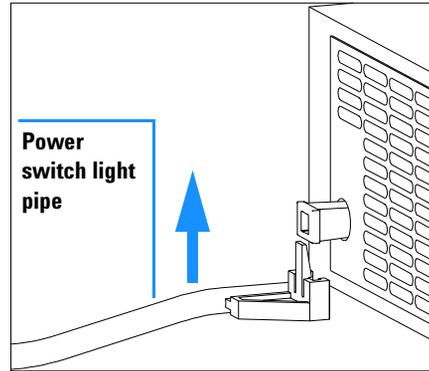
**The repair of the power supply assembly is comprised of exchanging the complete assembly. No serviceable parts are inside.**

## 5 Repairing the Fluorescence Detector

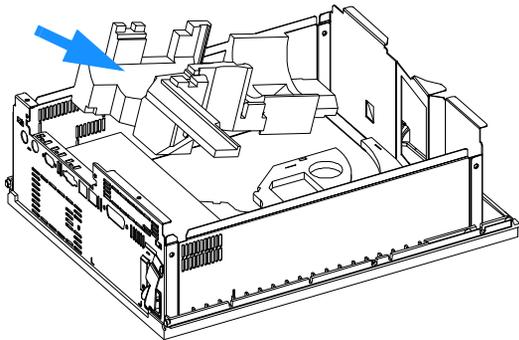
**5** Insert the power supply into its location and fix it with the screws at the rear panel.



**6** Press down and clip in the power switch light pipe into the power supply.



**7** Reinstall bottom foam piece. Slide it in underneath the leak drain.



**8** Reinstall the processor board, see ["Exchanging the Processor Board"](#) on page 159.

**9** Reinstall the fan assembly, see ["Exchanging the Fan"](#) on page 163.

**10** Reinstall the optical unit, see ["Installing the Optical Unit"](#) on page 194.

**11** Reinstall the front cover, top cover, see ["Replacing the Foams and Covers"](#) on page 196.

**12** Replace detector into the stack.

**13** Reconnect the power cable and turn on the detector.

## Replacing Status Light Pipe

**When required**

If part was broken or removed

**Tools required**

Screwdriver POZI 1 PT3

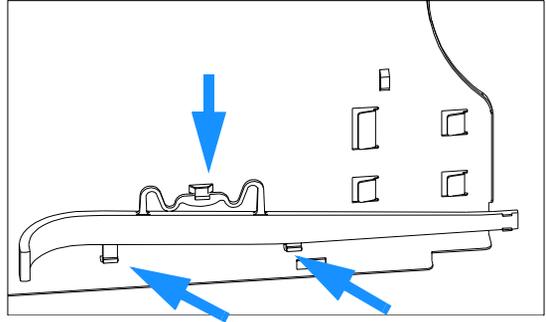
**Parts required**

Status light pipe 5041-8384

**Preparations for this procedure:**

- Remove the front cover and top cover, see ["Removing the Covers"](#) on page 144.

**1** The status light pipe is clipped into the top cover.



**2** Replace the top cover, see ["Replacing the Foams and Covers"](#) on page 196.

**3** Replace the detector into the stack and reconnect the cables and capillaries.

**4** Turn on the detector.

## 5 Repairing the Fluorescence Detector

### Installing the Optical Unit

**When required**  
**Tools required**

When all repairs on the optical unit have been completed  
Screwdriver POZI 1 PT3  
Hexagonal key, 4 mm

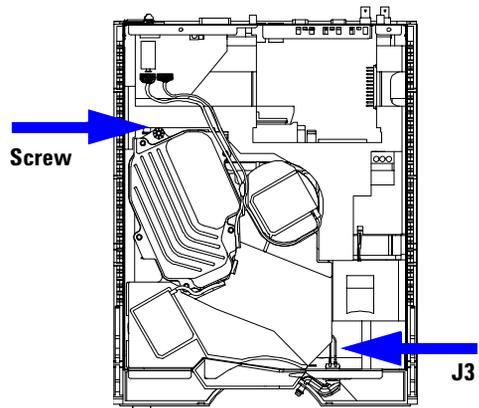
**WARNING**

For safe operation make sure that the ground cable is reconnected.

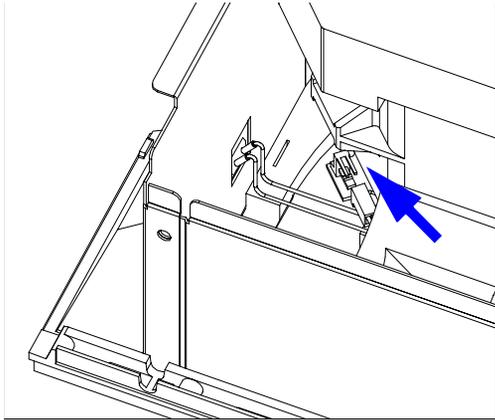
#### Preparations for this procedure:

- All previous work has been completed.
- Power supply has been installed.
- Fan has been installed.
- The optical unit has its foam cover attached.

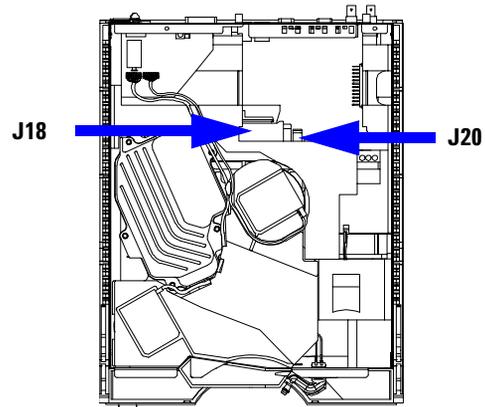
1 Place the optical unit in the instrument. Reconnect the grounding screw.



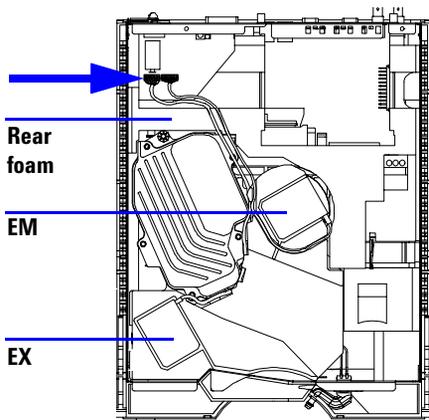
2 Reconnect the leak sensor cable (J3) using your left hand.



3 Reconnect the optical unit cable (J18) and the EM motor cable (J20).



4 Insert the rear foam and reconnect cables (EM monochromator) and (EX monochromator) to the detector main board (FLM).



**Next Steps:**

- Replace the covers as described in ["Replacing the Foams and Covers"](#) on page 196.
- Re-install the flow cell and the front panel
- Re-install the detector in the stack.
- Turn the lamp on.
- Perform a wavelength verification to check the correct positioning of the replaced assemblies, as described in chapter ["Wavelength Verification and Calibration"](#) on page 101.

## Replacing the Foams and Covers

Note:

If a new optical unit has been installed or assemblies within the optical unit have been replaced, then refer "Next Steps" on [page 189](#).

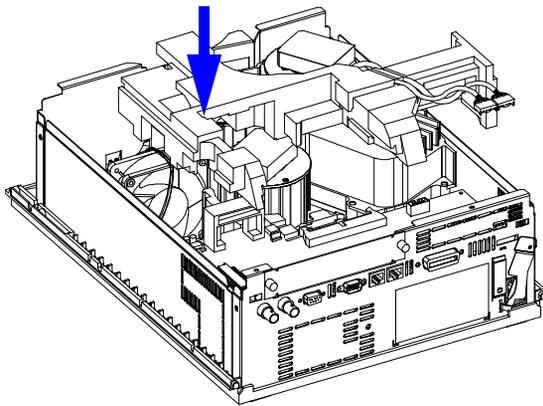
**When required** When all repairs have been completed  
**Tools required** Screwdriver POZI 1 PT3

**Prerequisites** The detector is open and other procedures have been carried out

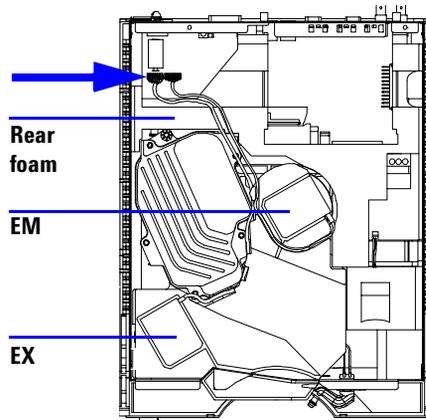
### NOTE

The front cover (across the optical unit) should only be removed when required during a procedure. This will keep dust away from optical components.

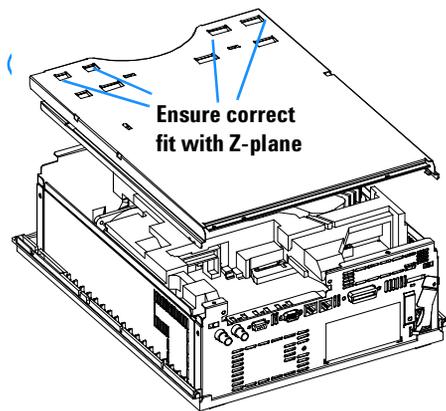
1 Carefully insert the rear foam.



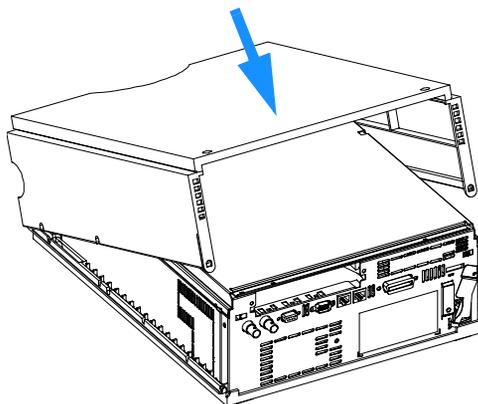
2 Route the cables towards the main board and reconnect to their connectors.



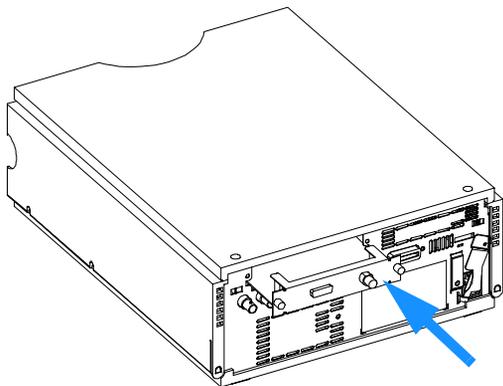
**3** Slide the top plate towards the rear and fix the top plate screws.



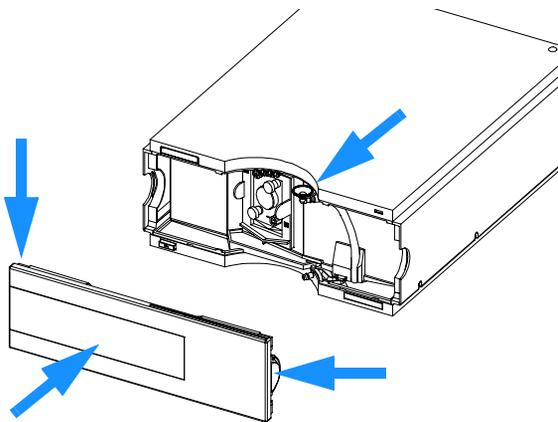
**4** Replace the cover.



**5** If required, insert the interface board and fix the screws.



**6** Replace the waste funnel assembly and the front panel.



**Next Steps:**

- Replace the detector into the stack and Reinstall the flow cell.
- Reconnect the power cable and turn on the detector.
- Turn the lamp on.

## 5 Repairing the Fluorescence Detector

### Assembling the Main Cover

<b>When required</b>	If cover is broken
<b>Tools required</b>	None
<b>Parts required</b>	Plastics kit 5062-8582 (includes base, top, left and right)

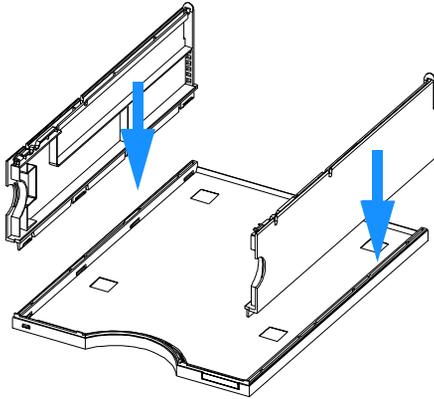
#### NOTE

The plastics kit contains all parts, but it is not assembled.

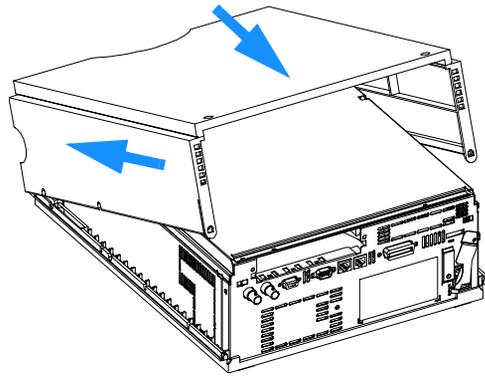
#### WARNING

If you mistakenly insert the left or right side in the opposite position, you may not be able to remove that side from the top part.

**1** Place the top part on the bench and insert the left and right sides into the top part.



**2** Replace the cover.



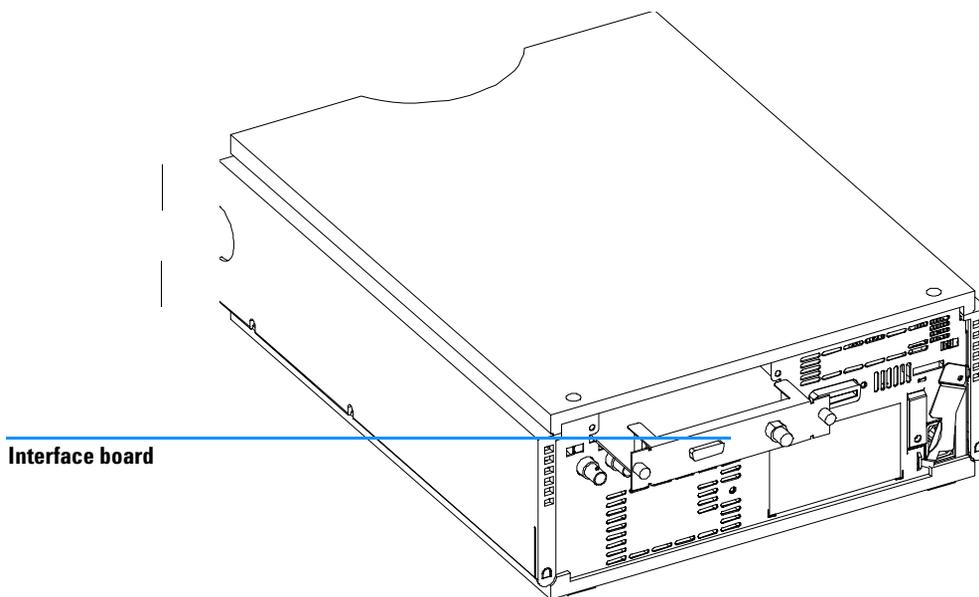
#### Next steps:

- Replace the detector in the stack and reconnect the cables and capillaries.
- Turn on the detector.

## Replacing the Interface Board

<b>When required</b>	For all repairs inside the detector or for installation of the board
<b>Part required</b>	Interface board (BCD) G1351-68701 with external contacts and BCD outputs Interface board (LAN) see "LAN Board" on page 284
<b>Tools required</b>	None

To replace the interface board unscrew the two screws, remove the board, slide in the new interface board and fix it with the board's screws.



**Figure 60** Location of the Interface Board

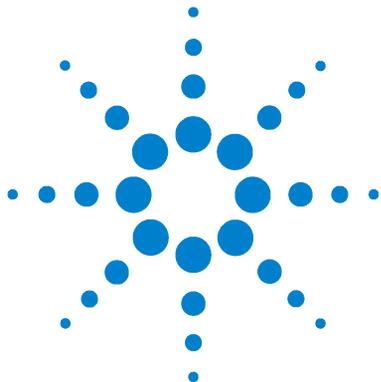
### Replacing the Detector's Firmware

The installation of new firmware is required:

- if new version solves problems of currently installed version, or
- if after exchange of the main board (FLM) the version on board is older than the previously installed one.

To upgrade the detector's firmware the following steps have to be taken:

- 1 Load the firmware into the detector, see help system of your user interface.
- 2 If FLM board was replaced, reenter the serial number information of the module through the user interface, see ["Entering the Serial Number using the Control Module"](#) on page 161 or ["Entering the Serial Number using the Agilent ChemStation"](#) on page 162.



## 6 Identifying Parts and Materials

Overview of Main Assemblies	202
Optical Unit Assembly	204
Lamp Cover Parts	207
Control Module	208
Sheet Metal Kit	209
Plastic Parts	210
Foam Parts	211
Power and Status Light Pipes	212
Leak Parts	213
Cuvette Kit	214
Spare Parts	215
Accessory Kit	216
Cable Overview	218
RS-232 Cable Kit	235
LAN Cables	236



## Overview of Main Assemblies

Figure 61 shows the main assemblies and their locations.

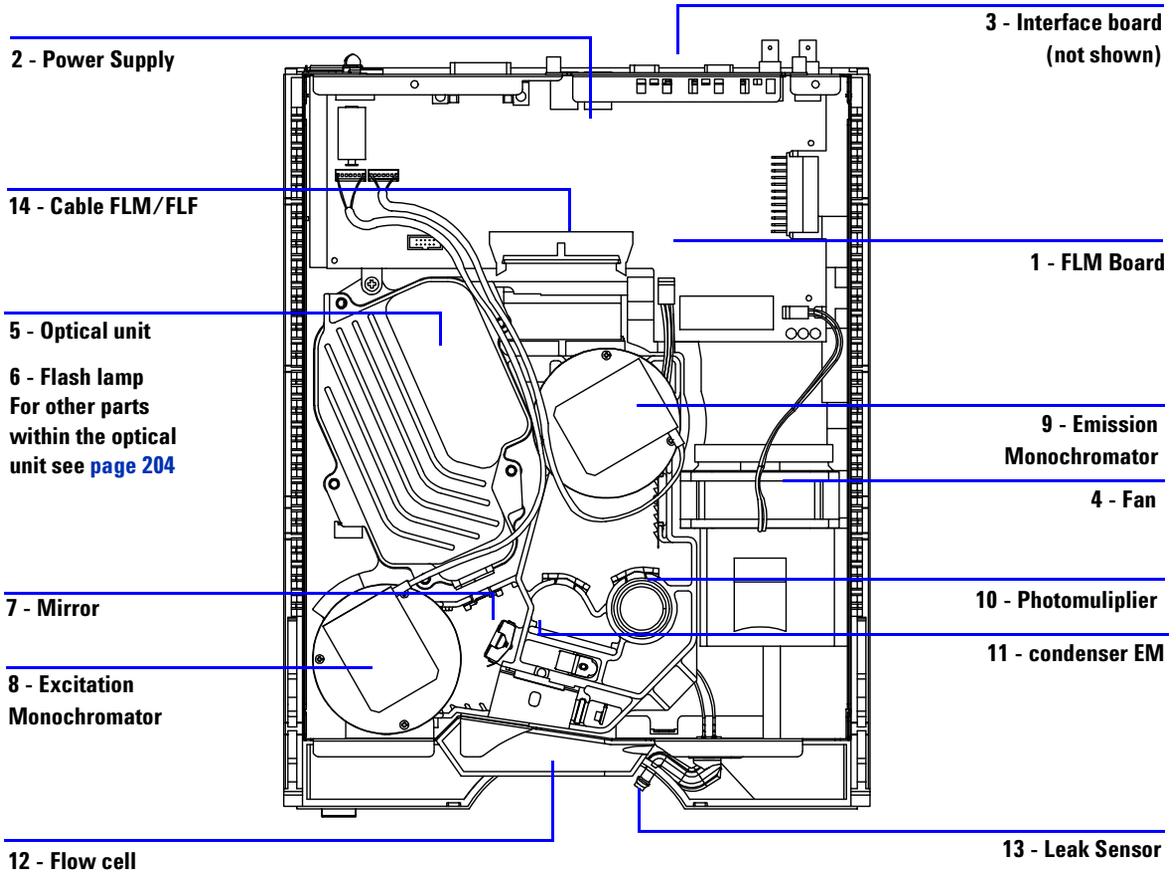


Figure 61 Main Assemblies

**Table 24** Main Assemblies

Item	Description	Part Number
1	Detector main board FLM ( <b>exchange assembly</b> )	G1321-69500
	Hexagonal nut for GPIB connector	0380-0643
	Hexagonal nut for RS-232C connector	1251-7788
	Nut for analog connector	2940-0256
	Washer for analog connector	2190-0699
	Cable CAN to Agilent 1100 Series modules (0.5 m), see <a href="#">page 233</a>	5181-1516
2	Power supply for power and status light parts, see <a href="#">page 212</a>	0950-2528
3	Interface board BCD (BCD/external contacts), optional	G1351-68701
3	Interface board MIO (LAN), optional, see <a href="#">page 284</a>	
	Fuse for BCD board, 250 mA (total of 4 are on the board)	2110-0004
4	Fan assembly	3160-1017
5	Optical unit ( <b>exchange assembly</b> ), for additional optical unit parts, see <a href="#">page 204</a>	G1321-69002
6	Xenon flash lamp	2140-0600
7	Mirror	1000-1000
8	Excitation Monochromator assembly (complete)	G1321-60003
9	Emission Monochromator assembly (complete)	G1321-60004
10	Photomultiplier tube assembly (PMT), see also <a href="#">page 180</a> for other types	1970-0201
11	Condenser EM, same as EX	1000-1124
12	Standard flow cell, 8 $\mu$ l, 20 bar, see also <a href="#">page 204</a> for cell screws	G1321-60005
13	Leak sensor assembly and Leak handling parts, see <a href="#">page 213</a>	5061-3356
14	Cable FLM - FLF board (comes with optical unit)	G1321-61600
	Front cover and plastic parts (housing), see <a href="#">page 210</a>	
	Sheet metal parts, see <a href="#">page 209</a>	
	Foam parts, see <a href="#">page 211</a>	

## Optical Unit Assembly

**Table 25** Optical Unit Assembly

Item	Description	Part Number
	Optical unit ( <b>exchange assembly</b> ), comes with foam part and CableFLM/FLF	G1321-69002
1	Flash Lamp Board (FLL), <b>includes</b> trigger pack	G1321-66512
2	Xenon flash lamp	2140-0600
3	Condenser Excitation	1000-1136
4	Slit (EX) 2x4	G1321-08103
5	Mirror	1000-1000
6	Excitation Monochromator assembly (complete)	G1321-60003
7	Emission Monochromator assembly (complete)	G1321-60004
8	Standard Flow Cell, 8 $\mu$ l, 20 bar, inlet i.d./length 0.17 mm/80 mm, outlet i.d./length 0.25 mm/80 mm	G1321-60005
	Cuvette, 8 $\mu$ l, 20 bar inlet i.d./length 0.5 mm/80 mm, outlet i.d./length 0.5 mm/80 mm	G1321-60007
	Screw for flow cell / cuvette	G1321-22400
9	Slit (EM) 2x8	G1321-08102
10	Photomultiplier tube assembly (PMT), see also <a href="#">page 180</a> for additional information	1970-0201
11	Condenser Emission	1000-1124
12	Front-end Board (FLF), underneath the optical ( <b>exchange assembly</b> ) (requires firmware revision A.04.06 or above)	G1321-69531
12a	Reference Diode Board (FLR)	G1321-66533
12b	Diffuser plate (in front of the reference diode board FLR)	1000-1121
13	Cutoff Filter 295 nm, for other type see <a href="#">page 215</a>	5062-8530
14	Carbon filter mat	01046-42701
15	Cable FLM - FLF board (comes with optical unit)	G1321-61600

**Table 25** Optical Unit Assembly (continued)

<b>Item</b>	<b>Description</b>	<b>Part Number</b>
	Foam Optics	G1321-40103
16	Cable chassis	G1321-61602
17	Screw M5 x 25 mm, for chassis cable	0515-2574
	Screw 2.5 mm hex, for monochromator, lamp pad, condenser #1, slits, etc.	0515-1052
	Screw M4 x 25 mm hex, for lamp housing	0515-2548
	Screw M4 x 8 mm, for FLF board	0515-2549

## 6 Identifying Parts and Materials

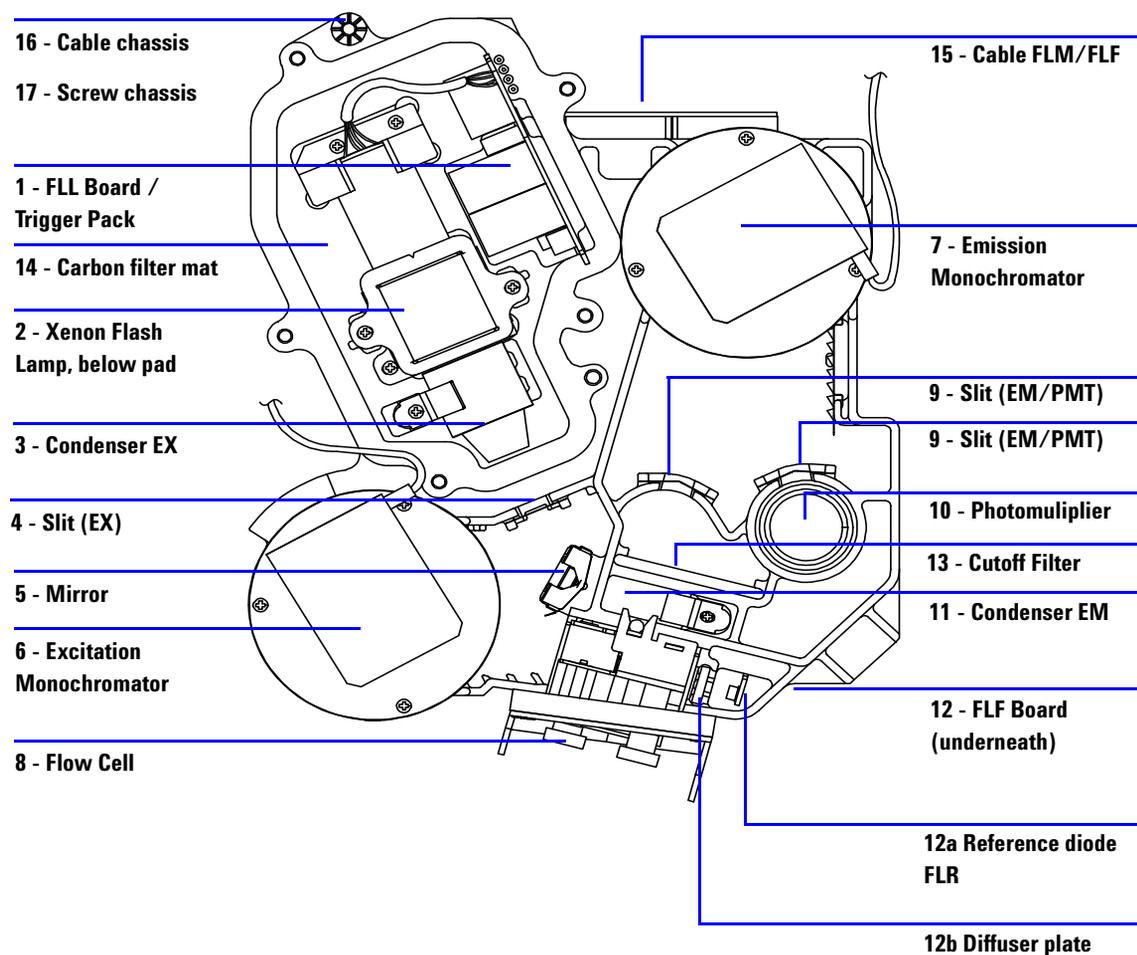
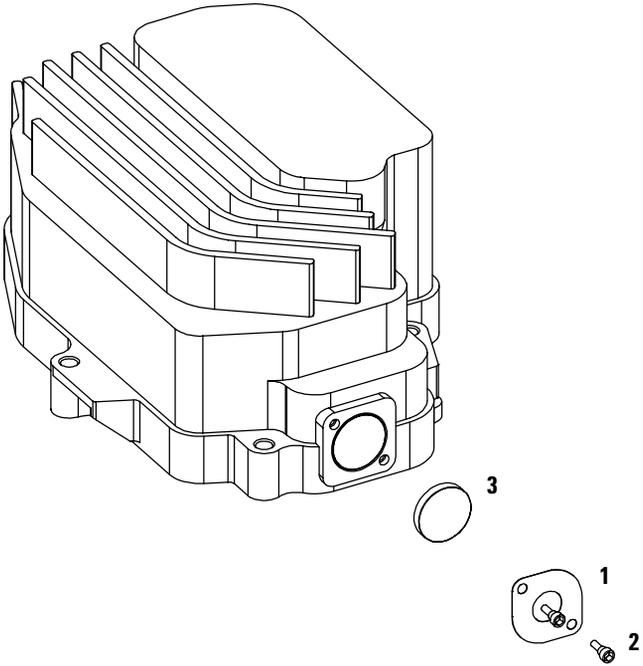


Figure 62 Optical Unit Parts

# Lamp Cover Parts

**Table 26** Lamp Cover Parts

Item	Description	Part Number
1	Plate	G1321-08100
2	Screw 2.5 mm hex	0515-1052
3	Quartz Window	1000-0999

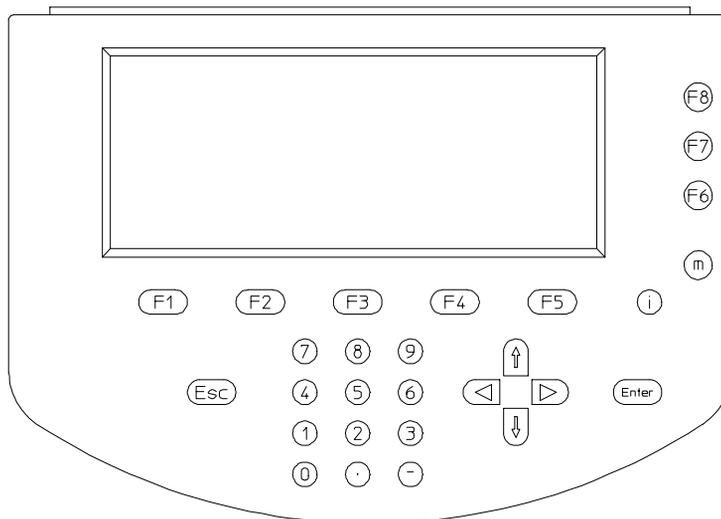


**Figure 63** Lamp Cover Parts

## Control Module

**Table 27** Control Module Parts

Item	Description	Part Number
	Control Module, replacement part including cable	G1323-67001
	Plastic Housing Kit, includes front, back and a clamp	5062-8583
	CAN cable Agilent 1100 module to control module	G1323-81600

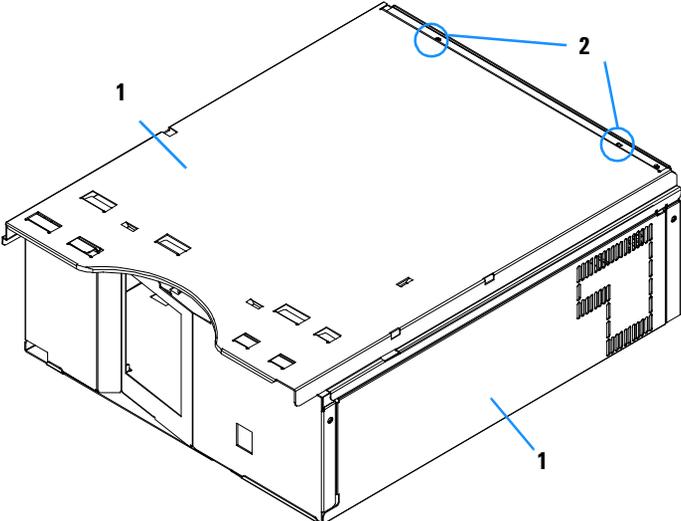


**Figure 64** Control Module

# Sheet Metal Kit

**Table 28** Sheet Metal Kit Parts

Item	Description	Part Number
1	Sheet metal kit includes case and top cover	G1321-68701
2	Screws M3 for cover and flow cell door	5022-2112



**Figure 65** Sheet Metal Kit Parts

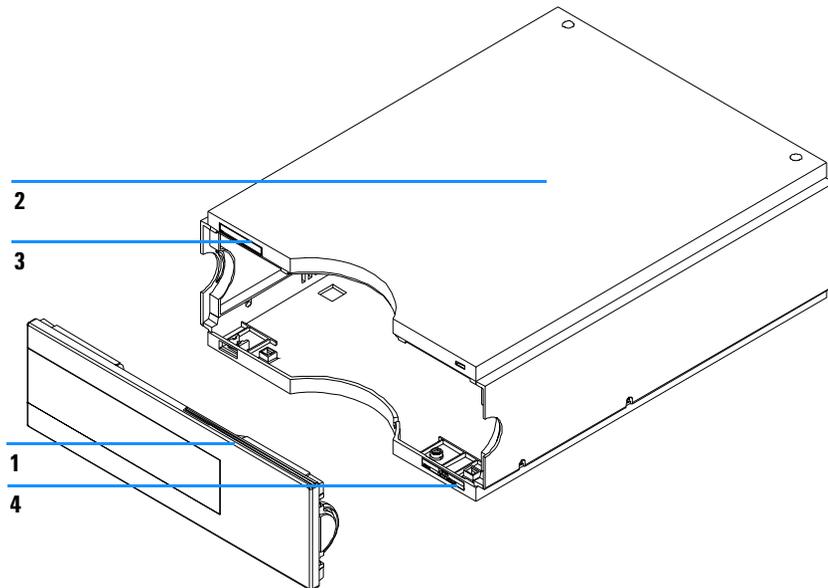
## Plastic Parts

**Table 29** Plastics Parts

Item	Description	Part Number
1	Front cover	5062-8582
2	Plastics, includes base, sides and top	5062-8565
3	Name plate Agilent 1100 Series	5042-1381
4	Name plate Serial Number (w/o serial number)	5042-1314

**NOTE**

For correct assembling of the top and sides, see [“Assembling the Main Cover”](#) on page 198.



**Figure 66** Plastic Parts

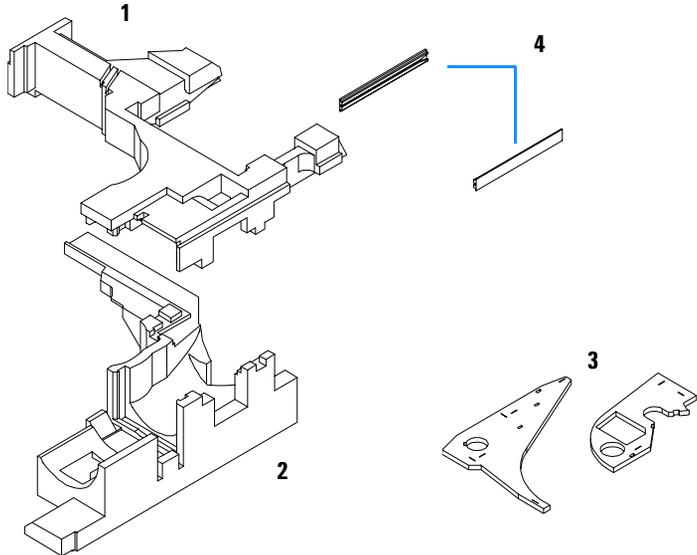
# Foam Parts

**Table 30** Foam Parts

Item	Description	Part Number
1, 2	EPP foam kit, includes bottom and top rear	G1321-68702
3	Foam Pads (located underneath the optical), set of two	G1321-68700
4	Guides for interface board	5041-8395

**NOTE**

Do not order the individual part numbers imprinted on the foam.

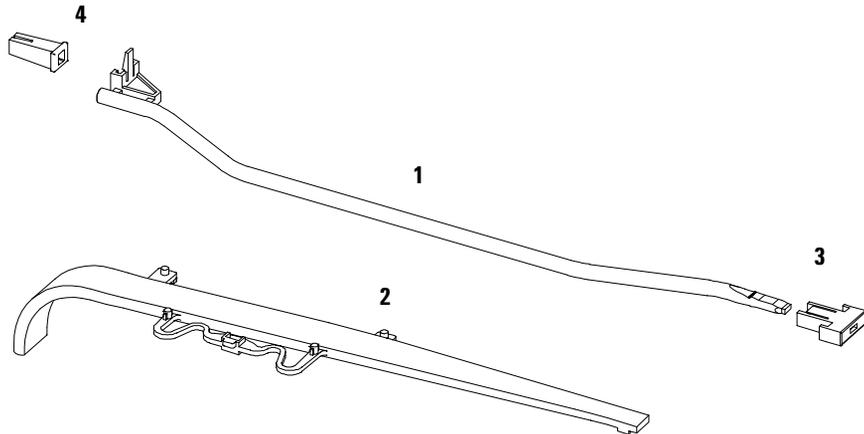


**Figure 67** Foam Parts

## Power and Status Light Pipes

**Table 31** Power and Status Light Pipes

Item	Description	Part Number
	Power supply assembly	0950-2528
	Screw M4 x 0.7, 8 mm lg, to fix power supply at rear panel	0515-0910
	Washer	2190-0409
1	Power light pipe	5041-8382
2	Status light pipe	5041-8384
3	Power switch button	5041-8381
4	Coupler for power supply actuator	5041-8383

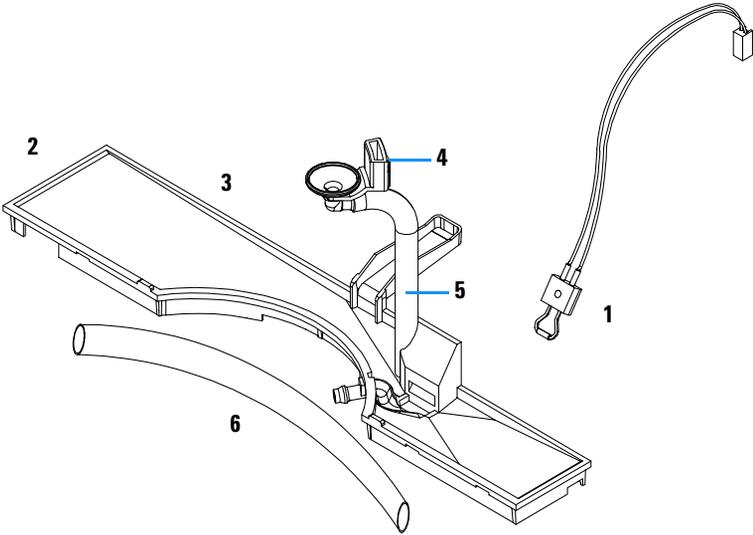


**Figure 68** Power and Status Light Pipe

# Leak Parts

**Table 32** Leak Parts

Item	Description	Part Number
1	Leak sensor assembly	5061-3356
2	Leak pan	G1321-45501
3	Leak funnel	5041-8388
4	Leak funnel holder	5041-8389
5	Corrugated tubing, 120 mm lg, re-order 5 m	5062-2463
6	Corrugated tubing, 1200 mm lg, re-order 5 m	5062-2463



**Figure 69** Leak Parts

## Cuvette Kit

**Table 33** Cuvette Kit

Item	Description	Part Number
	FLD Cuvette Kit, 8 $\mu$ l, 20 bar	G1321-60007
	includes	
	Tubing, flexible, 1 meter	
	SST Fitting, QTY=1	79814-22406
	SST front ferrule, QTY=1	0100-0043
	SST back ferrule, QTY=1	0100-0044
	PEEK fitting, QTY=1	0100-1516
	Needle-Syringe	9301-0407
	Glass-Syringe	9301-1446

## Spare Parts

The spare parts listed below allow changes to the standard hardware configuration to adapt to specific application needs (as it was possible on the HP 1046A Fluorescence detector).

### NOTE

The installation of these parts may affect the performance of the detector and may not fulfill the instruments specifications.

**Table 34** Spare Parts

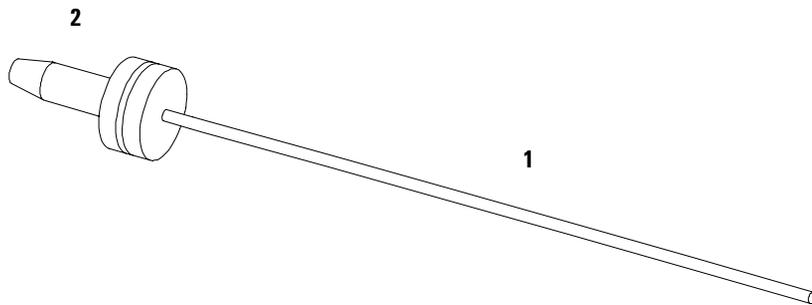
Description	Part Number
Cutoff filter kit: 389 nm, 408 nm, 450 nm, 500 nm, 550 nm	5061-3327
Cutoff filter kit: 380 nm, 399 nm, 418 nm, 470 nm, 520 nm	5061-3328
Cutoff filter kit: 280 nm, 295 nm, 305 nm, 335 nm, 345 nm	5061-3329
Cutoff filter 370 nm	1000-0822
Photo-multiplier Tube (PMT) R928HA (185 to 900 nm)	contact Hamamatsu dealers
Photo-multiplier Tube (PMT) R3788HA (185 to 750 nm)	

## Accessory Kit

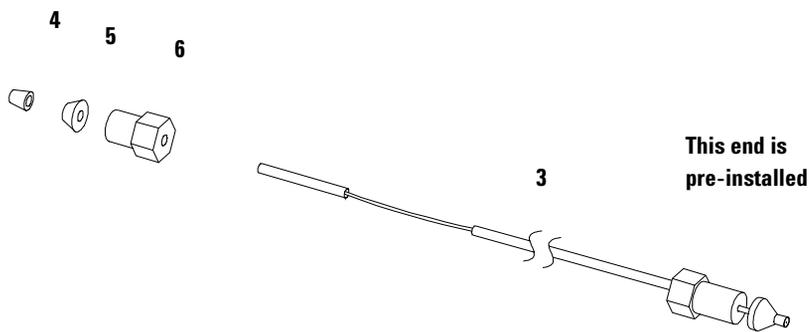
This kit contains some accessories and tools needed for the installation and repair/calibration of the detector.

**Table 35** Accessory Kit Parts

Item	Description	Part Number
	Accessory kit	G1321-68705
	includes	
	Corrugated tubing, 120 mm lg, re-order 5 m	5062-2463
1	Teflon Tubing flexible i.d. 0.8 mm (flow cell to waste), re-order 5 m	5062-2462
2	Fitting male PEEK, Qty=2	0100-1516
3	Capillary column – detector 380 mm lg, 0.17 i.d. includes items 4, 5 and 6 (not assembled)	G1315-87311
4	Ferrule front SST, qty=1	0100-0043
5	Ferrule back SST, qty=1	0100-0044
6	Fitting SST, qty=1	79814-22406
	Screwdriver hexagonal 4 mm, 100 mm long	5965-0027
	Screwdriver hexagonal 2.5 mm, 100 mm long	5965-0028
	Needle-Syringe	9301-0407
	Glass-Syringe	9301-1446
	Calibration Sample, Glycogen	5063-6597
	Sample filter, diameter=3 mm, pore size 0.45 $\mu\text{m}$ , QYT=5	5061-3367 (pack of 100)
	Hex key set 1 – 5 mm	8710-0641
	Wrench open end 1/4 – 5/16 inch	8710-0510
	ESD wrist strap	9300-1408



**Figure 70** Waste Tubing Parts



**Figure 71** Inlet Capillary (Column-Detector) Parts

## Cable Overview

**WARNING**

Never use cables other than the ones supplied by Agilent Technologies to ensure proper functionality and compliance with safety or EMC regulations.

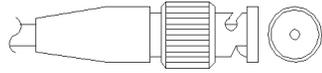
**Table 36** Cables Overview

Type	Description	Part Number
<b>Analog cables</b>	3390/2/3 integrators	01040-60101
	3394/6 integrators	35900-60750
	Agilent 35900A A/D converter	35900-60750
	General purpose (spade lugs)	01046-60105
	3390/2/3 integrators	01040-60101
<b>Remote cables</b>	3390 integrator	01046-60203
	3392/3 integrators	01046-60206
	3394 integrator	01046-60210
	3396A (Series I) integrator	03394-60600
	3396 Series II / 3395A integrator, see <a href="#">page 225</a>	
	3396 Series III / 3395B integrator	03396-61010
	Agilent 1100 / 1050 modules / HP 1046A FLD	5061-3378
	HP 1046A FLD	5061-3378
	Agilent 35900A A/D converter	5061-3378
	HP 1040 diode-array detector	01046-60202
	HP 1090 liquid chromatographs	01046-60202
	Signal distribution module	01046-60202

**Table 36** Cables Overview (continued)

<b>Type</b>	<b>Description</b>	<b>Part Number</b>
<b>BCD cables</b>	3392/3 integrators	18594-60510
	3396 integrator	03396-60560
	General purpose (spade Lugs)	18594-60520
<b>Auxiliary</b>	Agilent 1100 Series vacuum degasser	G1322-61600
<b>CAN cables</b>	Agilent 1100 module to module, 0.5 m	5181-1516
	Agilent 1100 module to module, 1 m	5181-1519
	Agilent 1100 module to control module	G1323-81600
<b>External contacts</b>	Agilent 1100 Series interface board to general purpose	G1103-61611
<b>GPIB cable</b>	Agilent 1100 module to Agilent ChemStation, 1 m	10833A
	Agilent 1100 module to Agilent ChemStation, 2 m	10833B
<b>RS-232 cable</b>	Agilent 1100 module to a computer This kit contains a 9-pin female to 9-pin female Null Modem (printer) cable and one adapter.	34398A
<b>LAN cable</b>	Twisted pair cross over LAN cable, 10 feet long (for point to point connection)	5183-4649
	Category 5 UTP cable, 8 m long (for hub connections)	G1530-61480

## Analog Cables



One end of these cables provides a BNC connector to be connected to Agilent 1100 Series modules. The other end depends on the instrument to which connection is being made.

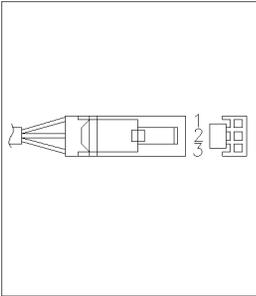
### Agilent 1100 to 3390/2/3 Integrators

Table 37

Connector 01040-60101	Pin 3390/2/3	Pin Agilent 1100	Signal Name
	1	Shield	Ground
	2		Not connected
	3	Center	Signal +
	4		Connected to pin 6
	5	Shield	Analog -
	6		Connected to pin 4
	7		Key
	8		Not connected

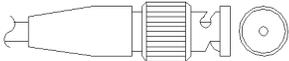
**Agilent 1100 to 3394/6 Integrators**

**Table 38**

Connector 35900-60750	Pin 3394/6	Pin Agilent 1100	Signal Name
	1		Not connected
	2	Shield	Analog -
	3	Center	Analog +

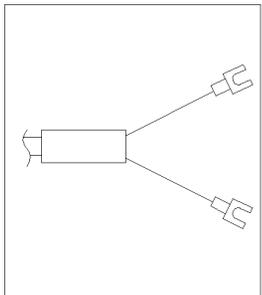
**Agilent 1100 to BNC Connector**

**Table 39**

Connector 8120-1840	Pin BNC	Pin Agilent 1100	Signal Name
	Shield	Shield	Analog -
	Center	Center	Analog +

**Agilent 1100 to General Purpose**

**Table 40**

Connector 01046-60105	Pin 3394/6	Pin Agilent 1100	Signal Name
	1		Not connected
	2	Black	Analog -
	3	Red	Analog +

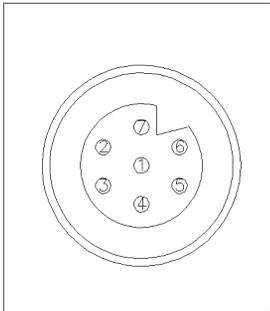
## Remote Cables



One end of these cables provides a Agilent Technologies APG (Analytical Products Group) remote connector to be connected to Agilent 1100 Series modules. The other end depends on the instrument it is to be connected to.

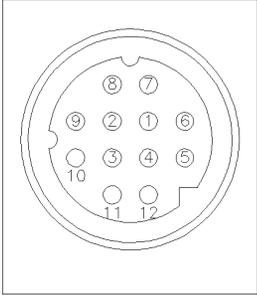
### Agilent 1100 to 3390 Integrators

Table 41

Connector 01046-60203	Pin 3390	Pin Agilent 1100	Signal Name	Active (TTL)
	2	1 - White	Digital ground	
	NC	2 - Brown	Prepare run	Low
	7	3 - Gray	Start	Low
	NC	4 - Blue	Shut down	Low
	NC	5 - Pink	Not connected	
	NC	6 - Yellow	Power on	High
	NC	7 - Red	Ready	High
	NC	8 - Green	Stop	Low
	NC	9 - Black	Start request	Low

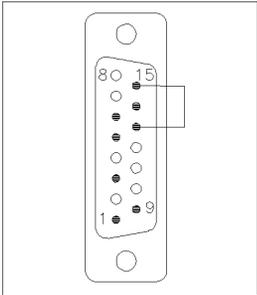
**Agilent 1100 to 3392/3 Integrators**

**Table 42**

Connector 01046-60206	Pin 3392/3	Pin Agilent 1100	Signal Name	Active (TTL)
 <p>4 - Key</p>	3	1 - White	Digital ground	
	NC	2 - Brown	Prepare run	Low
	11	3 - Gray	Start	Low
	NC	4 - Blue	Shut down	Low
	NC	5 - Pink	Not connected	
	NC	6 - Yellow	Power on	High
	9	7 - Red	Ready	High
	1	8 - Green	Stop	Low
	NC	9 - Black	Start request	Low

**Agilent 1100 to 3394 Integrators**

**Table 43**

Connector 01046-60210	Pin 3394	Pin Agilent 1100	Signal Name	Active (TTL)
	9	1 - White	Digital ground	
	NC	2 - Brown	Prepare run	Low
	3	3 - Gray	Start	Low
	NC	4 - Blue	Shut down	Low
	NC	5 - Pink	Not connected	
	NC	6 - Yellow	Power on	High
	5,14	7 - Red	Ready	High
	6	8 - Green	Stop	Low
	1	9 - Black	Start request	Low

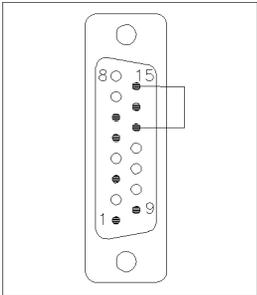
**Table 43**

Connector 01046-60210	Pin 3394	Pin Agilent 1100	Signal Name	Active (TTL)
	13, 15		Not connected	

**NOTE**

START and STOP are connected via diodes to pin 3 of the 3394 connector.

**Agilent 1100 to 3396A Integrators**
**Table 44**

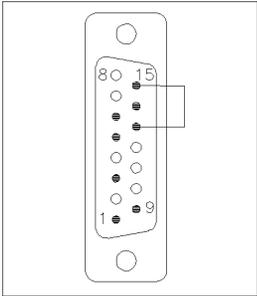
Connector 03394-60600	Pin 3394	Pin Agilent 1100	Signal Name	Active (TTL)
	9	1 - White	Digital ground	
	NC	2 - Brown	Prepare run	Low
	3	3 - Gray	Start	Low
	NC	4 - Blue	Shut down	Low
	NC	5 - Pink	Not connected	
	NC	6 - Yellow	Power on	High
	5,14	7 - Red	Ready	High
	1	8 - Green	Stop	Low
	NC	9 - Black	Start request	Low
	13, 15		Not connected	

**Agilent 1100 to 3396 Series II / 3395A Integrators**

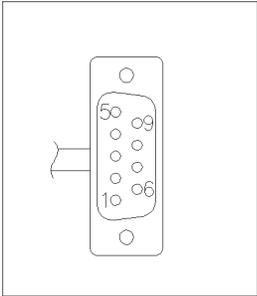
Use the cable 03394-60600 and cut pin #5 on the integrator side. Otherwise the integrator prints START; not ready.

**Agilent 1100 to 3396 Series III / 3395B Integrators**

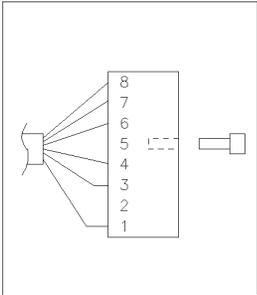
**Table 45**

Connector 03396-61010	Pin 33XX	Pin Agilent 1100	Signal Name	Active (TTL)
	9	1 - White	Digital ground	
	NC	2 - Brown	Prepare run	Low
	3	3 - Gray	Start	Low
	NC	4 - Blue	Shut down	Low
	NC	5 - Pink	Not connected	
	NC	6 - Yellow	Power on	High
	14	7 - Red	Ready	High
	4	8 - Green	Stop	Low
	NC	9 - Black	Start request	Low
	13, 15		Not connected	

**Agilent 1100 to HP 1050, HP 1046A or Agilent 35900 A/D Converters**
**Table 46**

Connector 5061-3378	Pin HP 1050 / ...	Pin Agilent 1100	Signal Name	Active (TTL)
	1 - White	1 - White	Digital ground	
	2 - Brown	2 - Brown	Prepare run	Low
	3 - Gray	3 - Gray	Start	Low
	4 - Blue	4 - Blue	Shut down	Low
	5 - Pink	5 - Pink	Not connected	
	6 - Yellow	6 - Yellow	Power on	High
	7 - Red	7 - Red	Ready	High
	8 - Green	8 - Green	Stop	Low
	9 - Black	9 - Black	Start request	Low

**Agilent 1100 to HP 1090 LC, HP 1040 DAD or Signal Distribution Module**
**Table 47**

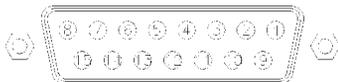
Connector 01046-60202	Pin HP 1090	Pin Agilent 1100	Signal Name	Active (TTL)
 <p>5 - Key</p>	1	1 - White	Digital ground	
	NC	2 - Brown	Prepare run	Low
	4	3 - Gray	Start	Low
	7	4 - Blue	Shut down	Low
	8	5 - Pink	Not connected	
	NC	6 - Yellow	Power on	High
	3	7 - Red	Ready	High
	6	8 - Green	Stop	Low
	NC	9 - Black	Start request	Low

**Agilent 1100 to General Purpose**

**Table 48**

Connector 01046-60201	Pin Universal	Pin Agilent 1100	Signal Name	Active (TTL)
		1 - White	Digital ground	
		2 - Brown	Prepare run	Low
		3 - Gray	Start	Low
		4 - Blue	Shut down	Low
		5 - Pink	Not connected	
		6 - Yellow	Power on	High
		7 - Red	Ready	High
		8 - Green	Stop	Low
		9 - Black	Start request	Low

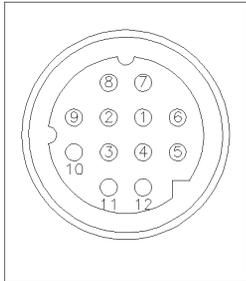
## BCD Cables



One end of these cables provides a 15-pin BCD connector to be connected to the Agilent 1100 Series modules. The other end depends on the instrument it is to be connected to.

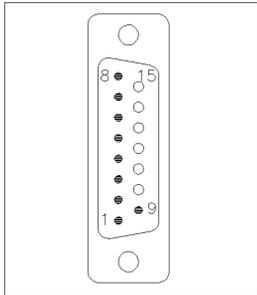
### Agilent 1100 to 3392/3 Integrators

Table 49

Connector 18584-60510	Pin 3392/3	Pin Agilent 1100	Signal Name	BCD Digit
 <p>6 - Key</p>	10	1	BCD 5	20
	11	2	BCD 7	80
	3	3	BCD 6	40
	9	4	BCD 4	10
	7	5	BCD0	1
	5	6	BCD 3	8
	12	7	BCD 2	4
	4	8	BCD 1	2
	1	9	Digital ground	
	2	15	+ 5 V	Low

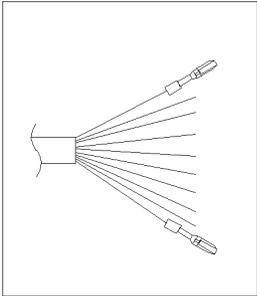
**Agilent 1100 to 3396 Integrators**

**Table 50**

<b>Connector 03396-60560</b>	<b>Pin 3392/3</b>	<b>Pin Agilent 1100</b>	<b>Signal Name</b>	<b>BCD Digit</b>
	1	1	BCD 5	20
	2	2	BCD 7	80
	3	3	BCD 6	40
	4	4	BCD 4	10
	5	5	BCD0	1
	6	6	BCD 3	8
	7	7	BCD 2	4
	8	8	BCD 1	2
	9	9	Digital ground	
	NC	15	+ 5 V	Low

## Agilent 1100 to General Purpose

Table 51

Connector 18594-60520	Wire Color	Pin Agilent 1100	Signal Name	BCD Digit
	Green	1	BCD 5	20
	Violet	2	BCD 7	80
	Blue	3	BCD 6	40
	Yellow	4	BCD 4	10
	Black	5	BCD0	1
	Orange	6	BCD 3	8
	Red	7	BCD 2	4
	Brown	8	BCD 1	2
	Gray	9	Digital ground	
	White	15	+5 Vt	Low

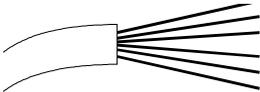
## Auxiliary Cable



One end of this cable provides a modular plug to be connected to the Agilent 1100 Series vacuum degasser. The other end is for general purpose.

### Agilent 1100 Series Degasser to general purpose

Table 52

Connector G1322-61600	Color	Pin Agilent 1100	Signal Name
	White	1	Ground
	Brown	2	Pressure signal
	Green	3	
	Yellow	4	
	Grey	5	DC + 5 V IN
	Pink	6	Vent

### CAN Cable

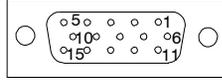


Both ends of this cable provide a modular plug to be connected to Agilent 1100 Series module's CAN-bus connectors.

**Table 53**

Agilent 1100 module to module, 0.5 m	5181-1516
Agilent 1100 module to module, 1 m	5181-1519
Agilent 1100 module to control module	G1323-81600

## External Contact Cable

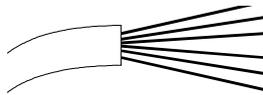


One end of this cable provides a 15-pin plug to be connected to Agilent 1100 Series module's interface board. The other end is for general purpose.

### Agilent 1100 Series Interface Board to general purpose

Table 54

Connector G1103-61611	Color	Pin Agilent 1100	Signal Name
	White	1	EXT 1
	Brown	2	EXT 1
	Green	3	EXT 2
	Yellow	4	EXT 2
	Grey	5	EXT 3
	Pink	6	EXT 3
	Blue	7	EXT 4
	Red	8	EXT 4
	Black	9	Not connected
	Violet	10	Not connected
	Grey/pink	11	Not connected
	Red/blue	12	Not connected
	White/green	13	Not connected
	Brown/green	14	Not connected
	White/yellow	15	Not connected



## RS-232 Cable Kit

This kit contains a 9-pin female to 9-pin female Null Modem (printer) cable and one adapter. Use the cable and adapter to connect Agilent Technologies instruments with 9-pin male RS-232 connectors to most PCs or printers.

### Agilent 1100 module to PC

#### RS-232 Cable Kit 34398As

Table 55

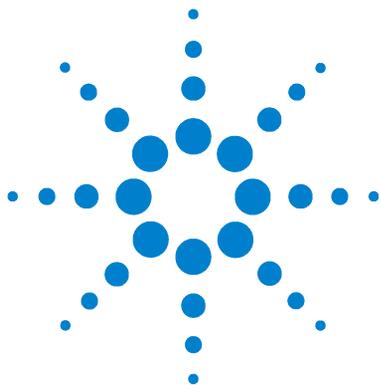
Instrument		PC	
DCD	1	1	DCD
RX	2	2	RX
TX	3	3	TX
DTR	4	4	DTR
GND	5	5	GND
DSR	6	6	DSR
RTS	7	7	RTS
CTS	8	8	CTS
RI	9	9	RI
DB9 Male	DB9 Female	DB9 Female	DB9 Male

## **LAN Cables**

### **Recommended Cables**

For point to point connection (not using a network hub) use a twisted pair cross over LAN cable (P/N 5183-4649, 10 feet long).

For standard network connections using a hub use category 5 UTP cables, (P/N G1530-61480, 8 m long).



## 7 Introduction to the Fluorescence Detector

Introduction to the Detector	238
How the Detector Operates	239
Raman Effect	242
Optical Unit	243
Analytical Information From Primary Data	251
Electrical Connections	256
Instrument Layout	258
Early Maintenance Feedback (EMF)	259

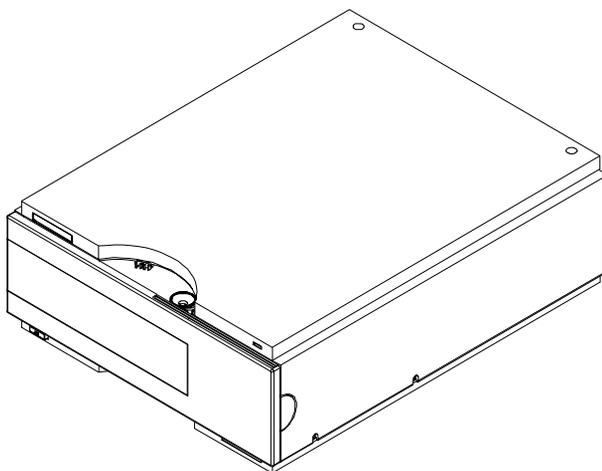


## Introduction to the Detector

The detector is designed for highest optical performance, GLP compliance and easy maintenance. It includes the following features:

- flash lamp for highest intensity and lowest detection limit
- multi-wavelength mode for on-line spectra
- spectra acquisition and simultaneous multi-signal detection
- optional cuvette is available and can be used for off-line measurements
- easy front access to flow cell for fast replacement and
- built-in wavelength accuracy verification.

For specifications, see [“Performance Specifications”](#) on page 328



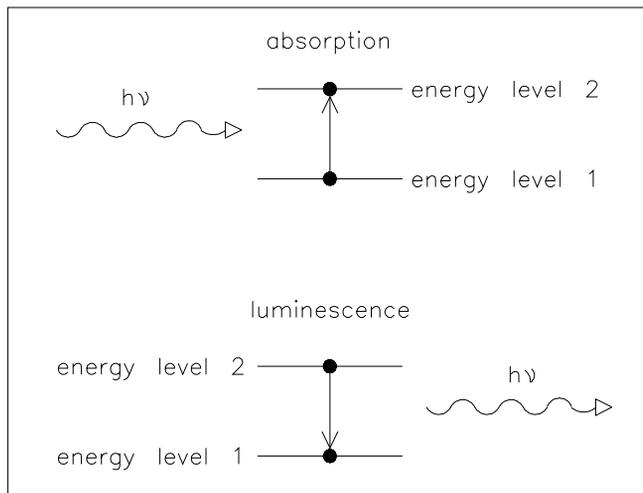
**Figure 72** The Agilent 1100 Series Fluorescence Detector.

## How the Detector Operates

### Luminescence Detection

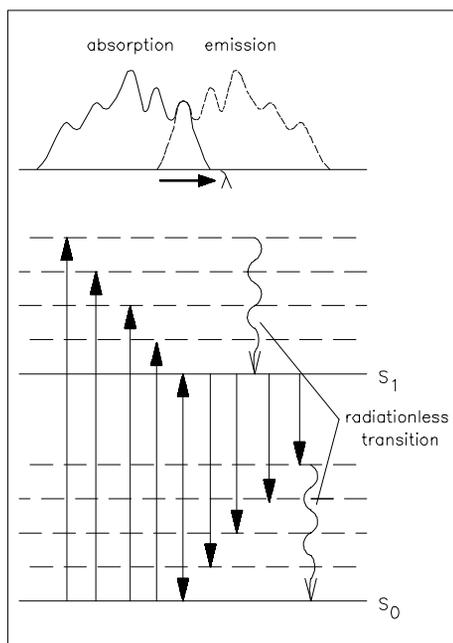
*Luminescence*, the emission of light, occurs when molecules change from an excited state to their ground state. Molecules can be excited by different forms of energy, each with its own excitation process. For example, when the excitation energy is light, the process is called *photoluminescence*.

In basic cases, the emission of light is the reverse of absorption, see [Figure 73](#). 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 73** Absorption of Light Versus Emission of Light

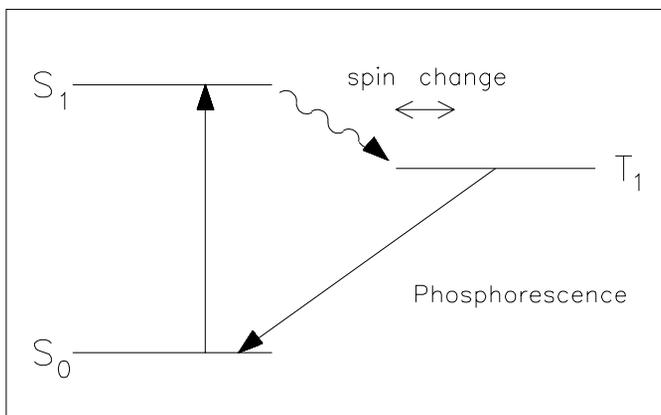
When a more complex molecule transforms from its ground energy state into an excited state, the absorbed energy is distributed into various vibrational and rotational sub-levels. When this, same molecule returns to the ground state, this vibrational and rotational energy is first lost by relaxation without 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 74](#). The characteristic maxima of absorption for a substance is its  $\lambda_{EX}$ , and for emission its  $\lambda_{EM}$ .



**Figure 74** 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 75.



**Figure 75** 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.

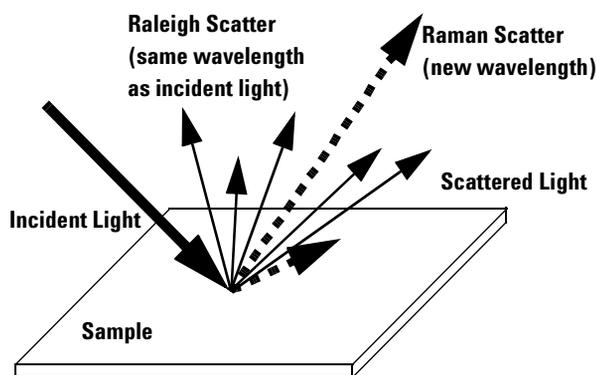
$$\text{Formula: } E = h \times \nu$$

In this equation:

- E is energy
- h is Planck's constant
- $\nu$  is the wavelength

## Raman Effect

The Raman effect arises when the incident light excites molecules in the sample which subsequently scatter the light. While most of this scattered light is at the same wavelength as the incident light, some is scattered at a different wavelength. This inelastically scattered light is called Raman scatter. It results from the molecule changing its molecular motions.



**Figure 76** Raman

The energy difference between the incident light ( $E_i$ ) and the Raman scattered light ( $E_s$ ) is equal to the energy involved in changing the molecule's vibrational state (i.e. getting the molecule to vibrate,  $E_v$ ). This energy difference is called the Raman shift.

$$E_v = E_i - E_s$$

Several different Raman shifted signals will often be observed; each being associated with different vibrational or rotational motions of molecules in the sample. The particular molecule and its environment will determine what Raman signals will be observed (if any).

A plot of Raman intensity versus Raman shift is a Raman spectrum.

## Optical Unit

All the elements of the optical system, shown in [Figure 77](#) on page 244, including Xenon flash lamp, excitation condenser, excitation slit, mirror, excitation grating, flow cell, emission condenser, cut-off filter, emission slit, emission grating and photo-multiplier tube are housed in the metal casting inside the detector compartment. The fluorescence detector has grating/grating optics, enabling the selection of both excitation and emission wavelengths. The flow cell can be accessed from the front of the fluorescence detector.

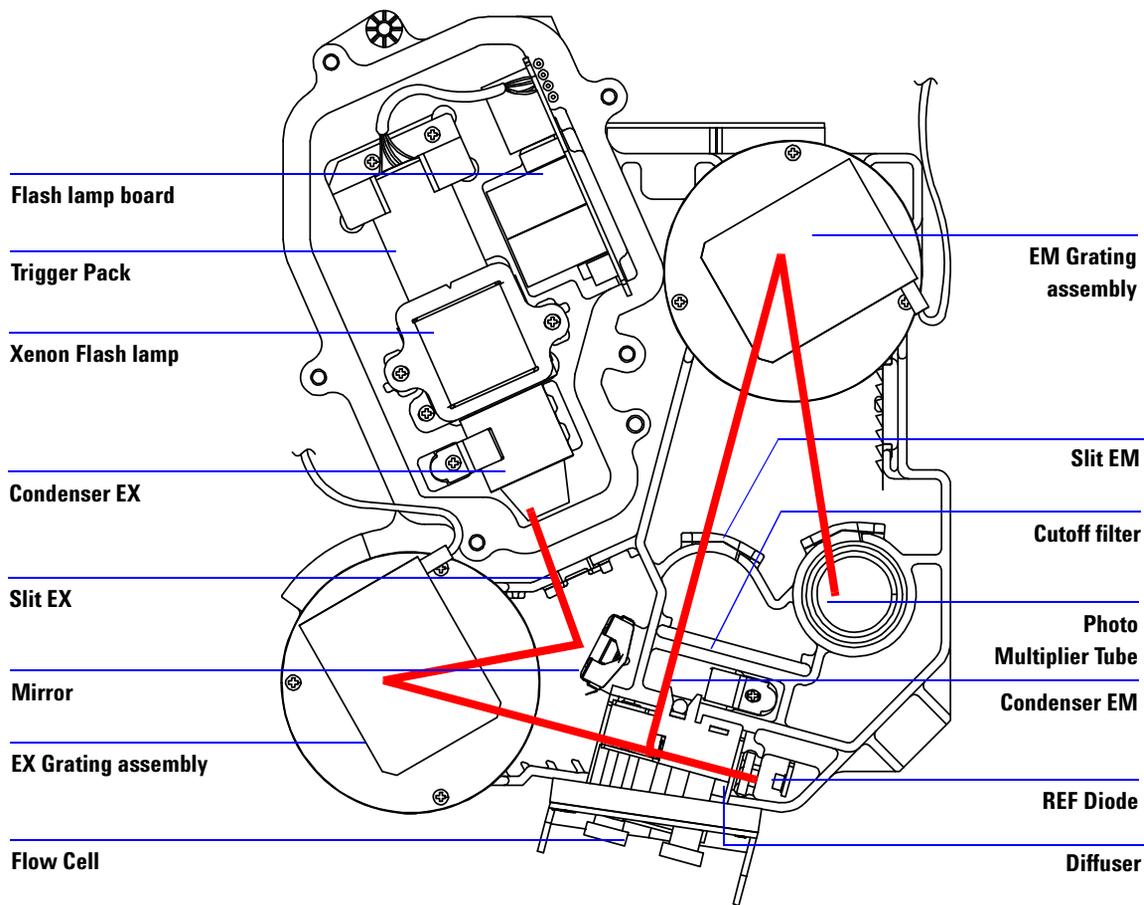
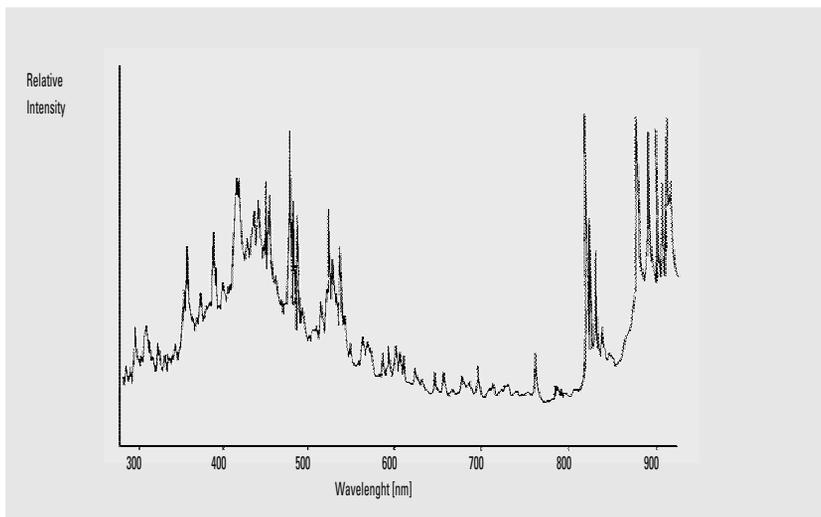


Figure 77 Optical Unit

The radiation source is a xenon flash-lamp. The 3  $\mu$ s flash produces a continuous spectrum of light from 200 nm to 900 nm. The light output distribution can be expressed as a percentage in 100 nm intervals, see [Figure 78](#). The lamp can be used for some 1000 hours depending on the sensitivity requirements. You can economize during automatic operation using keyboard setpoints, 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.

UV degradation, especially below 250 nm is significantly higher compared to Visible wavelength range. Generally the "LAMP ON during run" - setting or using "economy mode" will increase lamp life by a magnitude.



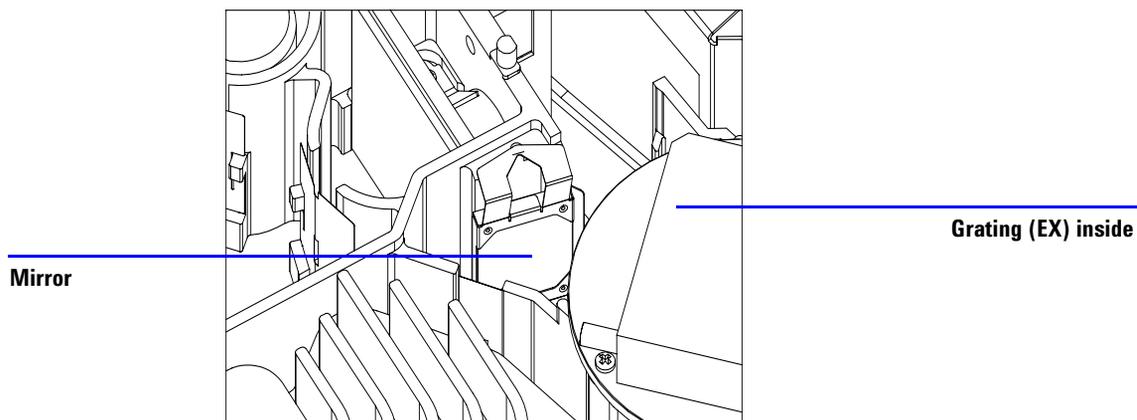
**Figure 78** Lamp Energy Distribution (vendor data)

## 7 Introduction to the Fluorescence Detector

The radiation emitted by the lamp is dispersed and reflected by the excitation monochromator grating onto the cell entrance slit.

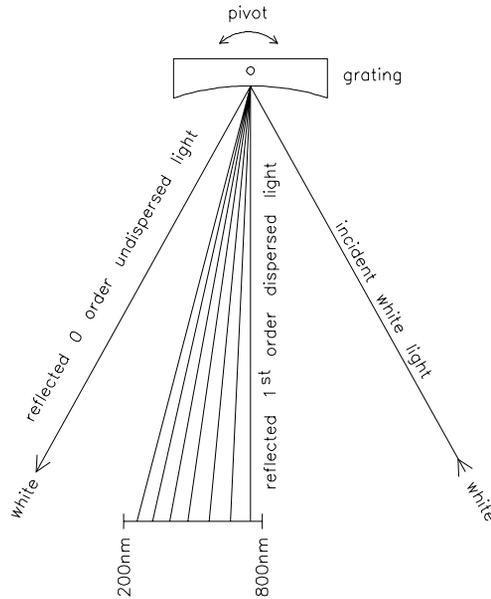
The holographic concave grating is the main part of the monochromator, dispersing and reflecting the incident light. The surface contains many minute grooves, 1200 of them per millimeter. The grating carries a blaze to show improved performance in the visible range.

Y



**Figure 79** Mirror Assembly

The geometry of the grooves is optimized to reflect almost all of the incident light, in the 1<sup>st</sup> 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 80** illustrates the light path at the surface of the grating.



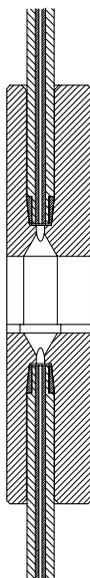
**Figure 80** Dispersion of Light by a Grating

The grating is turned using a 3-phase brushless DC 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.

For spectra acquisition and multi-wavelength detection, the grating rotates at 4000 rpm.

The excitation and emission gratings are similar in design, but have different blaze wavelengths. The excitation grating reflects most 1<sup>st</sup> order light in the ultra-violet range around 250 nm, whereas the emission grating reflects better in the visible range around 400 nm.

The flow cell is a solid quartz body with a maximum back pressure of 20 bar. Excessive back pressure will result in destruction of the cell. Operating the detector close to waste with low back pressure is recommended. A slit is integrated to the quartz body.

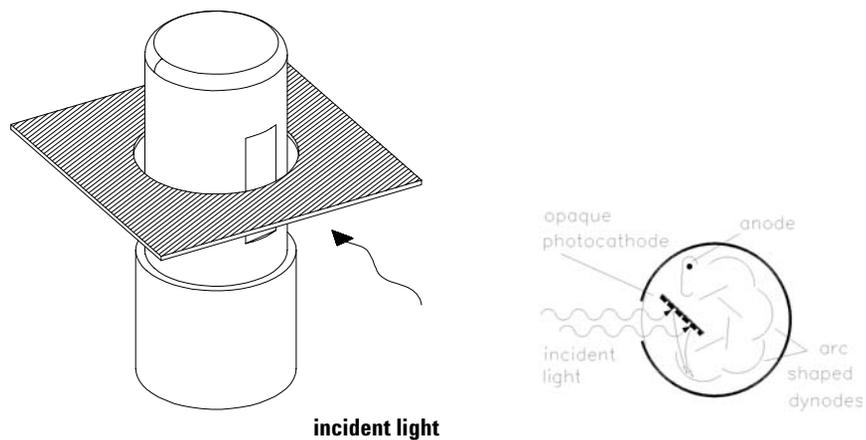


**Figure 81** Cross-Section of Flow Cell

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 1<sup>st</sup> order scatter and 2<sup>nd</sup> order stray light, see [Figure 80](#) on page 247.

The selected wavelength of light is reflected onto the slit in the wall of the photo-multiplier compartment of the optical unit. The bandwidth of the emitted light is 20 nm.

On the photocathode, [Figure 82](#), 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.



**Figure 82** Photo-multiplier Tube

This type of so-called side-on photo-multiplier is compact ensuring fast response, conserving the advantages of the short optical path shown in [Figure 77](#) on page 244.

PMTs are designed for specific wavelength ranges. The standard PMT offers optimum sensitivity from 200 to 600 nm. In the higher wavelength range a red-sensitive PMT can improve performance. For additional PMT types refer to "[Replacing PMT and/or FLF board](#)" on page 180.

### Reference System

A reference diode, located behind the flow cell, measures the excitation (EX) light transmitted by the flow cell and corrects flash lamp fluctuations and long-term intensity drift. Because of a non-linear output of the diode (depending on the EX-wavelength), the measured data are normalized.

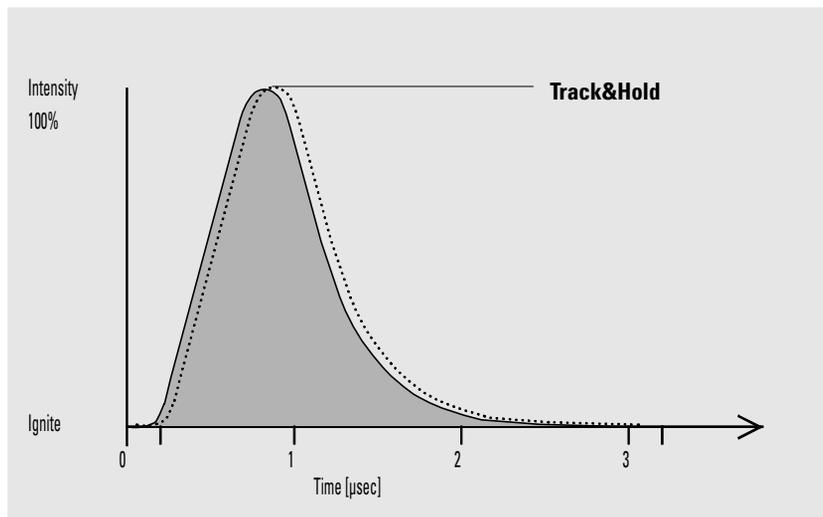
A diffuser is located in front of the reference diode (see [Figure 77](#) on page 244). This diffuser is made of quartz, reduces light and allows integral measurement of the light.

## Analytical Information From Primary Data

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

### Fluorescence Detection

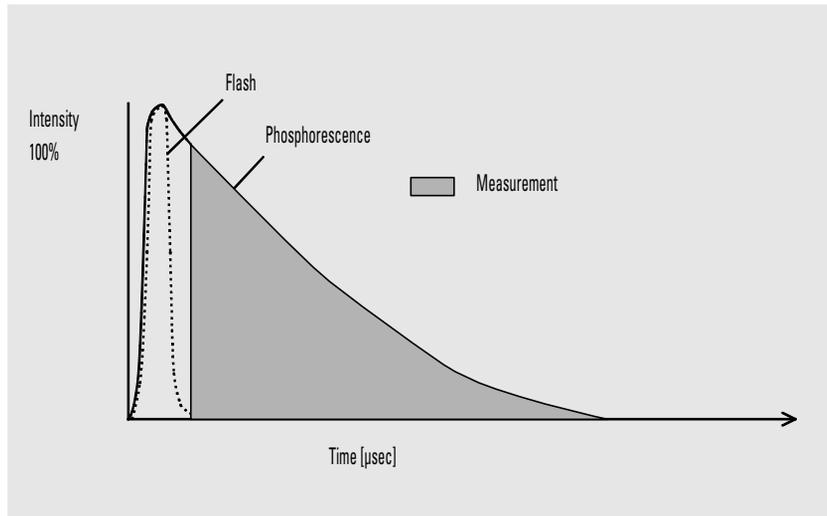
When the lamp flashes, the fluorescing compounds in your sample will luminesce almost simultaneously, see [Figure 83](#). The luminescence is short-lived, therefore the fluorescence detector need only measure over a short period of time after the lamp has flashed.



**Figure 83** Measurement of Fluorescence

## Phosphorescence Detection

An appropriate parameter set will be specified as soon as you chose the phosphorescence detection mode (special setpoints under FLD parameter settings).

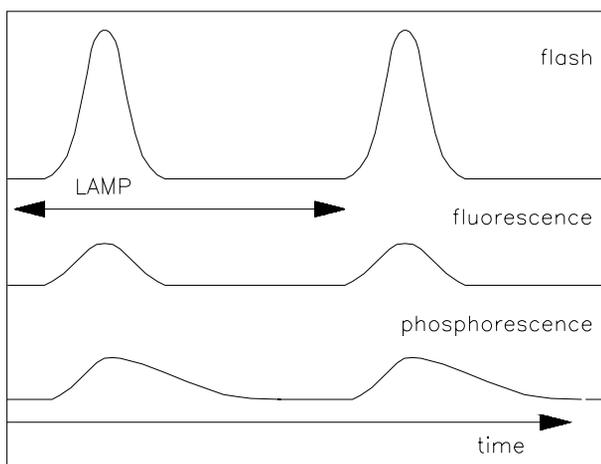


**Figure 84** Measurement of Phosphorescence

## Processing of Raw Data

If the lamp flashes at single wavelength and high-power, then the fluorescence data rate is 296 Hz. That means that your sample is illuminated 296 times per second, and any luminescence generated by the components eluted from the column is measured 296 times per second.

If the “economy” or multi-wavelength mode is set, then the flash frequency is 74 Hz.



**Figure 85** LAMP: Frequency of Flash, Fluorescence, and Phosphorescence

You can improve the signal-to-noise characteristics by disabling the “economy” mode.

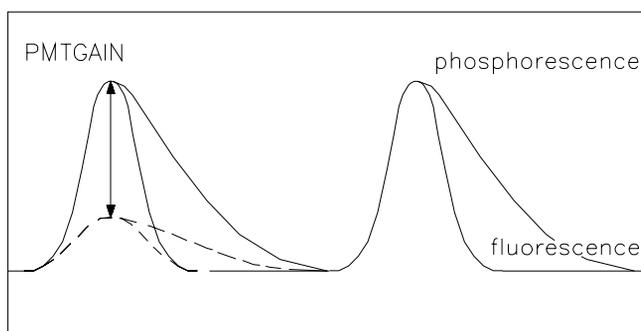
### NOTE

Disabling the “economy” mode will shorten the lifetime of the lamp significantly. Consider lifetime saving by switching off the lamp after the run is completed.

The data resolution is 20 bit at a response time of 4 seconds (default, which is equivalent to a time constant of 1.8 seconds and appropriate for standard chromatographical conditions). Weak signals may cause errors in

quantification because of insufficient resolution. Check your proposed PMTGAIN. If it is significantly distant from your setting, change your method or check the purity of your solvent.

You can amplify the signal using PMTGAIN. Depending on the PMTGAIN you have set, a multiple of electrons is generated for every photon falling on the photomultiplier. You can quantify large and small peaks in the same chromatogram by adding PMTGAIN changes during the run into a timetable.



**Figure 86** PMTGAIN: Amplification of Signal

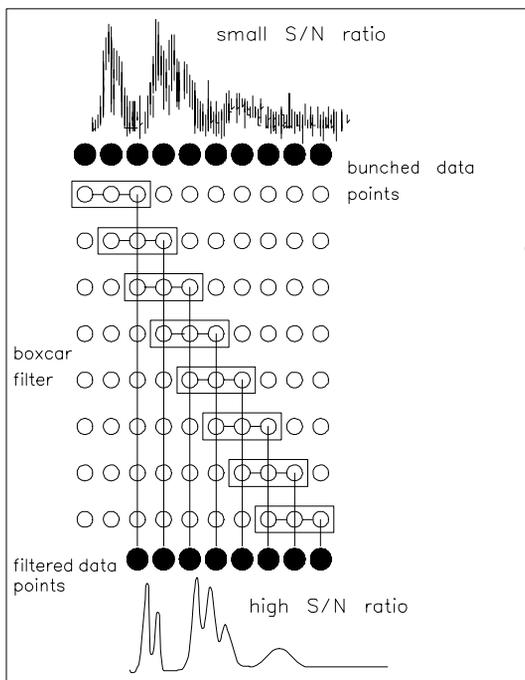
Check proposed PMTGAIN. Deviations of more than 2 PMT gains should be corrected in the method.

Each PMTGAIN step is increased approximately by a factor of 2 (range 0 - 18). To optimize your amplification for the peak with the highest emission, raise the PMTGAIN setting until the best signal-to-noise is achieved.

After the photons are converted and multiplied into an electronic signal, the signal (at present analog) is tracked and held beyond the photo-multiplier. After being held, the signal is converted by an A-to-D converter to give one raw data point (digital). Eleven of these data points are bunched together as the first step of data processing. Bunching improves your signal-to-noise ratio.

The bunched data, shown as larger black dots in [Figure 87](#), 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 sec to 4 sec) doubles the signal-to-noise ratio.



**Figure 87** RESPONSETIME: Signal-to-Noise Ratio

## Electrical Connections

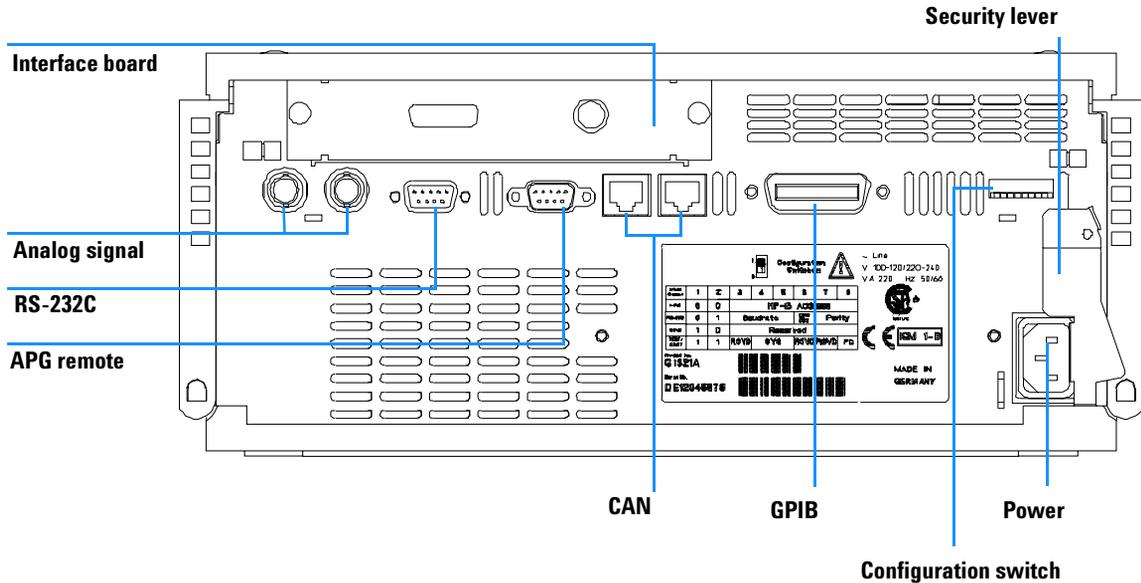
- The GPIB connector is used to connect the detector with a computer. The address and control switch module next to the GPIB connector determines the GPIB address of your detector. The switches are preset to a default address (see [“GPIB Default Addresses”](#) on page 291) which is recognized once the power is switched on.
- The CAN bus is a serial bus with high speed data transfer. The two connectors for the CAN bus are used for internal Agilent 1100 Series module data transfer and synchronization.
- Two independent analog outputs provide signals for integrators or data handling systems.
- The interface board slot is used for external contacts and BCD bottle number output or LAN connections.
- The REMOTE connector may be used in combination with other analytical instruments from Agilent Technologies if you want to use features such as start, stop, common shut down, prepare, and so forth.
- With the appropriate software, the RS-232C connector may be used to control the detector from a computer through a RS-232C connection. This connector is activated and can be configured with the configuration switch next to the GPIB connector (see [“Communication Settings for RS-232C Communication”](#) on page 292). See your software documentation for further information.

Together with a control module the RS-232C may be used to print screens to a connected printer.

- The power input socket accepts a line voltage of 100 – 120 V or 220 – 240 V AC  $\pm$  10 % with a line frequency of 50 or 60 Hz. Maximum power consumption is 220 VA. There is no voltage selector on your detector because the power supply has a wide-ranging capability. There are no externally accessible fuses, because automatic electronic fuses are implemented in the power supply. The security lever at the power input socket prevents removal of the detector cover when line power is still connected.

**WARNING**

Never use cables other than the ones supplied by Agilent Technologies to ensure proper functionality and compliance with safety or EMC regulations.



**Figure 88** Electrical Connections

### Instrument Layout

The industrial design of the detector incorporates several innovative features. It uses Agilent's E-PAC concept for the packaging of electronics and mechanical assemblies. This concept is based upon the use of expanded polypropylene (EPP) layers of foam plastic spacers in which the mechanical and electronic boards components of the detector are placed. This pack is then housed in a metal inner cabinet which is enclosed by a plastic external cabinet. The advantages of this packaging technology are:

- virtual elimination of fixing screws, bolts or ties, reducing the number of components and increasing the speed of assembly/disassembly,
- the plastic layers have air channels molded into them so that cooling air can be guided exactly to the required locations,
- the plastic layers help cushion the electronic and mechanical parts from physical shock, and
- the metal inner cabinet shields the internal electronics from electromagnetic interference and also helps to reduce or eliminate radio frequency emissions from the instrument itself.

## Early Maintenance Feedback (EMF)

Maintenance requires the exchange of components which are subject to wear or stress. Ideally, the frequency at which components are exchanged should be based on the intensity of usage of the detector and the analytical conditions, and not on a predefined time interval. The early maintenance feedback (EMF) feature monitors the usage of specific components in the instrument, and provides feedback when the user-selectable limits have been exceeded. The visual feedback in the user interface provides an indication that maintenance procedures should be scheduled.

### EMF Counters

The detector provides three EMF counters for the lamp. The counters increment with lamp use, and can be assigned a maximum limit which provides visual feedback in the user interface when the limit is exceeded. The counters can be reset to zero after the lamp is exchanged. The detector provides the following EMF counters:

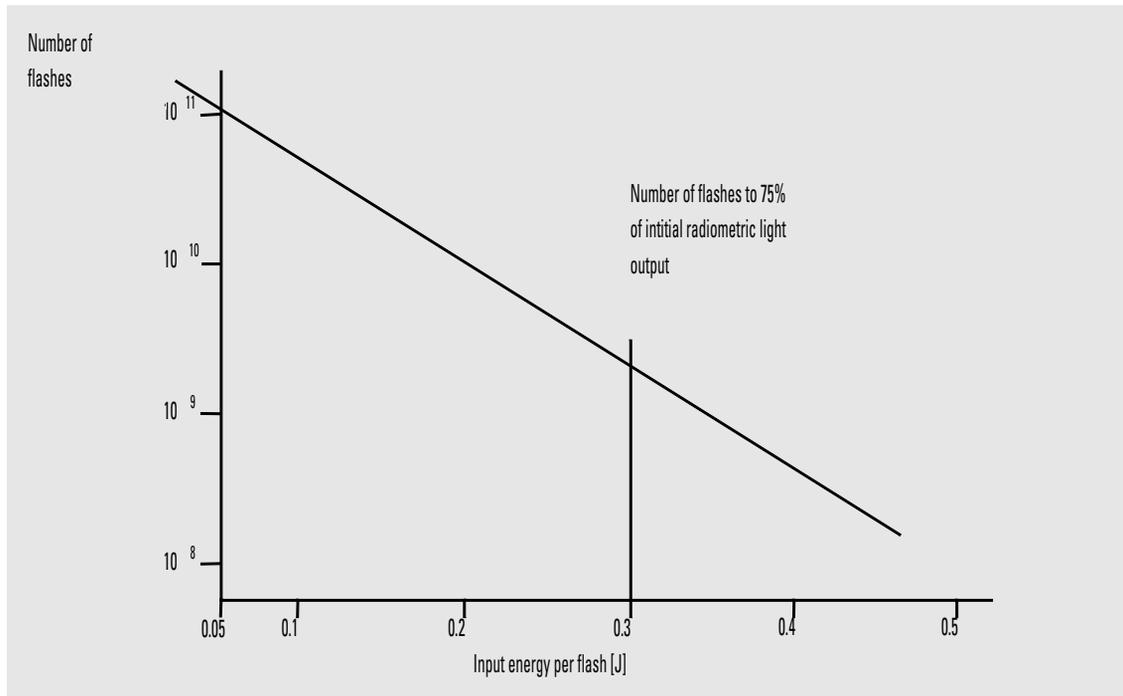
- number of flashes (low power mode, multiples of 1000 flashes)
- number of flashes (high power mode, multiples of 1000 flashes)
- Flash Lamp Life-Time (value of 0 – 100% as expected lifetime factor calculated from combined expected lifetime low power and high power flashes).

Figure 89 on page 260 shows the lamp life based on number of flashes vs. the input energy. The lamp flash frequency/energy can be changed into the following modes:

**Table 56** Flash Lamp Modes

Position	296 Hz (Standard), 560 V	63 mJoule (18.8 W)
	74 Hz (Economy), 560 V	63 mJoule (4.7 W)
Rotation (Multi Ex/Em)	74 Hz (Standard), 950 V	180 mJoule (13.3 W)
	74 Hz (Economy), 560 V	63 mJoule (4.7 W)

## 7 Introduction to the Fluorescence Detector



**Figure 89** Lamp life

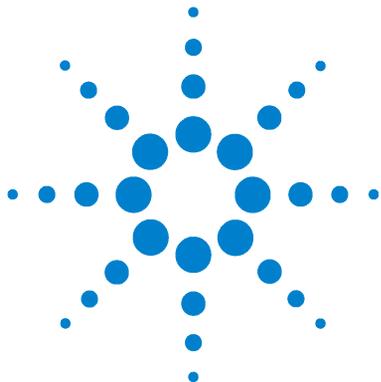
## Using the EMF Counters

The user-selectable EMF limits for the EMF counters enable the early maintenance feedback to be adapted to specific user requirements. The useful lamp burn time is dependent on the requirements for the analysis (high or low sensitivity analysis, wavelength etc.), therefore, the definition of the maximum limits need to be determined based on the specific operating conditions of the instrument.

### Setting the EMF Limits

The setting of the EMF limits must be optimized over one or two maintenance cycles. Initially, no EMF limit should be set. When instrument performance indicates maintenance is necessary, take note of the values displayed by lamp counters. Enter these values (or values slightly less than the displayed values) as EMF limits, and then reset the EMF counters to zero. The next time the EMF counters exceed the new EMF limits, the EMF flag will be displayed, providing a reminder that maintenance needs to be scheduled.

## **7 Introduction to the Fluorescence Detector**



## 8 Theory of Operation

Electronics	264
Detector Main Board (FLM)	265
Detector Frontend Board (FLF)	269
Detector Lamp Supply Board (FLL)	273
Firmware Description	274
Raw Data Conversion to Fluorescence (LU) Units	276
Optional Interface Boards	282
Interfaces	285
Setting the 8-bit Configuration Switch	290
The Main Power Supply Assembly	295



### Electronics

The electronics are comprised of four main components:

- **detector main board** (FLM), see [page 265](#).
- **detector frontend board** (FLF), see [page 269](#).
- **detector lamp board** (FLL), see [page 273](#).
- **power supply**, see [page 295](#).

Optional:

- **interface board** (BCD/external contacts), see [page 283](#).
- **interface board** (LAN), see [page 284](#).

## Detector Main Board (FLM)

This board controls all information and activities of all assemblies within the detector. Through interfaces (CAN, GPIB, RS-232C or LAN) connected to the user interface, the operator enters parameters, changes modes and controls the detector.

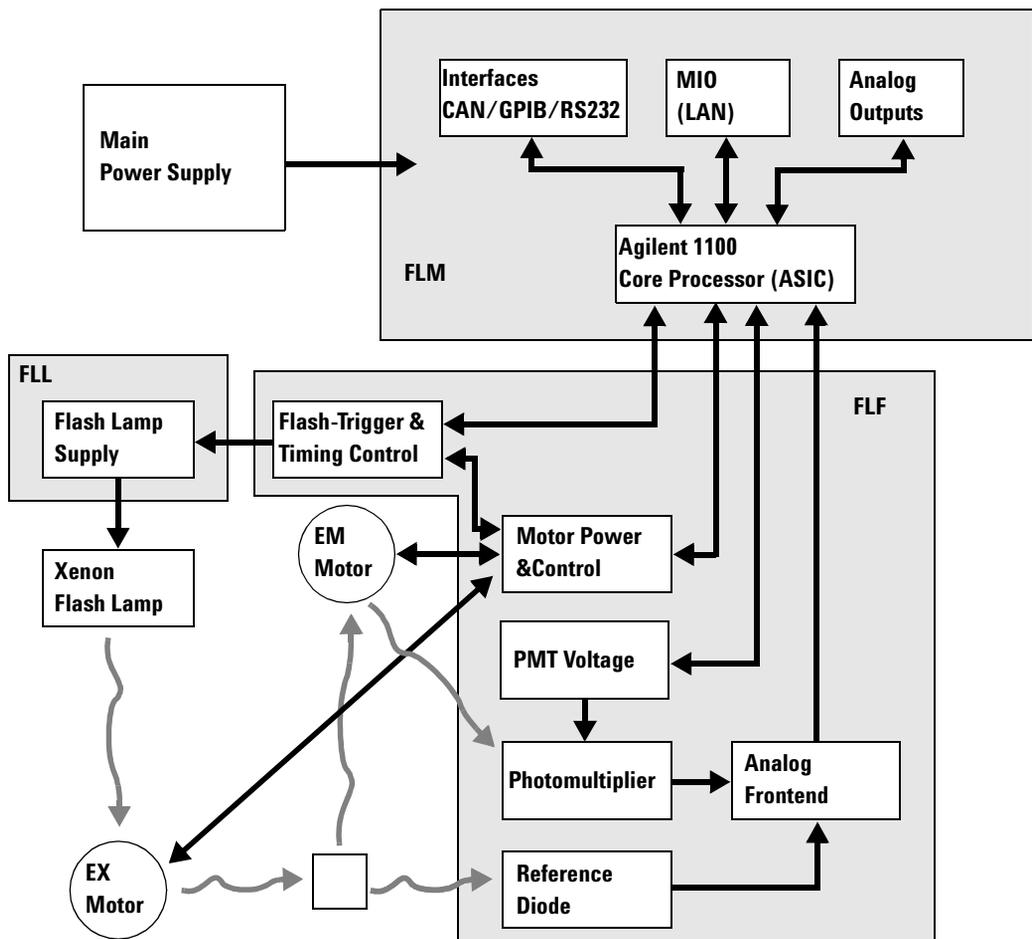


Figure 90 Detector Electronic Overview

### **ASIC — Application Specific Integrated Circuit**

The 304-pin application specific integrated circuit (ASIC) provides interfacing to external devices through drivers, including GPIB, CAN, APG remote, and RS-232C. It is connected directly to the four control LEDs located near the connectors on the board and the 8-bit configuration switch which is used to configure the address for the GPIB communication, the Baud rate for RS-232C transfer, and so on. For switch settings, refer to the *Installing Your Agilent 1100 Win System* handbook.

In addition, it controls the cooling fan via the PWM (pulse width modulation) driver. Movement of the cooling fan is sensed by the microprocessor.

### **Main processor**

The Motorola MC 68332 microprocessor acts as the main processor with a clock frequency of 16 MHz. It exchanges data with the ASIC through the core bus as well as with the SRAM (static random access memory) of 4 × 512 kilobyte, the flash EEPROM (electrically erasable programmable read-only memory) of 1 MB and the 1 × 128 KB battery-backed NV-RAM (non-volatile). Battery life is designed to last for more than 25 years under normal operating conditions.

Control lines provide communication to the frontend board, which provides high voltage power supplies for the flash lamp and the photomultiplier tube (PMT) and contains the data acquisition section.

### **FPGA—Field Programmable Gate Array**

The FPGA is loaded with code to control the timing of the flash ignition and the data acquisition. Thus a realtime control of these critical functions is achieved without processor control.

### **Excitation/Emission Monochromator Motor Control**

A chopped motor driver IC provides the signal to run the excitation/emission monochromator motor in positioning or rotation mode.

### **Encoder Signal Acquisition**

Each monochromator includes an optical encoder to get the actual position of the device. The two encoder signals are amplified and prepared for A/D-conversion.

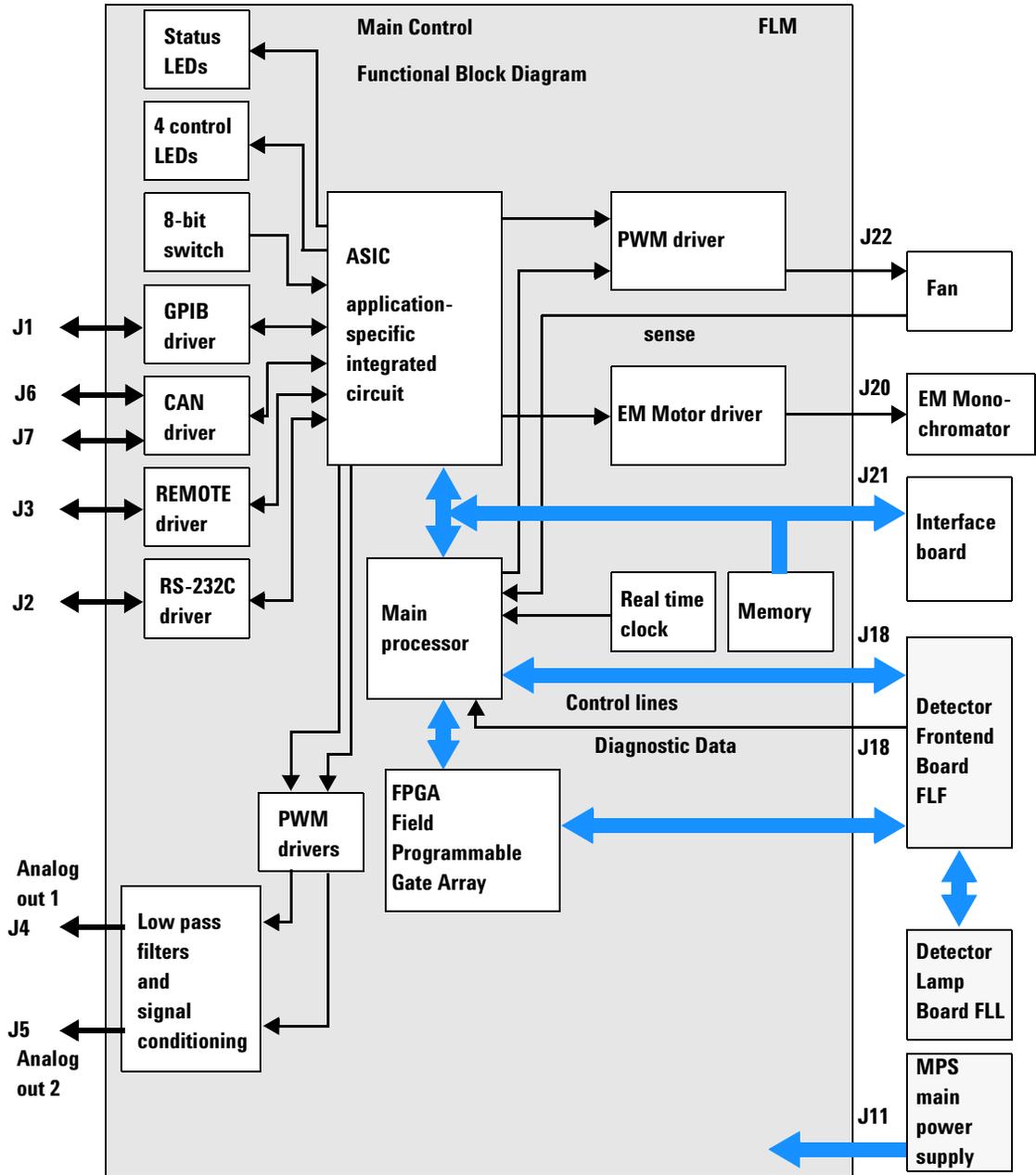


Figure 91 Block Diagram FLD Main Controller Functionality

### **Fan Drive**

The operation of the fan is controlled by the main processor and runs with constant revolution. The fan produces a sense signal which is derived from the revolution. This sense signal is used for diagnostics.

### **On-board Battery**

An on-board lithium battery buffers the electronic memory when the module is turned off.

For safety information on lithium batteries see "[Lithium Batteries Information](#)" on page 335.

### **Analog Outputs**

There are two independent analog outputs (0...1 V full scale). Digital data from the ASIC is converted into a pulse-width modulated signal (PWM). The PWM signal is then fed to an amplitude modulator, which precisely turns the reference signal on and off. This signal then passes to a low-pass filter section.

### **Interfaces**

For detailed information on interfaces, see "[Interfaces](#)" on page 285.

## Detector Frontend Board (FLF)

### Data Acquisition

The FLF board provides circuitry to measure three different physical effects:

- Fluorescence mode: peak value after flash
- Phosphorescence mode: integrated value after flash
- Chemoluminescence mode: amplified signal without flash operation

In parallel to the fluorescence signal the reference signal is acquired to get a compensation of lamp drift and noise effects. Both signals are amplified and buffered and routed to the ADC input multiplexer.

The 16-bit A/D-converter performs a conversion of the selected signal and additionally of the corresponding reference signal and several other signals:

- monochromator positions
- board temperature
- leak sensor status

Then the digital data are polled by the FPGA on the FLM main board to be available for calculations.

### Flash Lamp Power Supply

The flash lamp is operated with discharge voltages of 500 V or 1250 V. The electronics design provides the voltages using a regulating pulse width modulator to switch a 24 V rail with a chopping frequency of 40 kHz to a transformer. The resulting high voltage is rectified and fed back to the error amplifiers which generate a control feedback signal for the regulator to increase or decrease the duty cycle of the output signal. An additional softstart-circuitry and a limitation of the maximum voltage ramp-up reduces the resulting noise emission of the power supply. The complete flash voltage supply is isolated by optocouplers to avoid crosstalk to other circuits by the large switching currents.

### **Flash Trigger Voltage Supply**

The flash lamp trigger assembly includes an ignition transformer which needs a 160 V pulse to generate a short high voltage pulse ( $\sim 10$  kV) for a reliable lamp ignition. A second lamp power supply transformer output is used to generate this trigger voltage at both operating conditions.

### **Igniting the Flash Lamp**

The flash lamp is a plasma discharge lamp with an energy-controlled intensity operated at 74 Hz or 296 Hz. The applied voltage charges a capacitor array and forms a controlled discharge voltage difference between lamp anode and cathode. Then an additional ignition electrode is driven with a high ignition voltage pulse to build up a small ionized channel from the ignition electrode to the cathode. If this ionization is existent, the voltage difference between anode and cathode leads to a flash discharge within a very short time (1  $\mu$ s). After the flash the system pauses the regulator to force the ionized path to break down rapidly before charging the capacitors for the next cycle.

### **Flash Lamp Overcurrent Protection**

A special firmware-controlled diagnostic circuitry shuts down the regulator when the charge current exceeds a critical value. This may happen when the output voltage lines are shorted. When an overcurrent condition is found, the circuit stops the voltage regulator within the time between two flashes, and the firmware reports an error signal when a restart of the regulator fails again.

### **Flash Voltage Safety Switch**

A safety switch stops the voltage regulator when the lamp cover is removed to avoid high voltages on board when electronic parts are accessible.

### Photomultiplier Tube Voltage Supply

The photomultiplier tube needs an adjustable working voltage between -240 V and -820 V for correct operation. The PMT gains are realized by variable operating voltage of the device. A regulated pulse width modulator is used to generate the high voltage by switching the 24 V rail to a transformer. The feedback loop has a D/A-converter-controlled base voltage to set the desired output voltage. The PMT output delivers a current signal which is proportional to the detected light and to the actual working voltage.

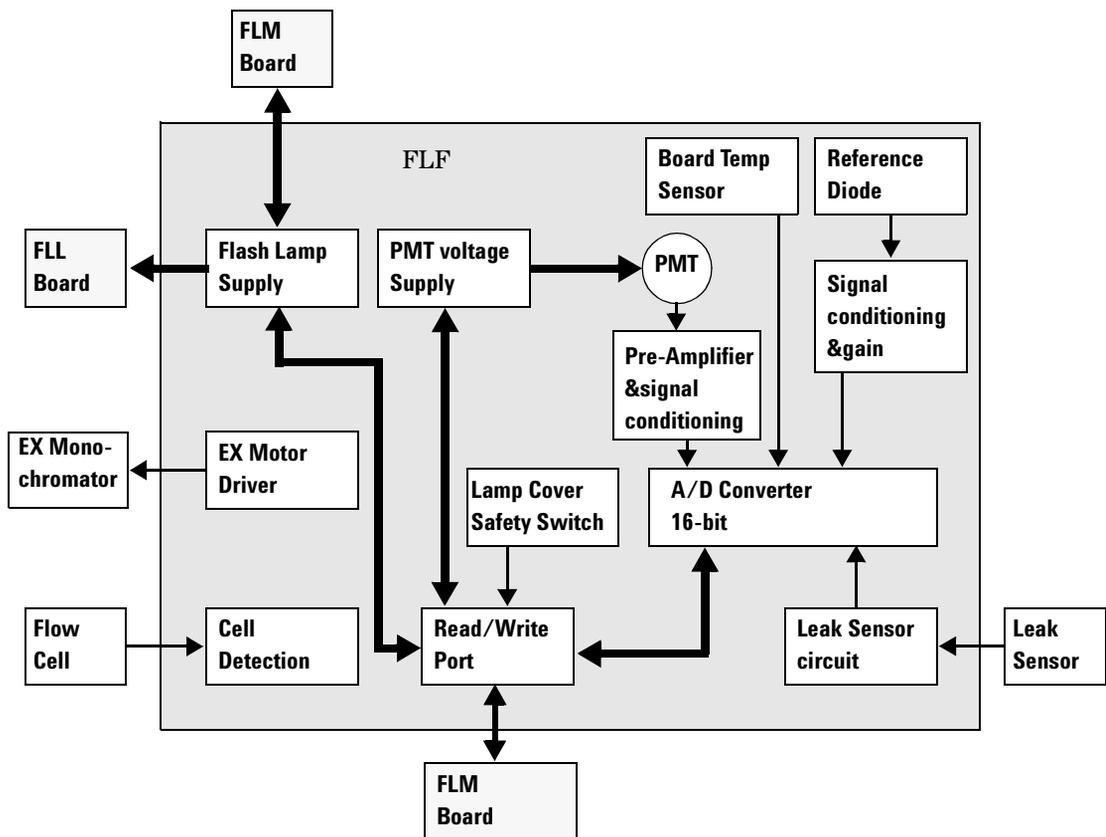


Figure 92 Block diagram FLF

### Reference System

A reference photodiode is located behind the cell and monitors the incoming radiation through a diffusor window. The data are used to compensate the fluorescence data for drift effects. The reference path provides multiple gain stages which are set automatically according to the actual intensity.

### Cell Detection

The system detects if a cell is inserted and switches off lamp operation, PMT high voltage and monochromator rotation if no cell is present to prevent injury (by UV radiation, high voltage or rotating parts) to the user.

### Emission Monochromator Motor Control

A chopped motor driver IC provides the signal to run the excitation monochromator motor in positioning or rotation mode.

### Leak Sensor

A leak sensor is mounted in the instrument's front chassis to detect leakage of the cell assy. In case of a leakage, the complete Agilent 1100 System is shut down to prevent system damage.

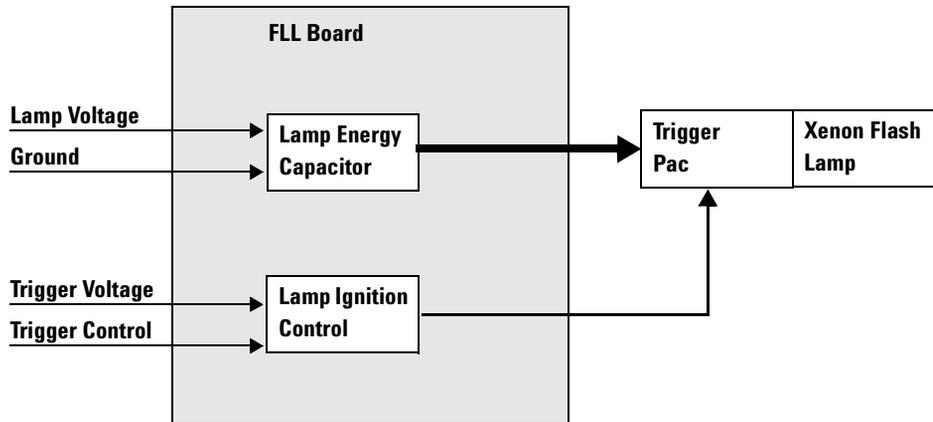
This block consists of a PTC for the leak detection and a NTC for the ambient temperature compensation. A leak would cool down the PTC and this change in resistance would generate a leak signal.

### Digital Interface

The control and data lines from and to the Fluorescence Detector Main Board (FLM) are buffered by Read- and Write ports. The signals from the main board allow setting of the signal multiplexers, reference gain stages, lamp current and to start the initial self-calibration of the A/D-converter. The port to the main board drives the A/D-converter data and several control lines like cell detection, overcurrent detection and lamp cover safety switch position.

The signals driving the lamp are derived from the FPGA on the Main Board and have separate connections. This allows the lamp timing control without processor interaction and guarantees a realtime control of the lamp operation.

## Detector Lamp Supply Board (FLL)



**Figure 93** Block Diagram FLL Board

### Lamp Energy Capacitors

The lamp high voltage circuit of the FLF board is used to charge the 4 energy capacitors of the FLL board. The energy per flash depends on the applied charging voltage ( $1/2 * C * U^2$ ). A power diode freewheeling circuit prevents the backflowing current from generating negative lamp discharge currents which may damage the flash lamp.

### Lamp Ignition Control

An ignition voltage is switched by a thyristor to the TriggerPac ignition transformer to build a small ionized channel (refer "[Igniting the Flash Lamp](#)" on page 270). A freewheeling diode forces the thyristor to switch off again to prevent lamp damage.

# Firmware Description

The firmware of the instrument consists of two independent sections:

- a non-instrument specific section, called **resident system**,
- an instrument specific section, called **main system**.

### Resident System

This resident section of the firmware is identical for all Agilent 1100 series modules. Its properties are:

- the complete communication capabilities (GPIB, CAN, LAN and RS-232C),
- memory management,
- ability to update the firmware of the 'main system'.

### Main System

Its properties are:

- the complete communication capabilities (GPIB, CAN, LAN and RS-232C),
- memory management,
- ability to update the firmware of the resident system.

In addition the main system comprises the instrument functions that are divided into common functions like

- run synchronization through APG remote
- error handling,
- diagnostic functions,
- or module specific functions like:
  - internal events such as lamp control, filter movements,
  - raw data collection and conversion to absorbance (see ["Raw Data Conversion to Fluorescence \(LU\) Units"](#) on page 276).

## Firmware Updates

Firmware updates can be done using your user interface:

- handheld control module with files from a PC-card or
- Agilent ChemStation with files from floppy disk

The file naming conventions are:

xxxx-vvv.DLB, where

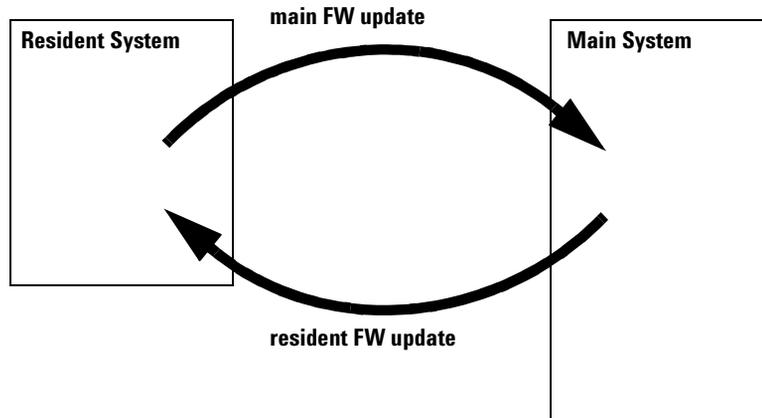
xxxx is the product number, for example, 1315 for the G13151A DAD, and vvv is the revision number, for example 104 is revision 1.04

For instructions refer to your user interface.

### NOTE

Update of main system can be done in the resident system only.

Update of the resident system can be done in the main system only.

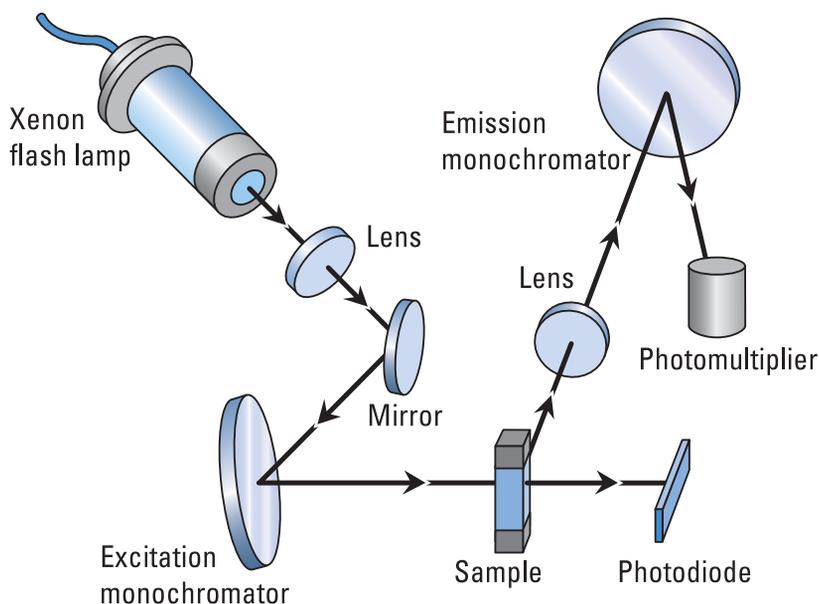


**Figure 94** Firmware Update Mechanism

## Raw Data Conversion to Fluorescence (LU) Units

LU (light or luminescence units) is used as new unit and will indicate the light intensity (not comparable with the physical unit Lumen!).

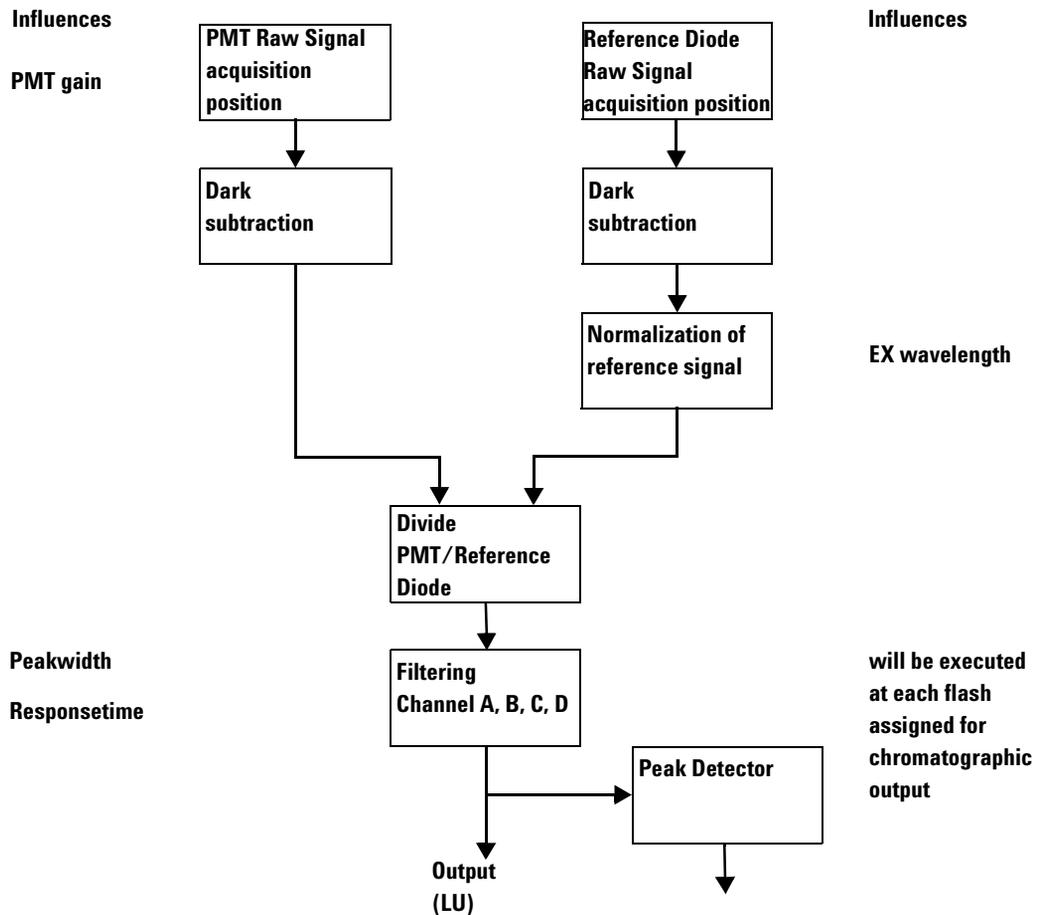
These units are calculated from the PMT (photomultiplier tube) in the emission path and a reference diode located behind the flow cell, see figure below.



**Figure 95** Optical Diagram of the FLD

## Data flow for chromatographic output

The data flow is shown below.

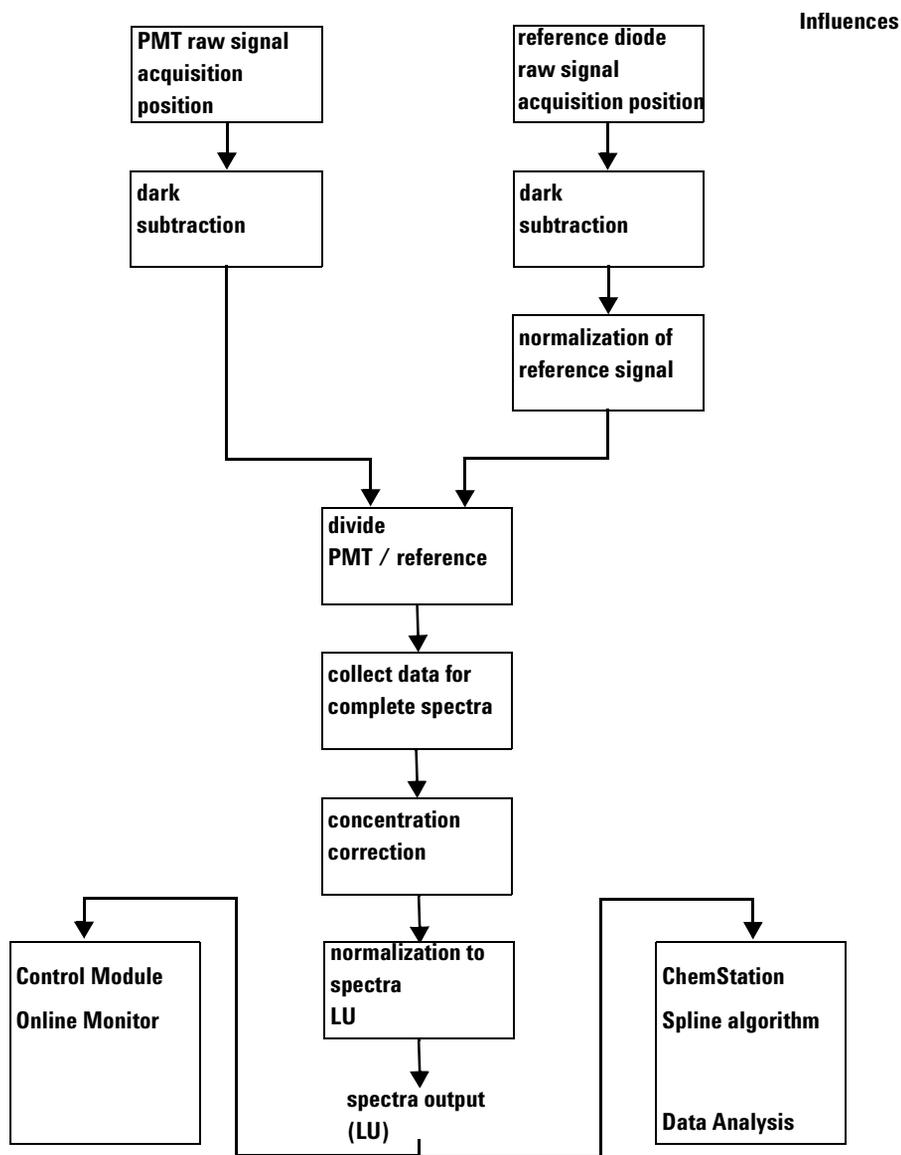


**Figure 96** Data flow for chromatographic output

For multi-channel output, any single flash data point will be processed in a separate independent filter.

### Data flow for spectral output

The data flow is shown below.



**Figure 97** Data flow for spectral output

### **Raw Signal Acquisition (PMT and Reference Diode)**

Each raw data value of the PMT and the reference diode signal is measured synchronized to the flashes of the xenon flash lamp with a 16-bit analog-to-digital converter.

### **Dark Subtraction**

The dark value of the PMT and the reference signal are measured during any power cycle of the detector without flash lamp on. These values are stored and subtracted from any PMT and reference diode value

### **Normalize Reference Diode Signal**

The reference diode, located behind the flow cell, measures the excitation (EX) light within the flow cell and corrects flash lamp fluctuations. Because of a non-linear output of the diode (depending on the EX-wavelength), the measured data are now normalized to get better real light level values within the flow cell.

### **Divide PMT/Reference Diode**

With this calculation the output of the detector will suppress lamp effects like lamp flash fluctuations or lamp lifetime.

### **Filtering**

FIR-filters (finite-impulse-response) are used to filter the given raw data and output it to a the digital data bus and the analog outputs.

### **Spectra: Collect data to complete spectra**

All flashes that are measured to get spectral information are collected to get one spectra.

### Concentration Correction

For every measured spectrum, a certain time is required. During this time the concentration within the flow cell may be changed. This can effect the spectra. To avoid this distortion every spectra point is corrected with the quasi-simultaneous measured concentration of the chromatographic channel A. As point of reference the mid-point of each spectra is used.

Concentration correction is not done

- If the cuvette is used, e.g. fluorescence scan.
- If 'all spectra without signal' is used as spectra acquisition mode.

### Peak Detector

The peak detector (PD) always uses signal A as the *pilot* signal. The PD is adjusted by means of two setpoints: peakwidth and threshold. Both setpoints are time-programmable. While the PD-threshold is a method parameter of its own, the PD-peakwidth parameter is derived from the general peakwidth (PKWD) parameter. At the start of the run, the value for the PD-peakwidth is set equal to the PKWD setpoint. The peak detector recognizes changes in the slope of this signal (upslope, apex, downslope and baseline) and stores spectra according to the operator's instructions. During the run the PD-peakwidth parameter can be changed by time-programming.

## Spectra Acquisition Modes

The spectra acquisition mode allows automatic storage of spectra during a run. The mode can be changed during the run by time-programming.

The modes in detail are:

**Table 57** Spectra Acquisition Modes

Mode	Description
none	no spectra is stored
apex	if apex of a peak is detected, one spectra is measured and stored. In addition the next baseline spectra is stored.
all in peak	The peak detectors will start and stop spectrum acquisition at begin and end of a detected peak. In addition the next baseline spectra is stored.
all	periodically all spectra are stored. The period depends on the spectra range and step and the number of selected chromatographical outputs (signals).
all without signals	<ul style="list-style-type: none"> <li>- for screening all flashes are used for spectra acquisition.</li> <li>- no concentration connection is done.</li> <li>- as chromatographical signal the mean of each spectra is output/stored.</li> </ul>

## Optional Interface Boards

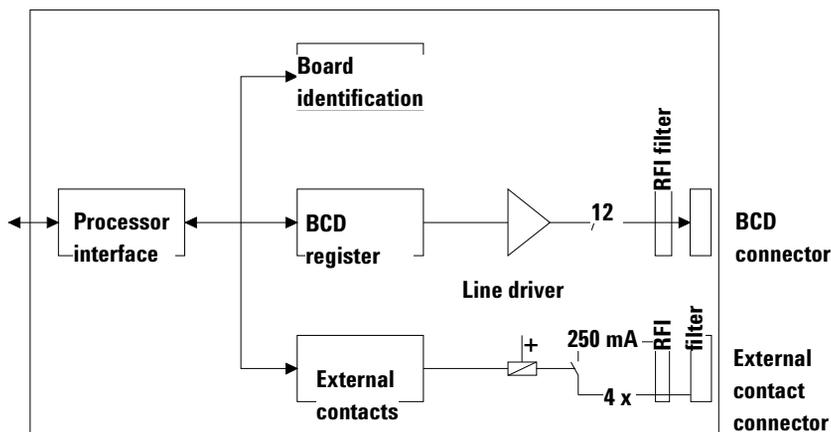
The Agilent 1100 Series modules have one optional board slot that allows addition of an interface board to the modules.

**Table 58** Optional Interface Boards

Description	Part Number
BCD Board	G1351-68701
Fuse 250 mA (four are on the board)	2110-0004
LAN Board (see next page for details)	

## BCD Board

The BCD board provides a BCD output for the bottle number of the Agilent 1100 Series autosampler and four external contacts. The external contact closure contacts are relay contacts. The maximum settings are: 30 V (AC/DC); 250 mA (fused). There are general purpose cables available to connect the BCD output, see "BCD Cables" on page 229 and the external outputs, see "External Contact Cable" on page 234 to external devices.



**Figure 98** Block Diagram BCD Board

## LAN Board

The HP JetDirect cards are network interface cards used in HP printers.

### NOTE

One board is required per Agilent 1100 stack. It is recommended to add the LAN board to the detector with highest data rate.

### NOTE

The LAN board can only be used together with:

a main board version G13XX-66520 (DAD/MWD/VWD/Pump/ALS) or G13XX-66500 (FLD/RID) and above.

a DOS-ChemStation software revision A.06.01 or above.

The following cards can be used with the Agilent 1100 modules.

**Table 59** LAN Boards

Agilent Order Number	Supported networks
J4106A	Ethernet/802.3, RJ-45 (10Base-T)
J4105A	Token Ring/802.5, DB9, RJ-45 (10Base-T)
J4100A	Fast Ethernet, Ethernet/802.3, RJ-45 (10/100Base-TX) + BNC (10Base2)

### NOTE

Minimum firmware of the JetDirect cards is A.05.05.

## Recommended Cables

For point to point connection (not using a network hub) use a twisted pair cross over LAN cable (P/N 5183-4649, 10 feet long).

For standard network connections using a hub use category 5 UTP cables, (P/N G1530-61480, 8 m long).

## Interfaces

The Agilent 1100 Series modules provide the following interfaces:

**Table 60** Agilent 1100 Series Interfaces

Interface Type	Pumps	Autosampler	DA Detector MW Detector FL Detector	VW Detector RI Detector	Thermostatted Column Compartment	Vacuum Degasser
CAN	Yes	Yes	Yes	Yes	Yes	No
GPIB	Yes	Yes	Yes	Yes	Yes	No
RS-232C	Yes	Yes	Yes	Yes	Yes	No
Remote	Yes	Yes	Yes	Yes	Yes	Yes
Analog	Yes	No	2 ×	1 ×	No	Yes*
Interface board	Yes	Yes	Yes	Yes	No	No

\* The vacuum degasser will have a special connector for specific use. For details see description of main board.

- CAN connectors as interface to other Agilent 1100 Series modules,
- GPIB connector as interface to the Agilent ChemStation,
- RS-232C as interface to a computer,
- REMOTE connector as interface to other Agilent products,
- Analog Output connector(s) for signal output, and
- Interface slot for specific interfacing (external contacts, BCD, LAN and so on).

For identification and location of the connectors [Figure 6](#) on page 23.

### WARNING

**Never use cables other than the ones supplied by Agilent Technologies to ensure proper functionality and compliance with safety or EMC regulations, see “[Cable Overview](#)” on page 218.**

## Analog Signal Output

The analog signal output can be distributed to a recording device. For details refer to the description of the module's main board.

## GPIB Interface

The GPIB connector is used to connect the module with a computer. The address and control switches next to the GPIB connector determine the GPIB address of your module. The switches are preset to a default address and recognized by the operating software from Agilent Technologies.

**Table 61** Default Addresses

Autosampler	28	Autosampler	28
Pump	22	RID	29
FLD	23		
VWD	24	Autosampler (HP 1050)	18
Agilent 8453A	25	Pump (HP 1050)	16
DAD / MWD	26	VWD (HP 1050)	10
Column Compartment	27	DAD (HP 1050)	17

## CAN Interface

The CAN is an intermodule communication interface. It is a 2-wire serial bus system supporting high speed data communication and real-time requirement.

## Remote Interface

The APG remote connector may be used in combination with other analytical instruments from Agilent Technologies if you want to use features such as common shut down, prepare, and so on.

Remote control allows easy connection between single instruments or systems to ensure coordinated analysis with simple coupling requirements.

The subminiature D connector is used. The module provides one remote connector which is inputs/outputs (wired-or technique).

To provide maximum safety within a distributed analysis system, one line is dedicated to SHUT DOWN the system's critical parts in case any module detects a serious problem. To detect whether all participating modules are switched on or properly powered, one line is defined to summarize the POWER ON state of all connected modules. Control of analysis is maintained by signal readiness READY for next analysis, followed by START of run and optional STOP of run triggered on the respective lines. In addition, PREPARE and START REQUEST may be issued. The signal level is defined as:

- standard TTL levels (0 V is logic true, + 5 V is false),
- fan-out is 10,
- input load is 2.2 kOhm against + 5 V, and
- outputs are open collector type, inputs/outputs (wired-or technique).

**Table 62** Remote Signal Distribution

Pin	Signal	Description
1	DGND	Digital ground
2	PREPARE	(L) Request to prepare for analysis (for example, calibration, detector lamp on). Receiver is any module performing pre-analysis activities.
3	START	(L) Request to start run / timetable. Receiver is any module performing run-time controlled activities.
4	SHUT DOWN	(L) System has serious problem (for example, leak: stops pump). Receiver is any module capable to reduce safety risk.
5		Not used

**Table 62** Remote Signal Distribution (continued)

Pin	Signal	Description
6	POWER ON	(H) All modules connected to system are switched on. Receiver is any module relying on operation of others.
7	READY	(H) System is ready for next analysis. Receiver is any sequence controller.
8	STOP	(L) Request to reach system ready state as soon as possible (for example, stop run, abort or finish and stop injection). Receiver is any module performing run-time controlled activities.
9	START REQUEST	(L) Request to start injection cycle (for example, by start key on any module). Receiver is the autosampler.

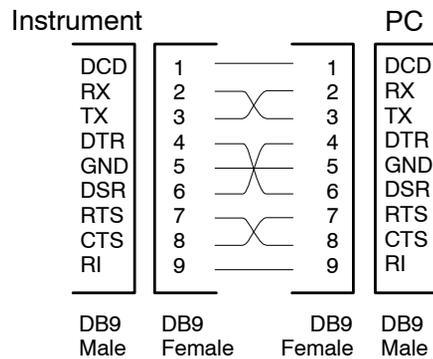
### RS-232C

The RS-232C connector is used to control the instrument from a computer through RS-232C connection, using the appropriate software. This connector can be activated by the configuration switch module next to the GPIB connector.

The RS-232C is designed as DCE (Data Communication Equipment) with a 9-pin male SUB-D type connector. The pins are defined as follows:

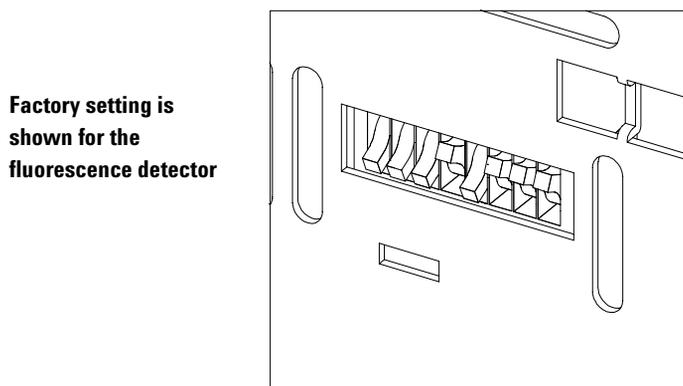
**Table 63** RS-232C Connection Table

Pin	Direction	Function
1	In	DCD
2	In	RxD
3	Out	TxD
4	Out	DTR
5		Ground
6	In	DSR
7	Out	RTS
8	In	CTS
9	In	RI

**Figure 99** RS-232 Cable

## Setting the 8-bit Configuration Switch

The 8-bit configuration switch is located next to the GPIB connector. Switch settings provide configuration parameters for GPIB address, serial communication protocol and instrument specific initialization procedures.



**Figure 100** 8-bit Configuration Switch

**Table 64** 8-bit Configuration Switch

Mode Select	1	2	3	4	5	6	7	8
GPIB	0	0		GPIB Address				
RS-232C	0	1	Baud rate			Data Bits	Parity	
Reserved	1	0	Reserved					
TEST/BOOT	1	1	RSVD	SYS		RSVD	RSVD	FC

Switches 1 and 2 define which set of parameters (for example, for GPIB, RS-232C, and so on) will be changed. Once the change has been completed, the instrument must be powered up again in order to store the values in the non-volatile memory.

In the non-volatile memory, the parameters are kept, regardless of whether you turn the instrument off and on again. They will be kept until the same set of parameters is changed and the power is reset. All other previously stored configuration settings will still remain in the non-volatile memory.

In this way, you can store more than one set of parameters using the same 8-bit configuration switch twice, for example, for both GPIB and RS-232C.

## GPIB Default Addresses

If you just want to change the GPIB address and need a detailed procedure, refer to the *Installing Your Agilent ChemStation System* handbook.

Default GPIB address is set to the following addresses:

**Table 65** Default Addresses for Agilent Series 1100 Modules

Module	Address	Binary Address
Pump	22	0 0 0 1 0 1 1 0
FLD	23	0 0 0 1 0 1 1 1
VWD	24	0 0 0 1 1 0 0 0
Agilent 8453A	25	0 0 0 1 1 1 0 1
DAD / MWD	26	0 0 0 1 1 0 1 0
Column compartment	27	0 0 0 1 1 0 1 1
Autosampler	28	0 0 0 1 1 1 0 0
RID	29	0 0 0 1 1 1 0 1

where 0 means that the switch is down and 1 means that the switch is up.

## Communication Settings for RS-232C Communication

The communication protocol used in this instrument supports only hardware handshake (CTS/RTS).

Switches 1 in down and 2 in up position define that the RS-232C parameters will be changed. Once the change has been completed, the instrument must be powered up again in order to store the values in the non-volatile memory.

**Table 66** Communication Settings for RS-232C Communication

Mode Select	1	2	3	4	5	6	7	8
RS-232C	0	1	Baud rate			Data Bits		

Use the following tables for selecting the setting which you want to use for RS-232C communication. The number 0 means that the switch is down and 1 means that the switch is up.

**Table 67** Baud Rate Settings

Switches			Baud Rate	Switches			Baud Rate
3	4	5		3	4	5	
0	0	0	9600	1	0	0	9600
0	0	1	1200	1	0	1	14400
0	1	0	2400	1	1	0	19200
0	1	1	4800	1	1	1	38400

**Table 68** Data Bit Settings

Switch 6	Data Word Size
0	7 Bit Communication
1	8 Bit Communication

**Table 69** Parity Settings

Switches		Parity
7	8	
0	0	No Parity
1	0	Odd Parity
1	1	Even Parity

One start bit and one stop bit are always used (not selectable).

Per default, the module will turn into 19200 baud, 8 data bit with no parity.

## Forced Cold Start Settings

Switches 1 and 2 do not force storage of this set of parameters in non-volatile memory. Returning switches 1 and 2 to other positions (other than being both up) will allow for normal operation.

### CAUTION

Forced cold start erases all methods and data stored in the non-volatile memory. Exceptions are diagnose and repair log books which will not be erased.

If you use the following switch settings and power the instrument up again, a forced cold start has been completed.

**Table 70** Forced Cold Start Settings

Mode Select	1	2	3	4	5	6	7	8
TEST/BOOT	1	1	0	0	0	0	0	1

To return to normal operation, set switches back to your GPIB or RS 232 configuration settings.

## Stay-Resident Settings

Firmware update procedures may require this mode in case of firmware loading errors.

Switches 1 and 2 do not force storage of this set of parameters in non-volatile memory. Returning switches 1 and 2 to other positions (other than being both up) will allow for normal operation.

If you use the following switch settings and power the instrument up again, the instrument firmware stays in the resident part, that is, it is not operable as a detector. It only uses basic functions of the operating system for example, for communication.

**Table 71** Stay Resident Settings

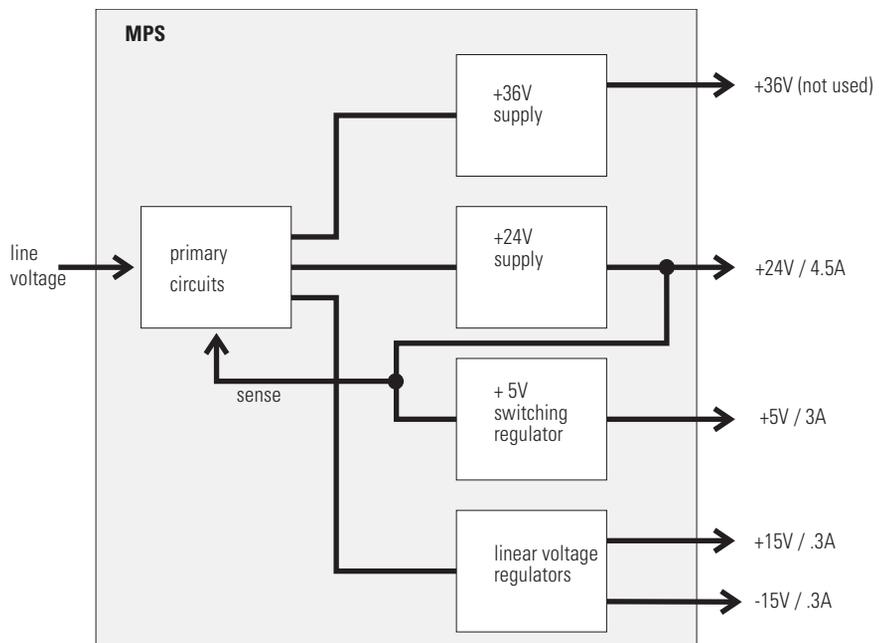
Mode Select	1	2	3	4	5	6	7	8
TEST/BOOT	1	1	0	0	1	0	0	0

To return to normal operation, set switches back to your GPIB or RS-232C configuration settings.

## The Main Power Supply Assembly

The main power supply comprises a closed assembly (no onsite repair possibility).

The power supply provides all DC voltages used in the module except for the voltages supplied by the lamp power supply to the deuterium and tungsten lamps in the detectors. The line voltage can vary in a range from 100 – 120 or 220 – 240 volts AC  $\pm 10\%$  and needs no manual setting.



**Figure 101** Main Power Supply (MPS) Blockdiagram

To disconnect the instrument from line, unplug the power cord. The power supply still uses some power, even if the power switch on the front panel is turned off.

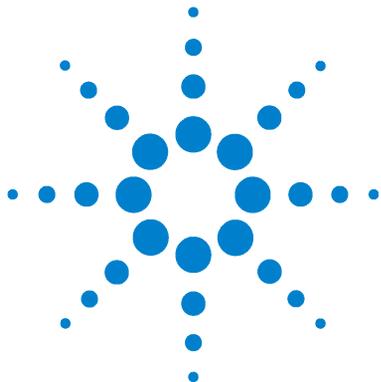
No accessible hardware fuse is needed because the main power supply is safe against any short circuits or overload conditions on the output lines. When overload conditions occur, the power supply turns off all output voltages. Turning the line power off and on again resets the power supply to normal operation if the cause of the overload condition has been removed.

An over-temperature sensor in the main power supply is used to turn off output voltages if the temperature exceeds the acceptable limit (for example, if the cooling fan of the instrument fails). To reset the main power supply to normal operating conditions, turn the instrument off, wait until it is approximately at ambient temperature and turn the instrument on again.

The following table gives the specifications of the main power supply.

**Table 72** Main Power Supply Specifications

Maximum power	130 W	Continuous output
Line Input	100 – 120 or 220 – 240 volts AC ± 10 %, line frequency of 50/60 Hz	Wide ranging
Output 1	+ 24 V / 4.5 A (maximum)	total power consumption of + 24 V and + 36 V must not exceed 107 W.
Output 2	+ 36 V / 2.5 A (maximum)	
Output 3	+ 5 V / 3 A	
Output 4	+ 15 V / 0.3 A	
Output 5	- 15 V / 0.3 A	



## 9 Introduction to the Control Module

Major keys on the Agilent 1100 Control Module [298](#)

Screens available from the Analysis screen [299](#)

Screens available from the System screen [315](#)

Screens available from the Records screen [318](#)

Diagnostics and Tests [323](#)



## Major keys on the Agilent 1100 Control Module

**Table 73**

ESC	Return to previous screen and scroll through top layer views (Analysis, Settings)
m	Open context sensitive menus
i	Information/help
Enter	Store changed parameters or execute the choice from a pull-down menu
On/Off	Switch on lamp(s)
Start	Start a run
Plot	View the chromatogram
Views	Change between view of analysis - status - system screens

**NOTE**

The screens shown on the next pages are based on:  
Control Module firmware revision B.01.01  
LC Module firmware revision 3.8x

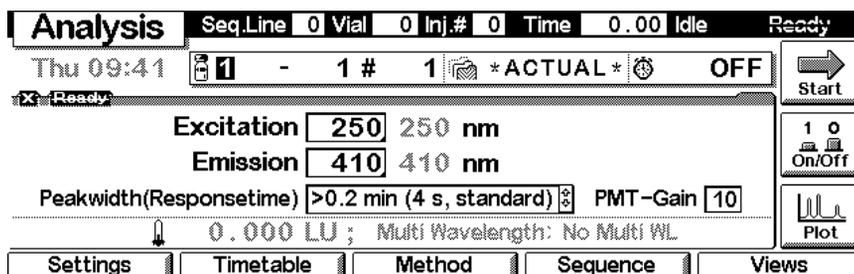
**NOTE**

In case the control module's display seems to be frozen (hang-up due to a communication problem on the CAN bus, unplug the control module from the LC module and reconnect.

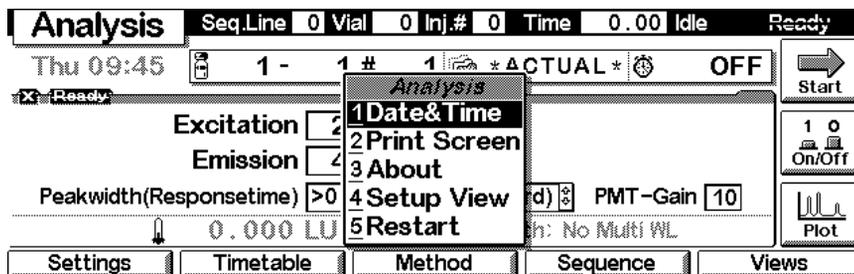
## Screens available from the Analysis screen

### The Analysis screen

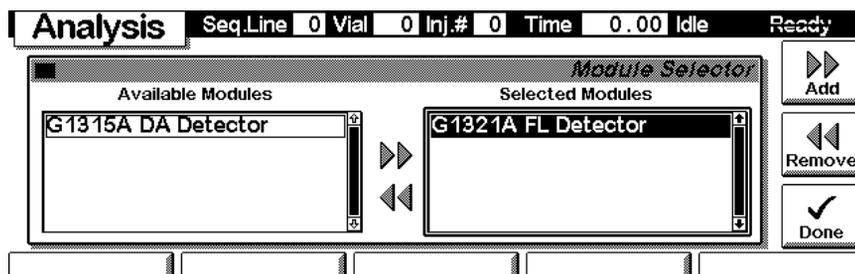
This is the wake-up screen, if the Agilent 1100 fluorescence detector is the only configured Agilent 1100 module. It is used to enter the most common FLD method parameters.



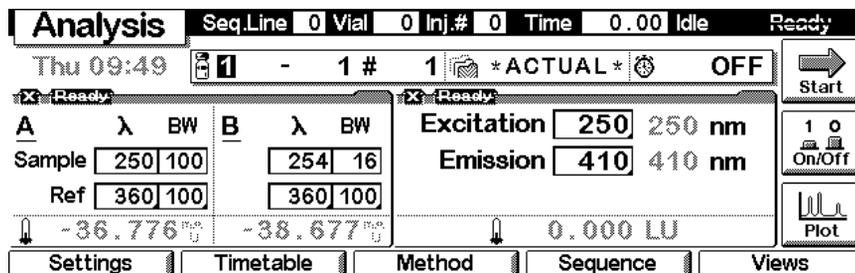
The **m**-key allows access to the context sensitive menu. **Setup view** leads you to add sections for additional Agilent 1100 modules. **Restart** re-boots the control module.



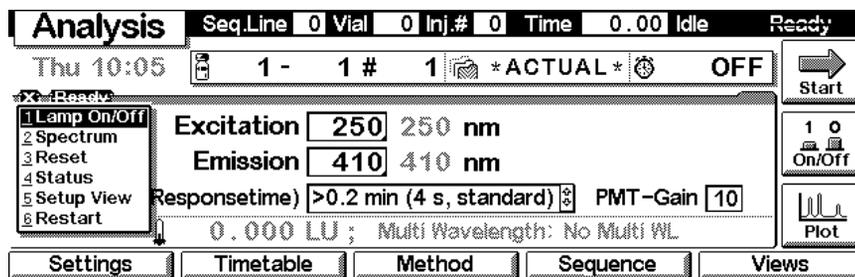
In the Setup view, e.g. the diode array detector can be added to the view.



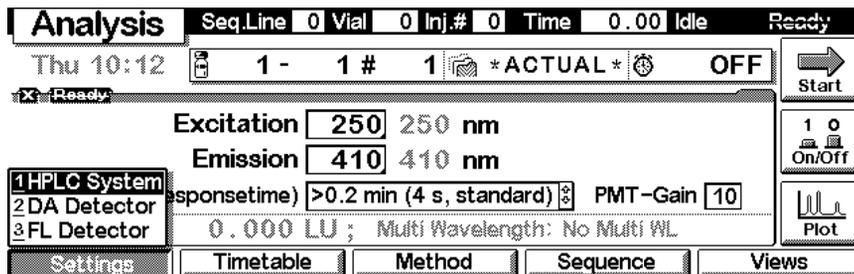
Here, e.g. the diode array detector parameters are shown on the display as well. The number of parameters on the display are restricted as additional modules are added. Maximum 4 modules are shown automatically. If more modules are in the system, you have to chose in Setup view. The number of parameters on the display are restricted as additional modules are added.



The **m**-key gives access to a context sensitive menu (e.g. when the cursor is positioned in the excitation window).

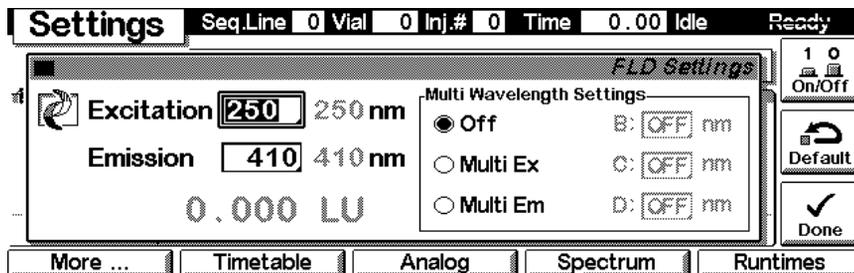


With the **Settings** key you open a pull-down menu where you can select the FLD module.

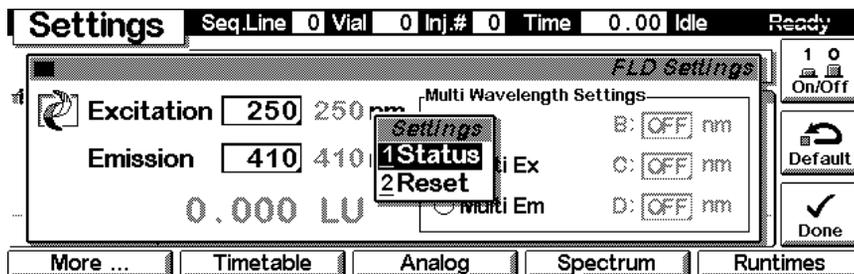


## Settings

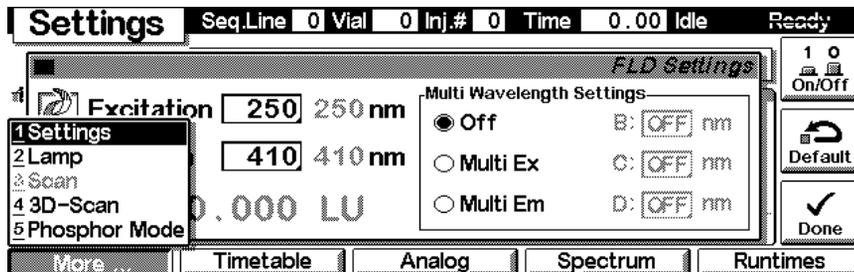
Within the **Settings** you can change the FLD parameters and with a different set of parameters available through the F1-5 keys. F7 key resets the FLD to default values. F8 opens a window to turn on the lamp.



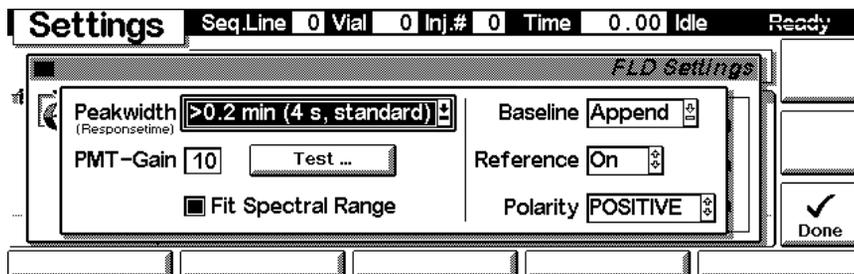
Use the **m**-key for the context sensitive menu. The **Status** command pulls up a monitor screen displaying signals and spectra as programmed. **Reset** will load the FLD default parameters.



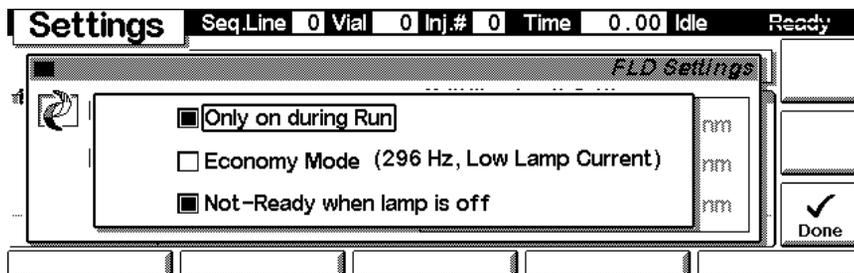
Use F1-key (**More**) opens a pull-down menu. The item **Scan** is available when Multi-Ex or Multi-Em mode has been selected. The **Phosphor** mode is not available with FLD firmware revisions A.03.6X and below.



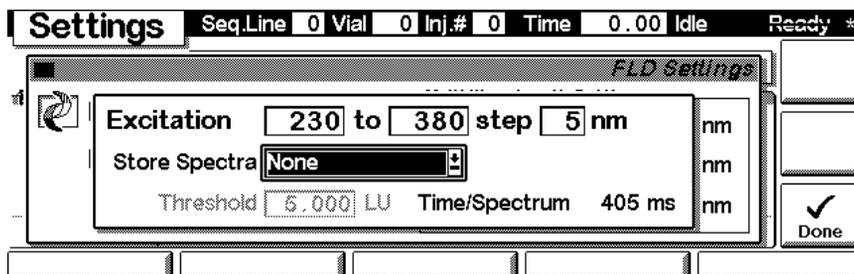
Selecting **More** and **Settings** pull down menu you can enter special FLD setpoints. The **PMT-Test** allows the automatic check for optimum signal/noise and dynamic range under current conditions in the flow cell.



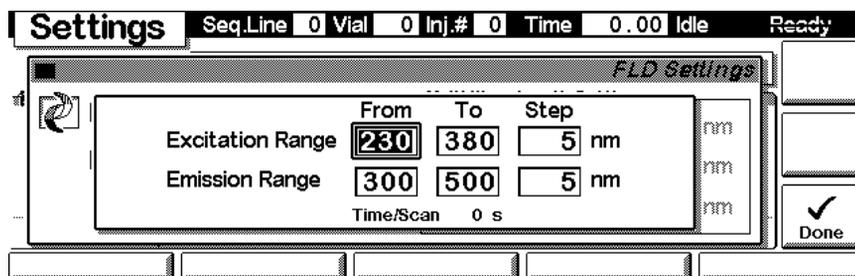
Selecting **More** and **Lamp** on the pull down menu you can enter different lamp operating modes. Deselect 'Not-Ready when lamp is off' when you keep the FLD on during analysis with another detector.



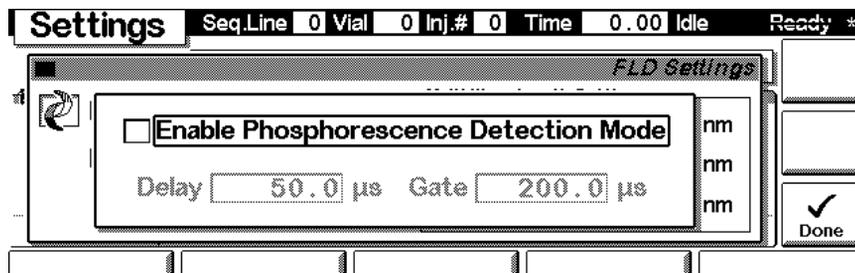
Selecting **More** and **Scan** on the pull down menu you can enter setpoints for spectrum acquisition (Multi-Ex or Multi-Em).



Selecting **More** and **3D-scan** on the pull down menu you can enter setpoints for taking for the 3D-fluorescence scans. Data can only be displayed using the 3D-Agilent ChemStation software.



Selecting **More** and **Phosphor** on the pull down menu you can enter setpoints for measuring phosphorescence. This mode is not available with FLD firmware revisions A.03.6X and below.



## Settings - Timetable

With the F2 key (**Timetable**) you can list the timetable for the FLD. Press F7 key (**Insert**) to add entries or F6 key (**Delete**) to remove entries.

Timetable				Seq.Line	0	Vial	0	Inj.#	0	Time	0.00	Idle	Ready *
Time	Module	Setting	Value										
1.00	FL Detector	Ex. Wavelength A	On, 230 nm										
5.00	FL Detector	Ex. Wavelength A	On, 250 nm										
*** end of timetable ***													

Press the F7 key (**Insert**) to add timetable events. Use the F6 key (**Done**) to view the entered lines of the timetable.

Timetable				Seq.Line	0	Vial	0	Inj.#	0	Time	0.00	Idle	Ready *
			<i>Timetable insert</i>										
Time [min]	Module	Setting											
<input type="text" value="0.00"/>	<input type="text" value="FL Detector"/>	<input type="text" value="Ex. Wavelength A"/>											
<input type="button" value="↻"/> <b>Excitation Wavelength A</b> <input type="text" value="230 nm"/>													
<i>fields change with setting selected</i>													

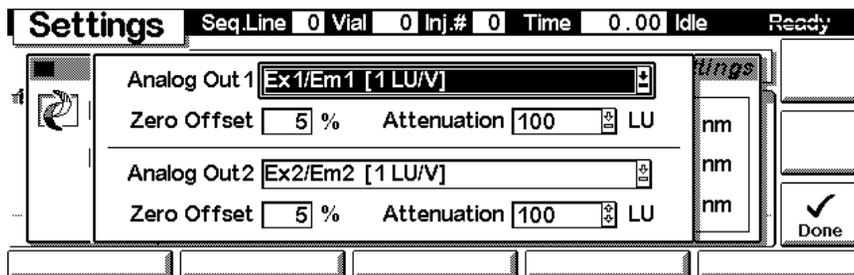
Use the **m**-key for the context sensitive menu. It gives you additional tools for the timetable.

Timetable				Seq.Line	0	Vial	0	Inj.#	0	Time	0.00	Idle	Ready *
Time	Module	Setting	Value										
1.00	FL Detector	Ex. Wav	nm										
5.00	FL Detector	Ex. Wav	nm										
*** end of timetable ***													

**Timetable**  
 1 Copy Line  
 2 Paste Line  
 3 Print Timetable

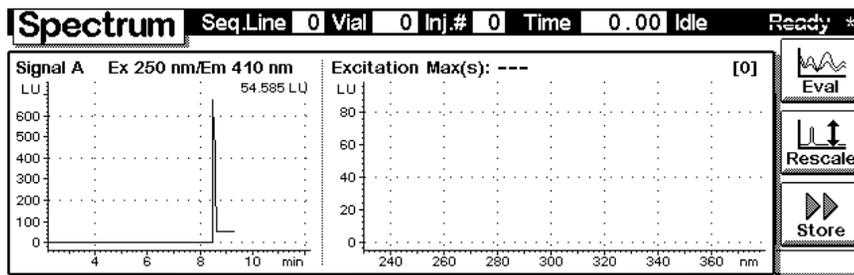
### Settings - Analog

With the F3 key (**Analog**) you can change the settings for the analog outputs.

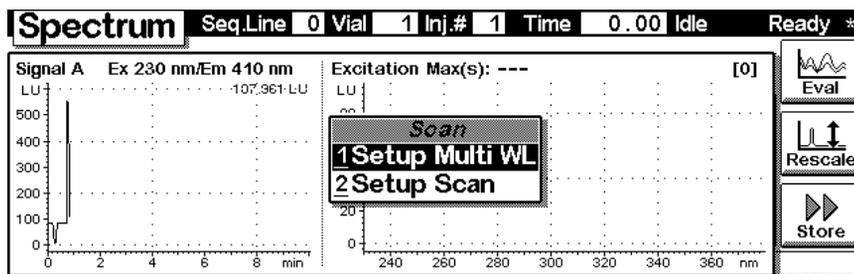


### Settings - Spectrum Monitor

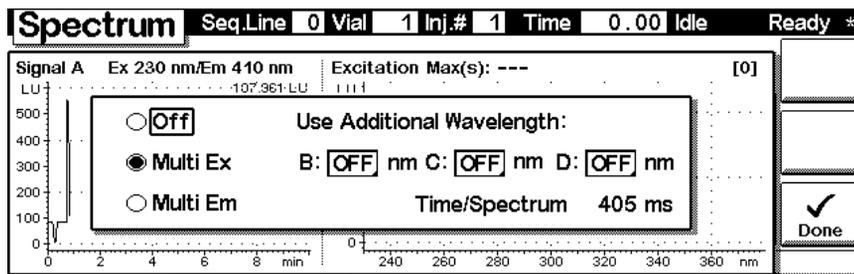
With the F4 key (**Spectrum**) you can watch the signals and spectra on-line as programmed. The F6 key (**Store**) will restart acquisition of spectra and loads the spectrum into memory in the control module. Spectra are lost when a new analysis is started or when the Control Module is switched off. The F8 key (**Eval**) leads to the next screen below.



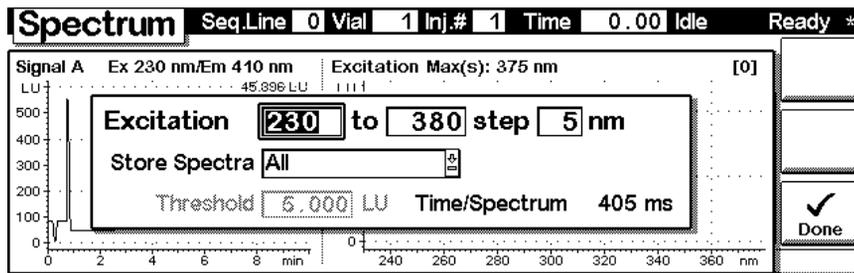
The m key starts the context sensitive menu to change setpoints for spectra acquisition.



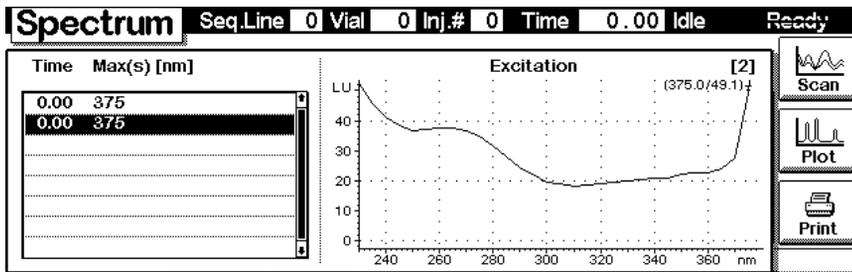
From Menu item 1 (**Setup Multi WL**) you can switch between Excitation and Emission spectra and choose simultaneous multi-wavelengths detection.



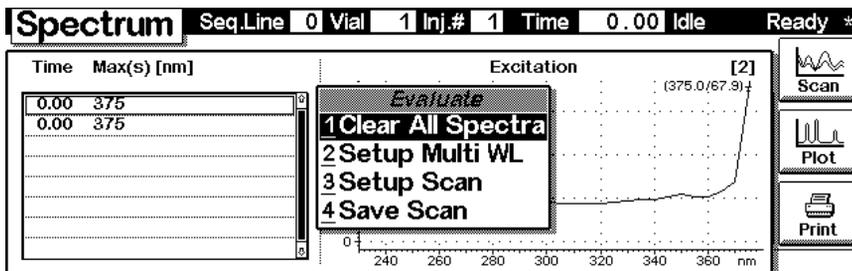
From Menu item 2 (**Setup Scan**) you can set spectra acquisition parameter. Store spectra is only valid if an Agilent Chemstation is connected.



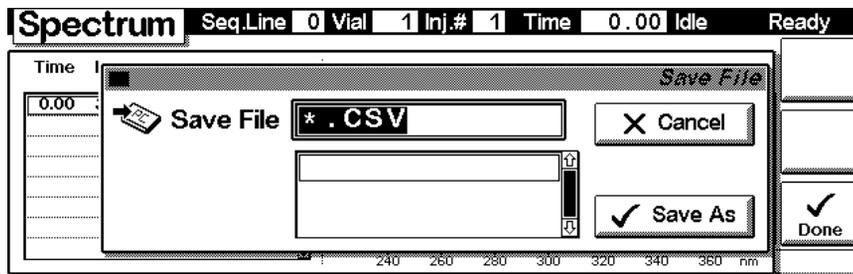
With the F8 key (**E**val) to call up the stored spectra. Use F7 key (**P**lot) to enlarge the spectrum plot. Use F6 key (**P**rint) to print the plot to a printer. The F8 key (**S**can) will return to the acquisition screen.



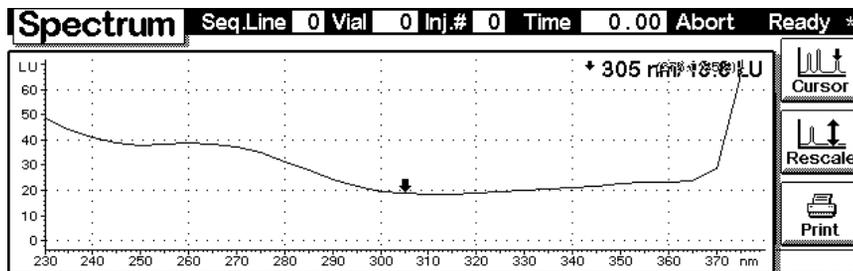
During spectra review press the **m**-key to open the context sensitive menu. The menu item 1 (Clear All Spectra) empties the memory in the Control Module. The menu item 4 (Save Spectrum) leads to the next screen.



With a PC card inserted into the Control Module spectra can be stored as \*.csv files and viewed with Microsoft Excel “ software. Letters of the filename can be entered by pressing several times the numerical keys (see *Reference Manual of the Control Module*).

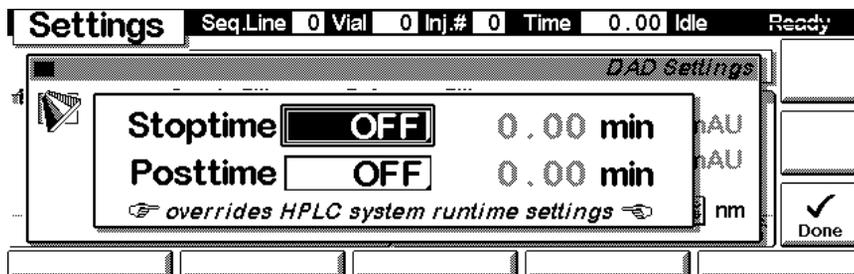


With the F8 key (**Cursor**) you can cruise with the cursor through the spectrum. The F6 key (**Print**) starts printing of the spectrum. Press **Esc** key to return to previous screen.

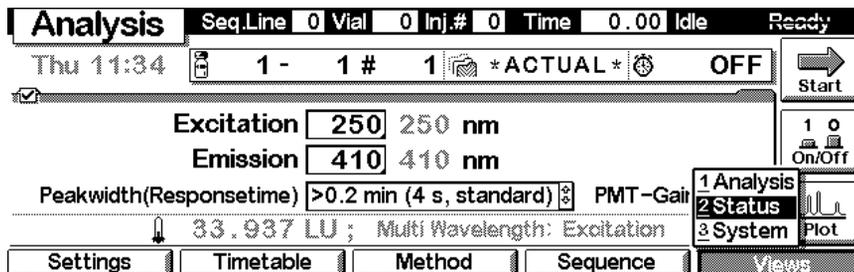


### Settings - Run times

With the F5 key (**Runtimes**) you can change the stop time and the post-run time.

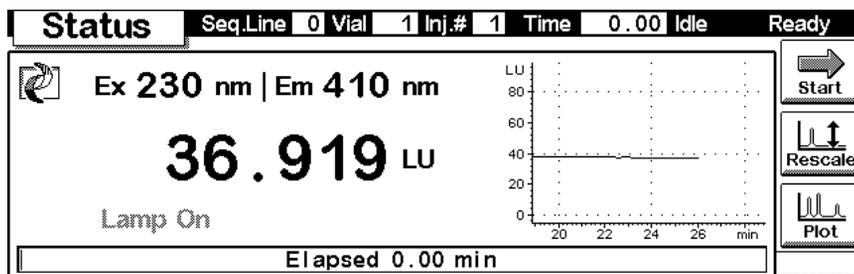


Press F5 key (**Views**) and select **Status**.

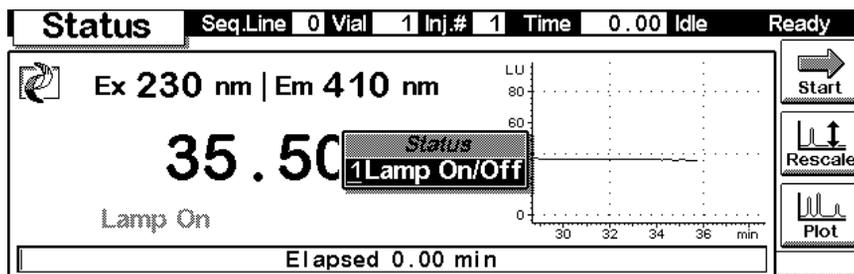


## Status

This is an example if an Agilent 1100 FLD is configured stand-alone.

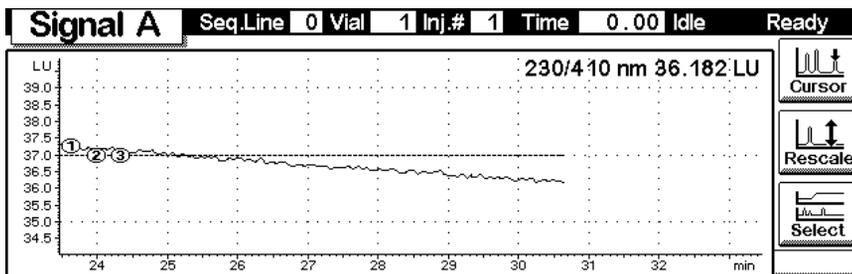


Press the **m**-key to open the context sensitive menu.

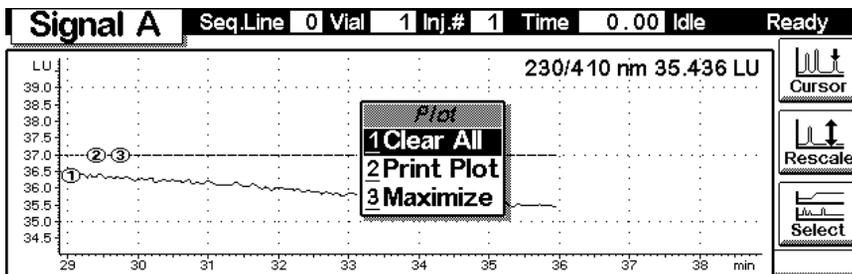


### Signal plot

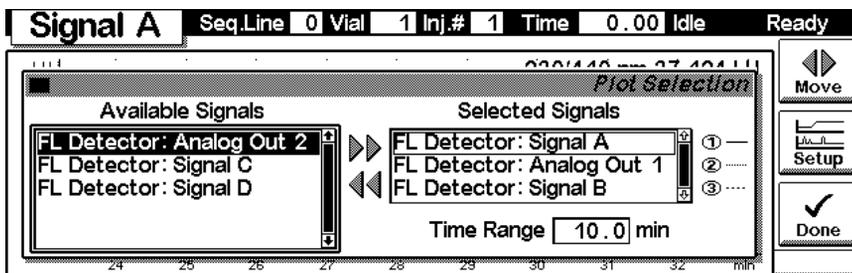
Press F6 key (**Plot**) to enter the plot screen (available also from the **Analysis** and **System** screen). Here you can observe the on-line signal(s). To add additional on-line signals (maximum 3), press F6 key (**Select**). If more than one signal is configured (see next), use the 1-2-3 number key to switch between the signals.



Press the **m**-key to open the context sensitive menu.

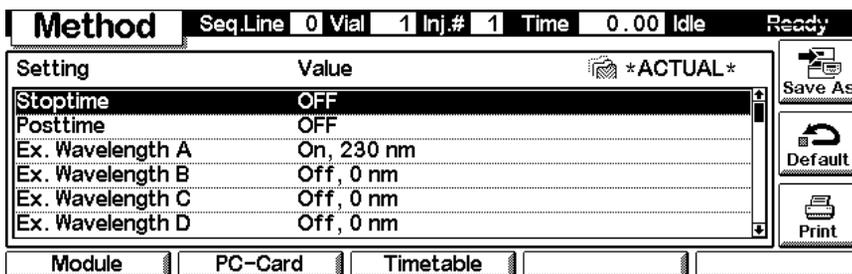


Press F6 key (**Select**). Here you can add additional on-line signals (maximum are 3). Additional signals could be also pressure or temperature signals from other modules. Use the Right/Left arrows to switch between Available and Selected Signals. Use the F8 key (**Move**) to enter available signals into the box for selected signals or vice versa.

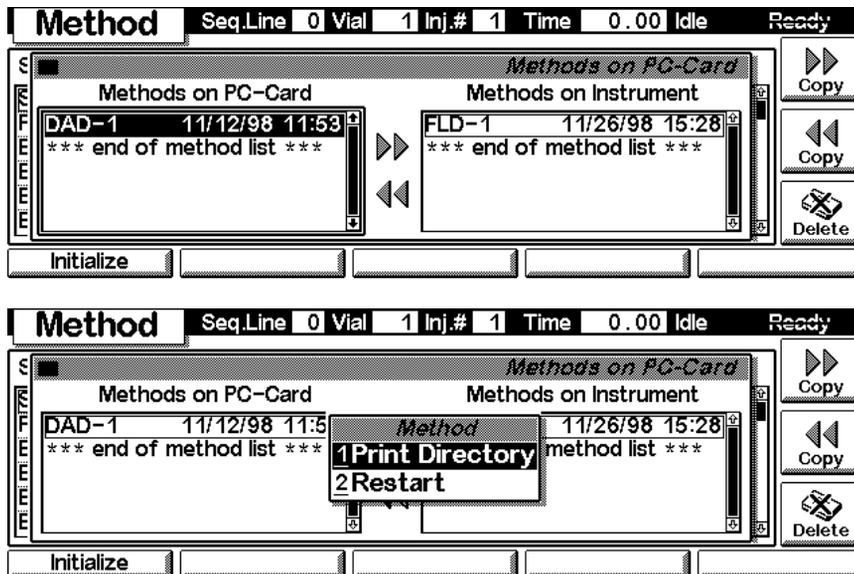


### Method screens

Use ESC to return to the **Analysis** screen. Use the F3 key (**Method**) to view the parameters in a method and F8 key (**Save As**) to save the method in the module(s). The PC-Card key is only active when a PCMCIA card is inserted in the control module.



Use F2 key (**PC-Card**) to save a method on a PCMCIA card. Use the Right/Left arrows to switch between PC-Card and Instrument window. Use the UP/Down arrows to select the method. Use the F7/F8 keys (**Copy**) to enter available signals into the box for selected signals or vice versa. Press the **m**-key to open the context sensitive menu.



## Screens available from the System screen

### System screen

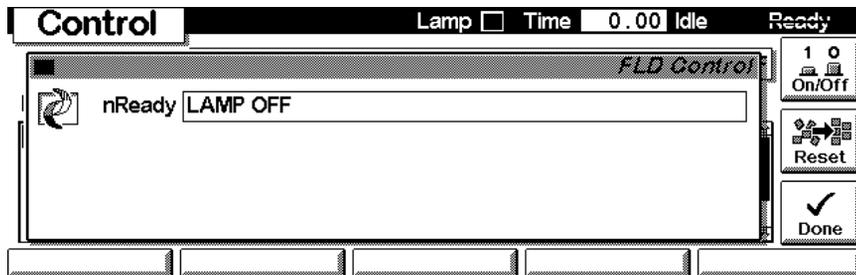
Use the Esc key to receive **Views** on the F5 key. Choose **System** from the pull-down menu. This screen shows the last activities in the system.

The screenshot shows the 'System' screen with the following layout:

- System** (Title)
- Lamp**  **Time** 0.00 **Idle** **Ready**
- Thu 15:39** **LAMP OFF** **EMF** **Start** (arrow button)
- Module** **Message** **Id** **Date** **Time**
- FL Detector** **Standard cell detected** **INFO** **11/26** **15:33:04**
- FL Detector** **Economy mode disabled** **STATE** **11/26** **15:33:04**
- FL Detector** **Calibration done** **STATE** **11/26** **15:33:04**
- FL Detector** **Reference on** **STATE** **11/26** **15:33:04**
- FL Detector** **Lamp off** **STATE** **11/26** **15:33:04**
- 1 0** **On/Off** (button)
- Plot** (button with graph icon)
- Control** **Configure** **Tests** **Records** **Views** (bottom navigation buttons)

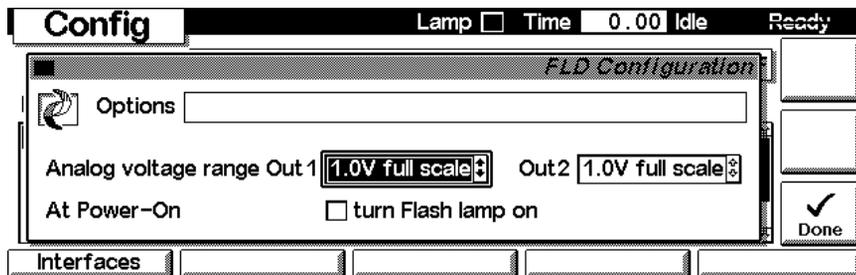
### System - Control

Use the F1 key (**Control**) to select the FL-Detector. Here you receive information about the not-ready conditions if needed. F2 key (**Reset**) does a re-initialization of the FLD. Use the F8 key (**On/Off**) to turn on the lamp(s).

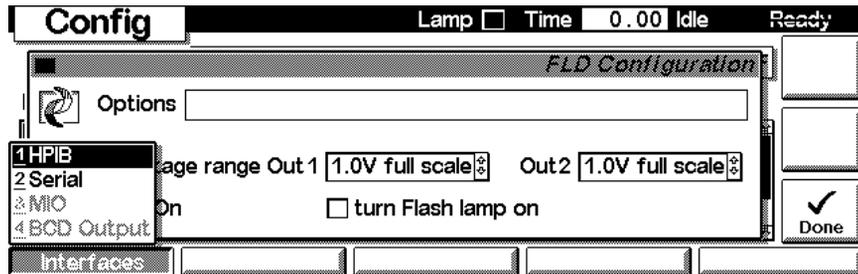


### System - Configuration

Use the **Esc** key to receive **Views** on the F5 key. Choose **System** from the pull-down menu. Use the F2 key (**Configure**) to select the FLD. Here you define further special setpoints for the FLD operation.



Use the F1 key (**Interfaces**) to access the interface settings (if required).



## Screens available from the Records screen

### Records screen

Use the Esc key to receive **Views** on the F5 key. Choose **System** from the pull-down menu. Use the F4 key (**Records**) to select the FLD. Errors are reported either into the **System Log** (F2) or **Error Log** (F3).

Records						Lamp <input type="checkbox"/>	Time 0.00	Idle	Ready
Module	Product#	Serial#	Version	On-Time	EMF				
Controller	G1323A*	DE53300105	A.02.04	0d 00:04h		<div style="text-align: center;">  Identify                 </div> <div style="text-align: center;">  Print                 </div>			
FL Detector	G1321A*	DE81700118	A.03.66	0d 07:27h					
						<div style="display: flex; justify-content: space-around;"> <span>EMF</span> <span>System Log</span> <span>Error Log</span> <span>Maint Log</span> <span>FW Update</span> </div>			

## System / Error Log

Use the F2 key (**System Log**) or F3 key (**Error Log**) to look for errors. For troubleshooting reasons they can be printed or saved to a file on the PCMCIA card (pressing the **m**-key).

Records			Lamp <input type="checkbox"/>	Time 0.00	Idle	Ready
<i>Fluorescence Detector System Logbook</i>						
Message	Date	Time				
No analysis	98/11/26	15:43:26				
Lamp off	98/11/26	15:43:26				
Reference on	98/11/26	15:43:26				
Calibration done	98/11/26	15:43:26				
Economy mode disabled	98/11/26	15:43:26				
						Print

Records			Lamp <input type="checkbox"/>	Time 0.00	Error	Ready
<i>Fluorescence Detector Error Logbook</i>						
Message	Date	Time				
Wavelength calibration (1) failed	05/12/99	07:55:22				
*** end of logbook ***						
Logbook						
1 Save Logbook						
						Print

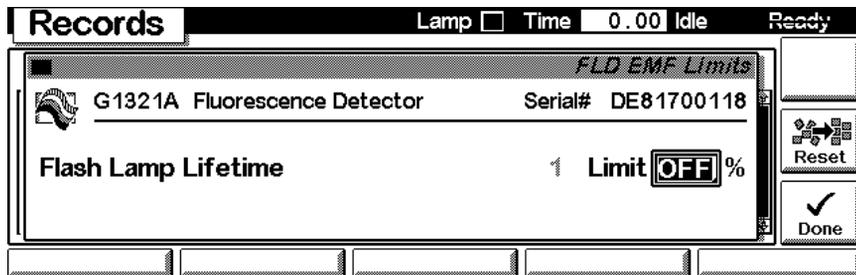
## Info Log

Use the **m**-key to receive a pop-up menu, Select **Info Log**. A list of the last events are listed. For troubleshooting reasons they can be printed or saved to a file on the PCMCIA card (pressing the **m**-key).

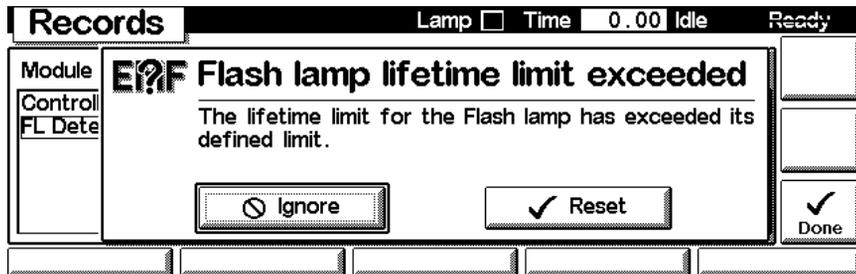
Records			Lamp <input type="checkbox"/>	Time 0.00	Idle	Ready
<i>Fluorescence Detector Info Logbook</i>						
Message	Date	Time				
Module method saved	11/26/98	15:28:22				
Info (0x00d6,10)	11/26/98	12:52:19				
Info 1(0x19d9,10)	11/26/98	12:02:41				
Info 1(0x19d9,10)	11/26/98	11:59:26				
Simulated chromatogram disabled	11/26/98	11:45:30				
						Print

### EMF (Early Maintenance Feedback)

Use the F1 key (**EMF**) to set EMF parameters. Choose menu item 1 (**Setup limits**) to select lamp-on level for the Xenon lamp at which you want to receive a warning.

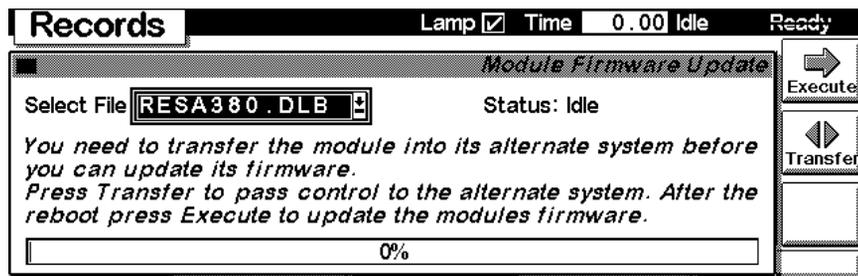


If a set limit has been exceeded, a message box will pop up. This will not stop a sequence or run (information only to plan maintenance activities). If you press **Reset**, the limits will be removed. **Ignore** will continue to keep the EMF flag set.

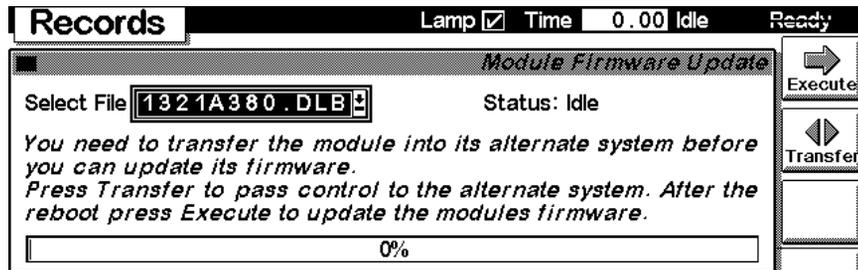


## Firmware Update

Use the Esc key to receive **Views** on the F5 key. Choose **System** from the pull-down menu. Use the F3 key (**Records**) to select the FLD. Use the F5 key (**FW Update**) to enter the Update section. If you want to update the resident firmware (together with specific main firmware revisions), select the a file from the PCMCIA card (RESnnnn.DLB) and press execute. If you want to update the main firmware, press F7 key (**Transfer**) to turn the module into the resident mode (LED on module should blink yellow).

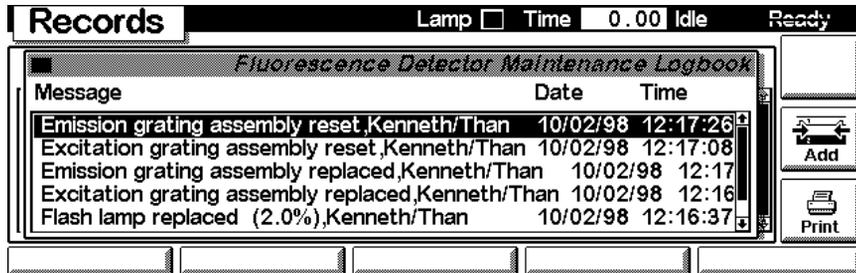


Use the Esc key to receive **Views** on the F5 key. Choose **System** from the pull-down menu. Use the F3 key (**Records**) to select the FLD. Use the F5 key (**FW Update**) to enter the Update section. Select the a file from the PCMCIA card (1321nnnn.DLB) and press execute. When the update has finished the update, press F7 key (**Transfer**) to return the module into the normal mode (LED on module should stays yellow).

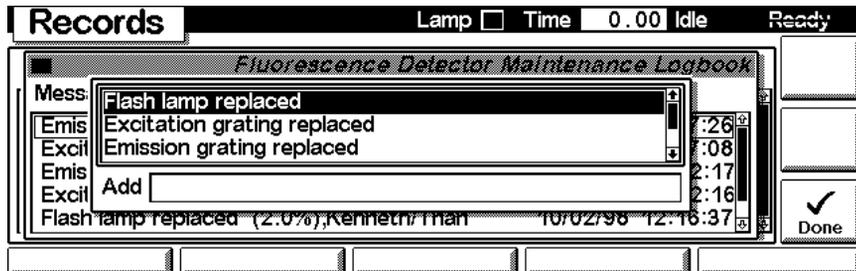


**Maintenance activities**

Use the Esc key to receive **Views** on the F5 key. Choose **System** from the pull-down menu. Use the F3 key (**Records**) to select the FLD. Use the F4 key (**Maint log**) to view and edit the maintenance logbook.



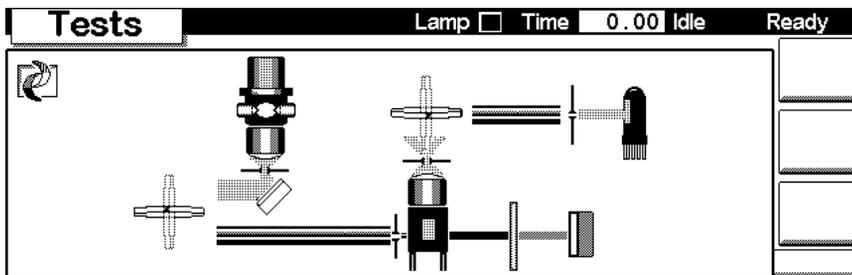
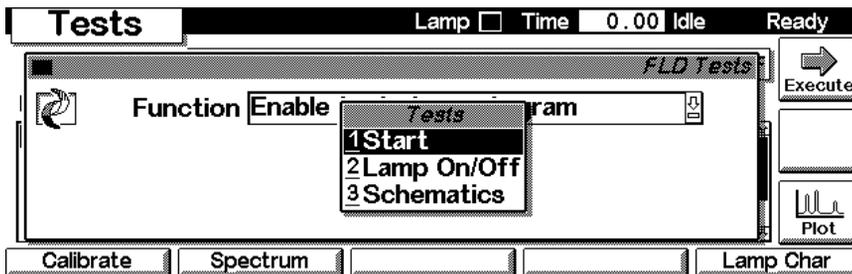
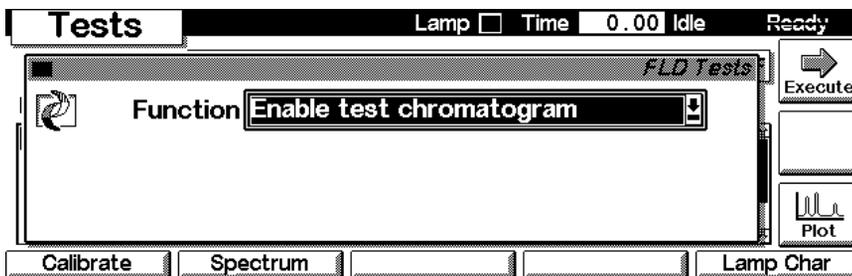
Use the F7 key (**Add**) to add new maintenance activities.



## Diagnostics and Tests

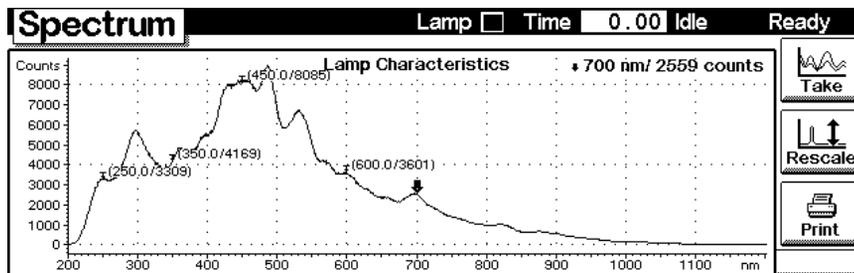
### Tests screen

Use the Esc key to receive **Views** on the F5 key. Choose **System** from the pull-down menu. Use the F3 key (**Tests**) to select the FLD. Several tests are available to test the Agilent 1100 FLD. Additional test are listed in the function box. Press the **m**-key to open the context sensitive menu. Menu item 3 (**Schematics**) will popup the FLD diagram.



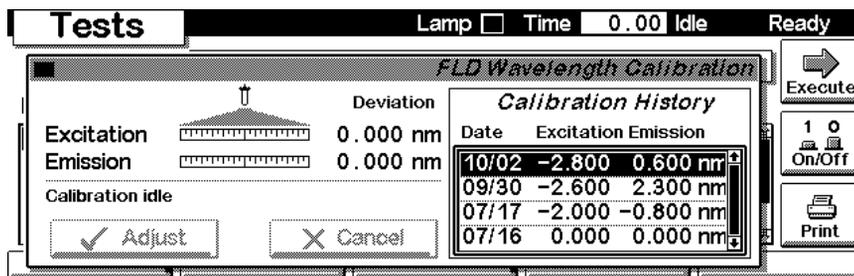
### Lamp Characteristic

Use the F5 key (**Lamp Char**) to get the instrument profile. Use F8 key (**Take**) to generate a new profile.



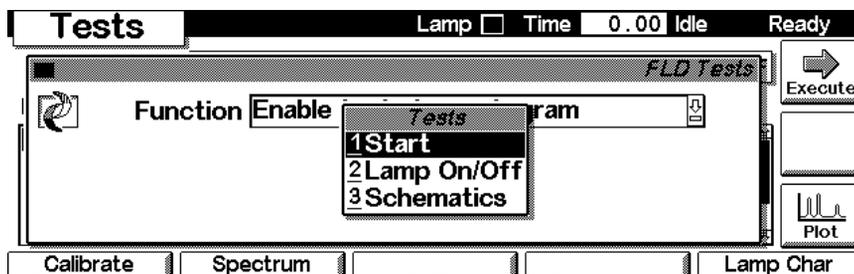
### Wavelength Calibration

Use the F1 key (**Calibrate**) and then F8 key (**Execute**) to start the FLD wavelength calibration (with Glycogen in the flow cell). If a deviation is found, press **Adjust**.

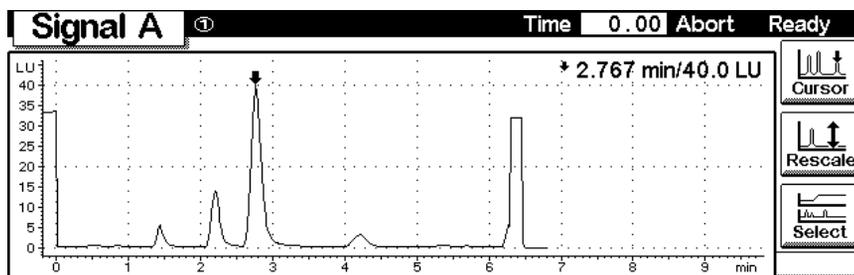


### Built-in Test Chromatogram (Simulation)

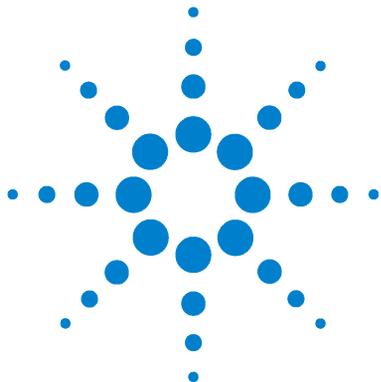
To start the built-in test chromatogram, use the F8 key (**Execute**) to enable the simulation. Then press the m-key to open the pop-up menu. Select **Start** to start a run (all modules in the Agilent 1100 system must be in ready state).



Use the F6 key (**Plot**) to view the signal. A simulated chromatogram (4 peaks) will repeat until the run is stopped. The signal is also available on the analog output. A change of the peakwidth will change the retention times.



## **9 Introduction to the Control Module**



## 10 Specifications

Performance Specifications [328](#)



## Performance Specifications

**Table 74** Performance Specifications Agilent 1100 Series Fluorescence Detector

Type	Specification	Comments
Detection type	Multi-signal fluorescence detector with rapid on-line scanning capabilities and spectral data analysis	
Performance Specifications	10 fg Anthracene, Ex=250 nm, Em=400 nm* RAMAN (H <sub>2</sub> O) > 200 (FLF rev A) RAMAN (H <sub>2</sub> O) > 400 (FLF rev >A) Ex=350 nm, Em=397 nm, dark value 450 nm, standard flow cell time constant=4 seconds (8 seconds responsetime)	see note below this table see "Raman ASTM Signal-to-Noise Test" on page 119
Light source	Xenon Flash Lamp, normal mode 20 W, economy mode 5 W	
Pulse frequency	296 Hz for single signal mode 74 Hz for spectral mode	
Excitation Monochromator	Range: 200 nm - 700 nm and zero-order Bandwidth: 20 nm (fixed) Monochromator: concave holographic grating, F/1.6, blaze: 300 nm	
Emission Monochromator	Range: 280 nm - 900 nm and zero-order Bandwidth: 20 nm (fixed) Monochromator: concave holographic grating, F/1.6, blaze: 400 nm	
Reference System:	in-line excitation measurement	
Timetable programing:	up to 4 signal wavelengths, response time, PMT Gain, baseline behavior (append, free, zero), spectral parameters	

**Table 74** Performance Specifications Agilent 1100 Series Fluorescence Detector

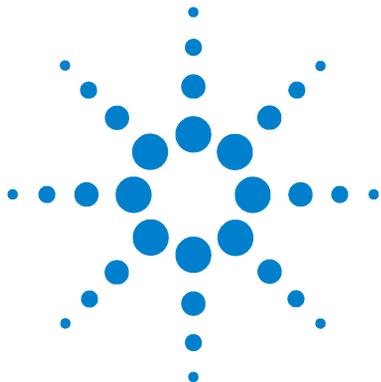
Type	Specification	Comments
Spectrum acquisition:	Excitation or Emission spectra Scan speed: 28 ms per datapoint (e.g. 0.6 s/spectrum 200-400 nm, 10 nm step) Step size: 1-20 nm Spectra storage: All	
Wavelength characteristic	Repeatability+/- 0.2 nm Accuracy+/- 3 nm setting	
Flow cells	Standard: 8 µl volume and 20 bar (2 MPa) pressure maximum, quartz Optional: Fluorescence cuvette for offline spectroscopic measurements with 1 ml syringe, 8 µl volume, quartz	
Control and data evaluation	Agilent ChemStation for LC, Agilent 1100 Control Module with limited spectral data analysis and printing of spectra	
Analog outputs	Recorder/integrator: 100 mV or 1 V, output range $>10^2$ luminescence units, two outputs	
Communications	Controller-area network (CAN), GPIB, RS-232C, LAN, APG Remote: ready, start, stop and shut-down signals	
Safety and maintenance	Extensive diagnostics, error detection and display (through control module and ChemStation), leak detection, safe leak handling, leak output signal for shutdown of pumping system. Low voltages in major maintenance areas.	

**Table 74** Performance Specifications Agilent 1100 Series Fluorescence Detector

Type	Specification	Comments
GLP features	Early maintenance feedback (EMF) for continuous tracking of instrument usage in terms of lamp burn time with user-settable limits and feedback messages. Electronic records of maintenance and errors. Verification of wavelength accuracy, using the Raman band of water.	
Housing	All materials recyclable.	
Environment:	0 to 40 °C constant temperature at <95% humidity (non-condensing)	
Dimensions:	140 mm x 345 mm x 435 mm (5.5 x 13.5 x 17 inches) (height x width x depth)	
Weight:	11.5 kg (25.5 lbs)	

**NOTE**

Reference conditions: standard cell 8 µl, response time 4 s, flow 0.4 ml/min LC-grade Methanol, 2.1 x 100 mm ODS column.



## A Safety Information

General Safety Information	332
Lithium Batteries Information	335
Radio Interference	336
Sound Emission	337
UV-Radiation	338
Solvent Information	339
Declaration of Conformity for HOX2 Filter	340
Agilent Technologies on Internet	341



# General 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. Agilent Technologies 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.

Make sure that only fuses with the required rated current and of the specified type (normal blow, time delay, and so on) 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

Table 75 shows safety symbols used on the instrument and in the manuals.

**Table 75** Safety Symbols

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.
	Eye damage may result from directly viewing the light produced by the deuterium lamp used in this product. Always turn off the deuterium lamp before opening the metal lamp door on the side of the instrument.

**WARNING**

**A warning alerts you to situations that could cause physical injury or damage to the equipment. Do not proceed beyond a warning until you have fully understood and met the indicated conditions.**

---

**CAUTION**

**A caution alerts you to situations that could cause a possible loss of data. Do not proceed beyond a caution until you have fully understood and met the indicated conditions.**

---

## Lithium Batteries Information

**WARNING**

Danger of explosion if battery is incorrectly replaced. Replace only with the same or equivalent type recommended by the equipment manufacturer. Lithium batteries may not be disposed-off into the domestic waste.

Transportation of discharged Lithium batteries through carriers regulated by IATA/ICAO, ADR, RID, IMDG is not allowed. Discharged Lithium batteries shall be disposed off locally according to national waste disposal regulations for batteries.

---

**WARNING**

Lithiumbatteri - Eksplosionsfare ved fejlagtig håndtering. Udskiftning må kun ske med batteri af samme fabrikat og type. Lever det brugte batteri tilbage til leverandoren.

---

**WARNING**

Lithiumbatteri - Eksplosionsfare. Ved udskiftning benyttes kun batteri som anbefalt av apparatfabrikanten. Brukt batteri returneres apparatleverandoren.

---

**NOTE**

Bij dit apparaat zijn batterijen geleverd. Wanneer deze leeg zijn, moet u ze niet weggoien maar inleveren als KCA.



## **Radio Interference**

Never use cables other than the ones supplied by Agilent Technologies to ensure proper functionality and compliance with safety or EMC regulations.

### **Test and Measurement**

If test and measurement equipment is operated with equipment unshielded 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.

## Sound Emission

### Manufacturer's Declaration

This statement is provided to comply with the requirements of the German Sound Emission Directive of 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 7779:1988/EN 27779/1991 (Type Test)

## UV-Radiation

Emissions of ultraviolet radiation (200-315 nm) from this product is limited such that radiant exposure incident upon the unprotected skin or eye of operator or service personnel is limited to the following TLVs (Threshold Limit Values) according to the American Conference of Governmental Industrial Hygienists:

**Table 76** UV-Radiation Limits

Exposure/day	Effective Irradiance
8 hours	0.1 $\mu\text{W}/\text{cm}^2$
10 minutes	5.0 $\mu\text{W}/\text{cm}^2$

Typically the radiation values are much smaller than these limits:

**Table 77** UV-Radiation Typical Values

Position	Effective Irradiance
Lamp installed, 50-cm distance	average 0.016 $\mu\text{W}/\text{cm}^2$
Lamp installed, 50-cm distance	maximum 0.14 $\mu\text{W}/\text{cm}^2$

## Solvent Information

Observe the following recommendations on the use of solvents.

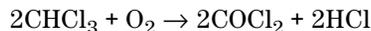
### Flow Cell

Avoid the use of alkaline solutions (pH > 11) 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.

## Declaration of Conformity for HOX2 Filter

We herewith inform you that the

*holmium oxide glass filter (type Hoya HY-1)*

meets the following specification of absorbance maxima positions:

360.8 nm – 418.5 nm – 536.4 nm (spectral bandwidth: 1 nm).

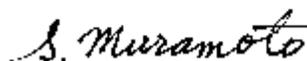
Agilent Technologies guarantees the traceability of the specified absorbance maxima to a National Institute of Standards & Technology (NIST) holmium oxide solution standard with a lot-to-lot tolerance of  $\pm 0.3$  nm.

The wavelength calibration filter built into the Agilent Technologies UV-visible detectors

**Table 78**

			Wavelength Accuracy
Product number	79853C	HP 1050 Series	$\pm 2$ nm
	G1314A	Agilent 1100 Series	$\pm 1$ nm

is made of this material and meets these specifications. It is, therefore, suitable for wavelength calibration of these detectors within the specified wavelength accuracy of the respective detector over its wavelength range.



November 1, 1995

Date Quality Manager

## Agilent Technologies on Internet

For the latest information on products and services visit our worldwide web site on the Internet at:

<http://www.agilent.com>

Select “Products” - “Chemical Analysis”

It will provide also the latest firmware of the Agilent 1100 series modules for download.

## **A Safety Information**

# Index

## A

A/D overflow, 94  
accessory kit, 18  
accessory kit parts, 216  
accuracy of wavelength, 328  
Agilent on internet, 341  
algea, 139  
analog output, 268  
analog signal output, 286  
APG remote interface, 287

## B

battery  
description, 268  
location on FLM board, 160  
safety information, 335  
BCD board, 283  
BCD/LAN board, 282  
bench space, 15  
boards  
interface board (BCD/LAN), 282  
location of connectors, 160

## C

cable  
connecting APG remote, 21  
connecting CAN, 21  
connecting GPIB, 21  
connecting LAN, 21  
connecting the ChemStation, 21  
connecting the power, 21  
overview and identification, 218  
calibration sample, 110  
CAN interface, 286  
check of Raman band of water, 104  
configuration switch  
default settings, 290  
description and factory settings, 290  
control module

Analysis screens, 299  
Config screens, 316  
Control screens, 316  
Error Log screen, 319  
Firmware Update screens, 321  
Info Log and EMF screen, 319  
introduction to FLD screens, 4  
Maintenance screens, 322  
Method screens, 313  
part numbers, 208  
Records screen, 318  
Run Times screen, 310  
Settings screens, 301  
Signal Pilot screens, 312  
Spectrum screens, 306  
Status screens, 310  
System screen, 315  
Tests and Diagnostic screens, 323  
Timetable screens, 305  
cut-off filter, 243  
cutoff filter  
other type, 215  
cuvette, 238  
how to use, 138

## D

DAC test, 127  
dark current test, 117  
degradation UV, 115, 245  
delivery checklist, 17  
dimensions and weight, 16  
dispersion of light, 247

## E

early maintenance feedback (EMF), 259  
electrical connections  
description of, 256  
EMF (early maintenance feedback), 259  
emission condenser, 243  
emission grating, 243

emission monochromator, 247  
emission slit, 243  
environment, 15  
error messages, 77  
A/D overflow, 94  
ADC not calibrated, 93  
compensation sensor open, 86  
compensation sensor short, 87  
encoder index not found, 89  
fan failed, 88  
flash lamp current overflow, 95  
flash trigger lost, 96  
FLF board not found, 92  
flow cell removed, 99  
lamp cover open, 91  
leak, 82  
leak sensor open, 84  
leak sensor short, 85  
motor encoder index wrong, 90  
motor errors, 89  
motor friction too high, 90  
motor or encoder not found, 89  
motor position not found or, 90  
motor speed too low or unstable, 90  
remote timeout, 80  
shutdown, 79  
synchronization lost, 81  
time-out, 78  
wavelength calibration failed, 97  
wavelength calibration lost, 98  
ESD (electrostatic discharge) strap, 132  
exchanging. see repairs  
excitation condenser, 243  
excitation grating, 243  
excitation monochromator, 246  
excitation slit, 243

## F

features  
safety and maintenance, 329  
firmware

## Index

- description, 274
- main system, 274
- raw data conversion, 276
- resident system, 274
- updates, 200, 275
- firmware updates, 275
- flash frequency, 253
- flat baseline - no peaks, 100
- FLM board
  - analog output, 268
  - battery, 268
  - diagram of main controller, 267
  - encoder signal acquisition, 266
  - EX/EM motor control, 266
  - fan drive, 268
  - interfaces, 268
  - processor, 266
- flow cell, 243, 248
- fluorescence and phosphorescence, 240
- fluorescence detection, 251
- fluorescence spectral libraries for peak confirmation, 50
- flushing of flow cell, 139
- front view of module, 22
- fuses
  - BCD board, 282
  - power supply, 296
- G**
- GLP features, 330
- glycogen, 110
- GPIB
  - default addresses, 286, 291
  - interface, 286
- H**
- holmium oxide
  - declaration of conformity, 340
- How the Detector Operates, 239
- how to use the cuvette, 138
- humidity, 16
- I**
- Identifying, 201
- information
  - on lithium batteries, 335
- installation
  - accessory kit, 18
  - bench space, 15
  - delivery checklist, 17
  - environment, 15
  - flow connections, 24
  - of flow cell and capillaries, 24
  - of the detector, 22
  - physical specifications, 16
  - power considerations, 14
  - power cords, 14
  - site requirements, 14
  - unpacking, 17
- instrument layout, 258
- interface board (BCD/LAN), 282
- interfaces
  - analog signal output, 286
  - APG remote, 287
  - CAN, 286
  - GPIB, 286
  - overview, 285
  - RS-232C, 288
- internet, 341
- Introduction to the Detector, 238
- L**
- lamp intensity history, 116
- lamp intensity test, 115
- LAN
  - interface board, 282
- LAN cables, 236
- LAN interface board, 284
- leaks
  - correcting, 140
- line voltage and frequency, 16
- luminescence, 239
- M**
- message
  - A/D overflow, 94
  - ADC not calibrated, 93
  - compensation sensor open, 86
  - compensation sensor short, 87
  - encoder index not found, 89
  - fan failed, 88
  - flash lamp current overflow, 95
  - flash trigger lost, 96
- FLF board not found, 92
- flow cell removed, 99
- lamp cover open, 91
- leak, 82
- leak sensor open, 84
- leak sensor short, 85
- motor encoder index wrong, 90
- motor friction too high, 90
- motor messages, 89
- motor or encoder not found, 89
- motor position not found or lost, 90
- motor speed too low or unstable, 90
- remote timeout, 80
- shutdown, 79
- synchronization lost, 81
- time-out, 78
- wavelength calibration failed, 97
- wavelength calibration lost, 98
- method development, 37
  - 1 - check the LC system for impurities, 38
  - 2 - optimize limits of detection and selectivity, 39
  - 3 - set up routine methods, 48
- fluorescence spectral libraries for peak confirmation, 50
- multi wavelength detection, 49
  - take a fluorescence scan, 40
- mirror, 243
- monochromator
  - EM, 243, 247
  - EX, 243, 246
- multi wavelength detection, 49
- N**
- no peaks, 100
- O**
- off-line measurements, 238
- operation of the detector, 239
- operation temperature, 16
- operation, theory of electronics, 263
- optical unit overview, 243
- optimization
  - example, 52

**P**

parts identification, 201  
 accessory kit, 216  
 cable overview, 218  
 cables - analog, 220  
 cables - APG remote, 223  
 cables - auxiliary, 232  
 cables - BCD, 229  
 cables - CAN, 233  
 cables - external contact, 234  
 cables - LAN cables, 236  
 control module, 208  
 foams and pads, 211  
 leak panels, 213  
 main assemblies, 203  
 optical unit, 204  
 overview, 202  
 plastics, 210  
 power and status, 212  
 sheet metal kit, 209

peak detector, 280

performance specifications, 328

phosphorescence detection, 252

photoluminescence, 239

photo-multiplier tube  
 figure, 249  
 location of PMT, 243  
 optional PMT types, 180  
 PMT, 248  
 replacing, 180

physical specifications, 16  
 humidity, 16  
 line voltage and frequency, 16  
 operation temperature, 16  
 power consumption, 16  
 safety standards, 16  
 weight and dimensions, 16

PMT  
 automatic test, 303  
 gain, 248  
 gain steps, 254  
 gain test, 31  
 optional PMT types, 180  
 photo-multiplier tube, 248  
 range, 254

power considerations, 14  
 power consumption, 16  
 power cords, 14

power supply  
 description, 295  
 specifications, 296

**R**

Raman, 242

Raman S/N test, 119

rear view of module, 23, 257

reasons for doing a verification or  
 calibration, 101

recalibration of wavelength, 74, 101

reference diode, 250

reference system, 250

removing covers, 144, 146

repair  
 flushing of flow cell, 139

Repairing, 129

repairs  
 assembling main cover, 198  
 cleaning the instrument, 131  
 correction leaks, 140  
 definition of, 130  
 exchanging a flow cell, 135  
 exchanging a lamp, 147  
 exchanging EX condenser, 152  
 exchanging internal parts, 142  
 exchanging leak sensor, 165  
 exchanging main board (FLM), 159  
 exchanging power supply, 190  
 exchanging the fan, 163  
 installing the optical unit, 194  
 introduction, 130  
 of the detector, 3, 129  
 removing covers, 144, 146  
 removing optical unit, 178  
 removing rear foam, 146  
 replacing cutoff filter, 168  
 replacing EM and EX Grating, 187  
 replacing EM condenser, 168  
 replacing firmware, 200  
 replacing FLF board, 180  
 replacing FLL board, 183  
 replacing foams and covers, 196  
 replacing leak handling system, 141  
 replacing mirror assembly, 172  
 replacing PMT, 180  
 replacing slits, 176  
 replacing status light pipe, 193

replacing trigger pack, 183  
 simple repairs overview, 133  
 using the ESD strap, 132  
 warnings and cautions, 130

replacing interface board (BCD/LAN), 199

resistance of monochromators, 118

responsetime, 255

RS-232C  
 cable kit to PC, 235  
 communication settings, 292  
 interface, 288  
 settings, 292

**S**

safety information  
 on lithium batteries, 335  
 standards, 16

serial number  
 entered on ChemStation, 162  
 entered on control module, 161

site requirements, 14

spare parts  
 cutoff filters, 215

specifications  
 analog outputs, 329  
 communications, 329  
 flow cell, 329  
 GLP features, 330  
 monochromators, 328  
 performance, 328  
 pulse frequency, 328  
 safety and maintenance, 329  
 wavelength accuracy, 328

spectra acquisition modes, 281

spectra wavelength shift, 32

stack configuration, 20, 21  
 front view, 20  
 rear view, 21

**T**

test chromatogram, 124

test functions, 74, 114

tests  
 DAC, 127  
 dark current, 117  
 functions, 114  
 lamp intensity, 115

## Index

- lamp intensity history, 116
- PMT gain test, 31
- Raman AST S/N, 119
- resistance of monochromators, 118
- test chromatogram, 124
- theory of operation and electronics, 263
- troubleshooting
  - error messages, 74, 77
  - no peaks, 100
  - status indicators, 74, 75

## U

- unpacking, 17
- using EMF, 259
- UV degradation, 115, 245

## W

- wavelength
  - recalibration, 74, 101
- wavelength calibration, 101
- wavelength calibration procedure, 101, 110
- wavelength calibration steps, 103
- wavelength shift of spectra, 32
- weight and dimensions, 16

## X

- xenon flash lamp, 243, 245





## **In This Book**

This manual contains technical reference information about the Agilent 1100 Series fluorescence detector. The manual describes the following:

- installing the detector,
- optimization of the detector,
- diagnostics and troubleshooting,
- repairing the detector,
- parts and materials, and
- introduction to the detector and theory of operation.

