Agilent 1200 Series Fluorescence Detector

G1321A

User Manual

Agilent Technologies

Notices

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Manual Structure

The **User Manual G1321-90010** (English) and its localized versions contain a subset of the Service Manual and is shipped with the detector in printed matter.

The **Service Manual G1321-90110** (English) contains the complete information about the Agilent 1200 Series Fluorescence Detector. It is available as Adobe Reader file (PDF) only.

Latest versions of the manuals can be obtained from the Agilent web.

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1 Introduction to the Fluorescence Detector

This chapter gives an introduction to the detector, instrument overview and internal connectors.

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 37

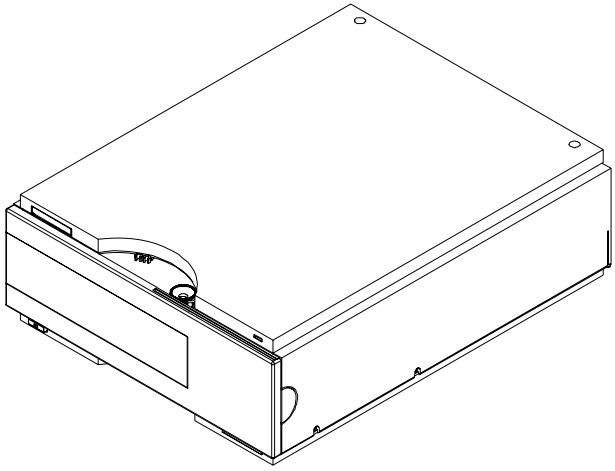


Figure 1 The Agilent 1200 Series Fluorescence Detector.

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 2. 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.

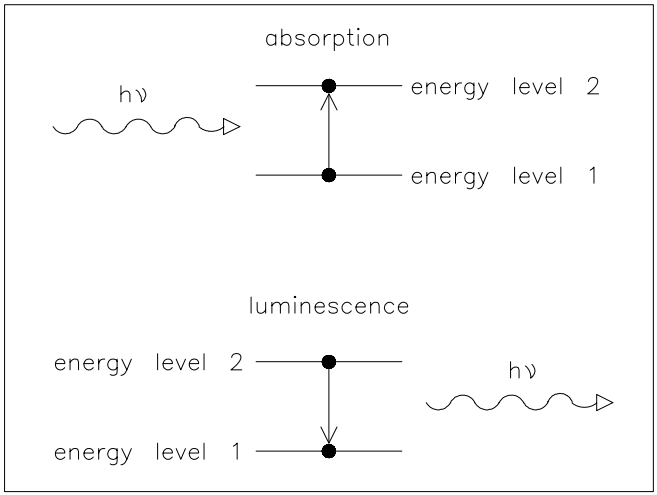


Figure 2 Absorption of Light Versus Emission of Light

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 3. The characteristic maxima of absorption for a substance is its λ_{EX}, and for emission its λ_{EM}.

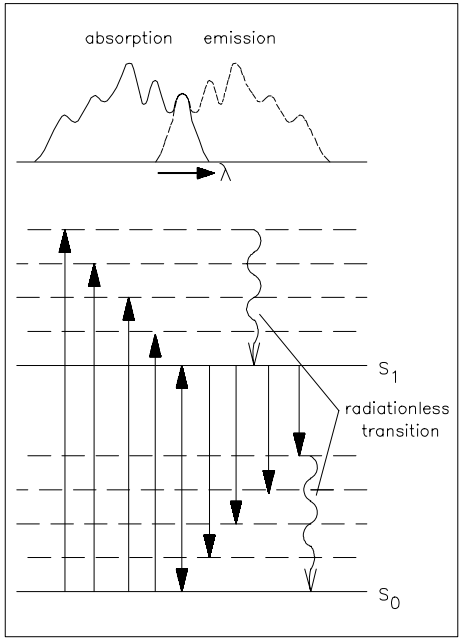


Figure 3 Relationship of Excitation and Emission Wavelengths

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 4.

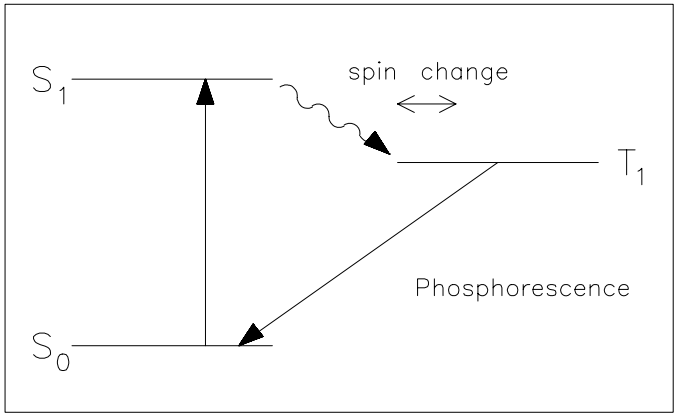


Figure 4 Phosphorescence Energy Transitions

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.

Formula: $E = h \times \lambda^{-1}$

In this equation:

E is energy
h is Planck's constant
λ 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 it's molecular motions.

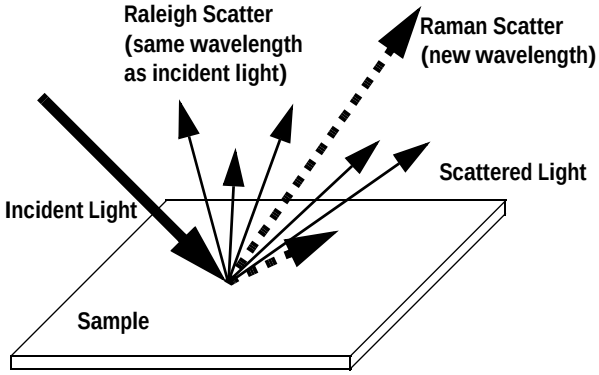


Figure 5 Raman

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 6 on page 15, 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.

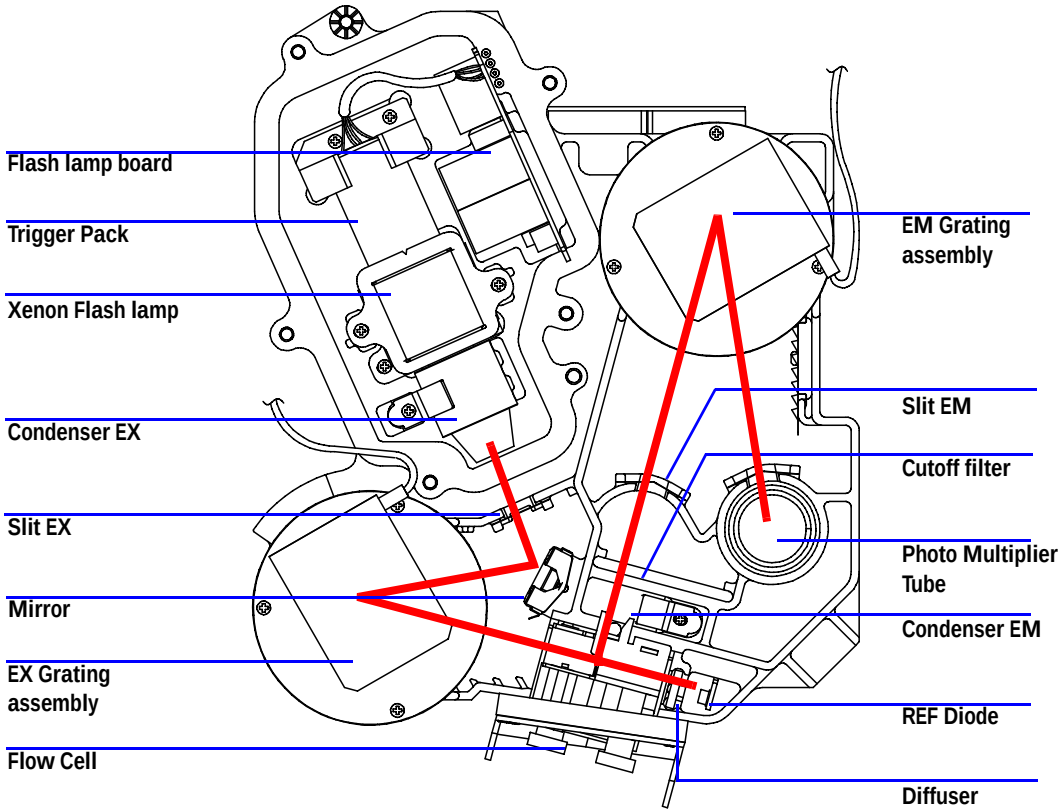


Figure 6 Optical Unit

The radiation source is a xenon flash-lamp. The 3 µs 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 7. 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.

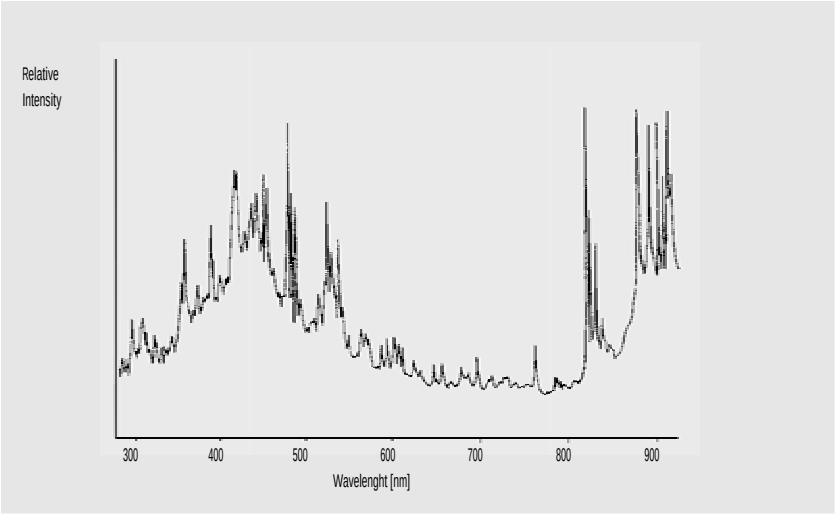


Figure 7 Lamp Energy Distribution (vendor data)

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.

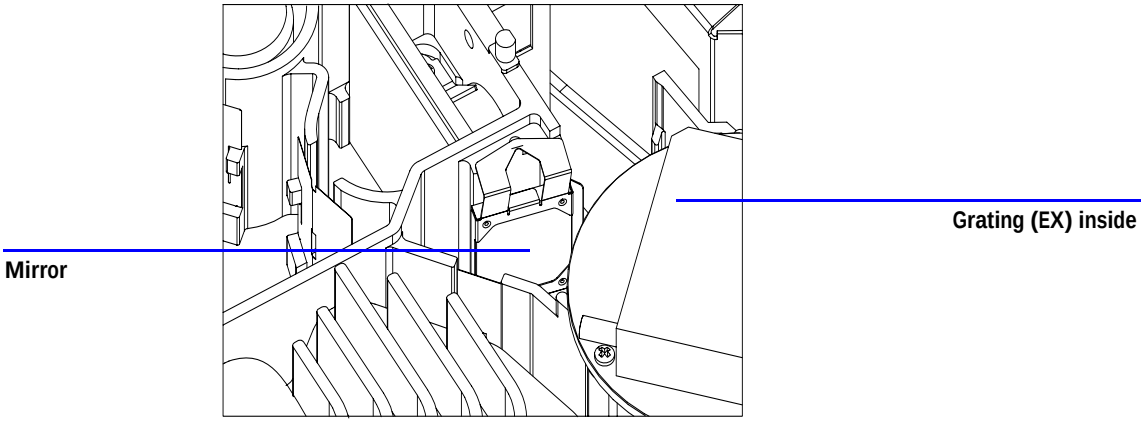


Figure 8 Mirror Assembly

The geometry of the grooves is optimized to reflect almost all of the incident light, in the 1st order and disperse it with about 70% efficiency in the ultra-violet range. Most of the remaining 30% of the light is reflected at zero order, with no dispersion. Figure 9 illustrates the light path at the surface of the grating.

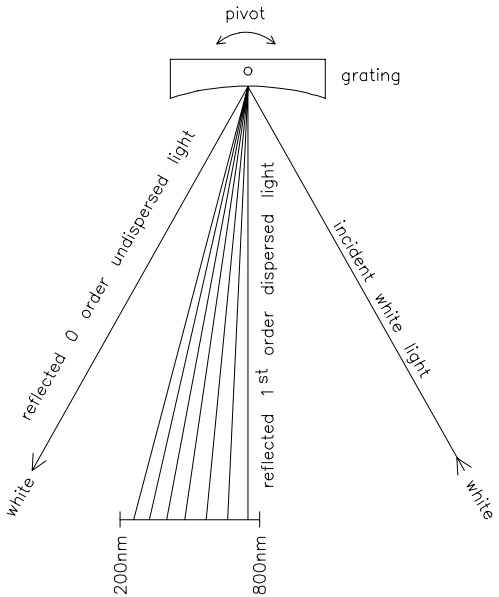


Figure 9 Dispersion of Light by a Grating

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 1st 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.

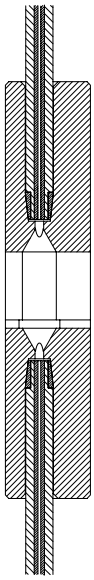


Figure 10 Cross-Section of Flow Cell

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 1st order scatter and 2nd order stray light, see Figure 9 on page 18.

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 11, 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.

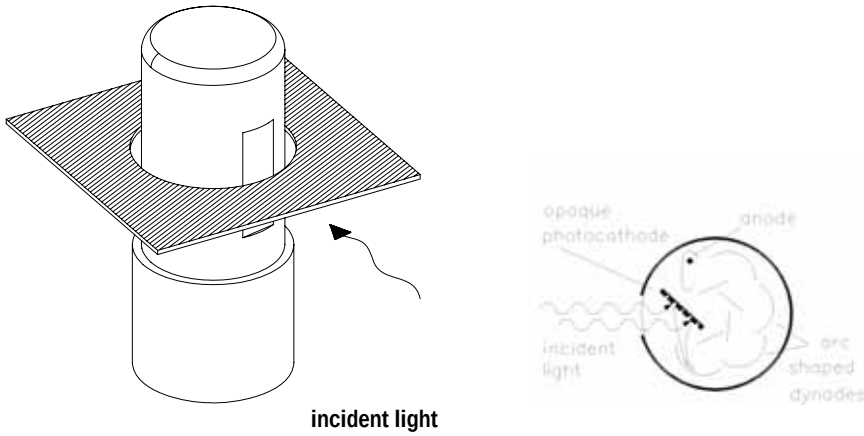


Figure 11 Photo-multiplier Tube

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 6 on page 15.

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"Spare Parts" on page 134.

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 6 on page 15). 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 12. The luminescence is short-lived, therefore the fluorescence detector need only measure over a short period of time after the lamp has flashed.

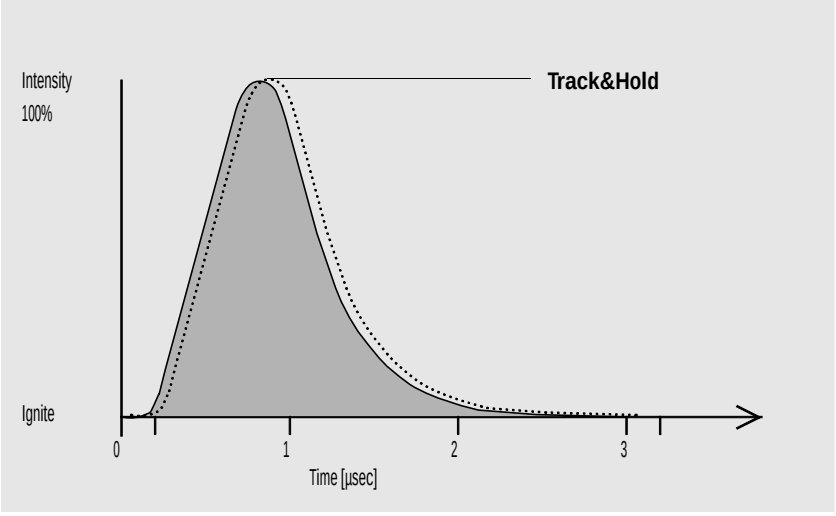


Figure 12 Measurement of Fluorescence

Phosphorescence Detection

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

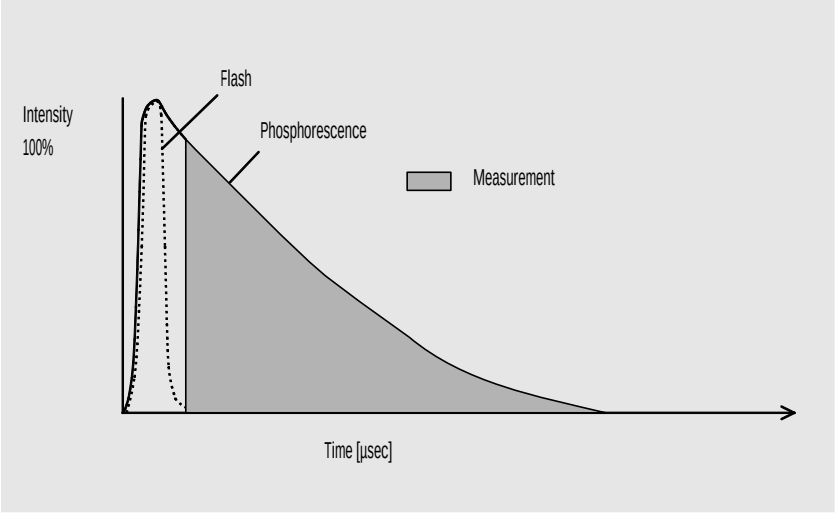


Figure 13 Measurement of Phosphorescence

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.

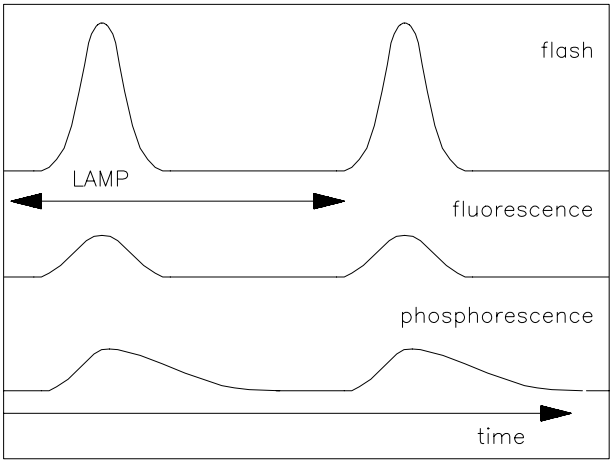


Figure 14 LAMP: Frequency of Flash, Fluorescence, and Phosphorescence

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.

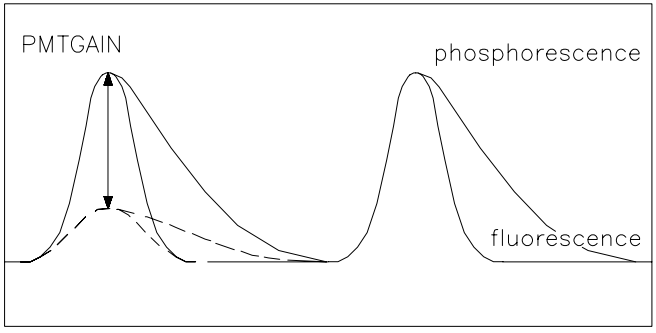


Figure 15 PMTGAIN: Amplification of Signal

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 16, 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.

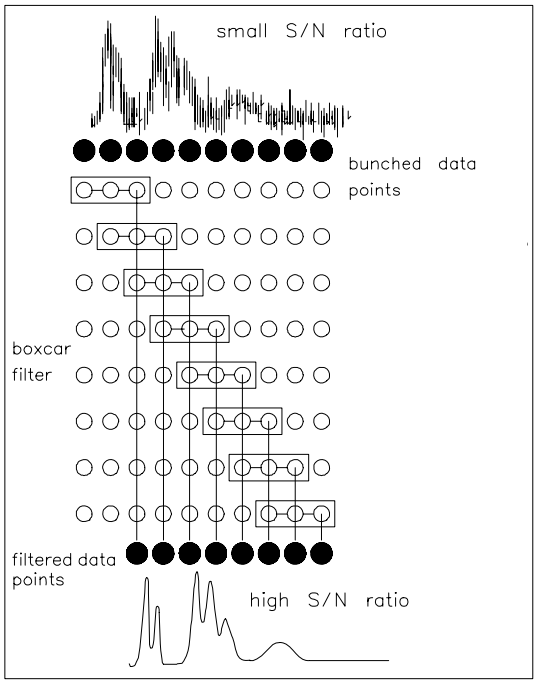


Figure 16 RESPONSETIME: Signal-to-Noise Ratio

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 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 1200 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 your software documentation for further information.

  Together with a Control Module G1323B the RS-232C may be used to print screens to a connected printer.
- The power input socket accepts a line voltage of 100 – 240 V AC ± 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.**

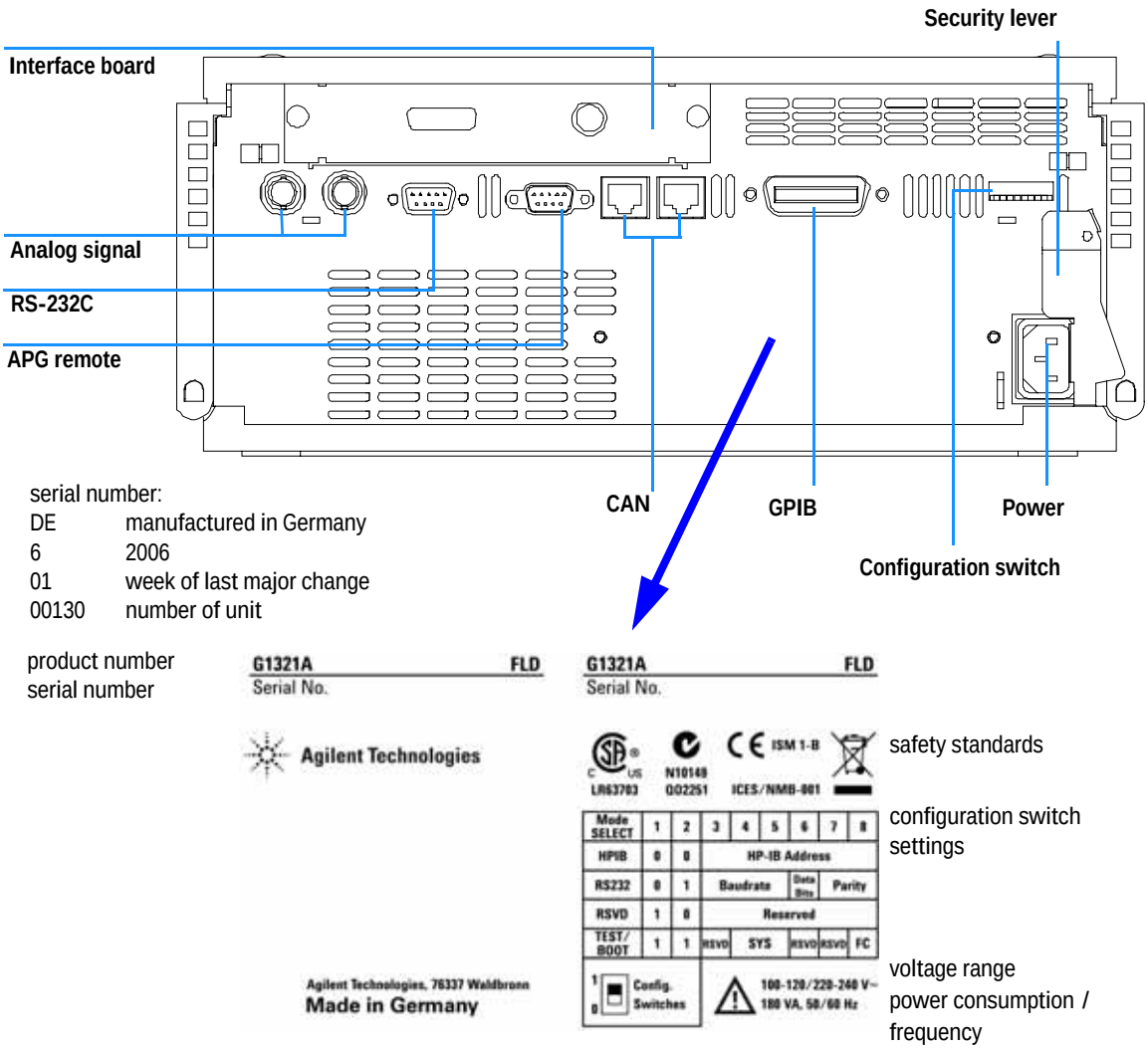


Figure 17 Electrical Connections

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 18 on page 31 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 1 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)

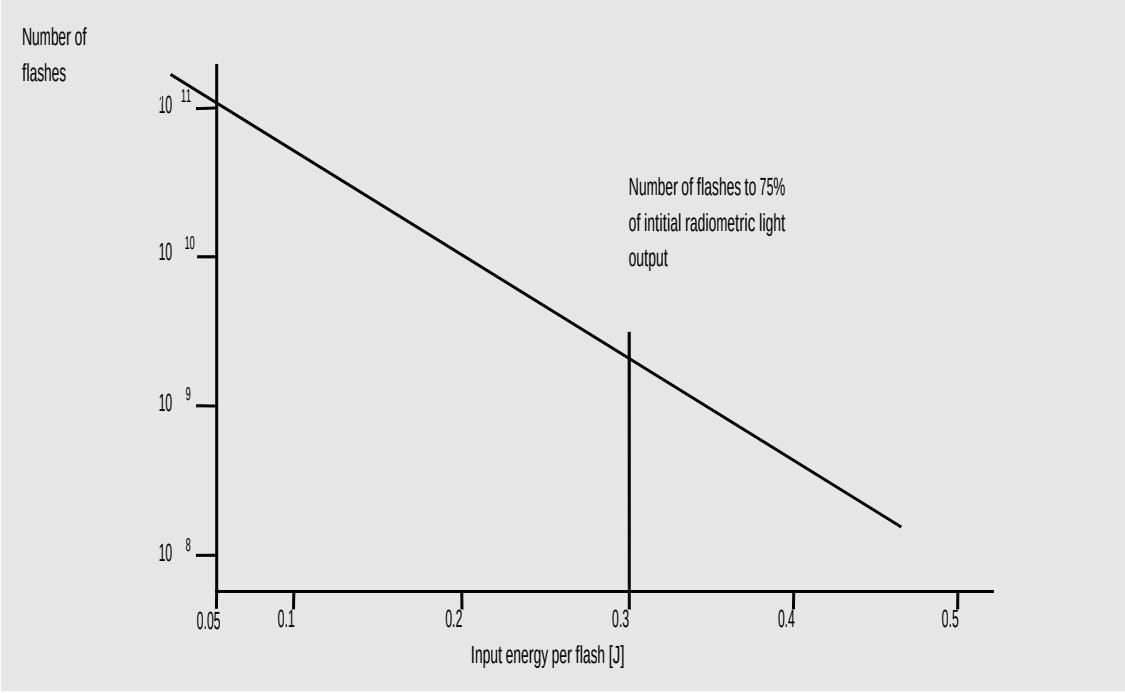


Figure 18 Lamp life

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.

2
Site Requirements and Specifications

This chapter gives information on environmental requirements, physical and performance specifications.

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 2 on page 36. 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.**

CAUTION Make sure to have easy access to the power cable of the instrument, in order to disconnect the instrument from line.

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.**

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

Bench Space

The detector dimensions and weight (see Table 2 on page 36) 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 1200 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 2 on page 36.

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 37) 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.

Physical Specifications

Table 2 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 – 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. For indoor use only.	

Performance Specifications

Table 3 Performance Specifications Agilent 1200 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*	see note below this table
	RAMAN single wavelength (H_2O) > 500 with Ex=350 nm, Em=397 nm, dark value 450 nm, standard flow cell time constant=4 seconds (8 seconds responsetime)	see Service Manual for details
	RAMAN dual wavelength (H_2O) > 300 with Ex=350 nm, Em=397 nm, dark value 450 nm, standard flow cell time constant=4 seconds (8 seconds responsetime)	see Service Manual for details
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	

Table 3 Performance Specifications Agilent 1200 Series Fluorescence Detector

Type	Specification	Comments
Timetable programing:	up to 4 signal wavelengths, response time, PMT Gain, baseline behavior (append, free, zero), spectral parameters	
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 Instant Pilot G4208A or Agilent Control Module G1323B 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	

Table 3 Performance Specifications Agilent 1200 Series Fluorescence Detector

Type	Specification	Comments
Safety and maintenance	Extensive diagnostics, error detection and display (through Instant Pilot G4208A, Control Module G1323B and ChemStation), leak detection, safe leak handling, leak output signal for shutdown of pumping system. Low voltages in major maintenance areas.	
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.

3
Installing the Detector

This chapter describes the installation of the detector.

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 4 Detector Checklist

Description	Quantity
Detector	1
Power cable	1
CAN cable	1
Flow cell	1 (built-in)
Optional flow cell/cuvette	as ordered
User Manual	1
Accessory kit (see Table 5 on page 43)	1

Detector Accessory Kit Contents

Table 5 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

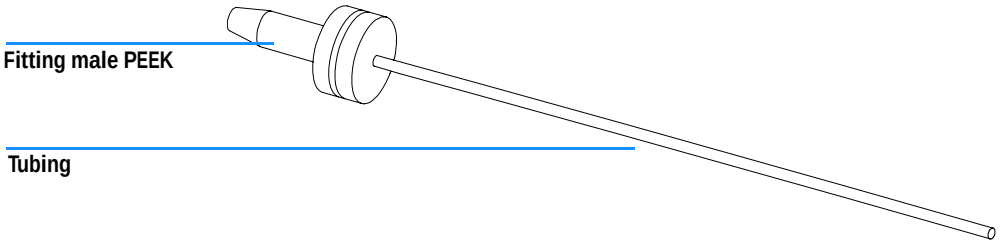


Figure 19 Waste Tubing Parts

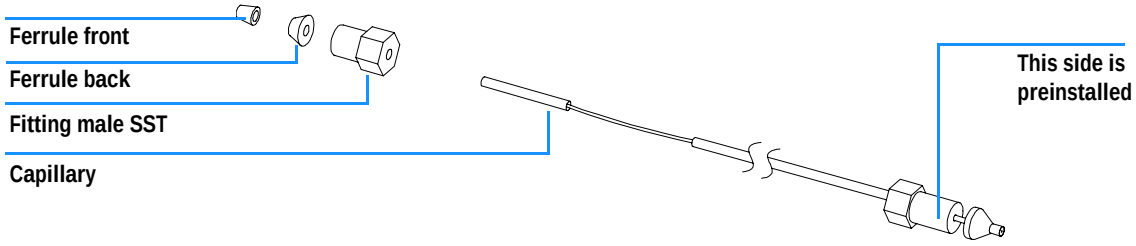


Figure 20 Inlet Capillary (Column-Detector) Parts

Optimizing the Stack Configuration

If your detector is part of a complete Agilent 1200 Series system, you can ensure optimum performance by installing the following configuration. This configuration optimizes the system flow path, ensuring minimum delay volume.

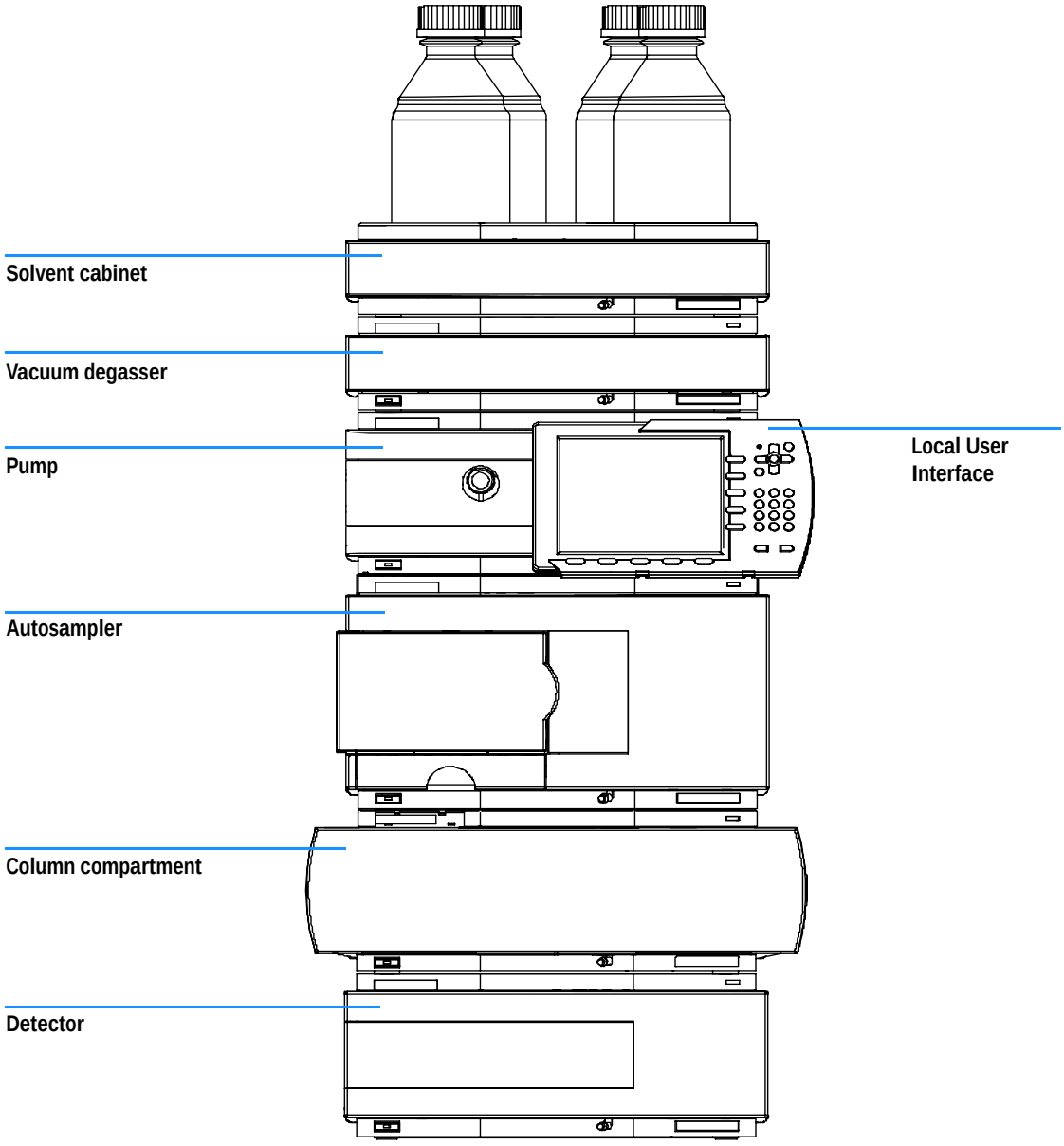


Figure 21 Recommended Stack Configuration (Front View)

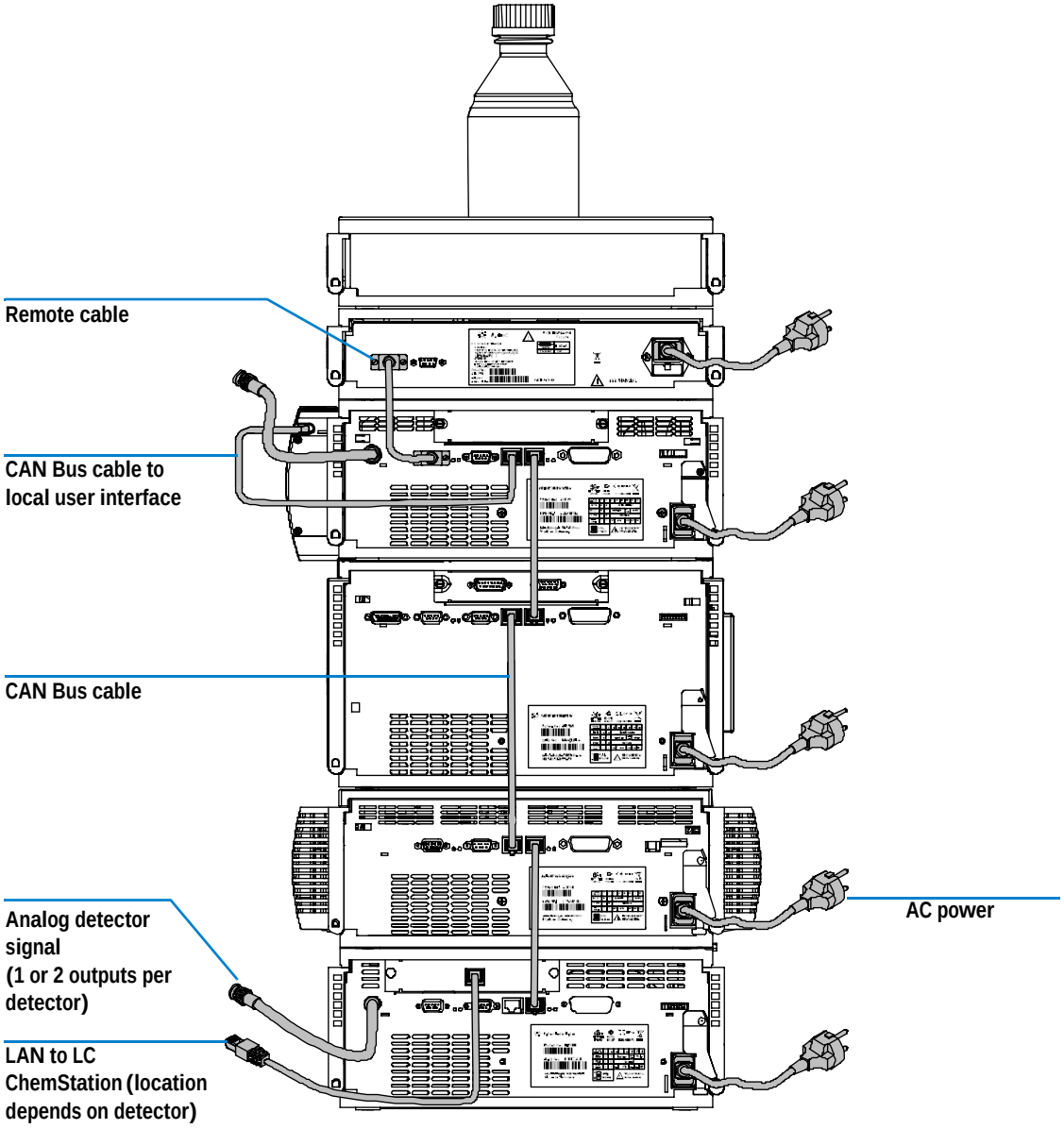


Figure 22 Recommended Stack Configuration (Rear View)

Installing the Detector

Preparations	Locate bench space Provide power connections Unpack the detector
Parts required	Detector Power cord, for other cables see below, Agilent ChemStation and / or Instant Pilot G4208A or Control Module G1323B.

1 Install the LAN interface board in the detector (if required), see "Replacing the Interface Board" on page 120.

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.

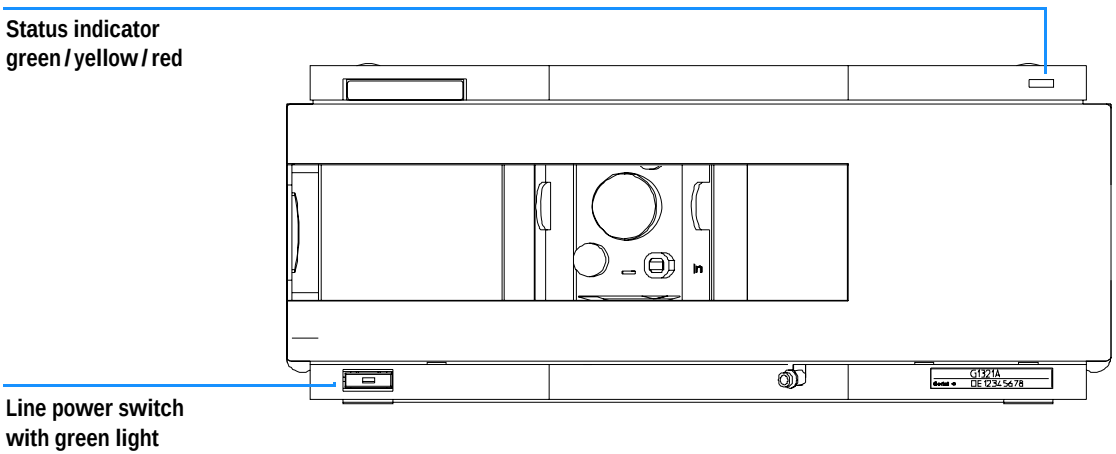


Figure 23 Front View of Detector

4 Connect the power cable to the power connector at the rear of the detector.

5 Connect the CAN cable to other Agilent 1200 Series modules.

6 If an Agilent ChemStation is the controller, connect either

- the LAN connection to the LAN interface board in the detector.

NOTE If an Agilent 1200 DAD/MWD/FLD is in the system, the LAN should be connected to the DAD/MWD/FLD (due to higher data load).

7 Connect the analog cable(s) (optional).

8 Connect the APG remote cable (optional) for non-Agilent 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.

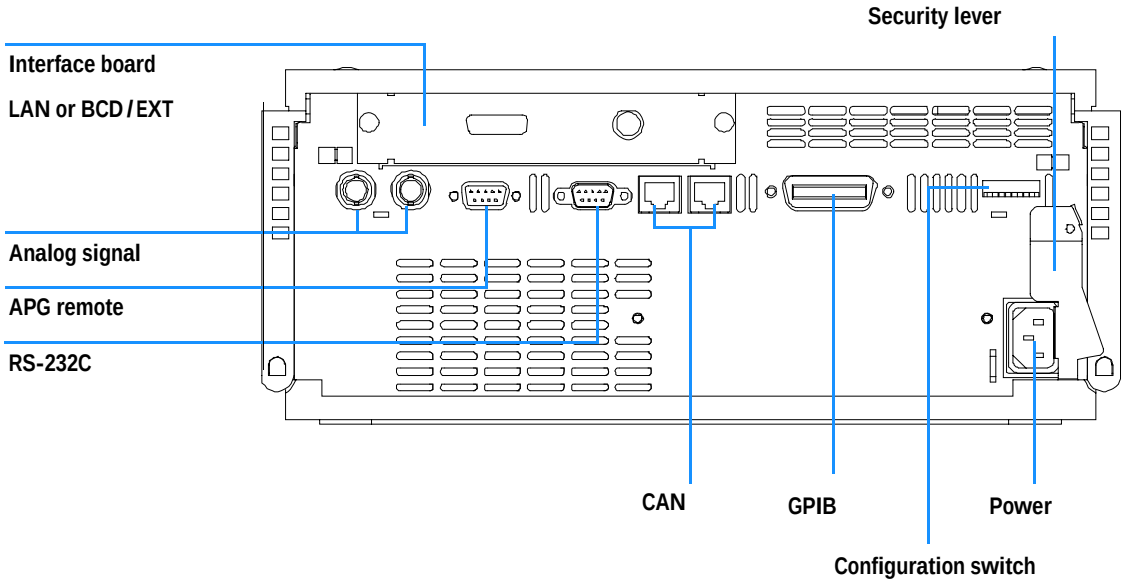


Figure 24 Rear View of Detector

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.

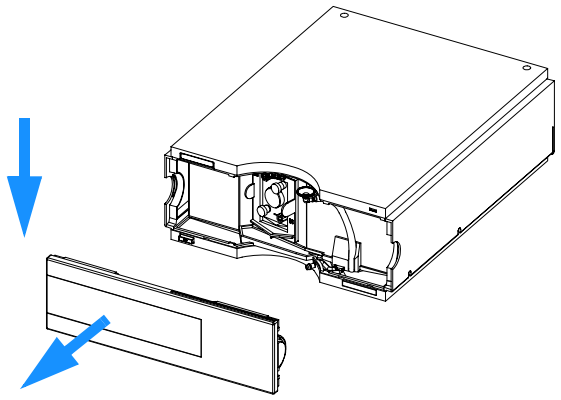
Flow Connections to the Detector

Preparations	Detector is installed in the LC system.
Parts required	Other modules Parts from accessory kit, see"Detector Accessory Kit Contents" on page 43. Two wrenches 1/4 – 5/16 inch for capillary connections

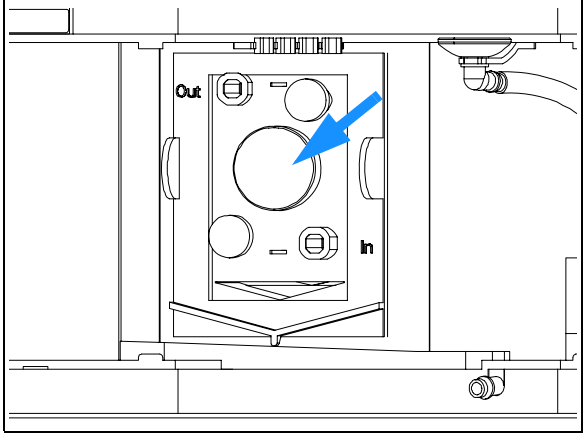
WARNING **When working with solvents please observe appropriate safety procedures (for example, goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet supplied by the solvent vendor, especially when toxic or hazardous solvents are used.**

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.

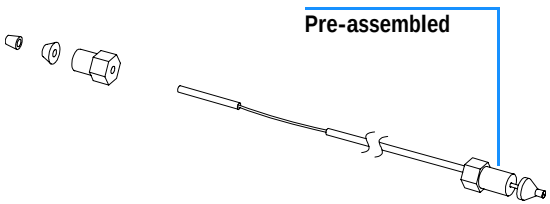
1 Press the release buttons and remove the front cover to gain access to the flow cell area.



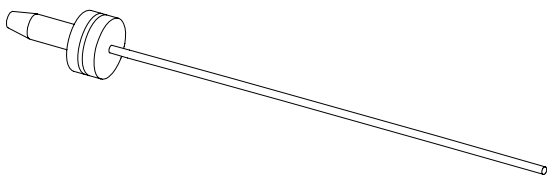
2 Locate the flow cell.



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) .

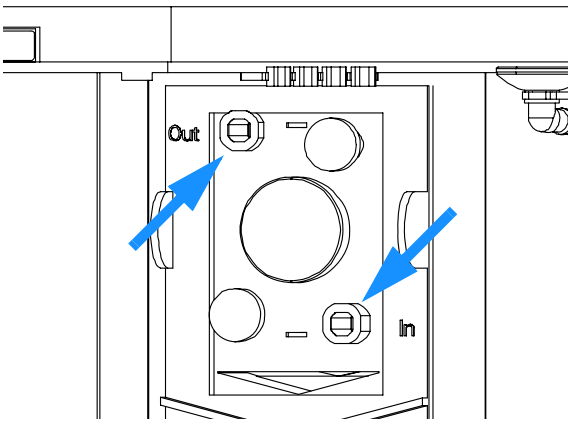
When working with detector behind the FLD (on own risk) determine the backpressure of this detector first by

- removing the column and the last detect and measuring system pressure at the application flow rate.

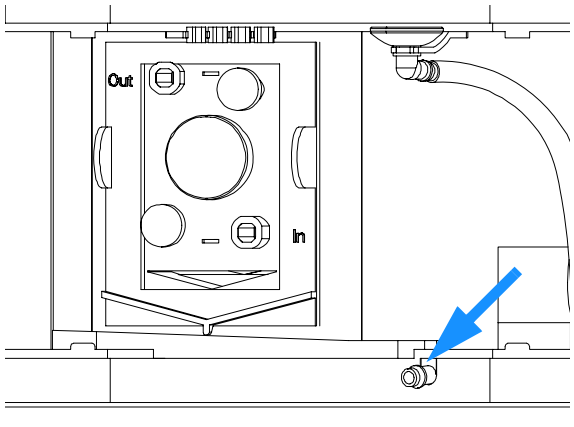
- connecting the last detector (without column and FLD) and measuring the system pressure with flow.

- the difference in measured pressure is due to the back pressure generated by the last detector and is the pressure seen by the FLD.

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

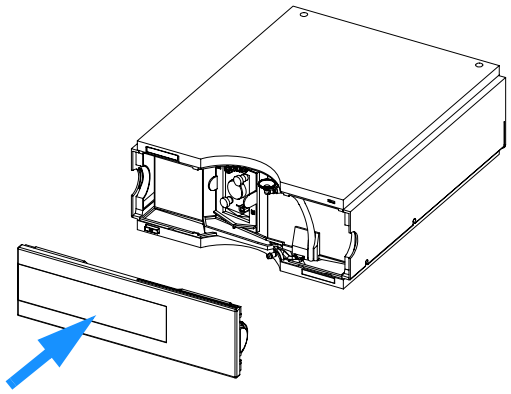


6 Connect the waste tubing to the bottom waste fitting.



7 Establish flow and observe if leaks occur.

8 Replace the front cover.



The installation of the detector is now complete.

NOTE

The detector should be operated with the front cover in place to protect the flow cell area against strong drafts from the ouside.

4
First Steps with the Detector

This chapter guides you how to start the work with the detector.

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.

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 1200 Series fluorescence detector using the Agilent isocratic checkout sample.

When required	If you want to checkout the detector
Hardware required	LC system with G1321A FLD
Parts required	Start-up Kit 5063-6528, includes LC cartridge Hypersil ODS, 5um, 125x4mm with CIS cartridge holder 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 6 Chromatographic Conditions

Mobile phases	A = water = 35% B = Acetonitrile = 65%
Column	OSD-Hypersil column, 125 mm x 4 mm i.d. with 5 µm 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 µl
Oven temperature (1200)	30°C
FLD Excitations/Emission Wavelength	EX = 246 nm, EM = 317 nm
FLD PMT Gain	PMT = 10
FLD Response time	4 seconds

2 Set the FLD setpoints according to Figure 25 (on the local Control Module G1323B, 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.

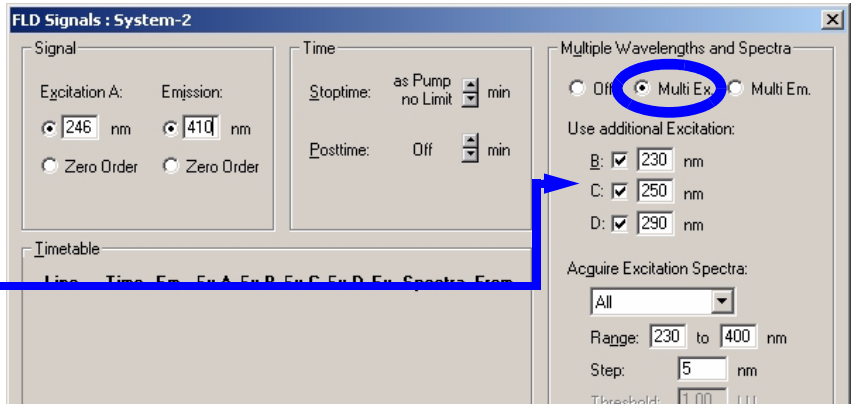


Figure 25 FLD Parameters

3 Start the run.

4 The resulting chromatograms are shown in Figure 26:

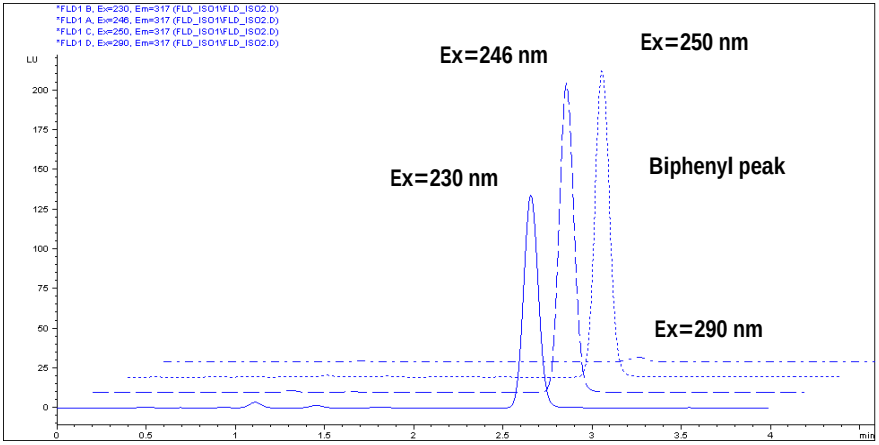


Figure 26 Biphenyl peak with different excitation wavelengths

5 The excitation maxima is around 250 nm.

Observe the maxima via the isoabsorbance plot

1 Load the data file (λ_{EX}=246 nm, λ_{EM}=317 nm) and open the isoabsorbance plot.

2 The maximum λ_{EX} will be found around 250 nm.

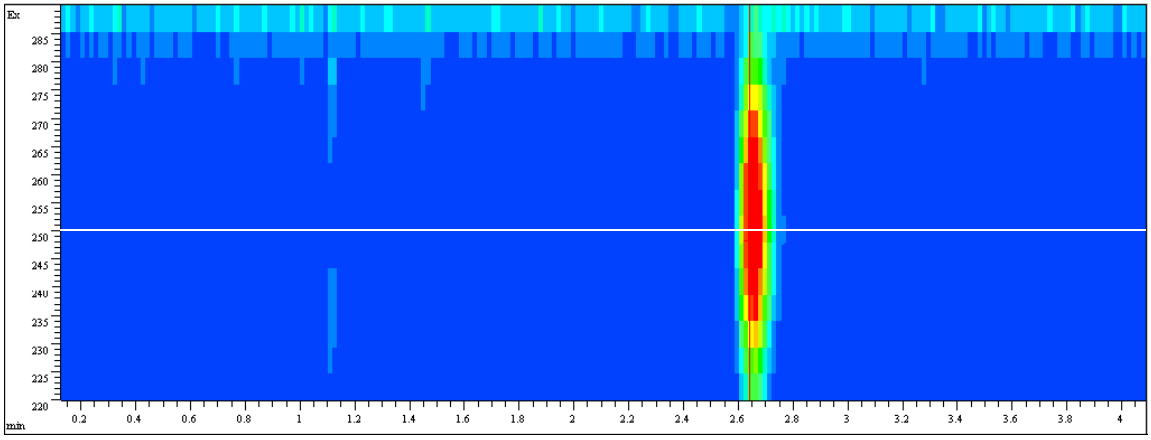


Figure 27 Isoabsorbance Plot

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 1200 Series fluorescence detector. Table 7 gives an overview of how to benefit from the operation modes during these steps.

Table 7 Steps for thorough method development

	Step 1: Check system	**Step 2: Optimize limits of detection and selectivity**	**Step 3: Set up routine methods**
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 28 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.

A pure water sample was put into the flow cell. Spectra were recorded at 5 nm step sizes.

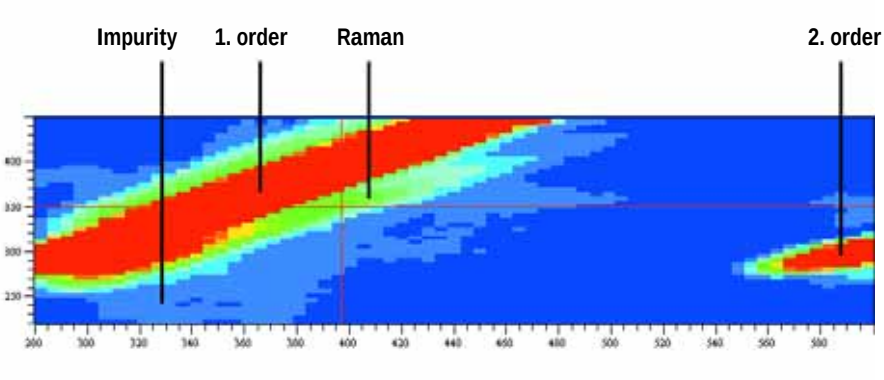


Figure 28 Isofluorescence plot of a mobile phase

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 29) 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.

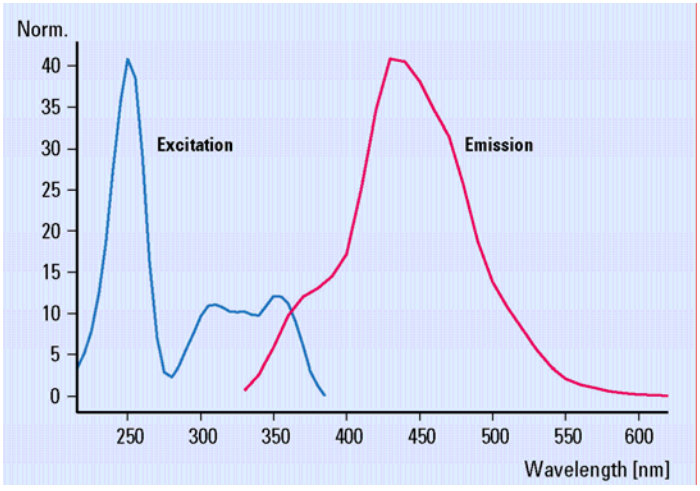


Figure 29 Excitation and emission spectra of quinidine

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 28 on page 64) 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 1200 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 1200 Series FLD to separate the compound mix under known conditions and acquire emission and excitation spectra separately.

Procedure III - Use an Agilent 1200 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 1200 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 30 on page 68 shows the complete information for quinidine as obtained with the Agilent 1200 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 30 on page 68:

All excitation and emission spectra of Quinidine (1 µg/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

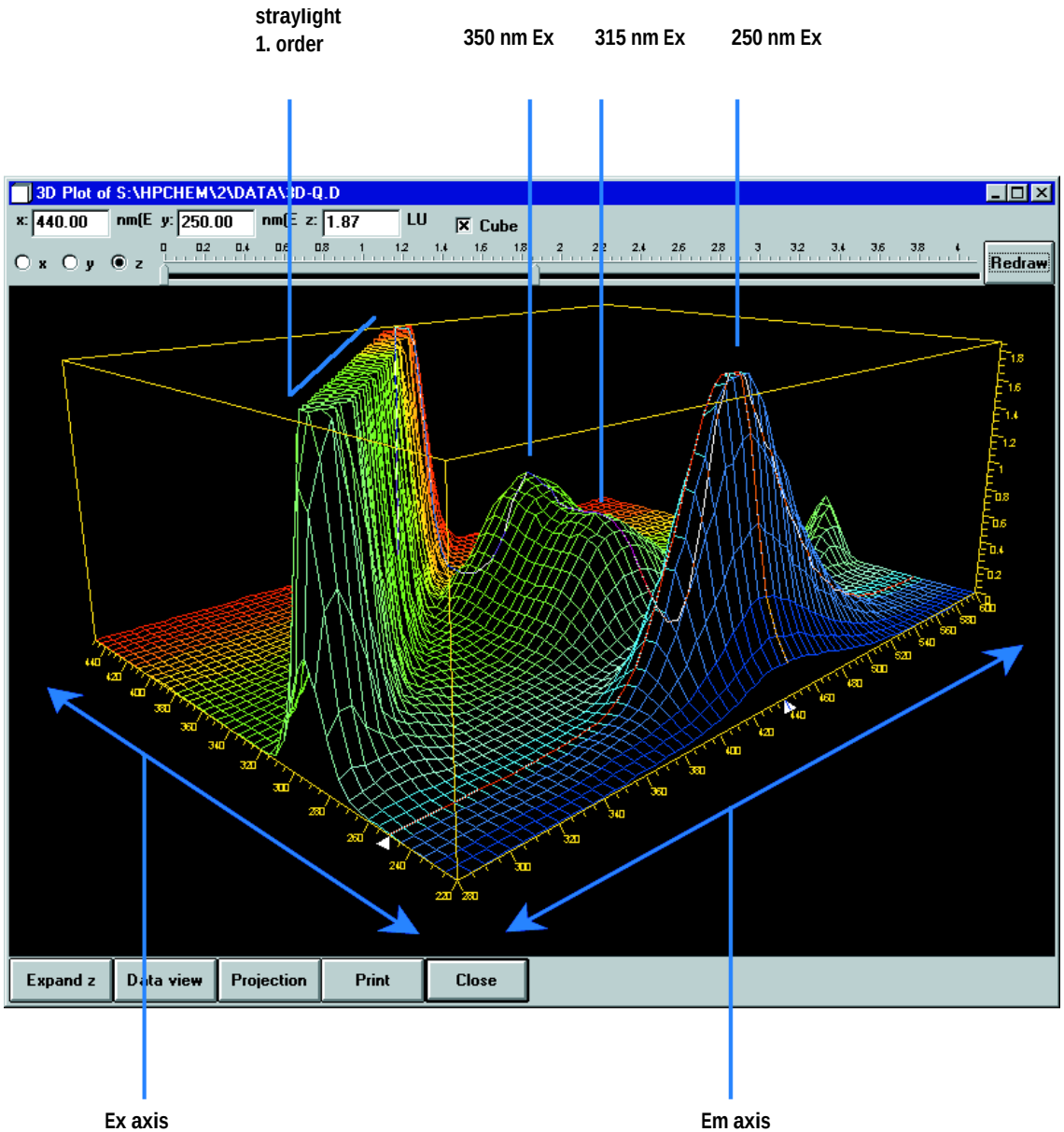


Figure 30 Characterization of a pure compound from a fluorescence scan

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 31 on page 70 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 8 Timetable for PNA analysis

0 min:	350 nm	for naphthalene to phenanthrene
8.2 min:	420 nm	for anthracene to benzo(g,h,I)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.

Table 9 Conditions for Figure 31 and Figure 32 on page 71

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).

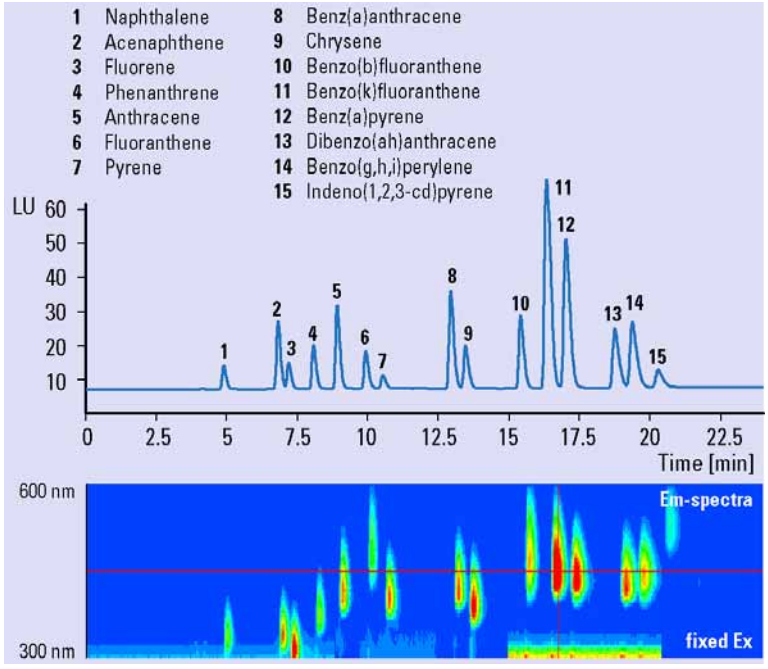


Figure 31 Optimization of the time-program for the emission wavelength

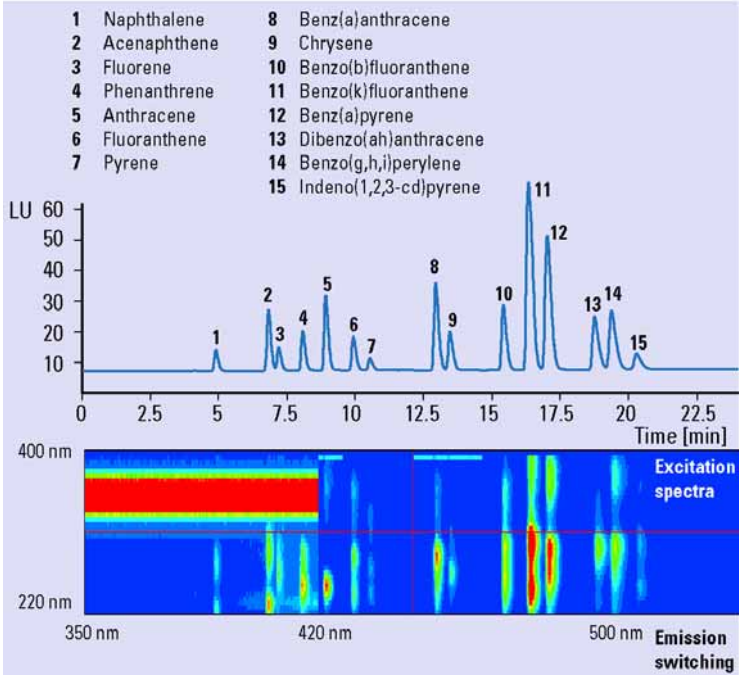


Figure 32 Optimization of the time-program for the excitation wavelength

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 10.

Table 10 Timetable for the analysis of 15 polynuclear aromatic hydrocarbons

Time [min]	Exitation 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 1200 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 34 on page 73 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.

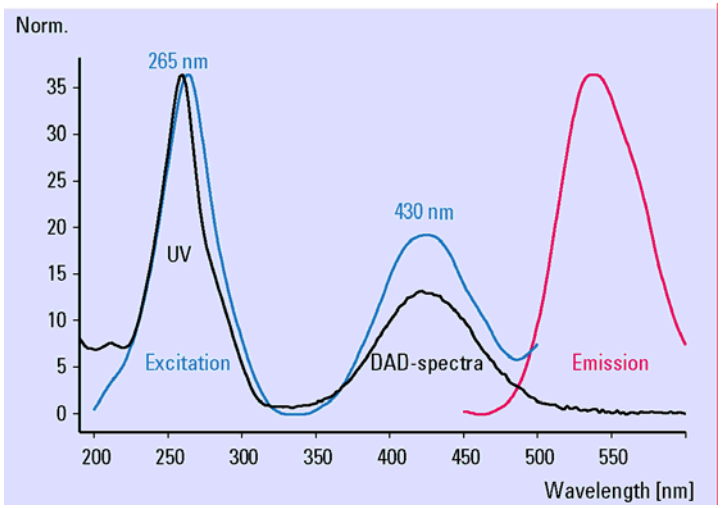


Figure 33 UV-spectrum and fluorescence spectra for 2,3-diaminophenazine (DAP)

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

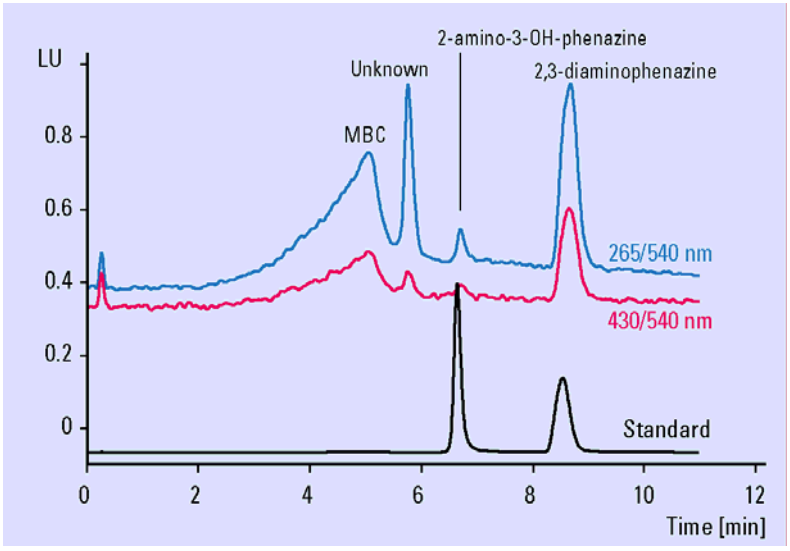


Figure 34 Qualitive analysis of MBC (2-benzimidazole carbamic acid methylester) and impurities

Table 11 Conditions for Figure 33 and Figure 34 on page 73

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 1200 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 1200 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 35 on page 76).

Table 12 Conditions for Figure 35 on page 76

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,

The upper trace was received with traditional wavelength switching.

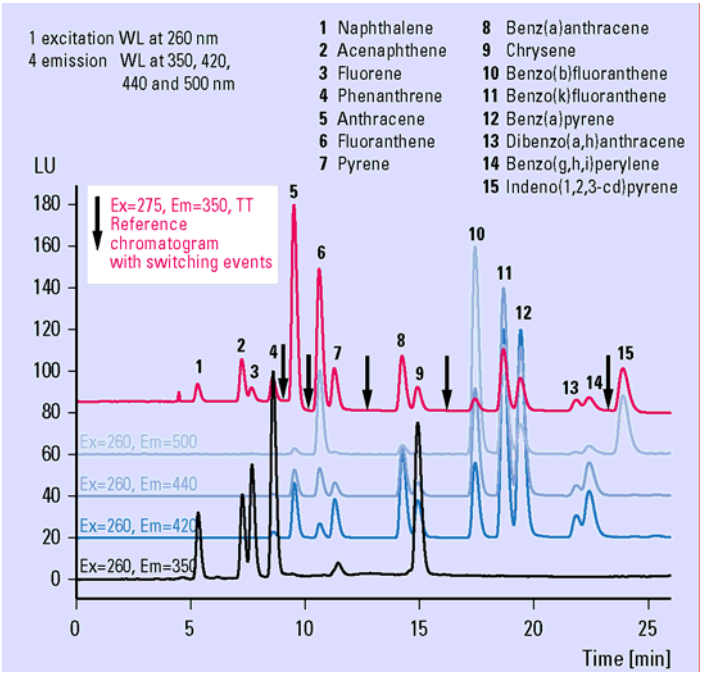


Figure 35 Simultaneous multi wavelength detection for PNA-analysis

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

Table 13 Peak confirmation using a fluorescence spectral library

Meas. RetTime [min]	Library [min]	CalTbl [min]	Signal	Amount [ng]	Purity Factor	#	Match	Libary Name
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 36 on page 79).

Table 14 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 (1200)	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.

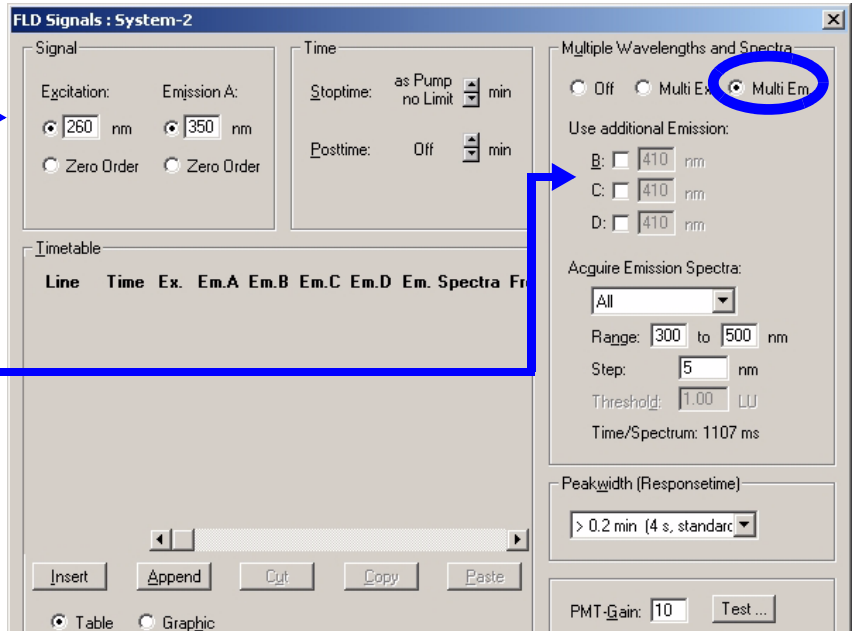


Figure 36 Detector Settings for Emission Scan

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).

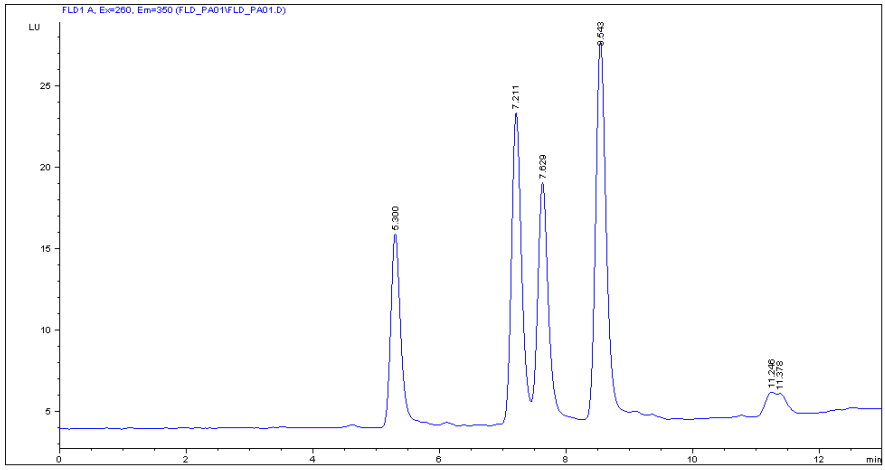


Figure 37 Chromatogram from Emissions Scan

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

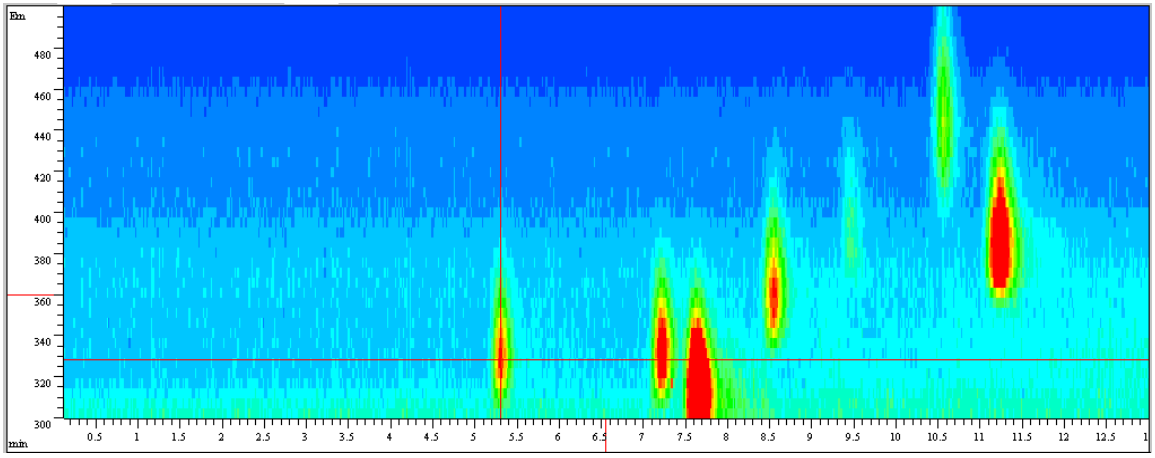


Figure 38 Isoabsorbance Plot from Emission Scan

Table 15

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	**11.23 min**	385 nm

5 Using the settings and the timetable (from previous page), do a second run for the evaluation of the optimal excitation wavelength. See Figure 39.

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

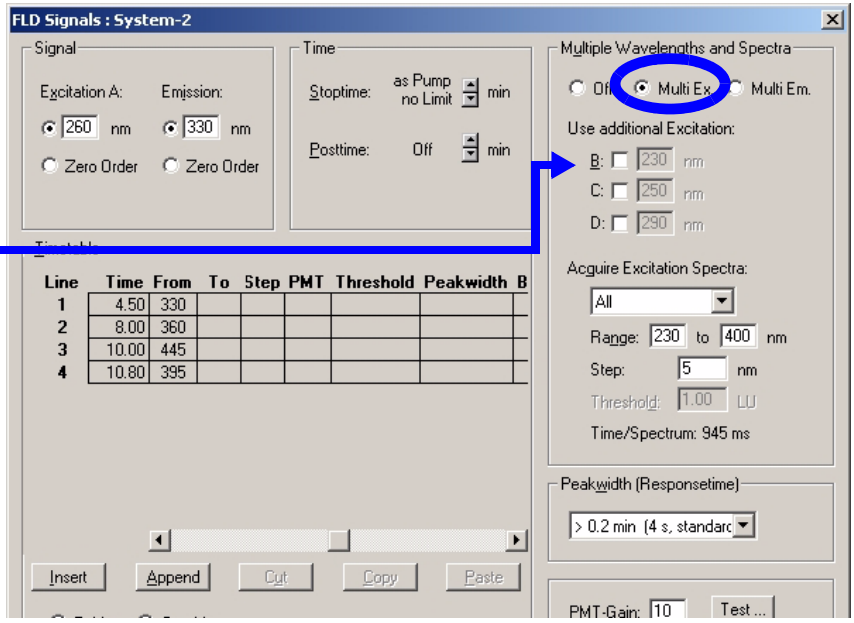


Figure 39 Detector Settings for Excitation Scan

6 Wait until the baseline stabilizes. Start the run.

7 Load the signal.

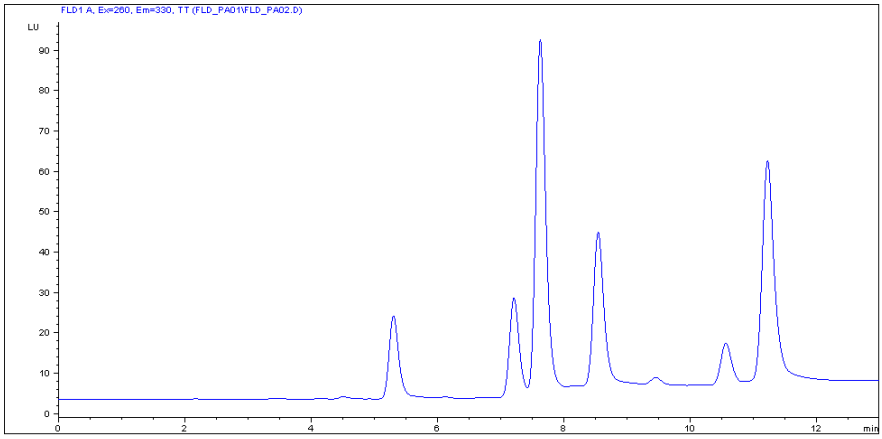


Figure 40 Chromatogram - excitation scan at reference wavelength 260/330 nm

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

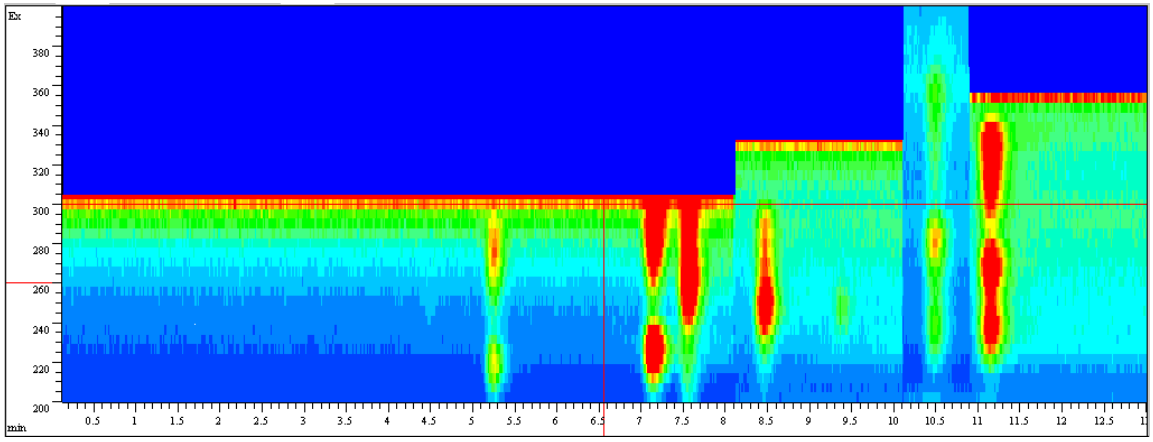


Figure 41 Isoabsorbance Plot - Excitation

The table below shows the complete information about emission (from Figure 38 on page 80) and excitation maxima.

Table 16

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	**11.3 min**	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

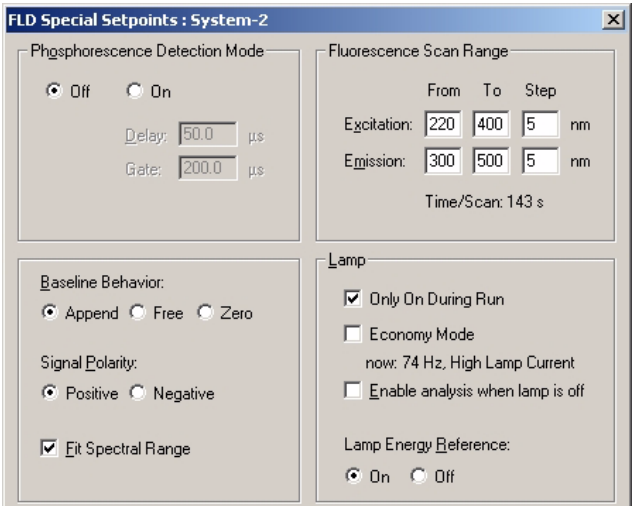


Figure 42 FLD special settings

4 Define a data file name and take a fluorescence scan. After the scan is completed, the isoabsorbance scan results appear, see Figure 43 on page 84.

NOTE A low background will improve the signal-to-noise, see also "Reducing Stray Light" on page 96.

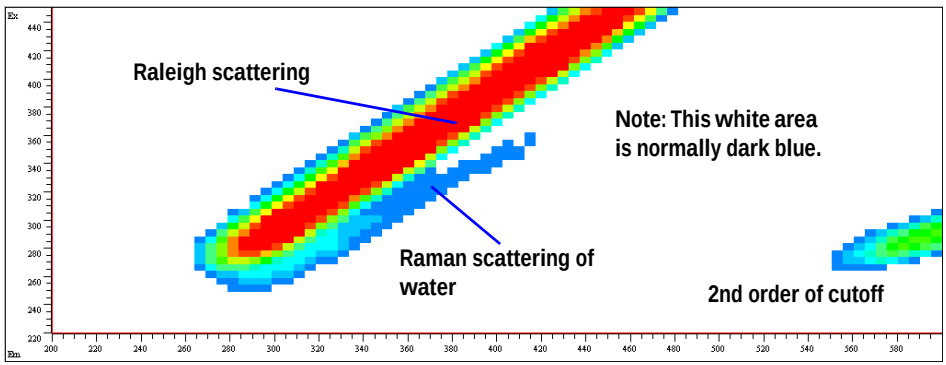


Figure 43 Fluorescence Scan of Water

5
How to optimize the Detector

This chapter provides information on how to optimize the detector.

Optimization Overview

Refer to “Optimization Overview” on page 57 for details.

Design Features Help Optimization

The Agilent 1200 Series fluorescence detector has several features you can use to optimize detection:

Table 17

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 1200 Series 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 1200 Series 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 1200 Series 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 1200 Series fluorescence detector scan of orthophthalaldehyde, a derivative of the amino acid alanine, (Figure 44 on page 89) shows a maximum between 220 nm and 240 nm.

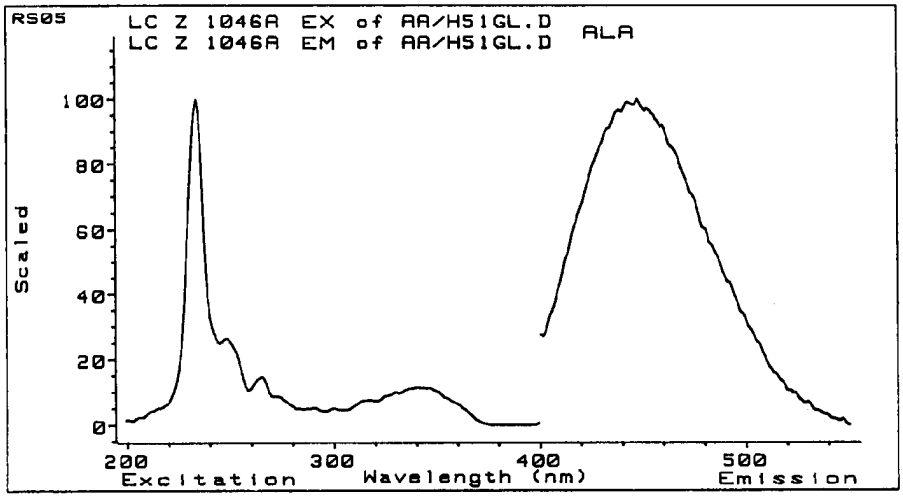


Figure 44 Scan Orthophthalaldehyde Derivative of Alanine

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 45 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.

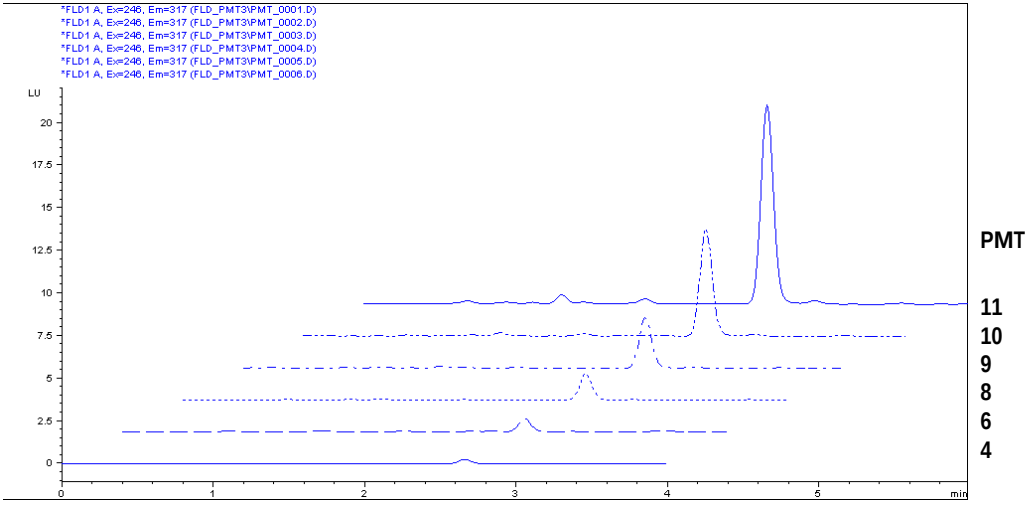


Figure 45 Finding Best PMTGAIN for Biphenyl

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 18 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 46.

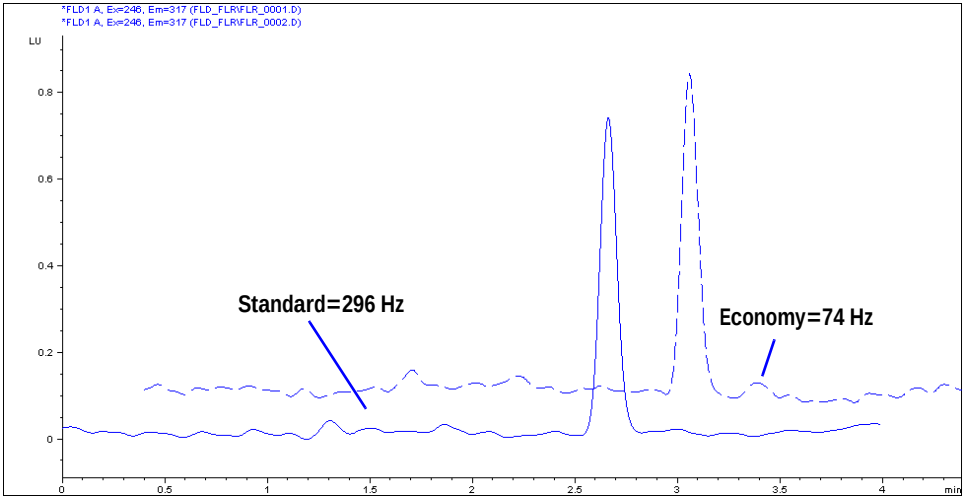


Figure 46 Xenon Flash Lamp Frequency

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 47.

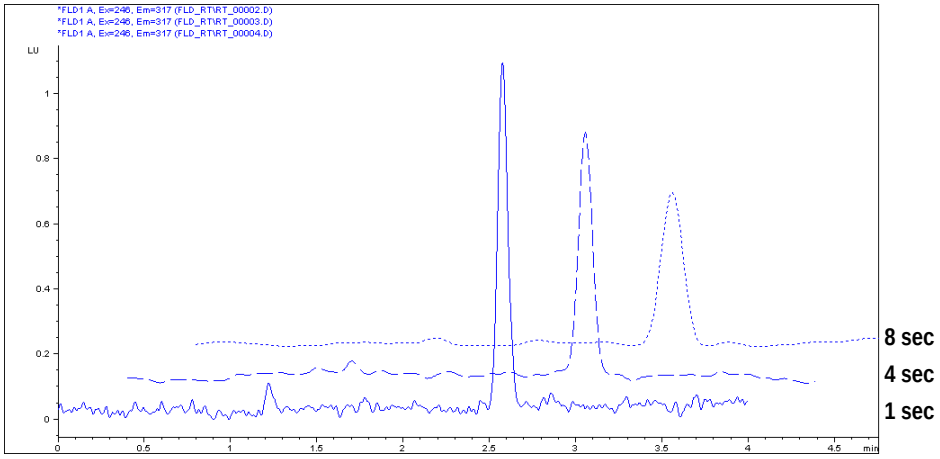


Figure 47 Finding Best Response Time

LC fluorescence detectors typically work with response times of 2 or 4 seconds. The default of the Agilent 1200 Series 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 48 Separation of Peaks using Responsetime

Reducing Stray Light

Cut-off filters are used to remove stray light and 2nd 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 2nd order light is the limiting factor. To explain the effect of such higher order light, assume the detector is on, but no sample is eluting through the flow cell.

The lamp sends 1 million photons into the flow cell at, for example 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 (1st order) and at 560 nm (2nd order). To remove this stray light, you need a cut-off filter around 280 nm.

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 49 on page 97).

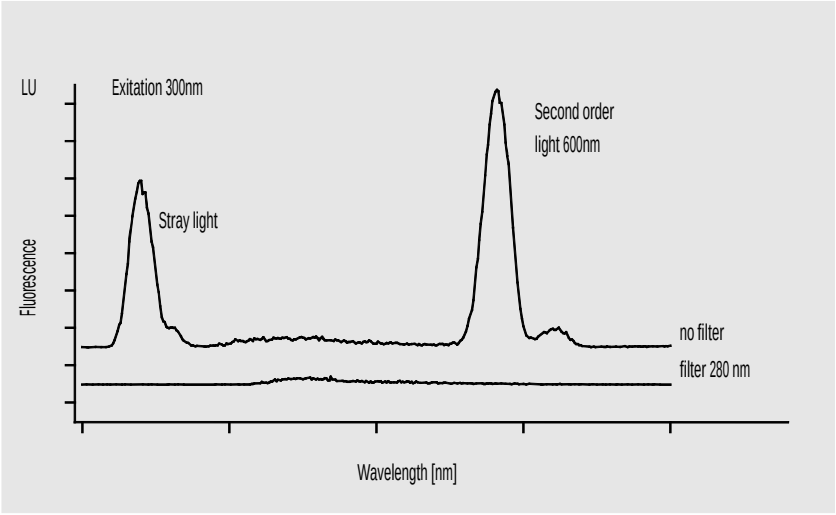


Figure 49 Reducing Stray Light

6
Troubleshooting and Test Functions

This chapter gives an overview about the troubleshooting and diagnostic features and the different user interfaces.

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 101).

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 Information" in the Service Manual.

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" in the Service Manual.

Test Functions

A series of test functions are available for troubleshooting and operational verification after exchanging internal components, see "Test Functions" in the Service Manual.

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.

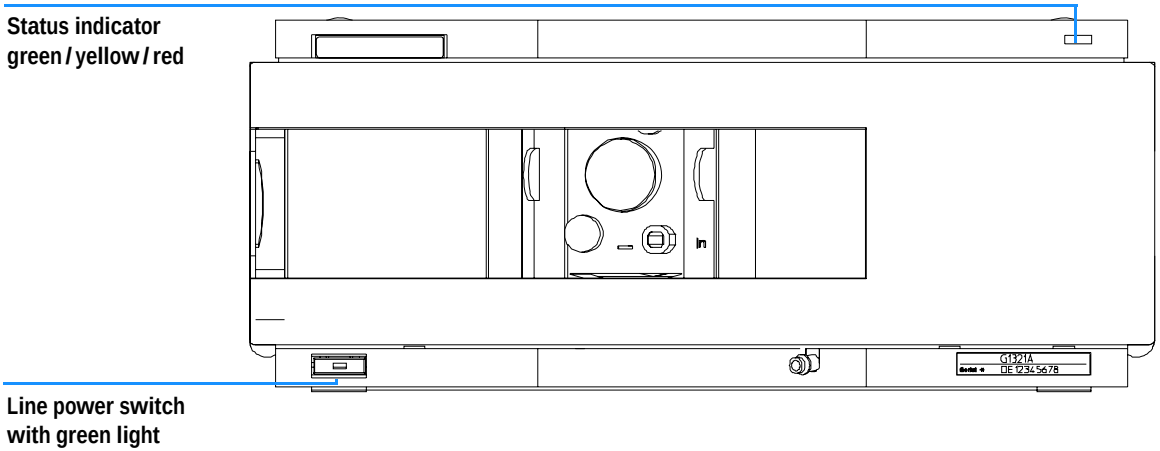


Figure 50 Location of Status Indicators

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.

User Interfaces

Depending on the user interface the available tests vary. All test descriptions are based on the Agilent ChemStation as user interface. Some descriptions are only available in the Service Manual.

Table 19 Test Functions avaible vs. User Interface

Test	ChemStation	Instant Pilot G4208A	Control Module G1323B
D/A Converter	No	No	Yes
Test Chromatogram	Yes (C)	No	Yes
Wavelength Calibration	Yes	Yes (M)	Yes
Lamp Intensity	Yes	No	Yes
Dark Current	Yes	No	No

C via command

M section Maintenance

D section Diagnose

NOTE The Agilent Control Module (G1323B) does not do any calculations. So there will be no reports generated with passed/failed information.

Agilent LC Diagnostic Software

The Agilent LC diagnostic software is an application independent tool that provides troubleshooting capabilities for the Agilent 1200 Series modules. It provides for all 1200 Series LC the possibility of a first guided diagnostic for typical HPLC symptoms and a status report stored as Adobe Acrobat pdf or as a printable file to assist users evaluating the instrument state.

At the introduction, following modules will be fully supported by the software, including module tests and calibrations as well as injector steps and maintenance positions.

- Agilent 1200 Series binary pump SL (G1312B)
- Agilent 1200 Series high performance autosampler SL (G1367B)
- Agilent 1200 Series thermostatted column compartment SL (G1316B)
- Agilent 1200 Series diode array detector SL (G1315C)

With further releases of the diagnostic software all Agilent 1200 Series HPLC modules will be fully supported.

This diagnostic software provides tests and diagnostic features that may differ from the descriptions in this manual. For details refer to the help files provided with the diagnostic software.

7
Maintenance and Repair

This chapter provides general information on maintenance and repair of the detector.

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 “Maintenance” on page 111.

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 “Repairs” in the Service Manual.

Warnings and Cautions

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.**

WARNING **To prevent personal injury, be careful when getting in contact with sharp metal areas.**

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.**

CAUTION Electronic boards and components are sensitive to electronic discharge (ESD). In order to prevent damage always use an ESD protection when handling electronic boards and components (see “Using the ESD Strap” on page 110).

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.**

Using the ESD Strap

Electronic boards are sensitive to electronic discharge (ESD). In order to prevent damage, always use an ESD strap 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.

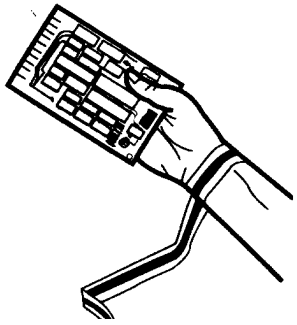


Figure 51 Using the ESD Strap

8 Maintenance

This chapter describes the maintenance of the detector and the required tests.

Overview of Maintenance

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

Table 20 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 "Wavelength Verification and Calibration" on page 125.
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

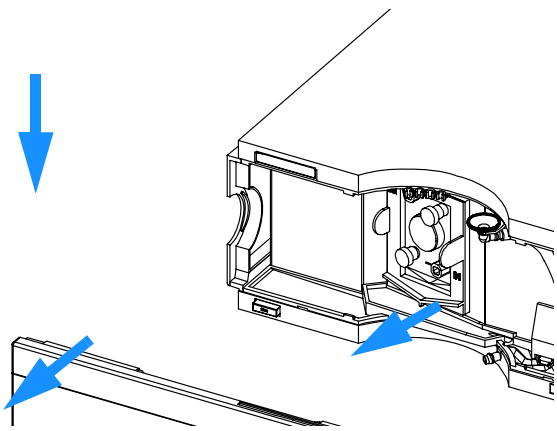
When required	If an application needs a different type of flow cell or the flow cell is defective (leaky).
Tools required	Two 1/4 inch wrenches for capillary connections
Parts required	Standard flow cell, 8 µl, 20 bar, G1321-60005 Cuvette for off-line measurements, 8 µl, 20 bar, G1321-60007, refer to "How to use the Cuvette" on page 116 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.

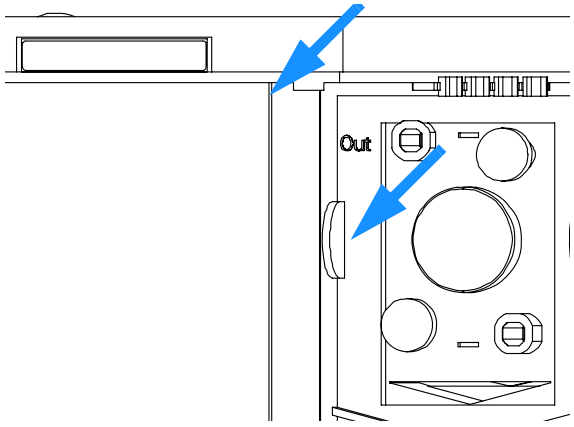
Preparations for this procedure:

- Turn off the flow.

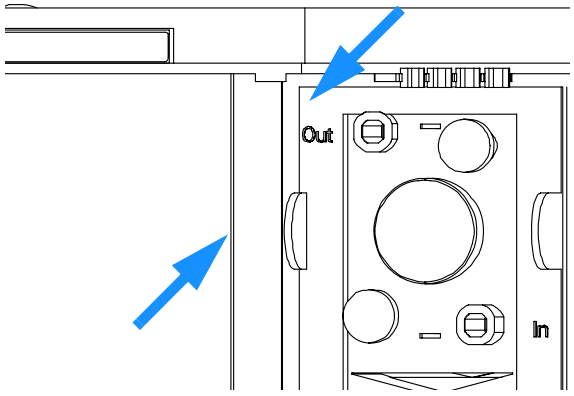
1 Press the release buttons and remove the front cover for access to the flow cell area.



2 Disconnect the capillaries from the flow cell.



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

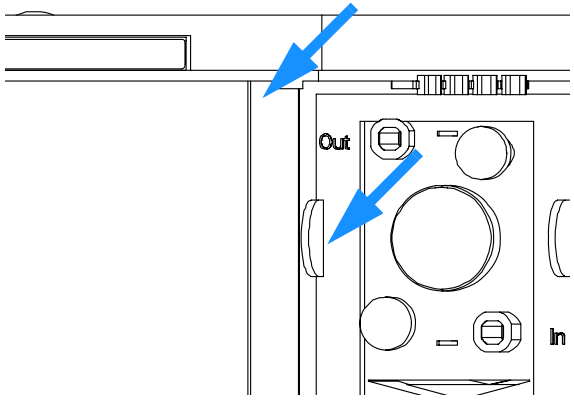


Note:

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:

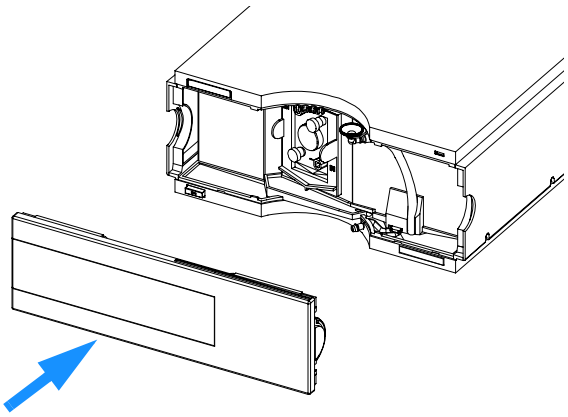
If an additional detector is added to the system, the fluorescence detector should be the last detector in the flow path except for evaporative detectors, like LC-MSD. Otherwise the back pressure generated by the other detector may overload the quartz flow cell and will lead to a defective cell (maximum 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 125.

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 133) 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

When required	If flow cell is contaminated
Tools required	Glass syringe, adapter
Parts required	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

When required	If a leakage has occurred in the flow cell area or at the capillary connections
Tools required	Tissue Two 1/4 inch wrenches for capillary connections
Parts required	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.

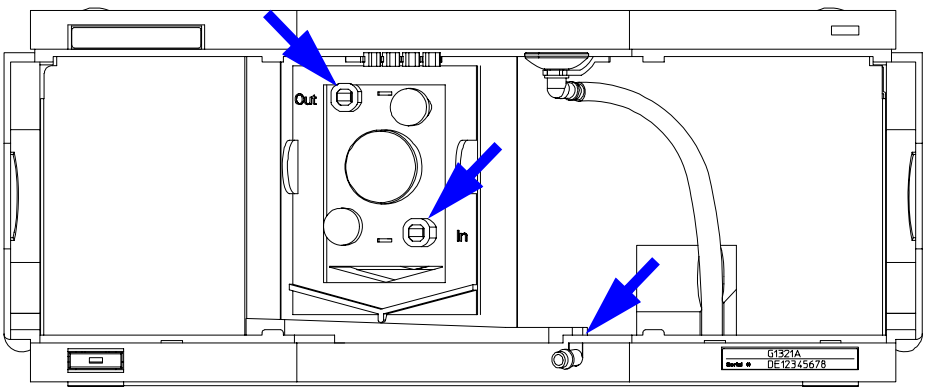


Figure 52 Observing for Leaks

Replacing Leak Handling System Parts

When required	If the parts are corroded or broken
Tools required	None
Parts required	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.

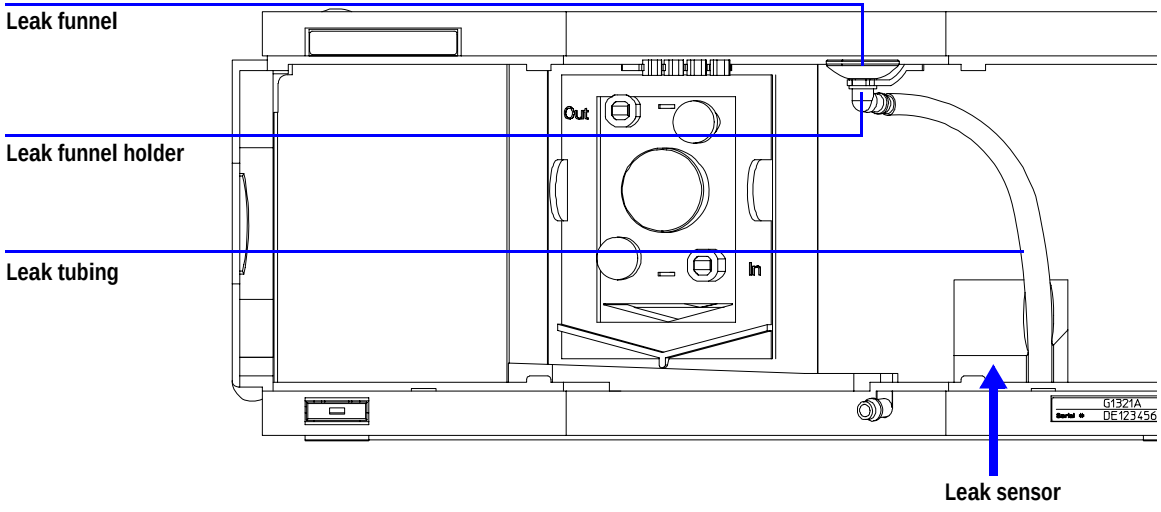


Figure 53 Replacing Leak Handling System Parts

Replacing the Interface Board

When required	For all repairs inside the detector or for installation of the board
Part required	Interface board (BCD) G1351-68701 with external contacts and BCD outputs LAN Communication Interface board G1369A or G1369-60001
Tools required	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.

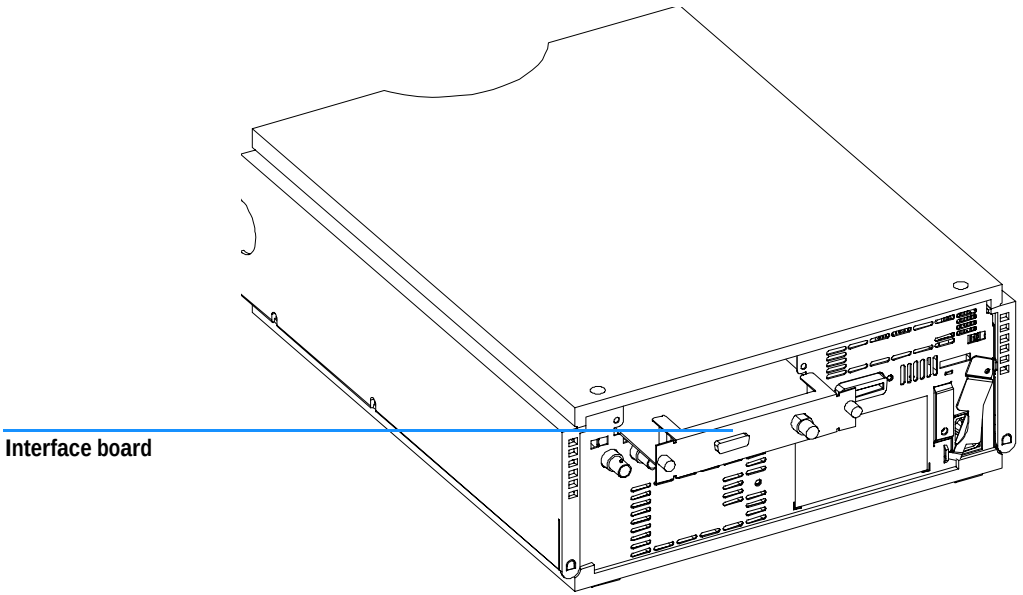


Figure 54 Location of the Interface Board

Replacing the Detector's Firmware

When required	If new version solves problems of currently installed version or after exchange of the detector main board (FLM) the version on board is older than previous installed one.
Tools required	LAN/RS-232 Firmware Update Tool, or Instant Pilot G4208A or Control Module G1323B
Parts required	Firmware, tools and documentation from Agilent web site
Preparations	Read update documentation provided with the Firmware Update Tool.

The installation of *older* firmware might be necessary:

- to keep all systems on the same (validated) revision, or
- if third part control software requires a special version.

To upgrade/downgrade the detector's firmware the following steps have to be performed:

1 Download the module's firmware, the LAN/RS-232 FW Update Tool Version 2.00 or above and the documentation from the Agilent web

http://www.chem.agilent.com/scripts/cag_firmware.asp.

2 Load the firmware into the detector as described in the documentation.

Tests & Calibrations

The following tests are required after maintenance of lamps and flow cells:

- “Lamp Intensity Test” on page 123.
- “Wavelength Verification and Calibration” on page 125.

Lamp Intensity Test

When required	If the flow cell or lamp has been replaced
Tools required	None
Pre-requisites	clean flow cell (flushed)

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).

```
Instrument:        G1321A
Serial Number:     DE92001563
Operator:          Wolfgang
Date:              09.01.2006
Time:              11:26:30
File:              C:\CHEM32\2\DIAGNOSE\FLD_INT.DGR
```

Intensity Plot

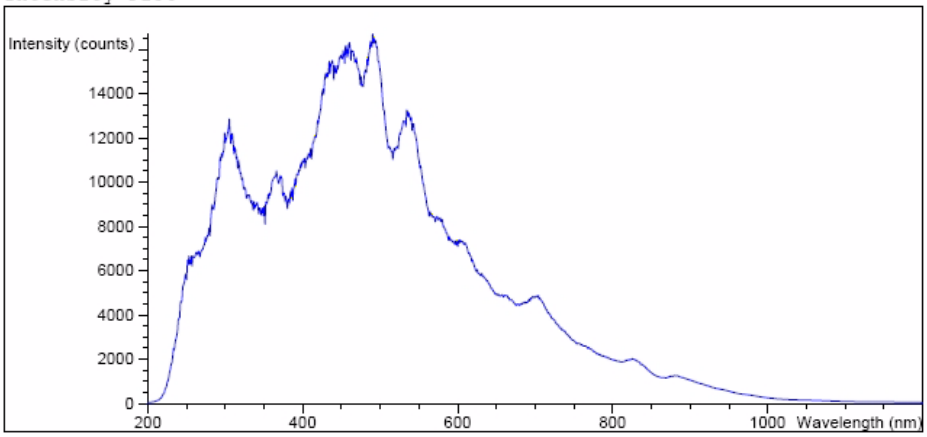


Figure 55 Lamp Intensity Test (Report)

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.

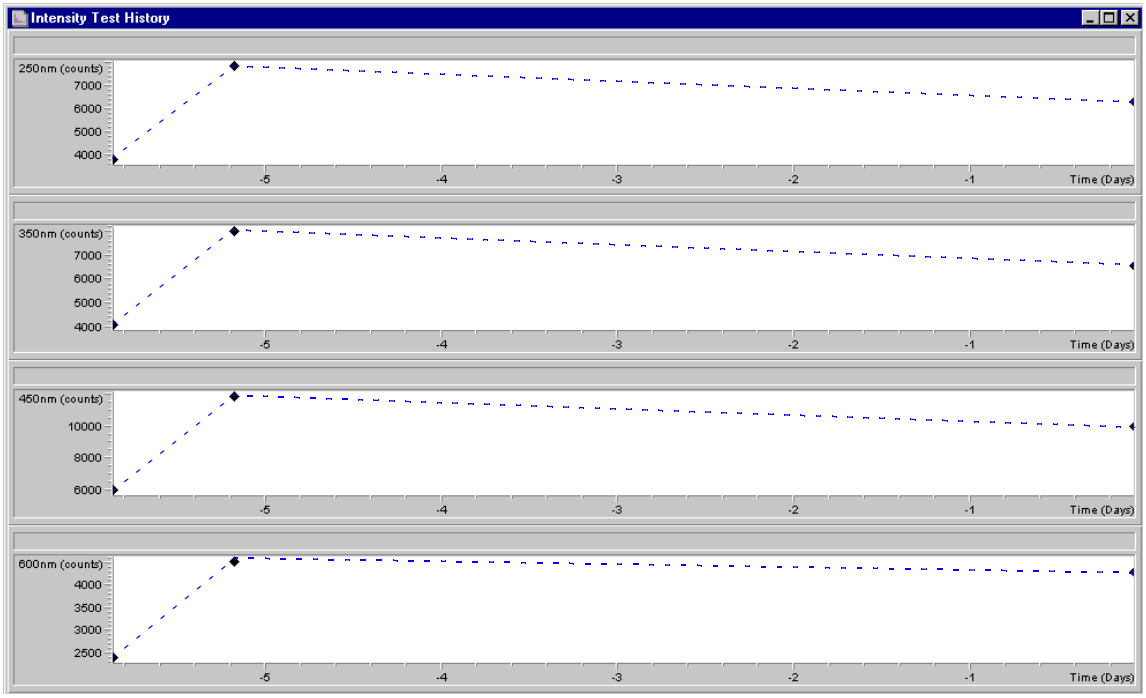


Figure 56 Lamp Intensity History

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

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.

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

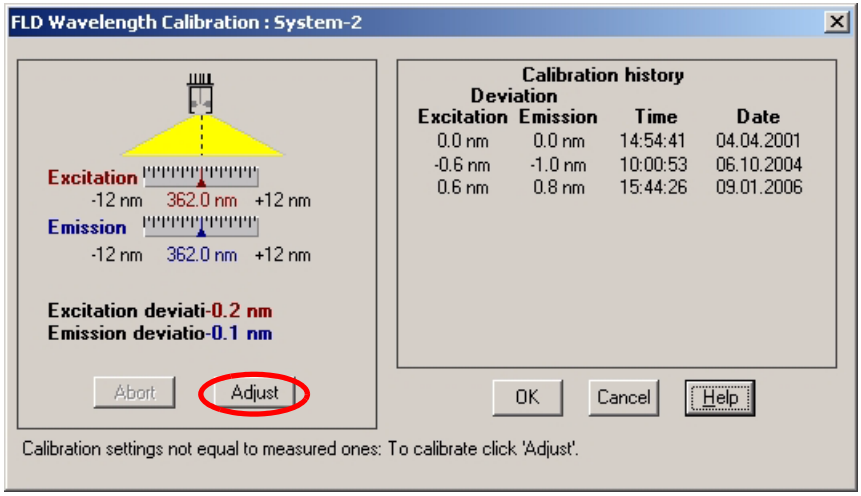


Figure 57 Wavelength Calibration

NOTE When the lamp is of, the calibration process will stop within the first two steps with “Wavelength Calibration Failed”.

Wavelength Calibration Procedure

When required	If application requires, or after replacement of flow cell or lamp.
Tools required	Laboratory balance
Parts required	Glycogen Calibration Sample, Syringe, needle, sample filter and PEEK fitting from the Accessory Kit, see "Accessory Kit" on page 135

Steps

1 Preparation of the Glycogen Calibration Sample.

2 Preparation of the Flow Cell.

3 Wavelength Calibration.

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 ±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 t#he needle.
7 Add the filter to the syringe and fit the needle to filter.

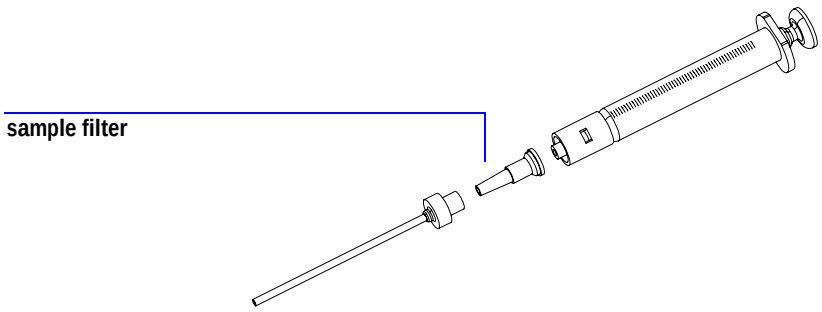


Figure 58 Syringe with Sample Filter

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.
Agilent ChemStation: **Diagnosis - Maintenance - FLD Calibration**
Instant Pilot G4208A: **Maintenance - FLD - Calibration**
Control Module G1323B: **System - Tests - FLD - Calibrate**.

NOTE If the wavelength calibration process fails, refer to "Wavelength Calibration Failed" in the Service Manual.

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.

8 Maintenance

9
Parts and Materials for Maintenance

This chapter provides information on parts for maintenance.

Overview of Maintenance Parts

Table 21 Maintenance Parts

Item	Description	Part Number
	Control Module G1323B or Instant Pilot G4208A	G1323-67001 G4208-67001
	Standard Flow Cell, 8 µl, 20 bar, inlet i.d./length 0.17 mm/80 mm, outlet i.d./length 0.25 mm/80 mm	G1321-60005
	Cuvette, 8 µl, 20 bar, see "Cuvette Kit" on page 133 inlet i.d./length 0.5 mm/80 mm, outlet i.d./length 0.5 mm/80 mm	G1321-60007
	Needle-Syringe	9301-0407
	Glass-Syringe	9301-1446
	Parts for wavelength calibration, see "Accessory Kit" on page 135	
	Front cover	5062-8592
	Leak funnel	5041-8388
	Leak funnel holder	5041-8389
	Clip	5041-8387
	Corrugated tubing, 120 mm lg, re-order 5 m	5062-2463
	Teflon Tubing flexible i.d. 0.8 mm (flow cell to waste)	5062-2462
	Cable CAN to Agilent 1200 Series modules (0.5 m)	5181-1516
	Cable CAN to Agilent 1200 Series modules (1 m)	5181-1519
	LAN Communication Interface Board (G1369A)	G1369-60001
	Cross-over network cable (shielded, 3 m long) for point to point connection	5023-0203
	Twisted pair network cable (shielded, 7 m long), for hub connections	5023-0202
	Analog cable (BNC to general purpose, spade lugs)	01046-60105
	Interface board BCD (BCD/external contacts)	G1351-68701

Cuvette Kit

Table 22 Cuvette Kit

Item	Description	Part Number
	FLD Cuvette Kit, 8 µl, 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 23 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 24 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 µm, 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

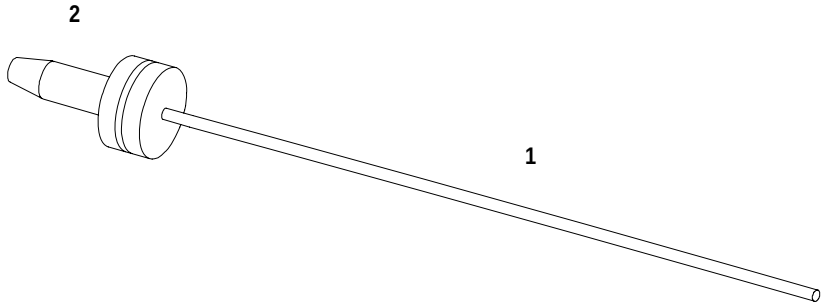


Figure 59 Waste Tubing Parts

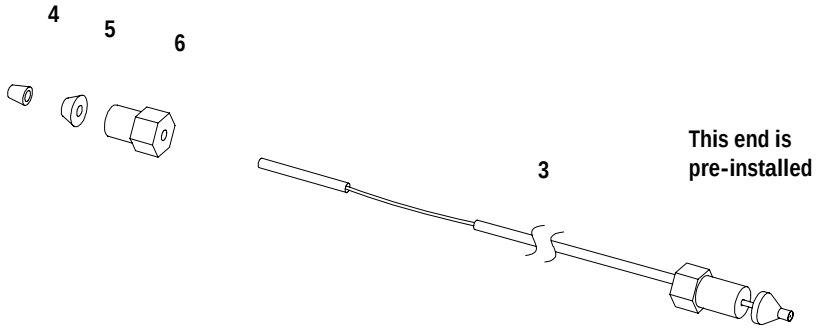


Figure 60 Inlet Capillary (Column-Detector) Parts

A
Appendix

This chapter provides safetey and other general information.

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.

This instrument is designed and certified as a general purpose laboratory instrument for research and routine application only. It is not certified for in-vitro or medical applications.

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.

CAUTION The operator of this instrument is advised that if the equipment is used in a manner not specified in this manual, the protection provided by the equipment may be impaired.

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 25 shows safety symbols used on the instrument and in the manuals.

Table 25 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.

Table 25 Safety Symbols (continued)

Symbol	Description
	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 leverandøren.**

WARNING **Lithiumbatteri - Eksplosionsfare. Ved udskiftning benyttes kun batteri som anbefalt av apparatfabrikanten. Brukt batteri returneres appararleverandoren.**

NOTE Bij dit apparaat zijn batterijen geleverd. Wanneer deze leeg zijn, moet u ze niet weggooien 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 unscreened cables and/or used for measurements on open set-ups, the user has to assure that under operating conditions the radio interference limits are still met within the premises.

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 Lp < 70 dB (A)
- At Operator Position
- Normal Operation
- According to ISO 7779:1988/EN 27779/1991 (Type Test)

UV-Radiation (UV-lamps only)

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 26 UV-Radiation Limits

Exposure / day	Effective Irradiance
8 hours	0.1 µW / cm^2
10 minutes	5.0 µW / cm^2

Typically the radiation values are much smaller than these limits:

Table 27 UV-Radiation Typical Values

Position	Effective Irradiance
Lamp installed, 50-cm distance	average 0.016 µW / cm^2
Lamp installed, 50-cm distance	maximum 0.14 µW / cm^2

Solvent Information

Observe the following recommendations on the use of solvents.

Flow Cell

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

Prevent any crystallization of buffer solutions. This will lead into a blockage/damage of the flow cell.

If the flow cell is transported while temperatures are below 5 degree C, it must be assured that the cell is filled with alcohol.

Aqueous solvents in the flow cell can built up algae. Therefore do not leave aqueous solvents sitting in the flow cell. Add small % of organic solvents (e.g. Acetonitrile or Methanol ~5%).

Solvents

Brown glass ware can avoid growth of algae.

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:

  $2CHCl_3 + O_2 \rightarrow 2COCl_2 + 2HCl$

  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.

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In This Book

This manual contains technical reference information about the Agilent 1200 Series fluorescence detector.

The manual describes the following:

- introcduction and specifications,
- installation,
- using and optimizing,
- troubleshooting,
- maintenance,
- parts identification,
- safety and related information.

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