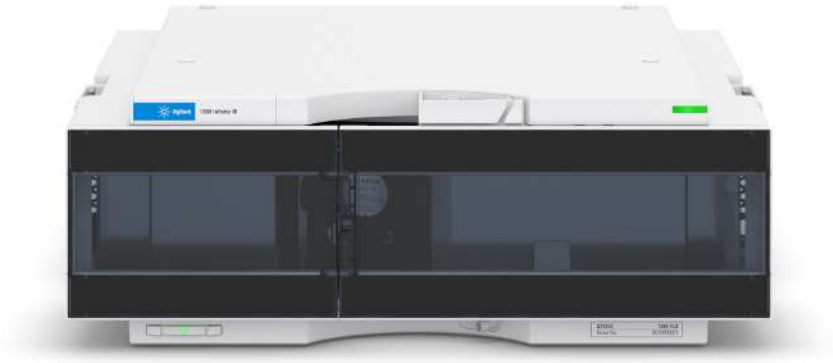
Agilent

Agilent InfinityLab LC Series

Fluorescence Detectors

User Manual



Notices

Document Information

The information in this document also applies to 1260 Infinity II and 1290 Infinity II modules.

Document No: SD-29000222 Rev. D
Edition: 10/2024

Copyright

Agilent Technologies
Hewlett-Packard-Strasse 8
76337 Waldbronn, Germany

Warranty

The material contained in this document is provided "as is," and is subject to being changed, without notice, in future editions. Further, to the maximum extent permitted by applicable law, Agilent disclaims all warranties, either express or implied, with regard to this manual and any information contained herein, including but not limited to the implied warranties of merchantability and fitness for a particular purpose. Agilent shall not be liable for errors or for incidental or consequential damages in connection with the furnishing, use, or performance of this document or of any information contained herein. Should Agilent and the user have a separate written agreement with warranty terms covering the material in this document that conflict with these terms, the warranty terms in the separate agreement shall control.

Technology Licenses

The hardware and/or software described in this document are furnished under a license and may be used or copied only in accordance with the terms of such license.

Restricted Rights Legend

U.S. Government Restricted Rights. Software and technical data rights granted to the federal government include only those rights customarily provided to end user customers. Agilent provides this customary commercial license in Software and technical data pursuant to FAR 12.211 (Technical Data) and 12.212 (Computer Software) and, for the Department of Defense, DFARS 252.227-7015 (Technical Data - Commercial Items) and DFARS 227.7202-3 (Rights in Commercial Computer Software or Computer Software Documentation).

Safety Notices

CAUTION

A **CAUTION** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a **CAUTION** notice until the indicated conditions are fully understood and met.

WARNING

A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

Contents

In This Book

This manual covers the following Agilent InfinityLab LC Series modules:

- Agilent 1260 Infinity III Fluorescence Detector (G7121A)
- Agilent 1260 Infinity III Fluorescence Detector Spectra (G7121B)

1 Introduction

This chapter gives an introduction to the module and instrument overview.

Overview of the Module

Table 1: Detector versions

Version	Description
G7121A	Introduced as 1260 Infinity III FLD without spectra and multi-signal capabilities. Maximum data rate is 74 Hz.
G7121B SPECTRA	Introduced as 1260 Infinity III FLD with spectra and multi-signal capabilities. Maximum data rate is 148 Hz. The G7121B can be converted to G7121A (emulation mode).

Product Description of the 1260 Infinity III Fluorescence Detector (G7121A)

The proven optical and electronic design of the Agilent 1260 Infinity III Fluorescence Detector provides highest sensitivity for the analysis of trace-level components. Time-programmable excitation and emission wavelength switching allows you to optimize the detection sensitivity and selectivity for your specific applications. High-speed detection with up to 74 Hz data rates keeping you pace with the analysis speed of fast LC.

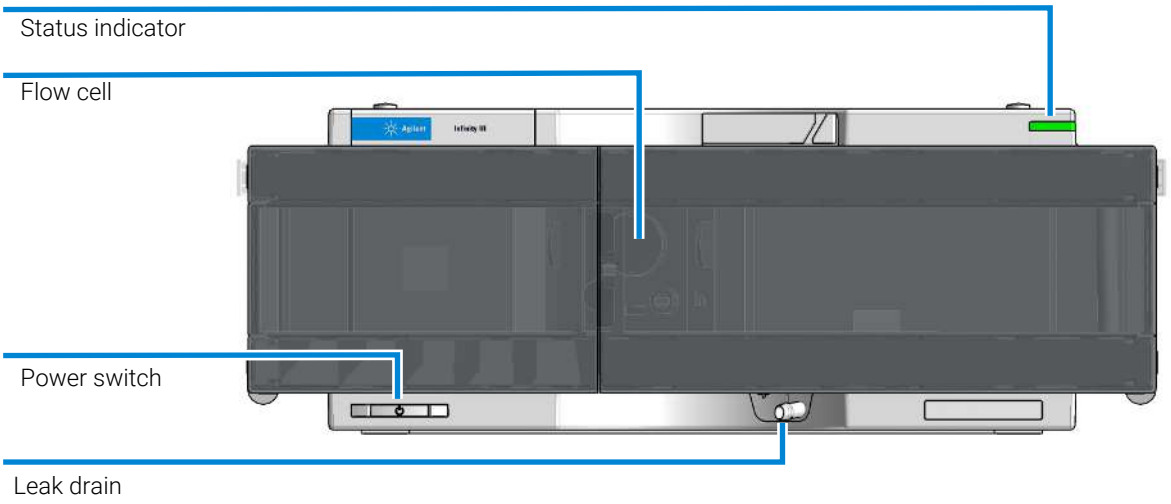


Figure 1: Overview of the G7121A Detector

Features of the 1260 Infinity III Fluorescence Detector (G7121A)

- Lowest limits of detection with a Raman S/N > 3000 (using dark signal noise reference). Simplified optical design for optimized baseline stability.
- Up to 100 % resolution gain in fast LC using a 74 Hz data acquisition rate.
- Long-life xenon lamp for highest sensitivity. The long-life (> 4000 hours) flash lamp, lamp reference system and efficient light collection ensure constant lamp energy for maximum excitation of fluorophores.
- Easy front access enables fast inspection or exchange of the flow cell.
- Automatic recognition of all flow cell cartridges provides documentation of instrument parameters and helps to comply with GLP.
- Extensive diagnostics, error detection and display with Instant Pilot controller and Agilent Lab Advisor software.

Product Description of the 1260 Infinity III Fluorescence Detector Spectra (G7121B)

The Agilent 1260 Infinity III Fluorescence Detector Spectra brings high-sensitivity fluorescence detection to your laboratory. This easy-to-use detector provides quantitative data and fluorescence spectra from a single run. Simultaneous multi-wavelength detection improves sensitivity and selectivity. Use the online spectral information for rapid method optimization and verification of separation quality. High-speed fluorescence detection with up to 148 Hz data rates keeping pace with the analysis speed of ultra-fast LC.

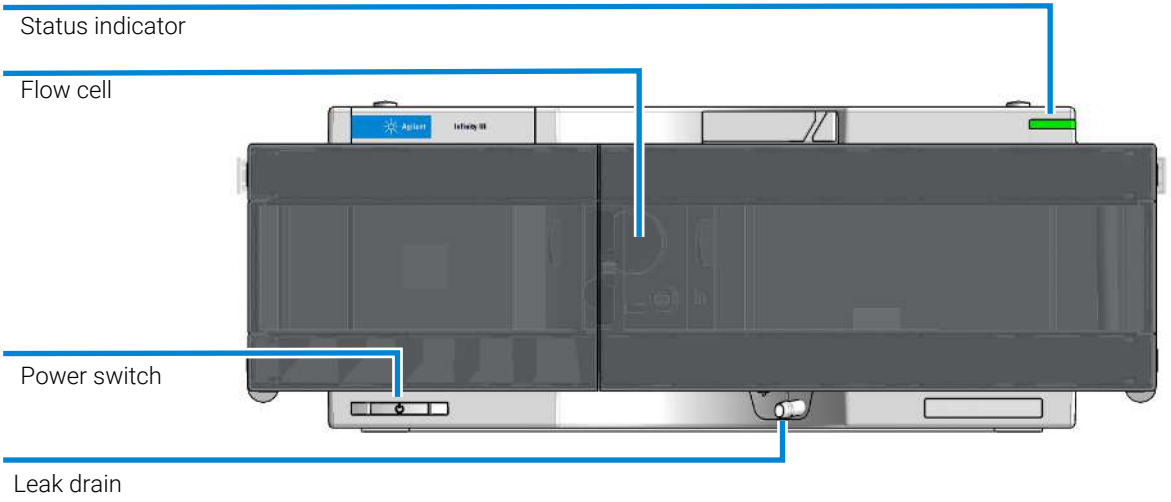


Figure 2: Overview of the G7121B Detector

Features of the 1260 Infinity III Fluorescence Detector Spectra (G7121B)

- Rotating gratings for multi-signal and online spectral data acquisition without loss in sensitivity.
- Lowest limits of detection with a Raman S/N > 3000 (using dark signal noise reference).
- Spectra and quantitative data from a single run.
- View online spectra without interrupting the chromatographic run.
- Simplified optical design for optimized baseline stability.
- Up to 100 % resolution gain in fast LC using a 148 Hz data acquisition rate.
- Long-life xenon lamp for highest sensitivity.
- The long-life (> 4000 hours) flash lamp, lamp reference system and efficient light collection ensure constant lamp energy for maximum excitation of fluorophores.
- Easy front access enables fast inspection or exchange of the flow cell.
- Automatic recognition of all flow cell cartridges provides documentation of instrument parameters and helps to comply with GLP.
- Extensive diagnostics, error detection and display with Instant Pilot controller and Agilent Lab Advisor software.

Operating Principle

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 3 on page 13. 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.

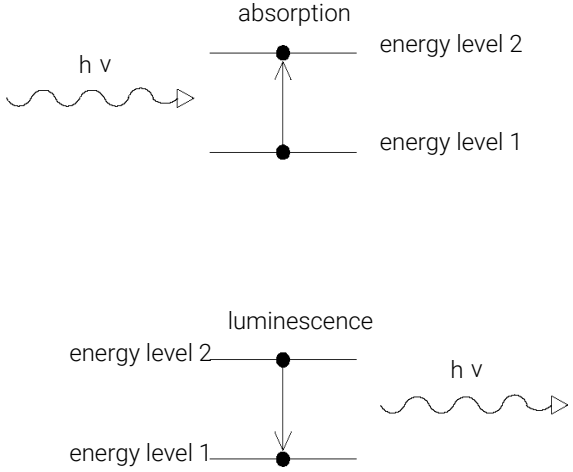


Figure 3: Absorption of Light Versus Emission of Light

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 4 on page 14. The characteristic maxima of absorption for a substance is its λ_{EX}, and for emission its λ_{EM}.

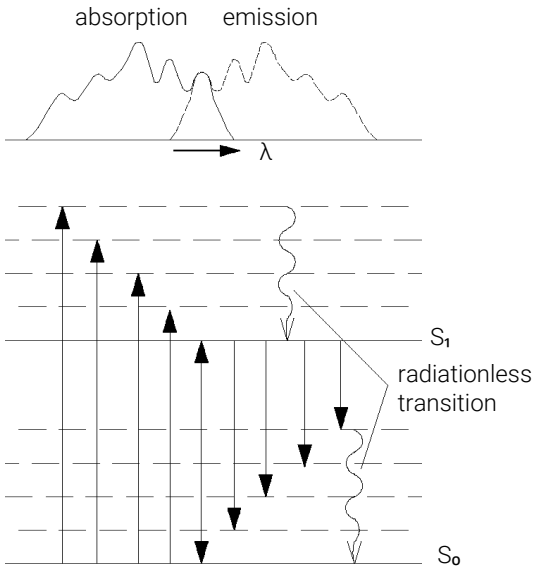


Figure 4: Relationship of Excitation and Emission Wavelengths

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

Phosphorescence is a longer process because one of the electrons involved in the excitation changes its spin, during a collision with a molecule of solvent, for example. The excited molecule is now in a so-called triplet state, T, see Figure 5 on page 15.

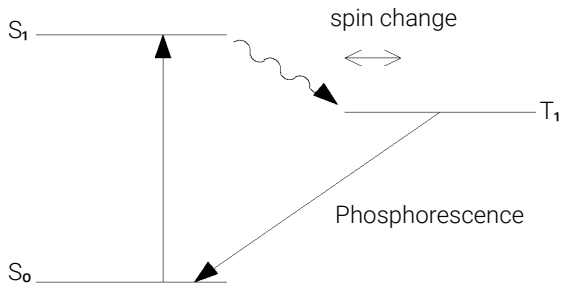


Figure 5: Phosphorescence Energy Transitions

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:

$$E = \frac{hc}{\lambda}$$

E	Energy
h	Planck's constant
λ	Wavelength
c	speed of light

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.

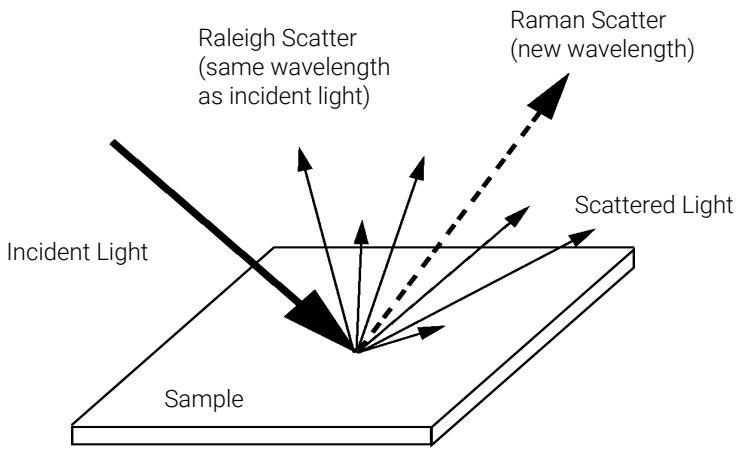


Figure 6: Raman

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 7 on page 17, including Xenon flash lamp, excitation condenser lens, excitation slit, mirror, excitation grating, flow cell, emission condenser lens, 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.

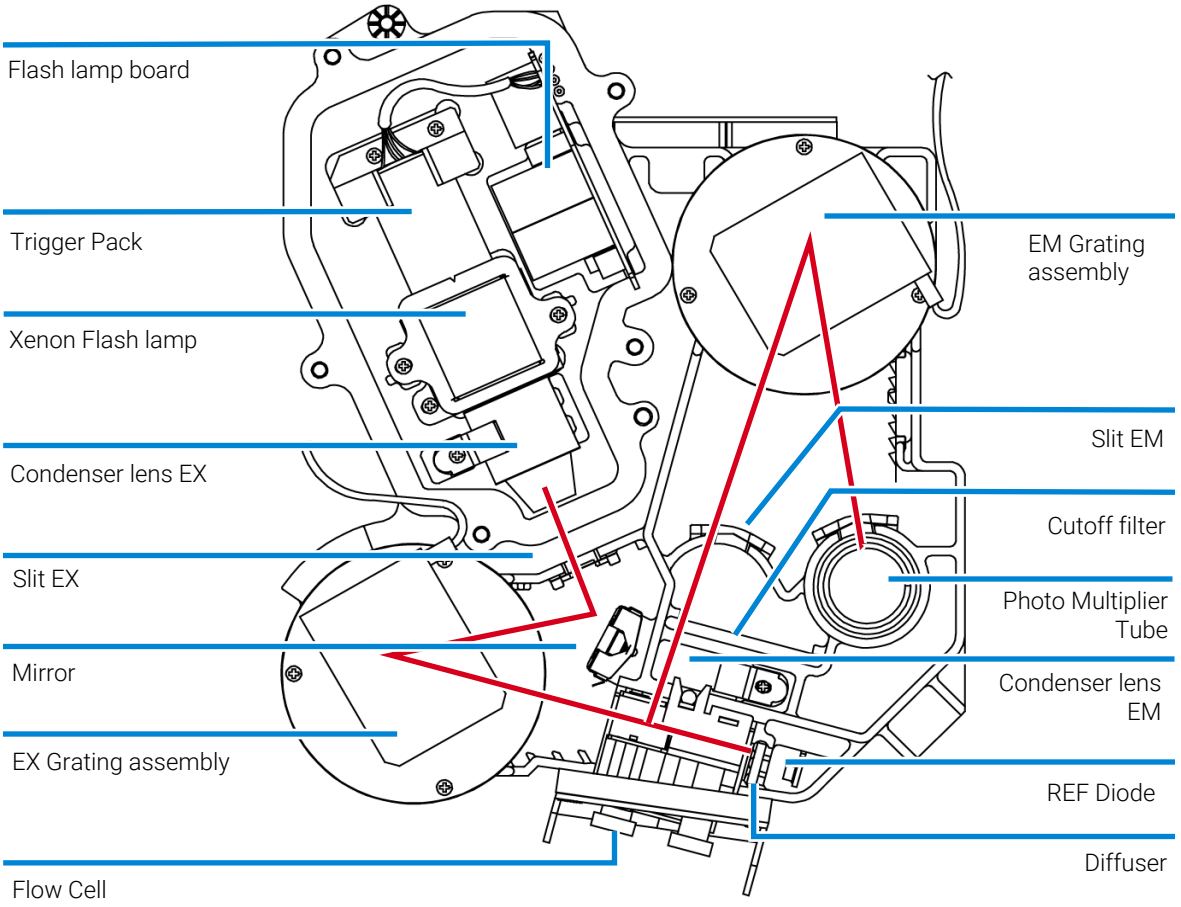


Figure 7: Optical Unit

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 8 on page 18. 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.

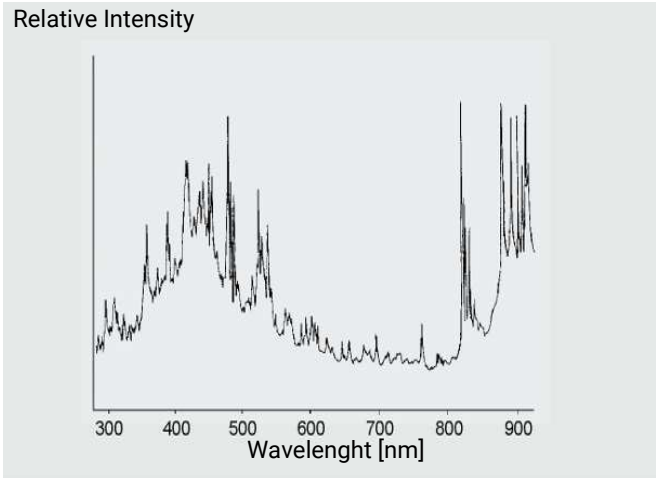


Figure 8: Lamp Energy Distribution (vendor data)

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.

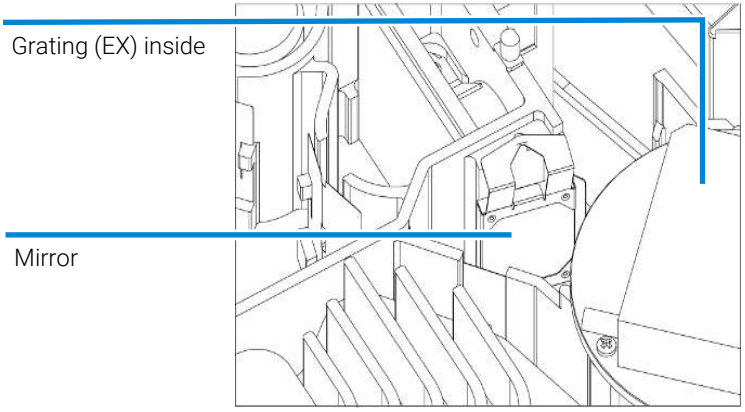


Figure 9: Mirror Assembly

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 10 on page 19 illustrates the light path at the surface of the grating.

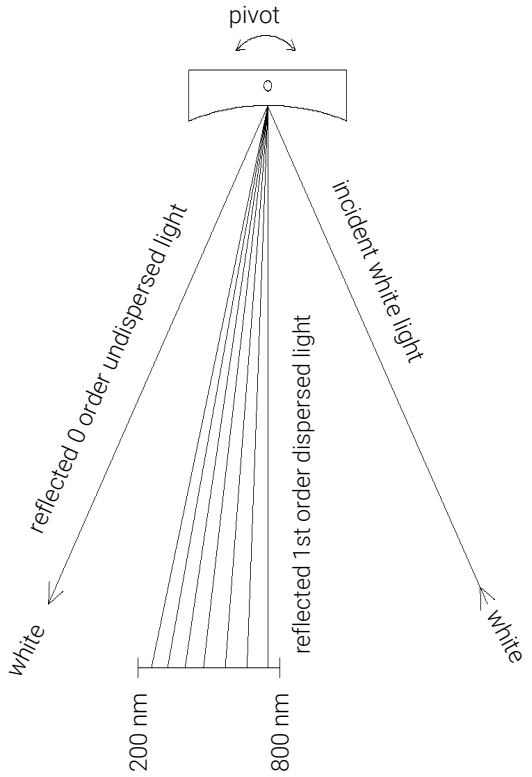


Figure 10: Dispersion of Light by a Grating

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 fused silica 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 body.

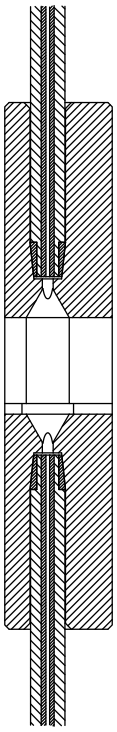


Figure 11: Cross-section of flow cell

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 10 on page 19.

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 12 on page 21, 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.

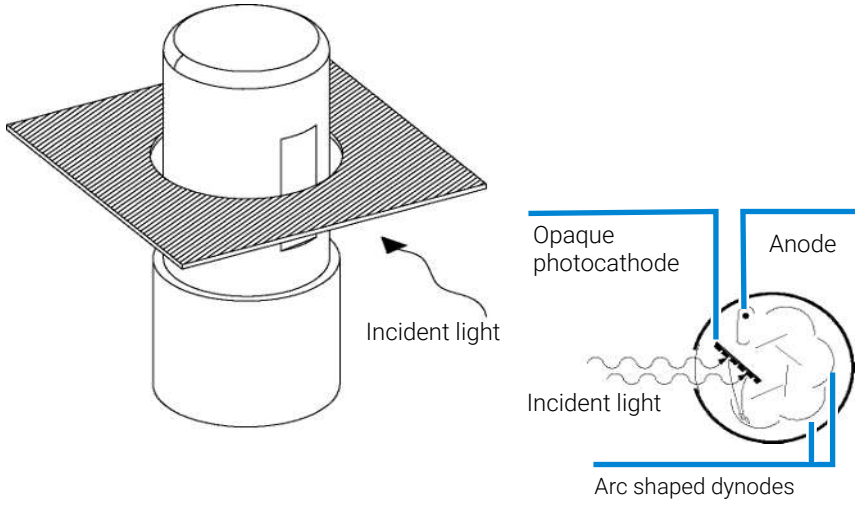


Figure 12: Photo-multiplier Tube

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 7 on page 17.

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.

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 7 on page 17). 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 13 on page 23. The luminescence is short-lived, therefore the fluorescence detector need only measure over a short period of time after the lamp has flashed.

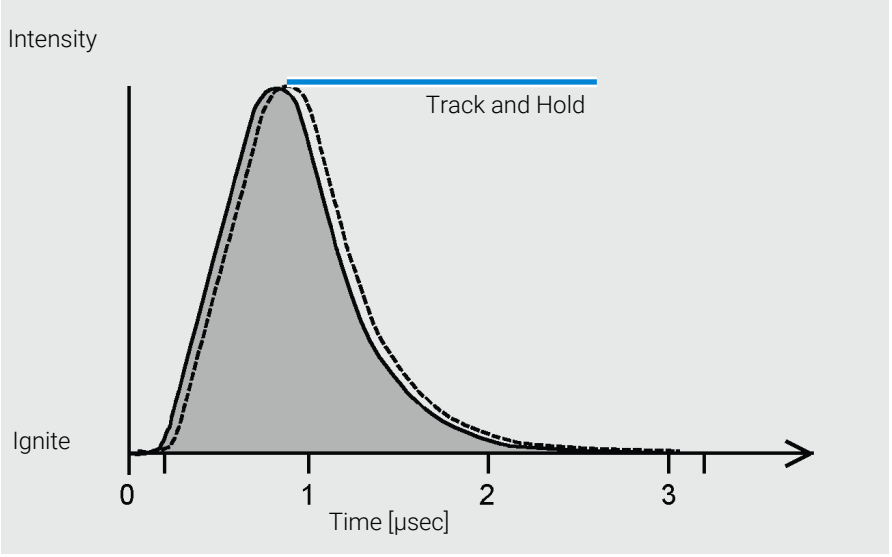


Figure 13: Measurement of Fluorescence

Phosphorescence Detection

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

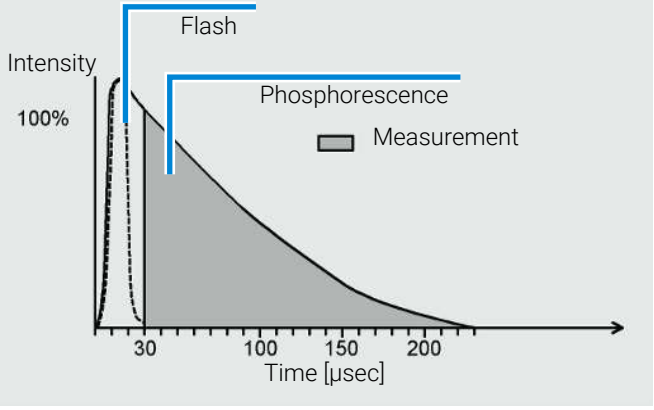


Figure 14: Measurement of Phosphorescence

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.

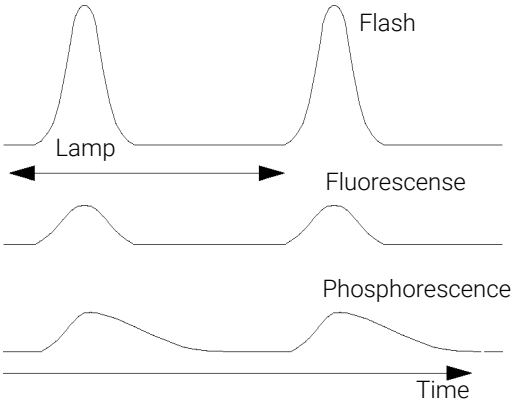


Figure 15: LAMP: Frequency of Flash, Fluorescence, and Phosphorescence

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 s (default, which is equivalent to a time constant of 1.8 s 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. See also Finding the Best Signal Amplification on page 113.

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.

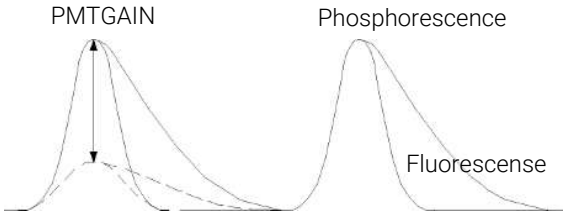


Figure 16: PMTGAIN: Amplification of Signal

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 17 on page 26, 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.

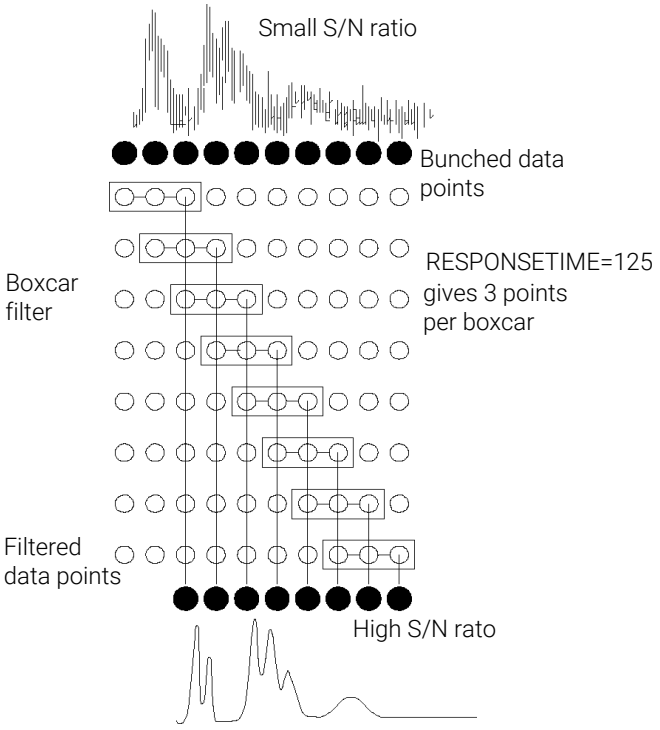


Figure 17: RESPONSETIME: Signal-to-Noise Ratio

System Overview

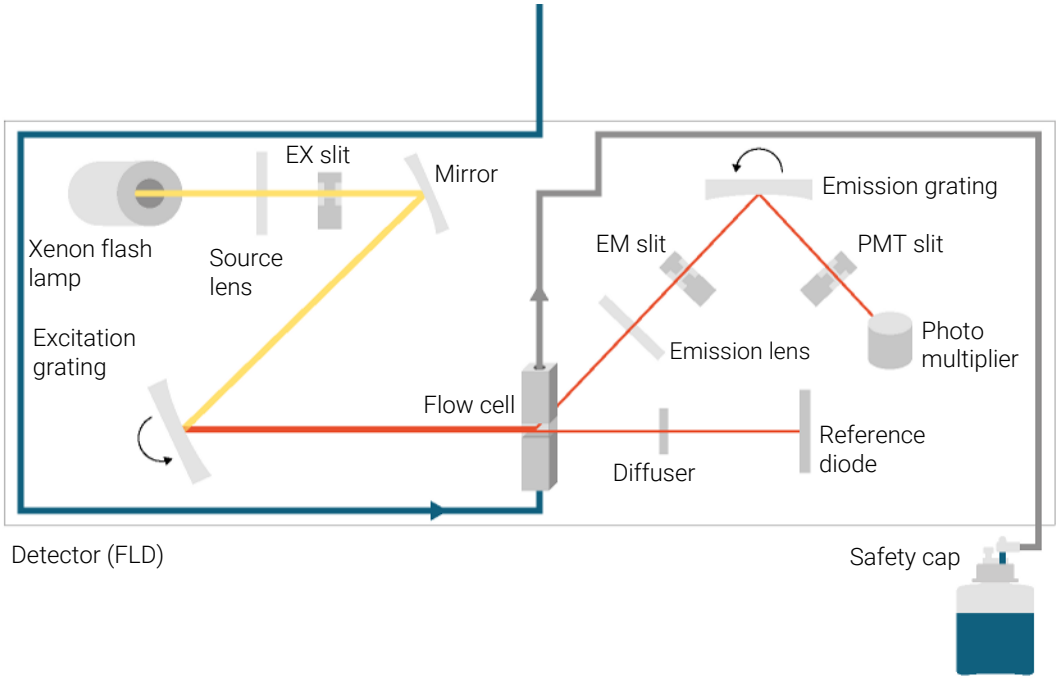


Figure 18: Optical path of the FLD

2 Site Requirements and Specifications

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

Site Requirements

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

Power Considerations

The module power supply has wide ranging capability. It accepts any line voltage in the range described in Physical Specifications. Consequently there is no voltage selector in the rear of the module. There are also no externally accessible fuses, because automatic electronic fuses are implemented in the power supply.

WARNING **Inaccessible power plug.**

In case of emergency it must be possible to disconnect the instrument from the power line at any time.

- **Make sure the power connector of the instrument can be easily reached and unplugged.**
- **Provide sufficient space behind the power socket of the instrument to unplug the cable.**

WARNING **Incorrect line voltage at the module**

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

- **Connect your module to the specified line voltage.**

WARNING **Module is partially energized when switched off, as long as the power cord is plugged in.**

Repair work at the module can lead to personal injuries, e.g. shock hazard, when the cover is opened and the module is connected to power.

- **Make sure that it is always possible to access the power plug.**
- **Remove the power cable from the instrument before opening the cover.**
- **Do not connect the power cable to the Instrument while the covers are removed.**

Power Cords

Country-specific power cords are available for the module. The female end of all power cords is identical. It plugs into the power-input socket at the rear. The male end of each power cord is different and designed to match the wall socket of a particular country or region.

Agilent makes sure that your instrument is shipped with the power cord that is suitable for your particular country or region.

WARNING **Unintended use of power cords**

Using power cords for unintended purposes can lead to personal injury or damage of electronic equipment.

- **Never use a power cord other than the one that Agilent shipped with this instrument.**
- **Never use the power cords that Agilent Technologies supplies with this instrument for any other equipment.**
- **Never use cables other than the ones supplied by Agilent Technologies to ensure proper functionality and compliance with safety or EMC regulations.**

WARNING **Absence of ground connection**

The absence of ground connection can lead to electric shock or short circuit.

- **Never operate your instrumentation from a power outlet that has no ground connection.**

WARNING

Electrical shock hazard

Solvents may damage electrical cables.

- **Prevent electrical cables from getting in contact with solvents.**
- **Exchange electrical cables after contact with solvents.**

Bench Space

The module dimensions and weight (see Physical Specifications) allow you to place the module 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 shall carry a complete HPLC system, make sure that the bench is designed to bear the weight of all modules.

The module should be operated in a horizontal position.

NOTE

Agilent recommends that you install the HPLC instrument in the InfinityLab Flex Bench rack. This option helps to save bench space as all modules can be placed into one single stack. It also allows to easily relocate the instrument to another lab.

Condensation

CAUTION

Condensation within the module

Condensation can damage the system electronics.

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

Specifications of the 1260 Infinity III Fluorescence Detector (G7121A)

Table 2: Physical Specifications of the 1260 Infinity III Fluorescence Detector (G7121A)

Type	Specification	Comments
Weight	11.9 kg (26.2 lbs)	
Dimensions (height × width × depth)	140 x 396 x 436 mm (5.5 x 15.6 x 17.2 inches)	
Line voltage	100–240 V~, ±10%	Wide-ranging capability
Line frequency	50 or 60 Hz, ±5%	
Power consumption	70 VA, 60 W	
Ambient operating temperature	4–40 °C (39–104 °F)	
Ambient non-operating temperature	-40–70 °C (-40–158 °F)	
Humidity	< 95% r.h. at 40 °C (104 °F)	Non-condensing
Operating altitude	Up to 3000 m (9842 ft)	
Safety standards: IEC, EN, CSA, UL	Overvoltage category II, Pollution degree 2	For indoor use only
ISM Classification	ISM Group 1 Class B	According to CISPR 11

Table 3: Performance Specifications of the 1260 Infinity III Fluorescence Detector (G7121A)

Type	Specification	Comments
Detection type	One signal wavelength (excitation and emission)	Programmable single wavelength (excitation and emission) fluorescence detector
Designed for use with Agilent InfinityLab Assist	Intuitive User Interface, Automated Workflows, Predictive Maintenance & Assisted Troubleshooting	

Type	Specification	Comments
Single wavelength operation	• RAMAN (H_2O) > 500 (noise reference measured at signal) Ex=350 nm, Em=397 nm, dark value 450 nm, standard flow cell • RAMAN (H_2O) > 3000 (noise reference measured at dark value) Ex=350 nm, Em=397 nm, dark value 450 nm, standard flow cell	
Light source	Xenon Flash Lamp, normal mode 20 W, economy mode 5 W, lifetime 4000 h	
Pulse frequency	296 Hz for single signal mode 74 Hz for economy mode	
Maximum data rate	74 Hz	
Excitation monochromator	Range: settable 200 nm - 1200 nm and zero-order Bandwidth: 20 nm (fixed)	
Emission monochromator	Range: settable 200 nm - 1200 nm and zero-order Bandwidth: 20 nm (fixed)	
Reference system	In-line excitation measurement	
Timetable programming	Single signal wavelength, response time, PMT Gain, baseline behavior (append, free, zero)	
Wavelength characteristic	Repeatability +/- 0.2 nm Accuracy +/- 3 nm setting	
Flow cells	Standard: 8 µL volume and 20 bar (2 MPa) pressure maximum, fused silica block	
Analog output	Recorder/integrator: 100 mV or 1 V, output range > 100 LU, two outputs	100 LU is the recommended range
Instrument Control	LC & CE Drivers A.02.14 or above Instrument Control Framework (ICF) A.02.03 or above Lab Advisor software B.02.09 or above InfinityLab Assist (G7180A) with firmware D.07.40 or above Instant Pilot (G4208A) with firmware B.02.19 or above	For details about supported software versions refer to the compatibility matrix of your version of the LC and CE Drivers
Communications	Controller Area Network (CAN), USB, ERI: ready, start, stop and shut-down signals	

Type	Specification	Comments
Safety features and maintenance	Leak detection, safe leak handling, leak output signal for shutdown of the pumping system. No hazardous voltages in major maintenance areas. Extensive diagnostics, error detection and display with Agilent InfinityLab Assist and Agilent Lab Advisor software.	
GLP features	Early maintenance feedback (EMF) for continuous tracking of instrument usage in terms of lamp burn time with pre-defined and user settable limits and feedback messages. Electronic records of maintenance and errors.	
Housing	All materials are recyclable.	

Specifications of the 1260 Infinity III Fluorescence Detector Spectra (G7121B)

Table 4: Physical Specifications of the 1260 Infinity III Fluorescence Detector (G7121B)

Type	Specification	Comments
Weight	11.9 kg (26.2 lbs)	
Dimensions (height × width × depth)	140 x 396 x 436 mm (5.5 x 15.6 x 17.2 inches)	
Line voltage	100–240 V~, ±10%	Wide-ranging capability
Line frequency	50 or 60 Hz, ±5%	
Power consumption	70 VA, 60 W	
Ambient operating temperature	4–40 °C (39–104 °F)	
Ambient non-operating temperature	-40–70 °C (-40–158 °F)	
Humidity	< 95% r.h. at 40 °C (104 °F)	Non-condensing
Operating altitude	Up to 3000 m (9842 ft)	
Safety standards: IEC, EN, CSA, UL	Overvoltage category II, Pollution degree 2	For indoor use only
ISM Classification	ISM Group 1 Class B	According to CISPR 11

Table 5: Performance Specifications of the 1260 Infinity III Fluorescence Detector (G7121B)

Type	Specification	Comments
Detection type	Multi-signal wavelength fluorescence detector with rapid on-line scanning capabilities and spectral data analysis	
Designed for use with Agilent InfinityLab Assist	Intuitive User Interface, Automated Workflows, Predictive Maintenance & Assisted Troubleshooting	

Type	Specification	Comments
Single wavelength operation	• RAMAN (H_2O) > 500 (noise reference measured at signal) Ex=350 nm, Em=397 nm, dark value 450 nm, standard flow cell • RAMAN (H_2O) > 3000 (noise reference measured at dark value) Ex=350 nm, Em=397 nm, dark value 450 nm, standard flow cell	
Dual wavelength operation	• RAMAN (H_2O) > 300 Ex = 350 nm, Em = 397 nm, standard flow cell • RAMAN (H_2O) > 300 Ex =350 nm, Em = 450 nm, standard flow cell.	
Light source	Xenon Flash Lamp, normal mode 20 W, economy mode 5 W, lifetime 4000 h	
Pulse frequency	296 Hz for single signal mode 74 Hz for economy/multi-wavelength/ spectra mode	
Maximum data rate	74 Hz, 148 Hz	
Excitation monochromator	Range: settable 200 - 1200 nm and zero-order Bandwidth: 20 nm (fixed)	
Emission monochromator	Range: settable 200 - 1200 nm and zero-order Bandwidth: 20 nm (fixed)	
Reference system	in-line excitation measurement	
Timetable programming	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	

Type	Specification	Comments
Flow cells	Standard: 8 µL volume and 20 bar (2 MPa) pressure maximum, fused silica block Optional: • Bio-inert: 8 µL volume and 20 bar (2 MPa) pressure maximum, (pH 1-12) • Micro: 4 µL volume and 20 bar (2 MPa) pressure maximum	
Analog output	Recorder/integrator: 100 mV or 1 V, output range > 100 LU, two outputs	100 LU is the recommended range
Instrument Control	LC & CE Drivers A.02.14 or above Instrument Control Framework (ICF) A.02.03 or above Lab Advisor software B.02.09 or above InfinityLab Assist (G7180A) with firmware D.07.40 or above Instant Pilot (G4208A) with firmware B.02.19 or above	For details about supported software versions refer to the compatibility matrix of your version of the LC and CE Drivers
Communication	Controller Area Network (CAN), USB ERI: ready, start, stop and shut-down signals	
Safety features and maintenance	Leak detection, safe leak handling, leak output signal for shutdown of the pumping system. No hazardous voltages in major maintenance areas. Extensive diagnostics, error detection and display with Agilent InfinityLab Assist and with Agilent Lab Advisor software.	
GLP features	Early maintenance feedback (EMF) for continuous tracking of instrument usage in terms of lamp burn time with pre-defined and user settable limits and feedback messages. Electronic records of maintenance and errors.	
Housing	All materials are recyclable.	

Specification Conditions

- Standard flow cell
- Standard Photomultiplier
- Using Agilent Lab Advisor, see Raman ASTM Signal-to-Noise Test on page 139.

3 Installation

The installation of the module will be done by an Agilent service representative. In this chapter, only installation of user-installable options and accessories are described.

Installing Capillaries

This section provides information on how to install capillaries and fittings.

Install Capillaries

Capillaries and connections depend on which system is installed.

NOTE As you move to smaller-volume, high-efficiency columns, you will want to use narrow id tubing, as opposed to the wider id tubing used for conventional HPLC instruments.

NOTE Agilent capillaries are color-coded for quick identification, see At-a-Glance Details About Agilent Capillaries on page 276.

Table 6: Capillary connections for 1260 Infinity III systems

p/n	From	To
G7120-60007 (Bottle Head Assembly)	Solvent Bottle	Infinity III Pump
5500-1246 (Capillary ST 0.17 mm x 500 mm SI/SI)	Pump	Sampler
5500-1217 (Capillary, ST, 0.17 mm x 900 mm SI/SX)	Pump	Vialsampler with ICC
5500-1246 (Capillary ST 0.17 mm x 500 mm SI/SI)	Multisampler	MCT Valve/Heat Exchanger
5500-1252 (Capillary, ST, 0.17 mm x 400 mm SL/SL)	Vialsampler	MCT Valve/Heat Exchanger
5500-1240 (Capillary ST 0.17 mm x 105 mm SL/SL)	Vialsampler	ICC Heat Exchanger
5500-1250 (Capillary, ST, 0.17 mm x 120 mm SL/SL, long socket)	ICC Heat Exchanger	Column
5500-1193 (InfinityLab Quick Turn Capillary ST 0.17 mm x 105 mm, long socket)	MCT Heat Exchanger	Column
5500-1191 (InfinityLab Quick Turn Capillary ST 0.12 mm x 280 mm, long socket)	Column/MCT Valve	Detector
5062-8535 (Waste accessory kit (Flow Cell to waste))	VWD	Waste
5062-2462 (Tube PTFE 0.7 mm x 5 m, 1.6 mm od)	DAD/FLD	Waste
G5664-68712 (Analytical tubing kit 0.25 mm i.d. PTFE-ESD)	Detector	Fraction Collector

Table 7: Capillary connections for 1260 Infinity III Bio-inert LC

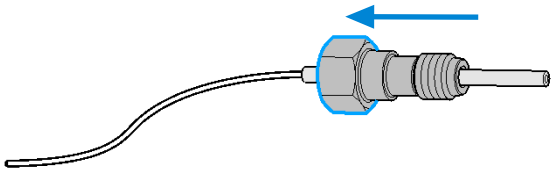
p/n	From	To
G7120-60007 (Bottle Head Assembly)	Solvent Bottle	Infinity III Pump

p/n	From	To
5500-1264 (Capillary Ti 0.17 mm x 500 mm, SL/SLV)	Pump	Multisampler
G5667-81005 (Capillary PK/ST 0.17 mm x 500 mm, RLO/RLO (Bio-inert))	Multisampler	MCT
5067-4741 (ZDV union (Bio-inert))	Capillary	Bio-inert Heat Exchanger
G7116-60041 (Quick Connect Heat Exchanger Bio-inert)		
0890-1763 (Capillary PEEK 0.18 mm x 1.5 m) and 5063-6591 (PEEK Fittings 10/PK)	Column/MCT Valve	Detector
5062-8535 (Waste accessory kit (Flow Cell to waste))	VWD	Waste
5062-2462 (Tube PTFE 0.7 mm x 5 m, 1.6 mm od)	DAD/FLD	Waste
G5664-68712 (Analytical tubing kit 0.25 mm i.d. PTFE-ESD)	Detector	Fraction Collector

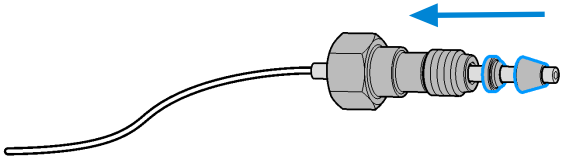
For correct installation of capillary connections it's important to choose the correct fittings, see Syntax for Capillary Description.

1 Select a nut that is long enough for the fitting you'll be using.

2 Slide the nut over the end of the tubing or capillary.



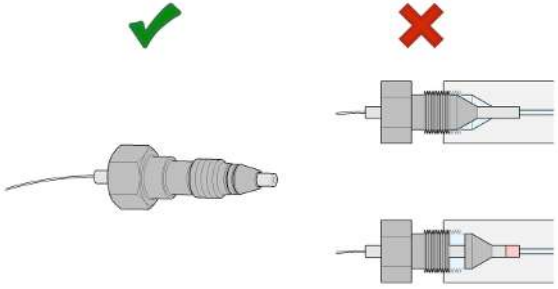
3 Carefully slide the ferrule components on after the nut and then finger-tighten the assembly while ensuring that the tubing is completely seated in the bottom of the end fitting.



4 Use a stable port installed to the module to gently tighten the fitting facing to the module. Or use the column to tighten the fitting facing to the column. This measure forces the ferrule to seat onto the tubing or capillary.

NOTE Do not overtighten. Over-tightening will shorten the lifetime of the fitting.

5 Loosen the nut and verify that the ferrule is correctly positioned on the tubing or capillary.



NOTE The first time that the Swagelok fitting is used on a column or an injection valve, the position of the ferrule is permanently set. If changing from a column or an injection valve to another, the fitting may leak or decrease the quality of the separation by contributing to band broadening.

For Bio and Bio-Inert Systems, the Swagelok instructions do not apply.

Handling Leak and Waste

The Agilent InfinityLab LC Series has been designed for safe leak and waste handling. It is important that all security concepts are understood and instructions are carefully followed.

The solvent cabinet is designed to store a maximum volume of 8 L solvent. The maximum volume for an individual bottle stored in the solvent cabinet should not exceed 2 L. For details, see the usage guideline for the Agilent Infinity III Solvent Cabinets (a printed copy of the guideline has been shipped with the solvent cabinet, electronic copies are available on the Internet).

All leak plane outlets are situated in a consistent position so that all Infinity and Infinity II/III modules can be stacked on top of each other. Waste tubes are guided through a channel on the right hand side of the instrument, keeping the front access clear from tubes.

The leak plane provides leak management by catching all internal liquid leaks, guiding them to the leak sensor for leak detection, and passing them on to the next module below, if the leak sensor fails. The leak sensor in the leak plane stops the running system as soon as the leak detection level is reached.

Solvent and condensate is guided through the waste channel into the waste container:

- from the detector's flow cell outlet
- from the Multisampler needle wash port
- from the Sample Thermostat (condensate)
- from the pump's Seal Wash Sensor (if applicable)
- from the pump's Purge Valve or Multipurpose Valve

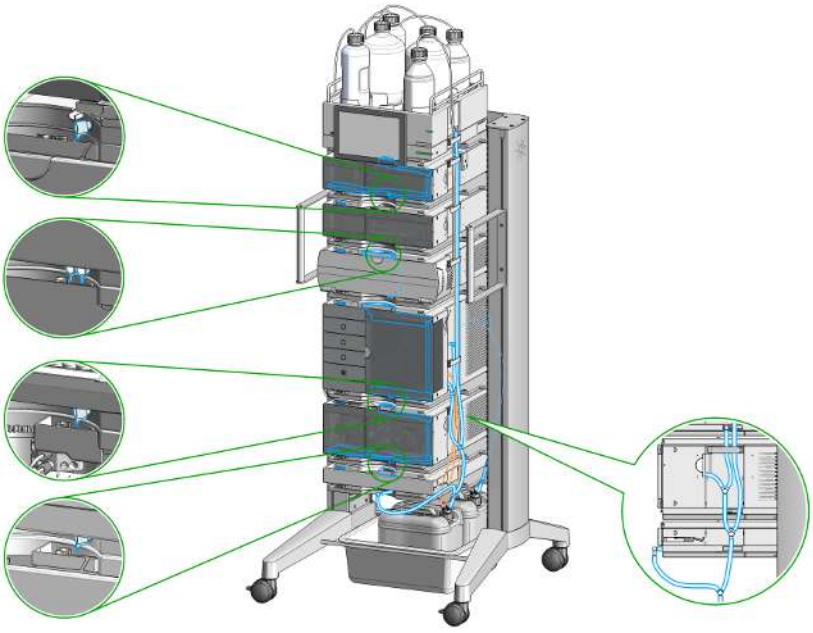


Figure 19: Infinity III Leak Waste Concept (Flex Bench installation)

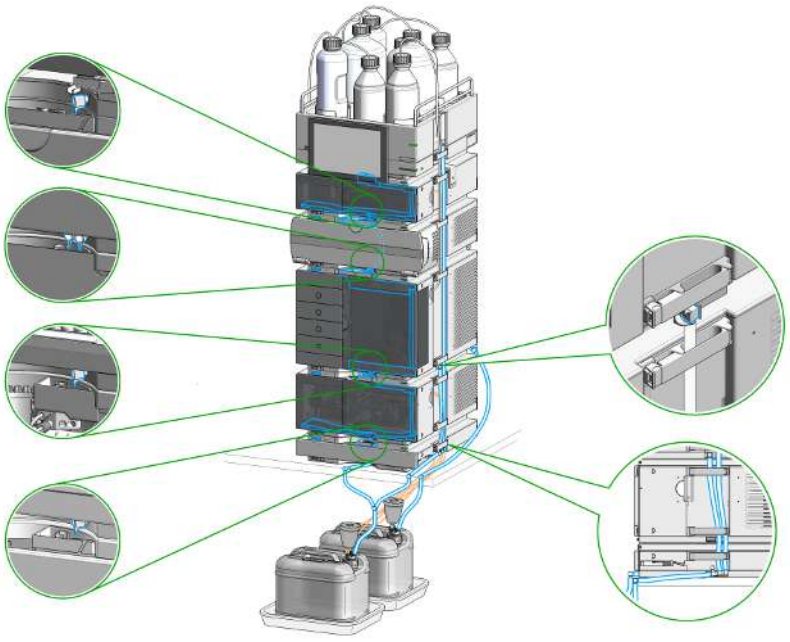


Figure 20: Infinity III Single Stack Leak Waste Concept (bench installation)

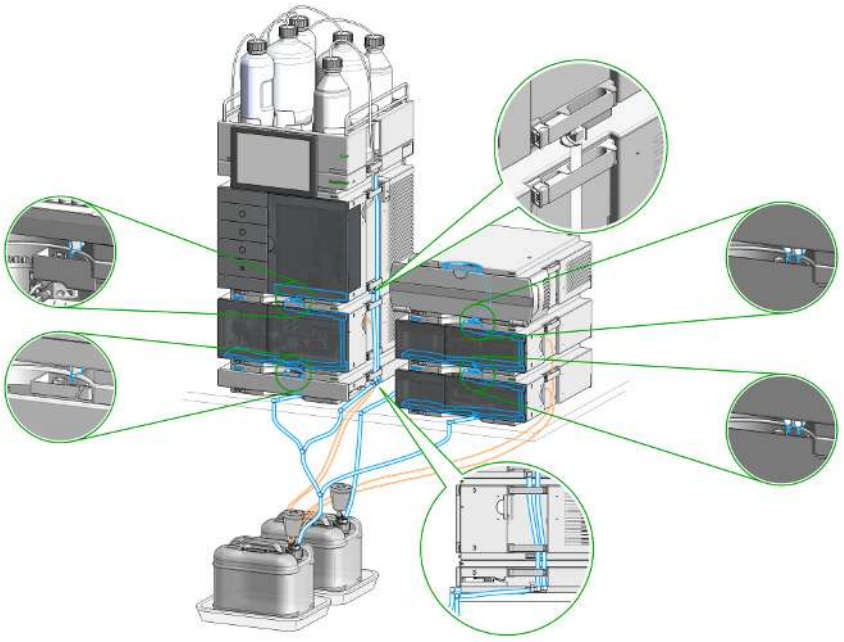


Figure 21: Infinity III Two Stack Leak Waste Concept (bench installation)

The waste tube connected to the leak plane outlet on each of the bottom instruments guides the solvent to a suitable waste container.

Drain Connectors Installation

Drain Connectors (available only as Drain Connectors Kit 5004-0000) have been developed to improve leak drainage for low flow leaks of high viscosity solvents (for example, isopropanol) in Agilent InfinityLab LC Series Systems. Install these parts to modules where they are missing (usually preinstalled).

- Make sure that dripping adapters are correctly installed on each module in the LC stack, excluding lowest module.
- Remove the dripping adapter if it is appeared to be installed on the lowest module in the LC stack and connect waste tube instead.
- Consider 5004-0000 (Drain Connectors Kit) if drain adaptor is missing on some module(s).

For illustration, see Handling Leak and Waste on page 44.

Parts required

Qty.	p/n	Description
	5004-0000	Drain Connectors Kit

Content of Drain Connectors Kit (p/n 5004-0000)

Parts can be ordered only as a complete kit.

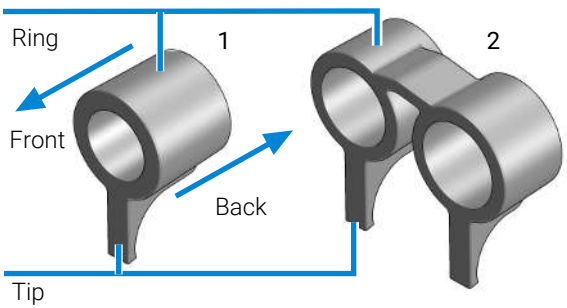


Figure 22: Overview of Drain Connectors: Single (left) and Double (right)

#	Qty.	p/n	Description
1	3		Single Drain Connector
2	1		Double Drain Connector

Table 8: Compatibility of drain connectors and modules

Drain Connector Type	Compatible Module	Compatible Module Type
Double	G7116A/B	Column Compartment
Single	G7114A/B	Detector
	G7115A	
	G7117A/B/C	
	G7121A/B	
	G7162A/B	
	G7165A	
	G7129A/B/C	Sampler
	G7167A/B/C	
	G5668A	
	G7137A	
	G7157A	
	G4767A	
	G7122A	Degasser
	G7104A/C	Pump
	G7110B	
	G7111A/B	
	G7112B	
	G7120A	
	G7131A/C	
	G7132A	
	G5654A	
	G4782A	

Preparations

- Leak drains of LC modules are clean and free of salt or solvent residuals.

NOTE

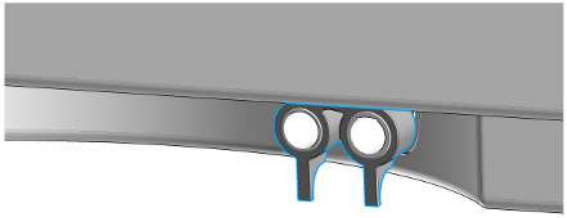
Do not install drain connectors on the bottom modules of the stack. Drain outlet of the bottom module has to be connected via waste tubing to a suitable waste container (see Leak and Waste Handling in the manual for a respective module).

NOTE In case of incorrect installation, drain connectors cannot fully perform the intended function.

NOTE It is not required to power off the HPLC stack to install Single and Double Drain Connectors. The installation of the connectors does not affect the analysis performed during the installation.

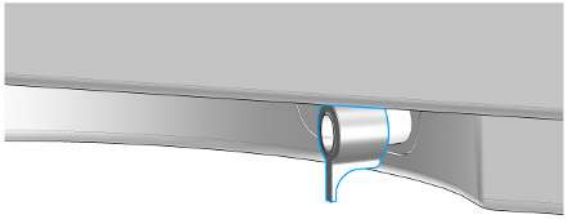
Install the Double Drain Connector on the leak drain of the 1260 Infinity III Multicolumn Thermostat (G7116A)/ 1290 Infinity III Multicolumn Thermostat (G7116B)

1 Align the rings with the leak drain outlets of the module, press slightly with the fingers, and slide the connector along the leak drain outlets until it is aligned with the front of the leak drain.



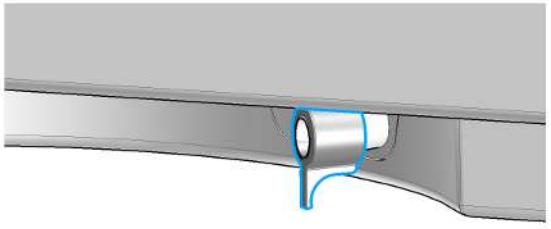
Install Single Drain Connectors on other modules in the LC stack

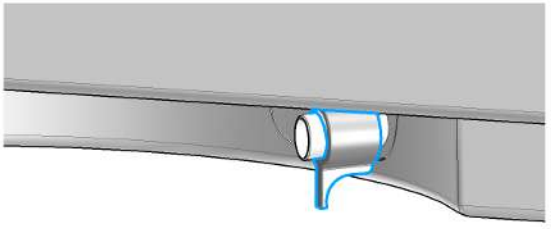
1 Align the ring with the leak drain outlet of the module, press slightly with the fingers, and slide the connector along the leak drain outlet until it is aligned with the front of the leak drain.



Make sure that the following requirements are covered:

- The tip of the drain connector points straight down.
- The leak drain outlets and the drain connectors are aligned properly.

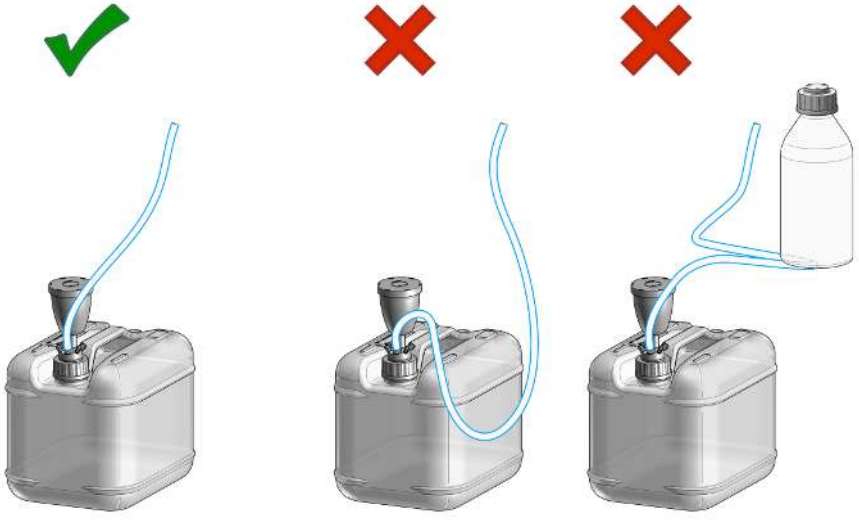




Waste Concept

Agilent recommends using the 5043-1221 (6 L waste can with 1 Stay Safe cap GL45 with 4 ports) for optimal and safe waste disposal. If you decide to use your own waste solution, make sure that the tubes don't immerse in the liquid.

Waste Guidance



NOTE The waste drainage must go straight into the waste containers. The waste flow must not be restricted at bends or joints.

Leak Sensor

CAUTION Solvent incompatibility

The solvent DMF (dimethylformamide) leads to corrosion of the leak sensor. The material of the leak sensor, PVDF (polyvinylidene fluoride), is incompatible with DMF.

- Do not use DMF as mobile phase.
- Check the leak sensor regularly for corrosion.

Connecting Modules and Control Software

WARNING Use of unsupplied cables

Using cables not supplied by Agilent Technologies can lead to damage of the electronic components or personal injury.

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

4 Using the Module

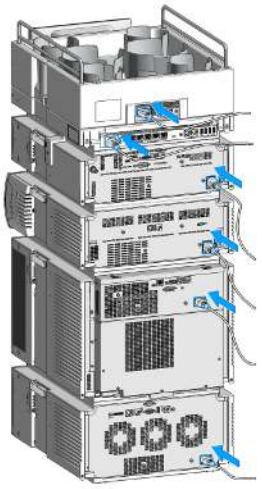
This chapter provides information on how to use the module.

General Information

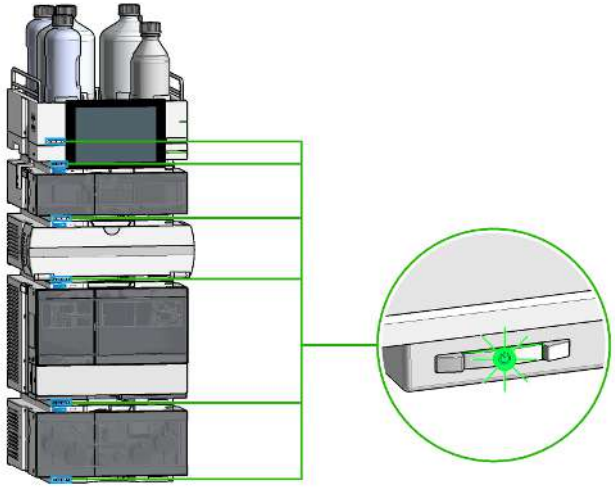
Turn On/Off

This procedure exemplarily shows an arbitrary LC stack configuration.

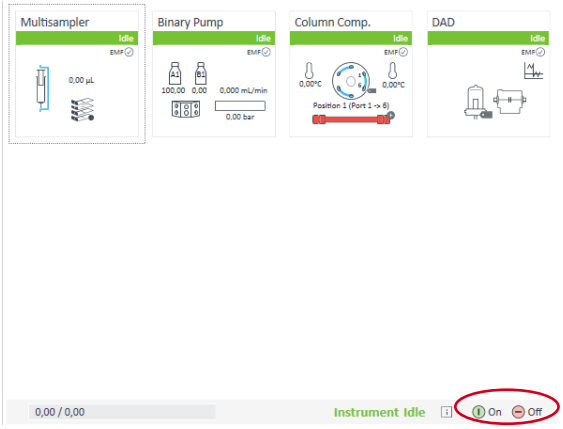
1



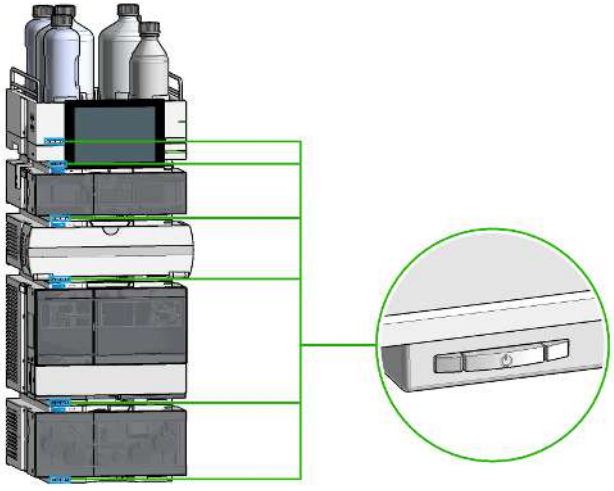
2 On/Off switch: On



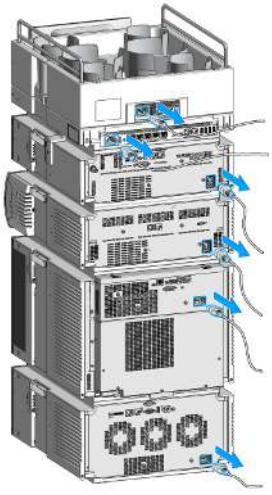
3 Turn instrument **On/Off** with the control software.



4 On/Off switch: Off



5



Status Indicators

The module status indicator indicates one of six possible module conditions.

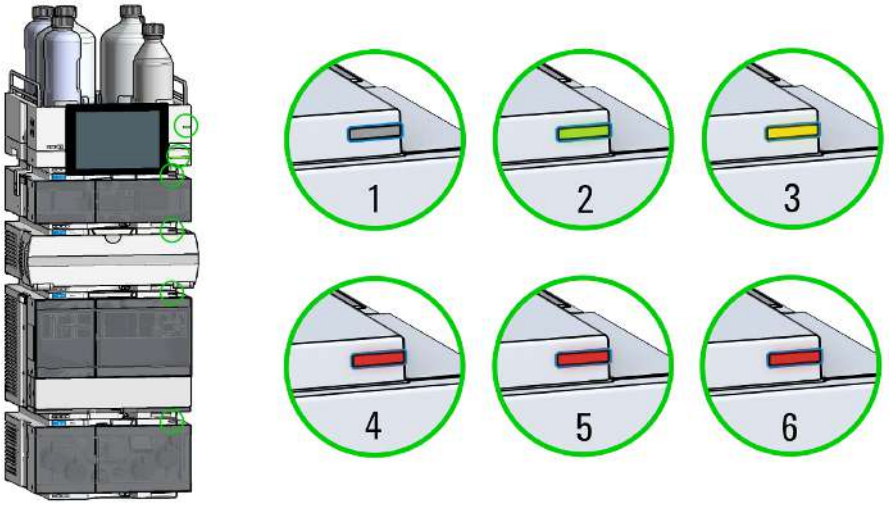


Figure 23: Arbitrary LC stack configuration (example)

1	Idle
2	Run mode
3	Not-ready. Waiting for a specific pre-run condition to be reached or completed.
4	Error mode - interrupts the analysis and requires attention (for example, a leak or defective internal components).
5	Resident mode (blinking) - for example, during update of main firmware.
6	Bootloader mode (fast blinking). Try to re-boot the module or try a cold-start. Then try a firmware update.

InfinityLab Assist Hub Status Indicator

The Assist Hub status indicator displays the status of the entire system. If a module in the system is not ready (yellow), the Assist Hub status indicator also shows not ready (yellow). The same applies for the module conditions Idle, Run mode, and Error mode.

Preparation of the System

Prepare a Run

This procedure exemplarily shows how to prepare a run. Parameters as shown in the screenshots may vary, depending on the system installed.

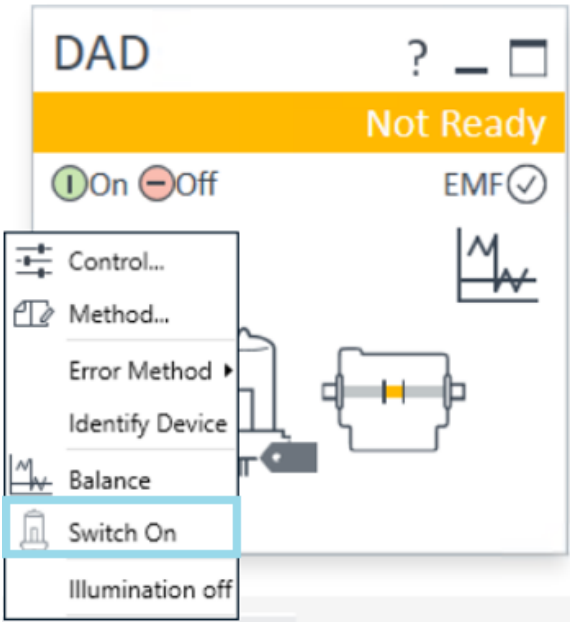
WARNING

Toxic, flammable and hazardous solvents, samples and reagents

The handling of solvents, samples and reagents can hold health and safety risks.

- **When working with these substances observe appropriate safety procedures (for example by wearing goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet supplied by the vendor, and follow good laboratory practice.**
- **Do not use solvents with an auto-ignition temperature below 200 °C (392 °F). Do not use solvents with a boiling point below 56 °C (133 °F).**
- **Avoid high vapor concentrations. Keep the solvent temperature at least 40 °C (72 °F) below the boiling point of the solvent used. This includes the solvent temperature in the sample compartment. For the solvents methanol and ethanol keep the solvent temperature at least 25 °C (45 °F) below the boiling point.**
- **Do not operate the instrument in an explosive atmosphere.**
- **Do not use solvents of ignition Class IIC according IEC 60079-20-1 (for example, carbon disulfide).**
- **Reduce the volume of substances to the minimum required for the analysis.**
- **Never exceed the maximum permissible volume of solvents (8 L) in the solvent cabinet. Do not use bottles that exceed the maximum permissible volume as specified in the usage guideline for solvent cabinet.**
- **Ground the waste container.**
- **Regularly check the filling level of the waste container. The residual free volume in the waste container must be large enough to collect the waste liquid.**
- **To achieve maximal safety, regularly check the tubing for correct installation.**

1 Switch on the detector.

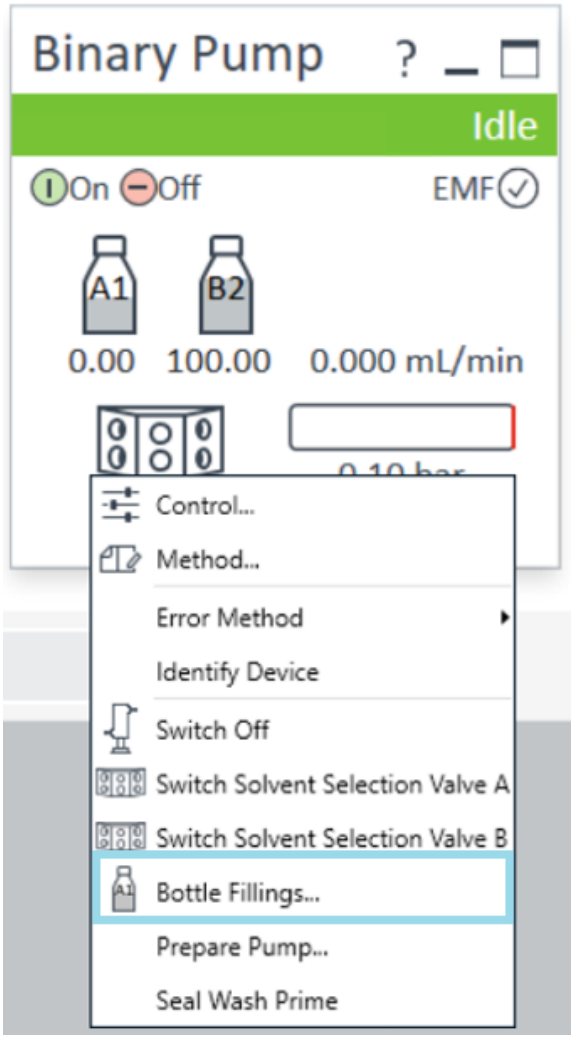


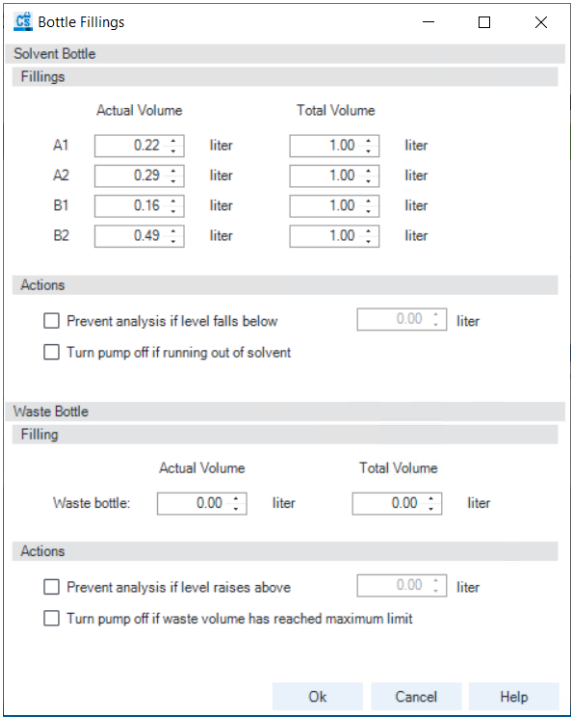
2 Fill the solvent bottles with adequate solvents for your application.

3 Place solvent tubings with bottle head assemblies into the solvent bottles.

4 Place solvent bottles into the solvent cabinet.

5 Solvent bottle filling dialog (in the software).





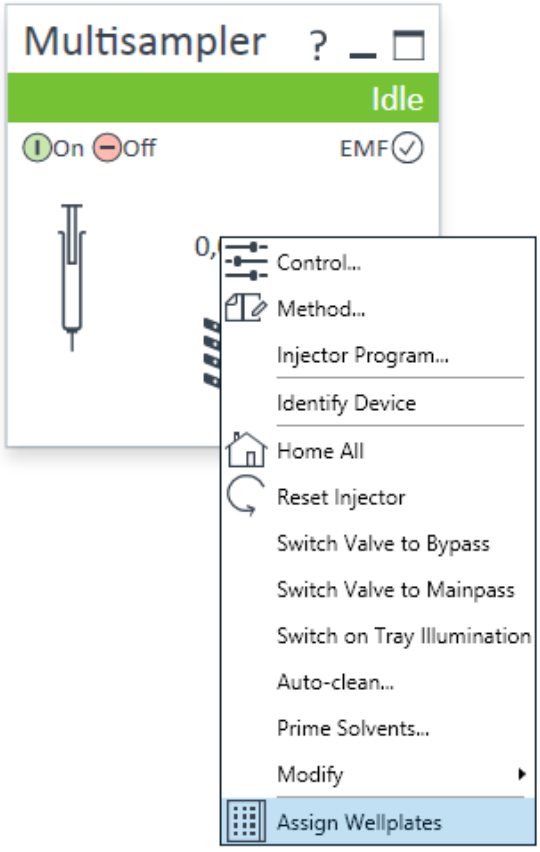
6 Purge the pump.

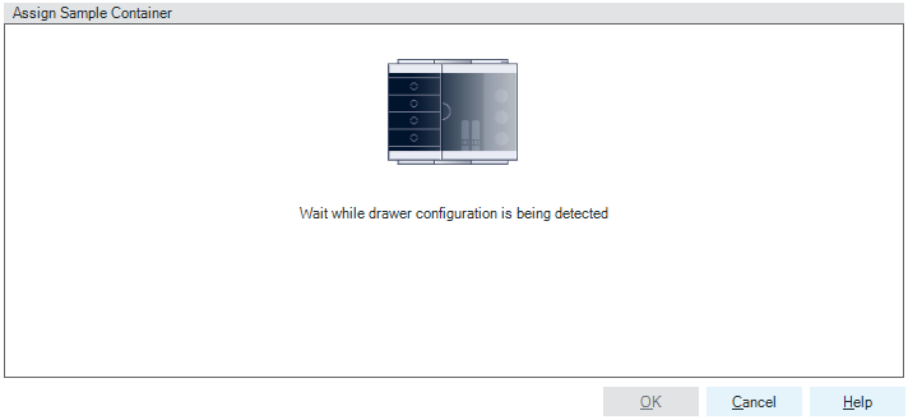
NOTE

For details on priming and purging, refer to the technical note *Best Practices for Using an Agilent LC System Technical Note (InfinityLab-BestPractice-en-SD-29000194.pdf, SD-29000194)*.

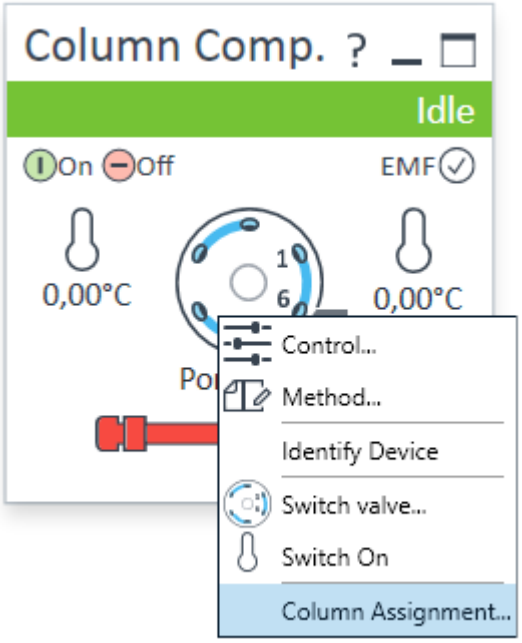
7 Change solvent type if necessary.

8 Choose the tray format of the sampler.

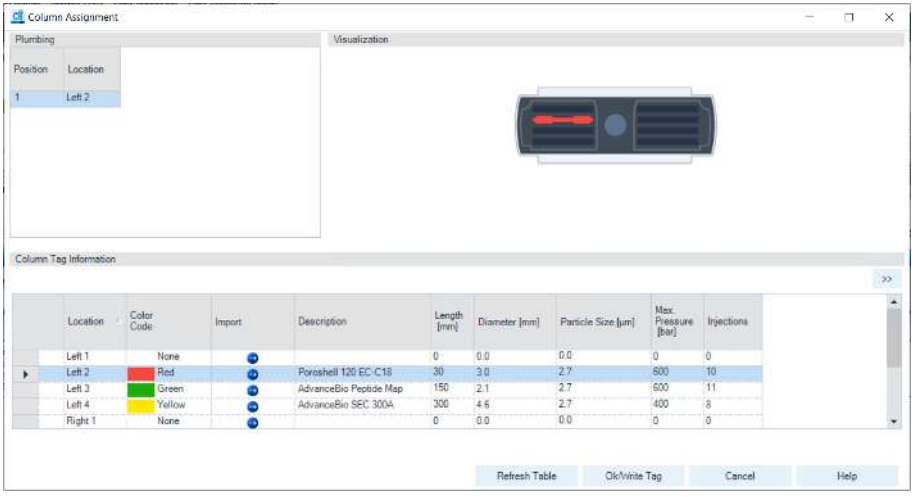




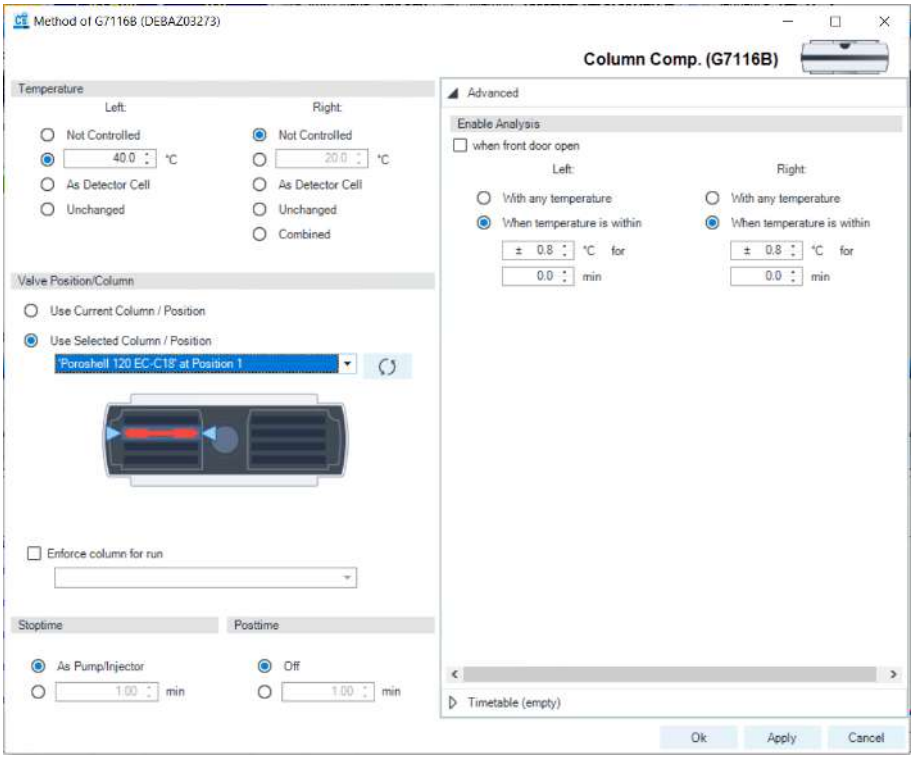
9 Add a new column.



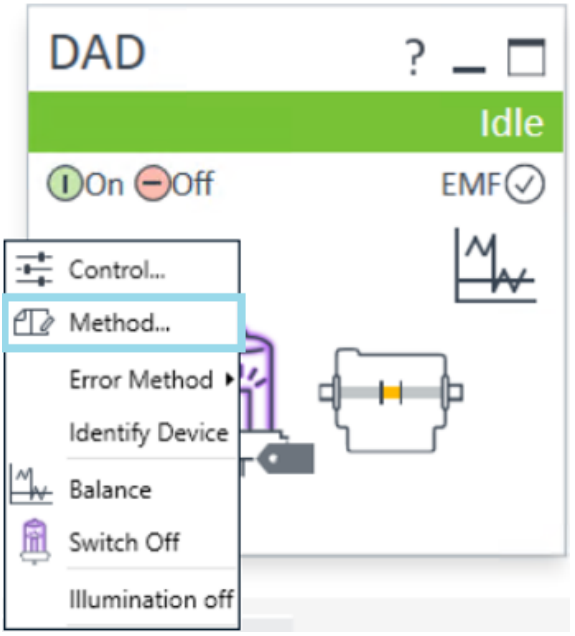
10 Enter the column information.



11 Select the column in the Method settings of the column compartment.



12 Set the detector parameters according to the needs of your method.


DAD
Idle
On
Off
EMF
Control...
Method...
Error Method
Identify Device
Balance
Switch Off
Illumination off

Prime and Purge the System

When the solvents have been exchanged or the pumping system has been turned off for a certain time (for example, overnight) oxygen will re-diffuse into the solvent channel between the solvent reservoir, vacuum degasser (when available in the system) and the pump. Solvents containing volatile ingredients will slightly lose these. Therefore priming of the pumping system is required before starting an application.

Table 9: Choice of priming solvents for different purposes

Activity	Solvent	Comments
After an installation	Isopropanol	Best solvent to flush air out of the system
When switching between reverse phase and normal phase (both times)	Isopropanol	Best solvent to flush air out of the system
After an installation	Ethanol or Methanol	Alternative to Isopropanol (second choice) if no Isopropanol is available
To clean the system when using buffers	Bidistilled water	Best solvent to re-dissolve buffer crystals
After a solvent change	Bidistilled water	Best solvent to re-dissolve buffer crystals
After the installation of normal phase seals (P/N 0905-1420)	Hexane + 5% Isopropanol	Good wetting properties

NOTE

The pump should never be used for priming empty tubings (never let the pump run dry). Use a syringe to draw enough solvent for completely filling the tubings to the pump inlet before continuing to prime with the pump.

1 Open the purge valve of your pump (by turning it counterclockwise) and set flow rate to 3 – 5 mL/min.

2 Flush all tubes with at least 30 mL of solvent.

3 Set flow to required value of your application and close the purge valve.

NOTE

Pump for approximately 10 minutes before starting your application.

Preparing the Module

Set Up the Detector with Agilent Open Lab ChemStation

The setup of the detector is shown with the Agilent OpenLab ChemStation C.01.07 and Driver A.02.14.

NOTE This section describes the detector settings only. For information on the Agilent OpenLab ChemStation or other 1200 Infinity modules refer to the corresponding documentation.

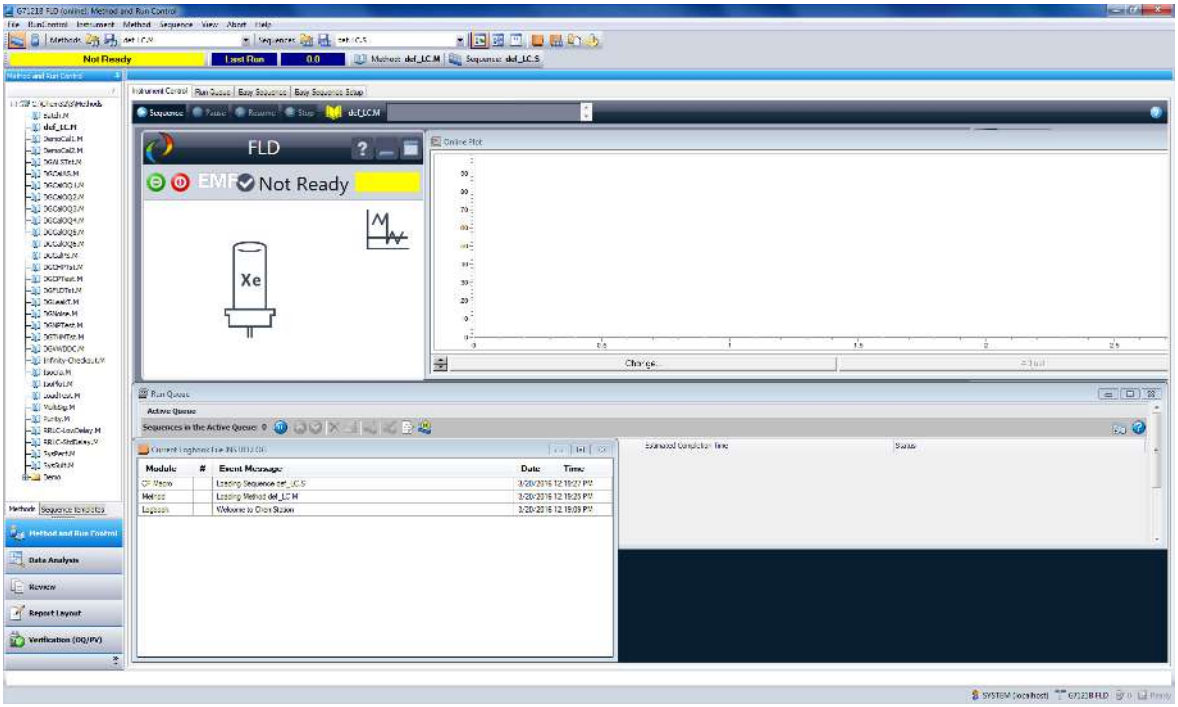
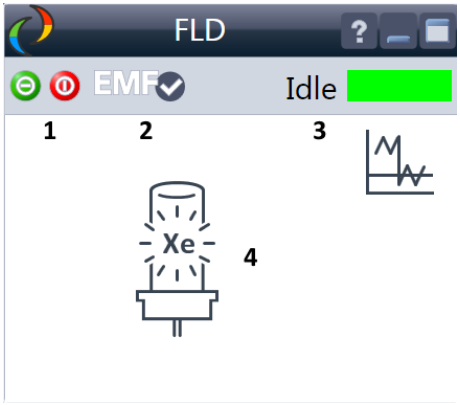


Figure 24: ChemStation Method and Run Control (just detector is shown)

After successful load of the OpenLab ChemStation, you should see the module as an active item in the graphical user interface (GUI).

The Detector User Interface



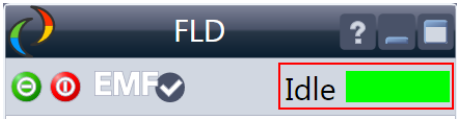
Within the detector GUI, there are active areas. If you move the mouse cursor across the icons the cursor will change.

1. Lamp: turn on and off of UV-lamp
2. EMF status
3. Detector status
4. Lamp status (on/off)

EMF Status shows Run / Ready / Error state and “Not Ready text” or “Error text”

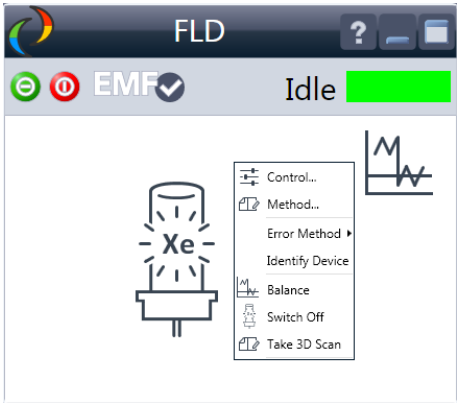
- Offline (gray)
- Ok. No Maintenance required (green)
- EMF warning. Maintenance might be required (yellow)
- EMF warning. Maintenance required (red)

Important: The EMF settings can be accessed via Agilent Lab Advisor. The limit(s) can be changed. Based on the limit, the User Interface displays the above status.



Module Status shows Run / Ready / Error state and “Not Ready text” or “Error text”

- Error (red)
- Not ready (yellow)
- Ready (green)
- Pre run, Post run (purple)
- Run (blue)
- Idle (green)
- Offline (dark gray)
- Standby (light gray)

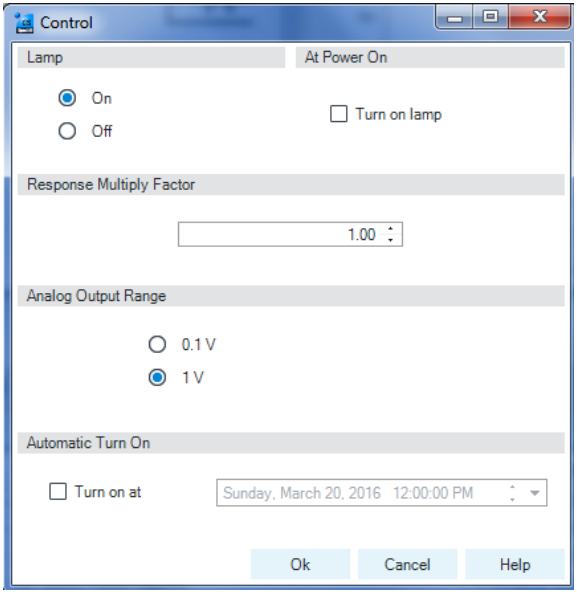


A right-click into the Active Area will open a menu to

- Show the Control Interface (special module settings)
- Show the Method interface (similar as via menu **Instrument** > **Setup Instrument Method**)
- Set Error Method
- Identify Module (Status LED will blink)
- Perform a Balance
- Switch the lamp on/off (same as click on button “Make Device Ready/ Turn device off (standby)”)

NOTE: The Balance icon is used for UV detectors and has no function on the FLD.

Detector Control Settings



The figure shows the default settings.

- **Lamps**: can be turned ON/OFF.
- **Response Multiply Factor**: 1
- **Analog Output Range**: can be set to either 100 mV or 1 Vfull scale, for additional settings see **Analog Output** (under Method Parameter Settings on page 72).
- **At Power On**: automatic lamp-on at power on.
- **Automatic Turn On**: automatic detector power on.

Method Parameter Settings

These settings are available via **Menu** > **Instrument** > **Set up Instrument Method** or via right click into the module's active area (does not show the Instrument Curves tab).

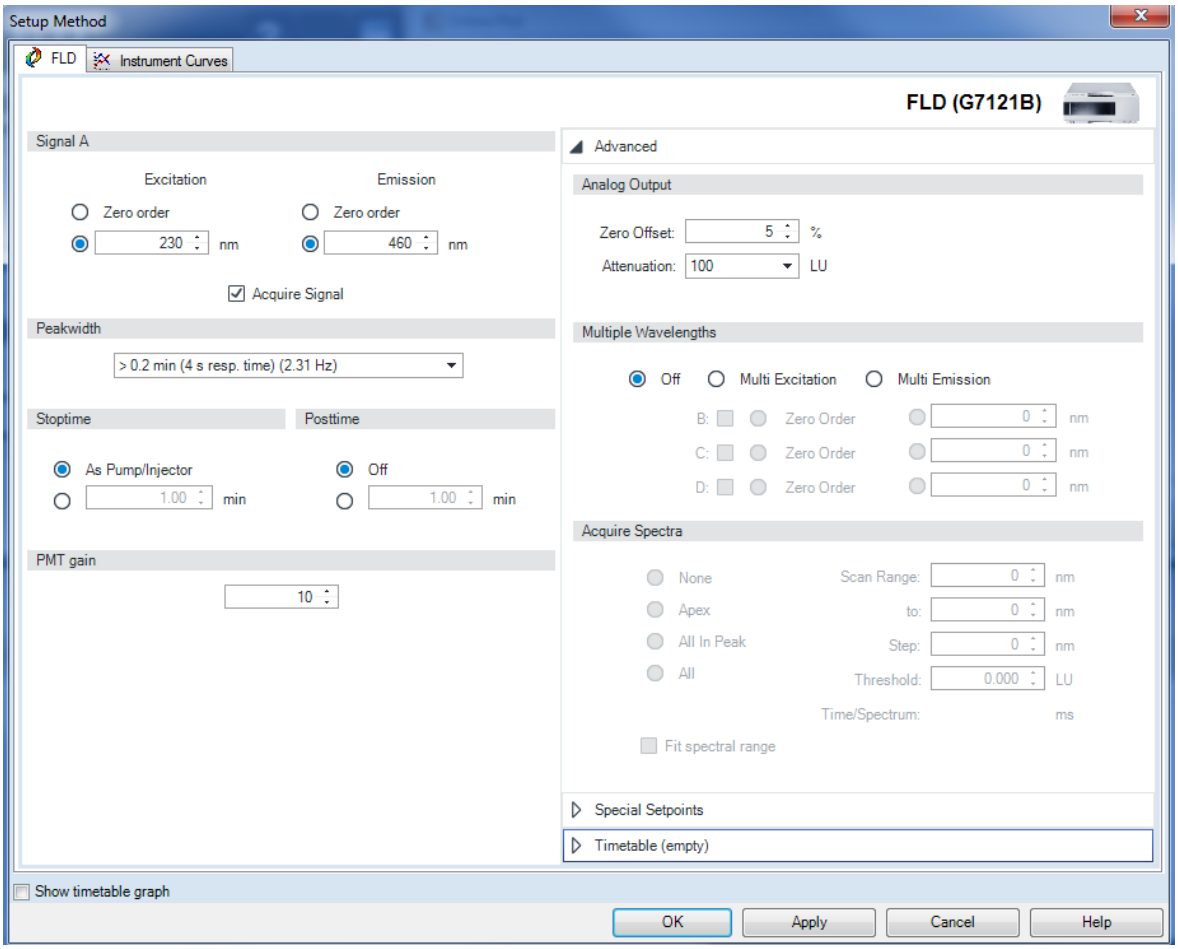
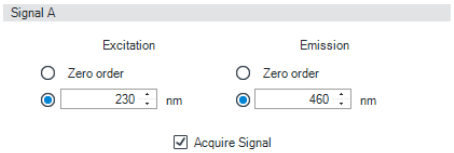


Figure 25: Method parameter settings

Advanced Settings



Signal A
You can define the wavelengths of the excitation and emission and specify signal acquisition.

Limits (Ex and Em): 200 to 1200 nm in steps of 1 nm.

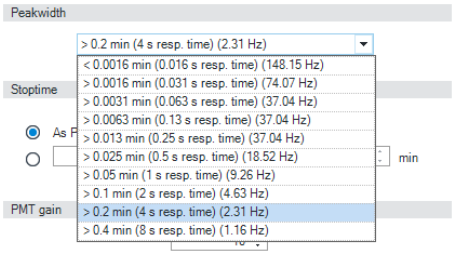
NOTE: The emission wavelength should be at least 10 nm greater than the excitation wavelength

NOTE: Addition signals B, C, D can be added via Mulitiple Wavelength mode (*G721B ONLY*).

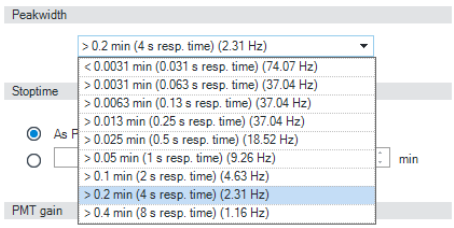
Zero Order (Ex)
The full spectrum of light from the Xenon lamp illuminates the flow cell. Each compound can absorb its characteristic wavelength of light and then emit maximum fluorescence. An increased stray light level is inherent in this setting, and this will decrease sensitivity (signal-to-noise).

Zero Order (Em)
Zero order sets the monochromator so that all light emitted from the sample will be reflected onto the detector.

Acquire Signal
Mark this check box to specify that the signal is stored in the CDS during data acquisition. When the check box is cleared, the signal is not stored.

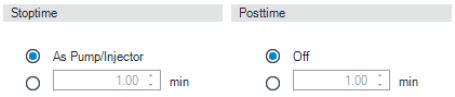


Peakwidth Settings (G7121B)

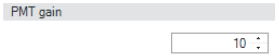


Peakwidth Settings (G7121A)

Peakwidth (Responsetime, Data Rate)
Peakwidth enables you to select the peak width (response time) for your analysis. The peak width is defined as the width of a peak, in minutes, at half the peak height. Set the peak width to the narrowest expected peak in your chromatogram. The peak width sets the optimum response time for your FLD. The peak detector ignores any peaks that are considerably narrower, or wider, than the peak width setting. The response time is the time between 10 % and 90 % of the output signal in response to an input step function.
Limits: When you set the peak width (in minutes), the corresponding response time is set automatically and the appropriate data rate for signal and spectra acquisition is selected as shown.

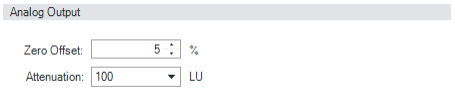


Stoptime/Posttime
The **Stoptime** is the time where either the complete system stops (As Pump/Injector) or the module (if different from system stop time). The data collection is stopped at this time. A **Posttime** period can be used to allow module's items to equilibrate (e.g. after gradient change or temperature change).



PMT Gain is the photomultiplier gain. Each step approximately doubles the signal. A typical setting is between 10 and 15 ; use lower settings for very high concentrations. Higher PMT Gain may improve signal-to-noise. See also FLD Scaling Range and Operating Conditions on page 114.

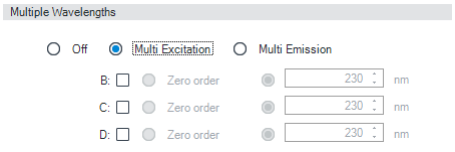
Limits: 0 to 18 in steps of 1 . Default value is 10



Analog Output

The range can be set to either 100 mV100 or 1 V1 full scale, see Detector Control Settings on page 72 .

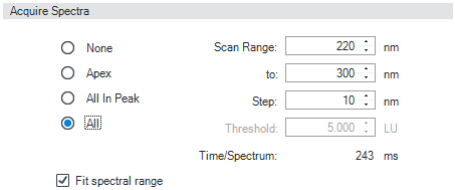
- Zero Offset: 1 – 99 % in steps of 1 % (5 % equal to 50 mV5).
- Attenuation: 0.195 – 1600 LU at discrete values for either 100 mV100 or 1 V full scale.



Multiple Wavelengths (*G7121B ONLY*)
You can choose to use three additional wavelengths, either excitation or emission wavelengths in your analysis. Choose **Off** to switch off multiple wavelengths, or **Multi Ex** or **Multi Em** to set up multiple wavelengths.

See also Signal A.

Acquire Spectra (G7121B Only)

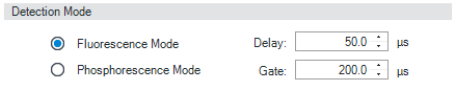


Acquire Spectra (G7121B ONLY)
If you choose multiple excitation or emission wavelengths, you can also choose to acquire spectra.
None: No spectra are taken.
Apex: A spectrum is acquired at the apex of the peak.
All in Peak: All spectra within the peak are acquired.
All w/o signal: All flashes are used for spectra acquisition. The mean value of all measured wavelengths is shown on channel A. This setting is useful for unknown spectra. Spectra are acquired continuously depending on the settings of the range and Step.
NOTE: If there are no peaks in Signal A, there are no spectra.
Scan Range: These two fields define the wavelength range for spectral storage.
Limits (Ex and Em): 200 – 1200 nm in steps of 1 nm.
Step: Step defines the wavelength resolution for spectral storage.
Limits: 1 – 20 nm in steps of 1 nm.
Threshold: The Threshold is the height in LU (Luminescence Units) of the smallest expected peak. The peak detector ignores any peaks that are lower than the threshold value and does not save spectra.
Limits: 0.001 – 1000 LU.
Acquisition time/rate
The *acquisition time* (in ms) for a single spectrum under the specified conditions is calculated and displayed.

The *acquisition rate* depends on:
- the number of additional signals specified
- the scan range of spectral acquisition
- the step size (wavelength resolution)

Fit Spectral Range
Select Fit Spectral Range to adjust the spectral range so that there is always a difference of at least 25 nm between the excitation wavelength and emission wavelength. This ensures that no first order stray light is measured due to the excitation and emission wavelengths being too close. If the range of the emission spectra overlaps with the excitation wavelength, first order stray light adds spectral bands in the emission spectra.
Multi Emission
The lower emission wavelength of the resulting spectra is set to be at least 25 nm higher than the excitation wavelength. The upper wavelength is adjusted according to the chosen step width.
Example: Ex: 280 nm, Em: 300 – 400 LU step 10 — Resulting spectra will have 300 – 395 nm
Multi Excitation
The upper excitation wavelength of the resulting spectra is set to be at least 25 nm lower than the emission wavelength. The upper wavelength is adjusted according to the chosen step width.
Example: Em: 350 nm, Ex: 300 – 400 nm step 10 — Resulting spectra will have 300 – 320 nm
Data rate
The data rate is calculated according to the specified scan conditions.

Special Settings



Detection Mode
Default: Fluorescence
Choose the Fluorescence Mode option to measure luminescence from samples that emit fluorescence.

Choose the Phosphorescence Mode option to measure luminescence from samples that emit phosphorescence. When you choose to switch on the Phosphorescence detection mode, the two parameters Delay and Gate are activated.

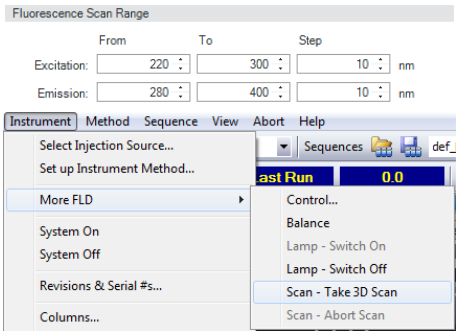
The luminescence that you measure with the fluorescence detector has different characteristics depending on the chemistry of your application. The characteristic of your sample determines whether you need to use fluorescence detection mode or phosphorescence detection mode.

Delay
Sets a waiting period, during which the lamp flashes, before the FLD starts to measure.

Limits: 0 – 5000.0 µs in steps of 0.1 µs.

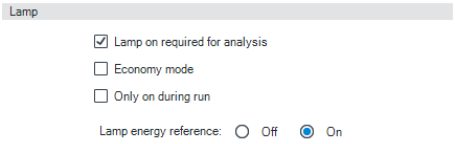
Gate
Sets a measurement time period after the lamp has flashed.

Limits: 20.0 – 5000.0 µs in steps of0.1 µs.



Fluorescence Scan Range (G7121B ONLY)
The **Fluorescence Scan Range** panel enables you to set up a fluorescence scan (3D scan). The fluorescence scan can be used to collect emission and excitation spectral information in a single task. It is most suitable for the characterization of single compounds, and for checking the purity of eluents. The contents of the flow cell must be kept constant during the measurement; use off-line measurements with the FLD cuvette or stopped-flow conditions in chromatography.

Enter the wavelength ranges for excitation and emission. The Step size determines the number of data points per spectrum. The time per scan (in seconds) is calculated automatically and displayed.



The **Lamp** section allows you to specify how the lamp is used in an analysis.

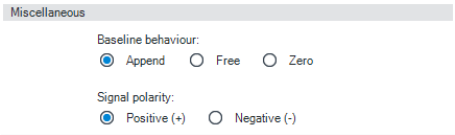
Select **Only On During Run** to specify that the lamp is switched on only during the run time, and is switched off when the run finishes.

Select **Economy Mode** to run the lamp in a low frequency, low current mode. This increases the lamp lifetime at the expense of sensitivity.

To prolong the life of the lamp, clear the check box if you have a multiple detector configuration and do not want to use the FLD for the next analyses.

NOTE: If the Lamp on required for analysis check box is marked, but the lamp is off when the run is started, the lamp is switched on automatically.

Lamp Energy Reference
Choose On to switch on a compensation for flash-to-flash variations in lamp intensity using the reference diode. This gives improved signal-to-noise.

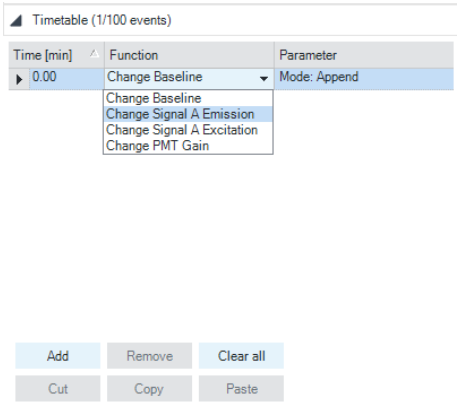


Baseline Behavior
Baseline behavior allows you to choose what happens to the baseline after a change in wavelength or PMT gain.

- Append: Resets the baseline to the previous baseline position.
- Free: Makes no adjustment to the baseline position; you may see a shift in baseline position.
- Zero: Resets the baseline to the baseline position at the start of the run. Note that this may not be 0 LU (Luminescence Units), depending on the analysis conditions.

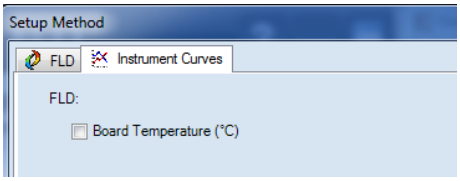
Signal Polarity
Select the Signal Polarity you expect from your data from Negative or Positive.

Time Table



Time Table
You may set up time events to change functions with their parameters over the run time. Add lines as required.
Time Limits: 0.00 – 99999.00 min in steps of 0.01 min.
Via the buttons in the bottom area, time table lines can be added, removed, cut copied, pasted or completely cleared.
Based on the chosen function, a certain parameter can be selected.

Instrument Curves



The detector has an internal temperatures signal that can be used for diagnosing problems. These can be baseline problems deriving from lamp wander / drift problems due to temperature changes.
This signals can be used in addition to the normal baseline signal to determine whether correlation to temperature of the lamp.
These signals are available via the Agilent ChemStation Online Plot/Data Signal and/or Agilent Lab Advisor Software.

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.

For optimal results refer to Optimizing the Performance of the Module on page 81.

5 Optimizing the Performance of the Module

This chapter provides information on how to optimize the module.

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 fluorescence detector. Table 10 on page 82 gives an overview of how to benefit from the operation modes during these steps.

Table 10: 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 wavelengths 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 26 on page 83 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.

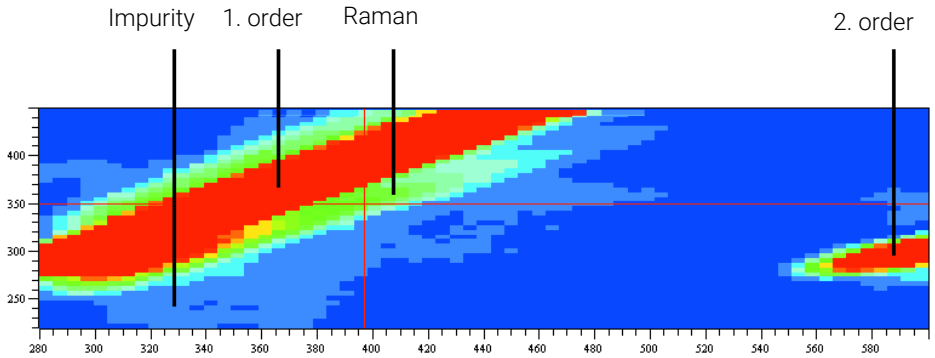


Figure 26: Isofluorescence plot of a mobile phase

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 27 on page 84) 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 µg/ml quinidine.

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

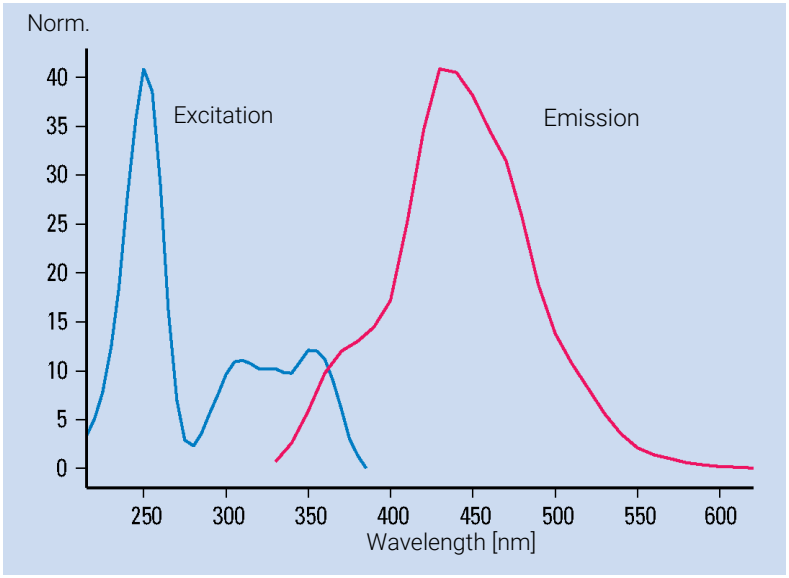


Figure 27: Excitation and emission spectra of quinidine

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 26 on page 83) 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 Infinity 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 1260 Infinity Fluorescence Detector to separate the compound mix under known conditions and acquire emission and excitation spectra separately.

Procedure III - Use an Agilent 1200 Infinty 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 1260 Infinity 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 28 on page 86 shows the complete information for quinidine as obtained with the Agilent 1260 Infinity Fluorescence Detector 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 28 on page 86:

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

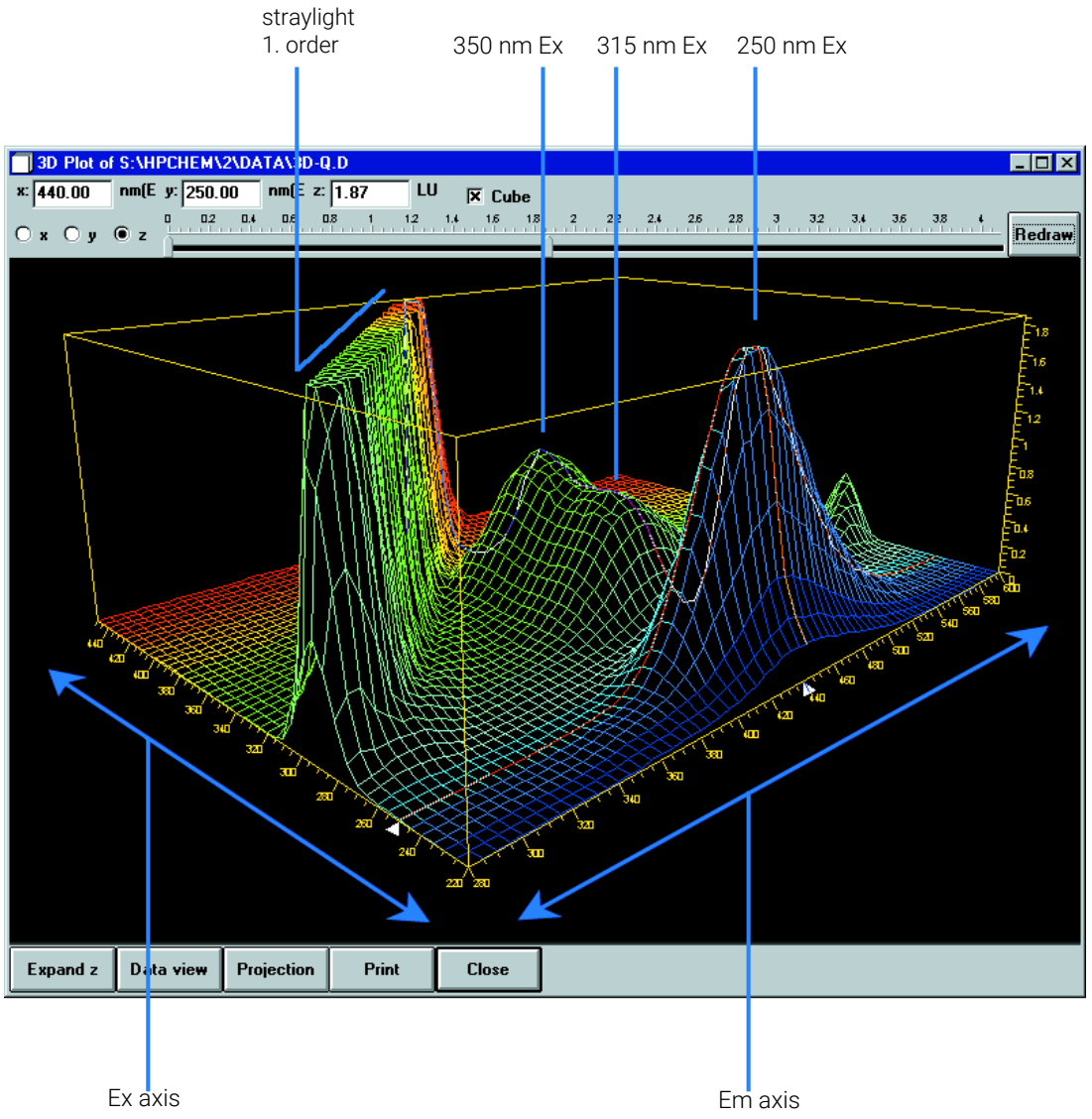


Figure 28: Characterization of a pure compound from a fluorescence scan

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 29 on page 88 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 11: 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-c,d)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 30 on page 89. 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 12: Conditions for Optimization of PNA analysis according to figures below

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 wavelength (260 nm).

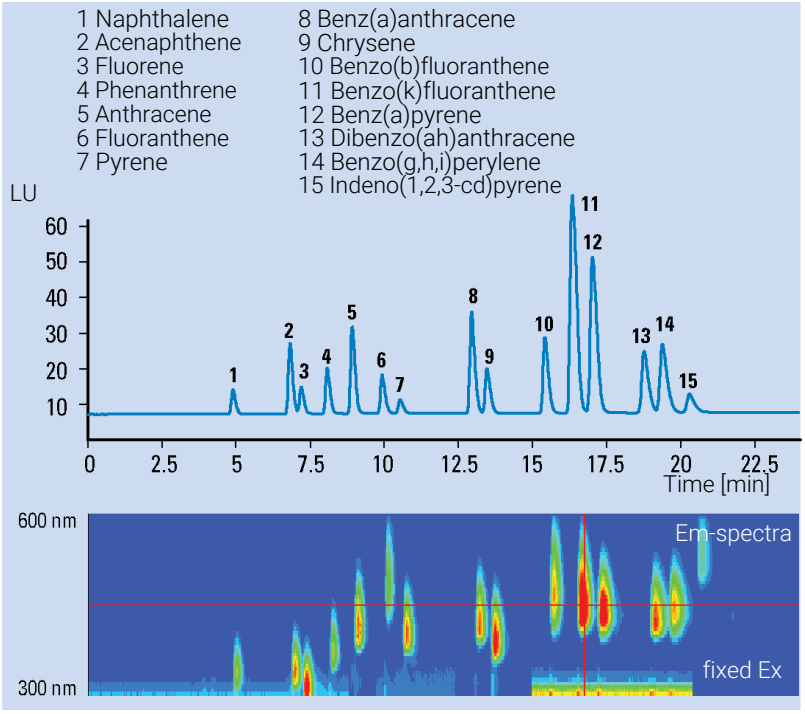


Figure 29: Optimization of the time-program for the emission wavelength

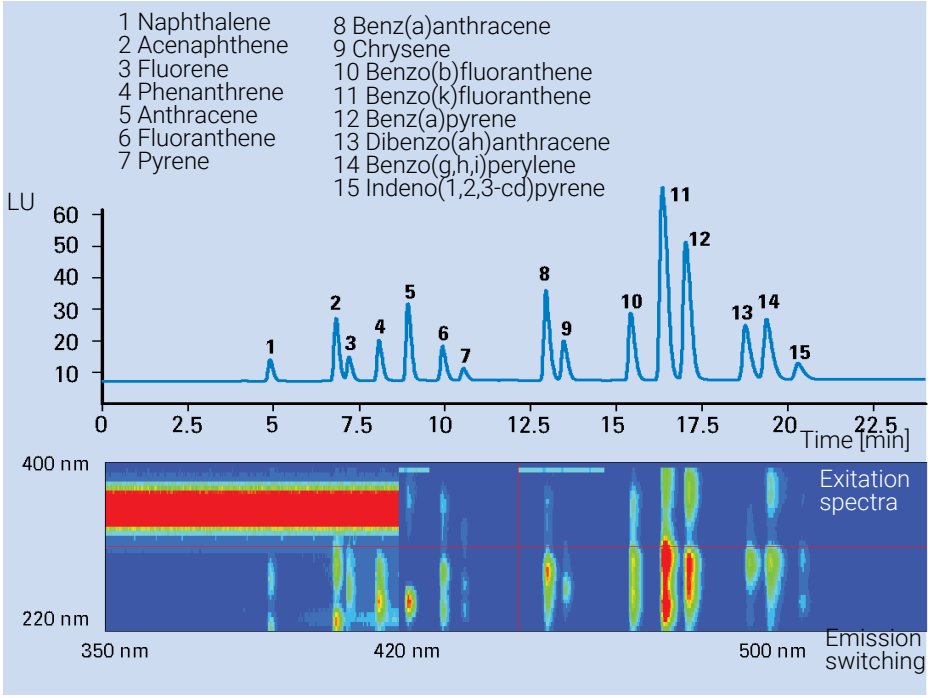


Figure 30: Optimization of the time-program for the excitation wavelength

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 13 on page 89.

Table 13: 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 a 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-diaminophenazine (DAP) and 2-amino-3-hydroxyphenazine (AHP). Reference samples of DAP and AHP were analyzed with diode array and fluorescence detection. Figure 31 on page 90 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 32 on page 91 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.

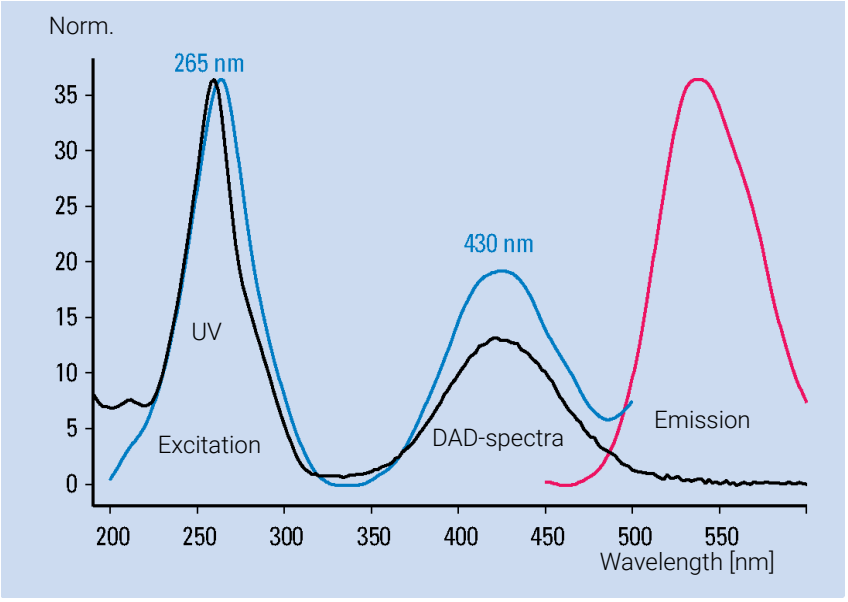


Figure 31: UV-spectrum and fluorescence spectra for 2,3-diaminophenazine (DAP)

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

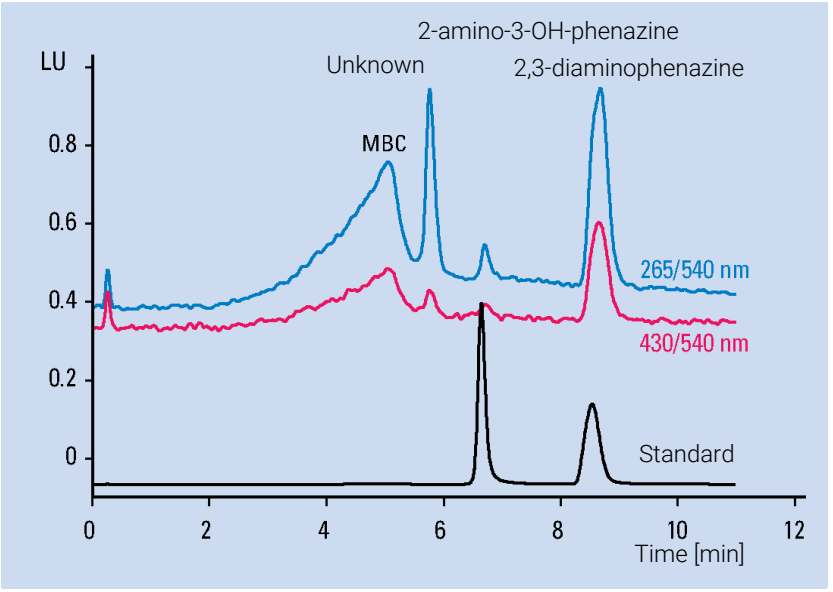


Figure 32: Qualitive analysis of MBC (2-benzimidazole carbamic acid methylester) and impurities

Table 14: Conditions for analysis of DAP and MBC according to figures above

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 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 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 33 on page 93).

Table 15: Conditions for simultanoeus multi wavelength detection for PNA-analysis (see figure below)

Column	Vydac, 2.1 x 250 mm, PNA, 5 µm
Mobile phase	A = water; B = acetonitrile (50 : 50)
Gradient	3 min, 60 % 14.5 min, 90 % 22.5 min, 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.

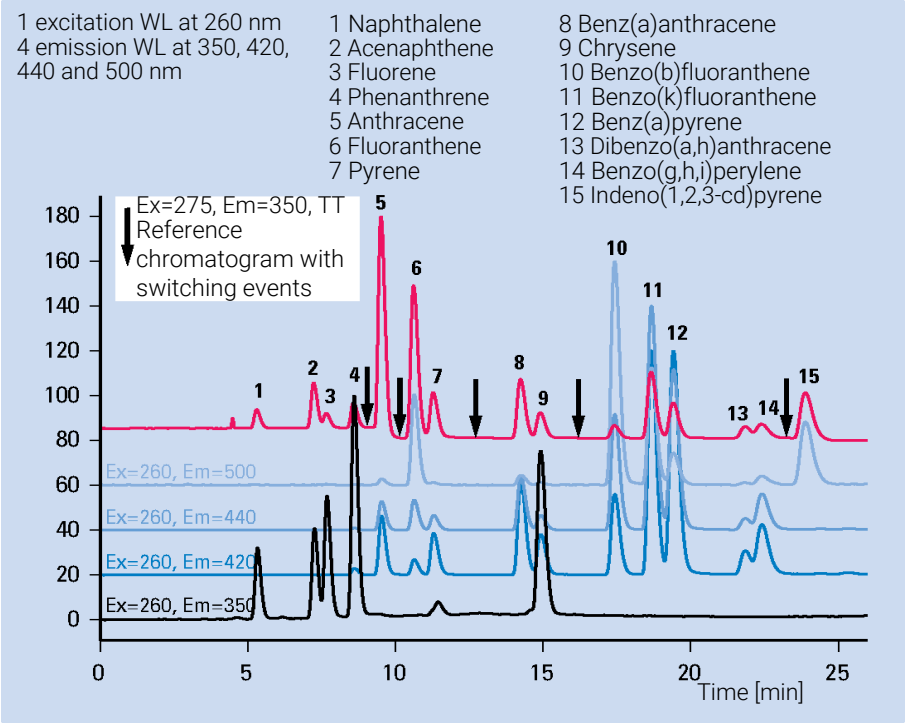


Figure 33: Simultaneous multi wavelength detection for PNA-analysis

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 16 on page 94 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 16 on page 94 shows an automated library search based on the emission spectra from a PNA reference sample.

Table 16: Peak confirmation using a fluorescence spectral library

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

Meas. RetTime	Library	CalTbl	Signal	Amount	Purity	#	Match	Libary Name
[min]	[min]	[min]		[ng]	Factor			
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-c,d)pyrene@em

Example: Optimization for Multiple Compounds

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

Setting the Chromatographic Conditions

This example uses the following chromatographic conditions (the detector settings are shown in Detector settings for emission scan).

Table 17: 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 min % B=50
	at 3 min % B=60
	at 14.5 min % B=90
	at 22.5 min % B=95
Stop time	26 min
Post time	8 min
Injection volume	1 µl
Oven temperature (1200)	30 °C
FLD PMT Gain	PMT = 15
FLD Response time	4 s

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.

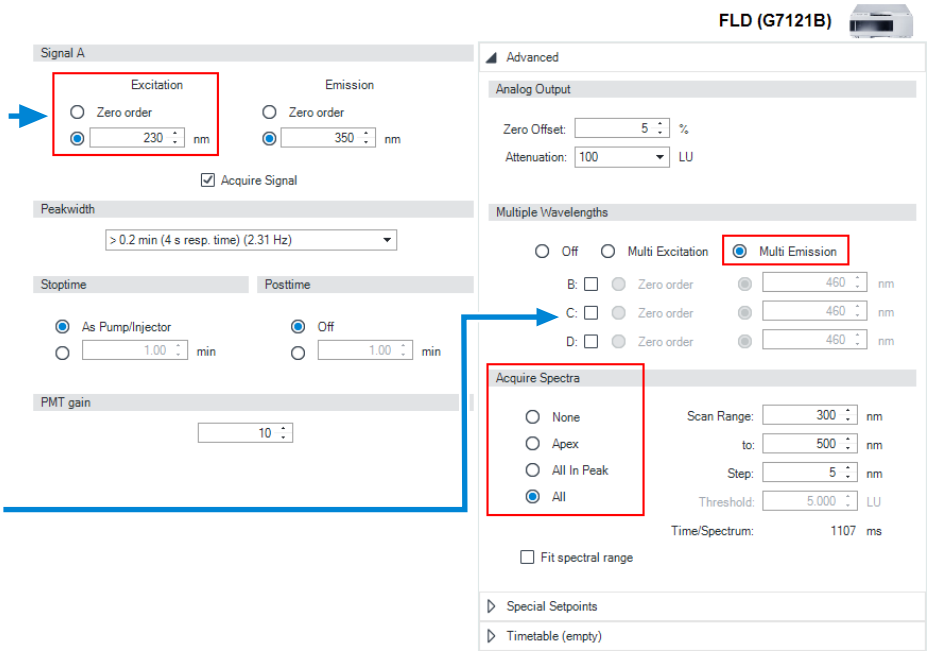


Figure 34: Detector settings for emission scan

1 Wait until the baseline stabilizes. Complete the run.

2 Load the signal. (In this example just the time range of 13 min is displayed).

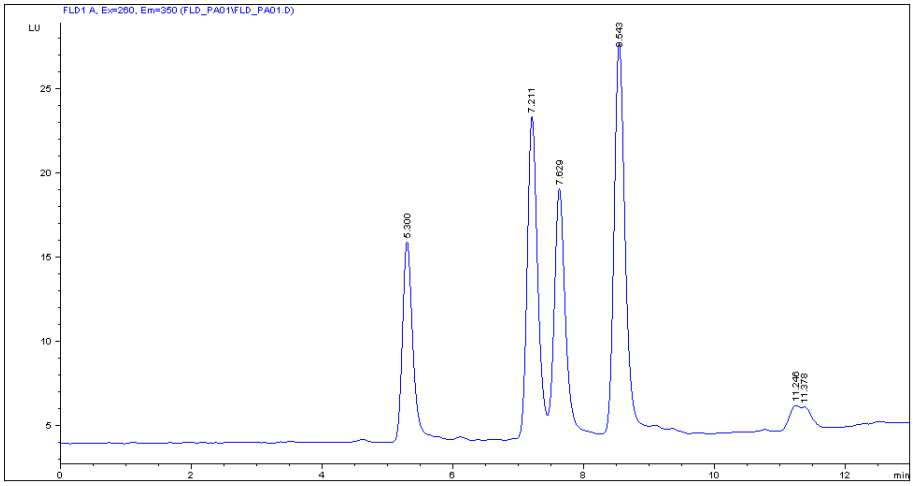


Figure 35: Chromatogram from Emissions Scan

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

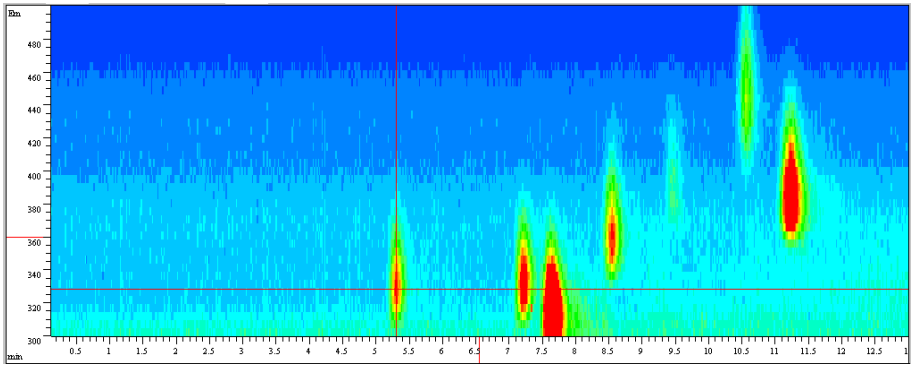


Figure 36: Isoabsorbance Plot from Emission Scan

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

Peak #	Time	Emission Wavelength
5	10.6 min	445 nm
6	11.23 min	385 nm

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

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

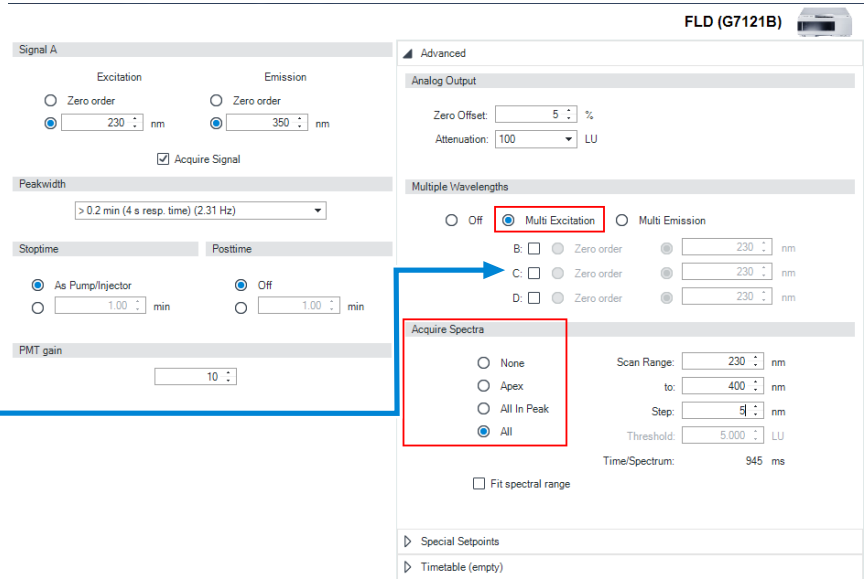


Figure 37: Detector settings for excitation scan

5 Wait until the baseline stabilizes. Start the run.

6 Load the signal.

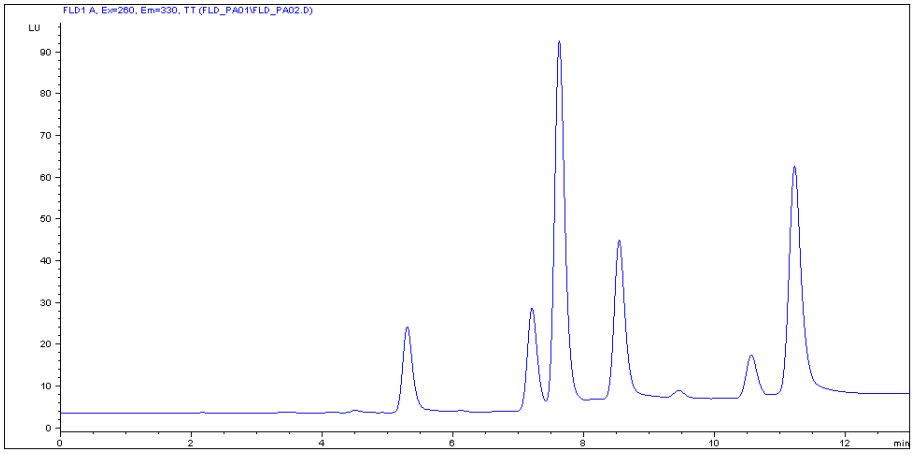


Figure 38: Chromatogram - Excitation Scan at Reference Wavelength 260/330 nm

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

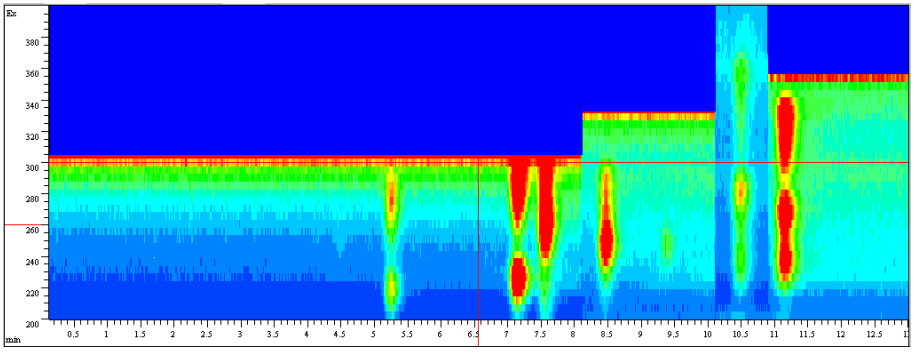


Figure 39: Isoabsorbance Plot - Excitation

The table below shows the complete information about emission (from Setting the Chromatographic Conditions, step 3 on page 99) and excitation maxima.

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

Peak #	Time	Emission Wavelength	Excitation Wavelength
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.

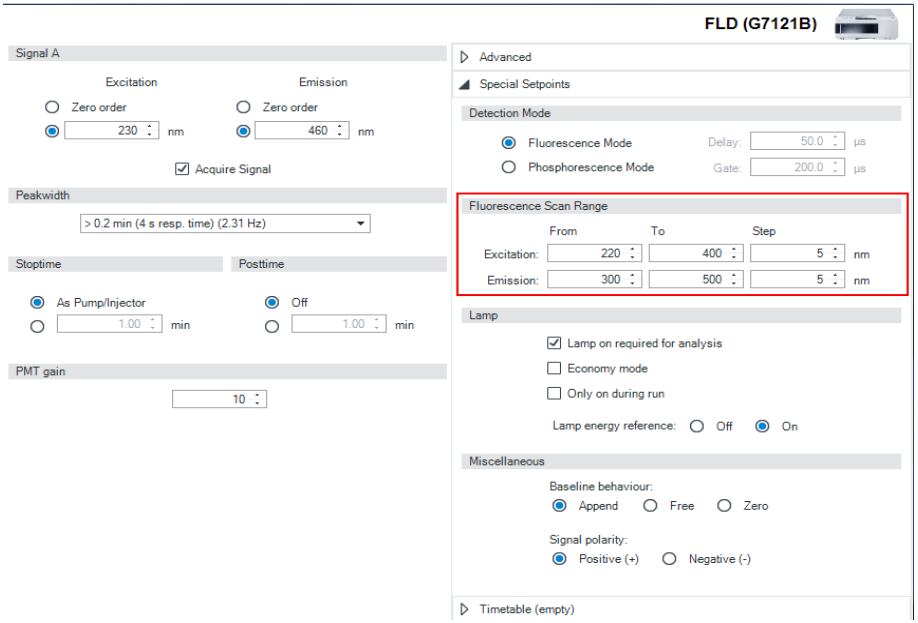


Figure 40: FLD special settings

4 Define a data file name and take a fluorescence scan. After the scan is completed, the isoabsorbance scan results appear, see Figure 41 on page 104.

NOTE A low background will improve the signal-to-noise, see also Reducing Stray Light on page 123.

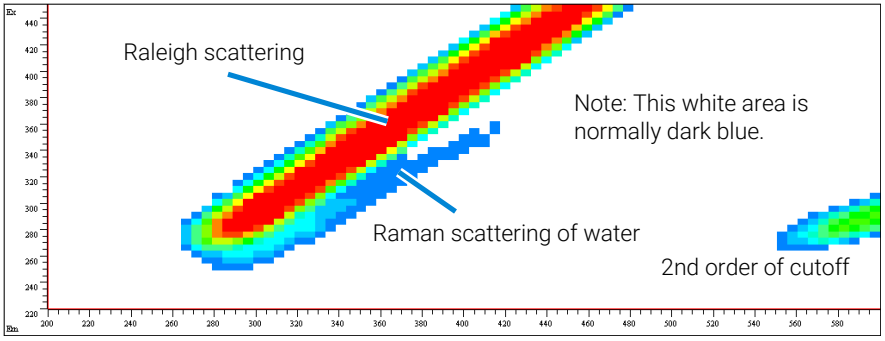


Figure 41: Fluorescence Scan of Water

Optimization Overview

NOTE Some features (e.g. spectrum acquisition, multi-wavelength detection) described in this chapter are not available on the 1260 Infinity III Fluorescence Detector (G7121A).

NOTE

PMT Gain Test
The PMT Gain test is not available in the Agilent CDS (OpenLAB CDS, OpenLAB CDS Chemstation Edition, OpenLAB EZChrom Edition) and G4208A Instant Pilot. The PMT Gain test is available in the Agilent Lab Advisor since B.02.04 [093]. The PMT Gain Test can be found under Instrument Control > Special Commands .

1. Setting the right PMT value

   For most applications a setting of 10 is adequate (see Finding the Best Signal Amplification on page 113). The FLD 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 (see Selecting the Best Response Time on page 121). 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 (see Finding the Best Wavelengths on page 111). 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 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 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 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.

How to Collect Spectra with Modes SPECTRA ALL IN PEAK and APEX SPECTRA ONLY

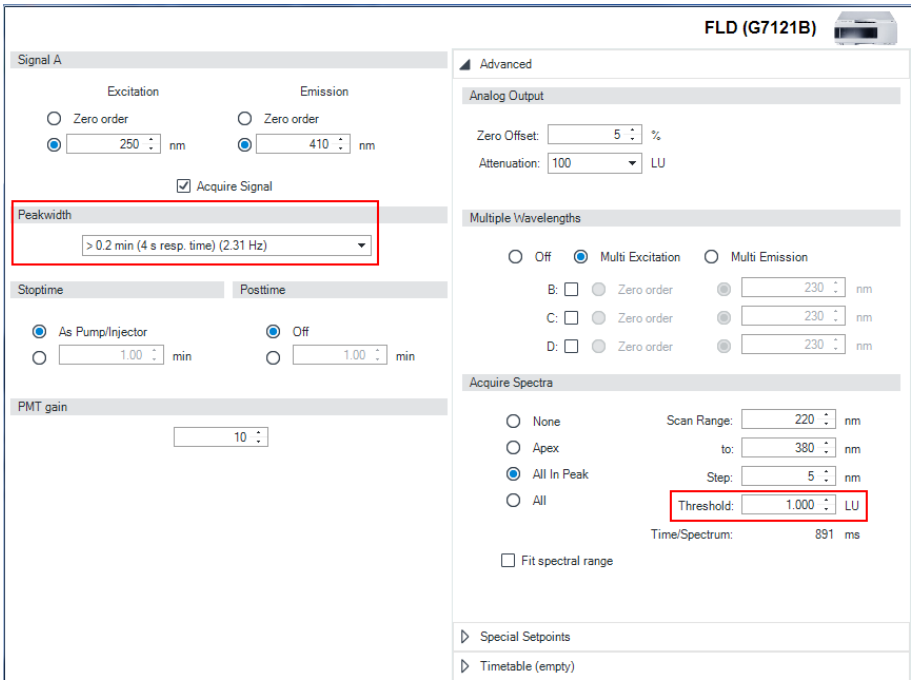
This section describes how to overcome a malfunction in the current implementation of the Agilent OpenLAB CDS with the Fluorescence Detector (G7121B). In these modes spectra intermittently are not collected into the data file.

The peak triggered spectra acquisition in the FLD is controlled by two parameters - THRS (Threshold) and PDPW (PeakDetector PeakWidth). In addition the parameter PKWD (Detector PeakWidth) only influences the filtering of the chromatogram.

1 Set the parameters THRS, PDPW and PKWD accordingly to the current chromatogram.

Best results for collecting peak triggered spectra are gathered when PDPW is 2 steps lower than PKWD, see Table 19 on page 122.

2 In the FLD's setup-screen there are 2 fields to enter the PKWD **Peakwidth (Responsetime)** and the THRS **Threshold** (visible when **Multi-EX** or **Multi-EM** is selected). Defaults are: PKWD = 6 (0.2 min); THRS = 5.000 LU.



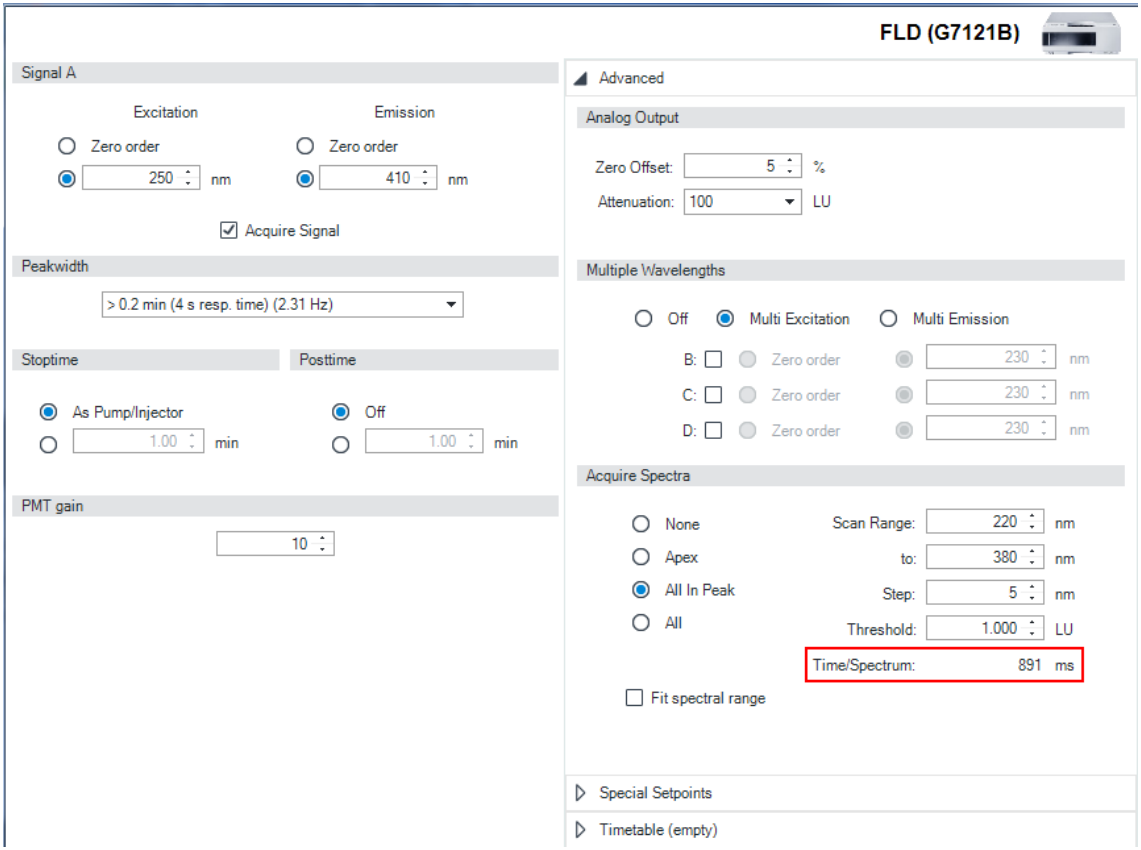
The selected values are fixed during the run.

THRS and PDPW influence the peak-triggered spectra-acquisition. You can change THRS in the FLD's setup-screen; PDPW can only be changed with the **Peakwidth**-field in the **Timetable**.

Notes:

- The peak-detection algorithm works best when a peak is reduced to 8 – 16 data points. The FLD collects the data points with an internal data rate of 74.08 Hz (= 13.50 ms) (1 signal only). The data reduction is only influenced by the PDPWparameter.

- The peak-detector works online on the current chromatogram. This means that begin/apex/end of a peak is recognized with delay. Additionally the points of spectra are sequentially acquired. This means that the acquisition of wide range spectra lasts much longer than the acquisition of a short-range spectrum. When you have a fast chromatography it is nearly impossible to collect a "clean" APEX-spectra: the first/last points of the spectra are acquired before/after you have the highest concentration in the detector's cell.
- How long the acquisition of single spectra lasts is shown in the FLD's setup screen.



Design Features Help Optimization

The module has several features you can use to optimize detection:

PMTGAIN	Amplification factor
LAMP	Flash frequency
RESPONSETIME	Data reduction interval

Check Performance Before You Start

Before you start you should check that your detector is performing according to the specifications published by 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 module 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 module 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 module 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 module scan of orthophthalaldehyde, a derivative of the amino acid alanine, (Figure 42 on page 112) shows a maximum between 220 nm and 240 nm.

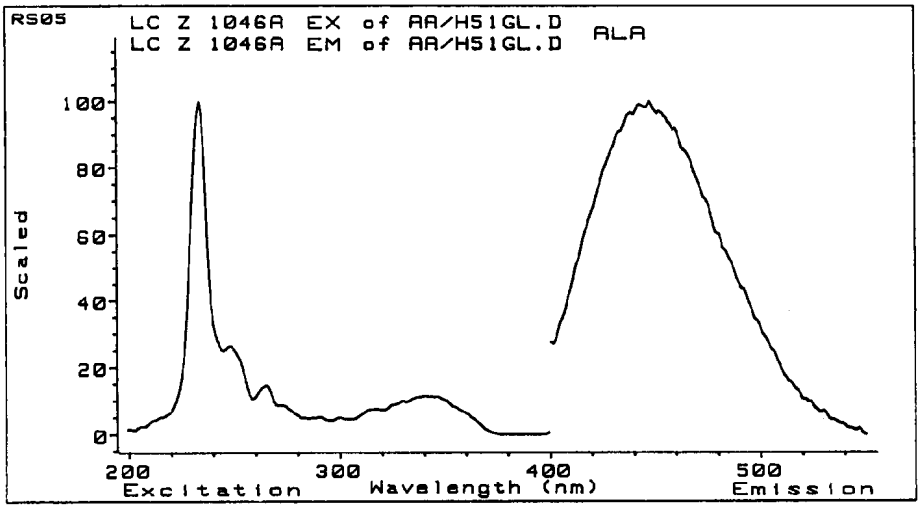


Figure 42: Scan Orthophthalaldehyde Derivative of Alanine

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 .

In Figure 43 on page 113 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.

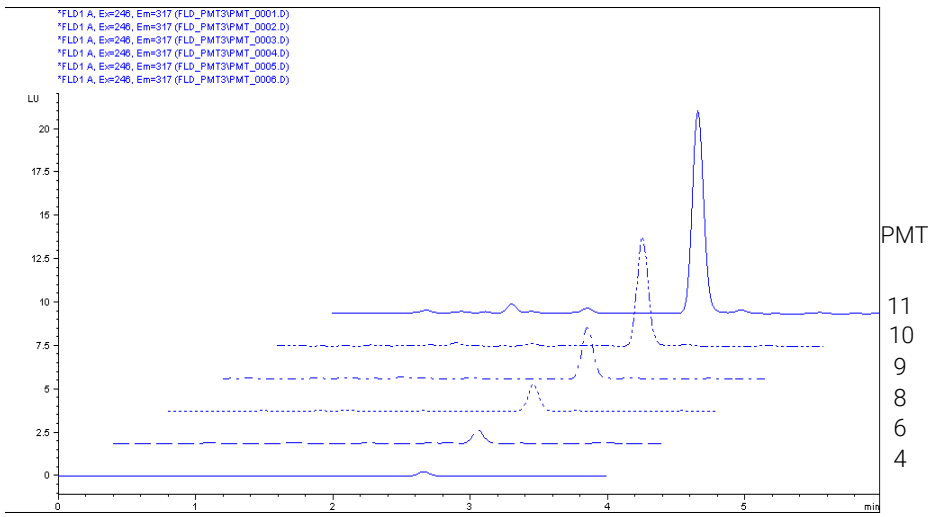


Figure 43: Finding Best PMTGAIN for Biphenyl

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.

FLD Scaling Range and Operating Conditions

When using different FLD

- The signal height of individual G7121 FLD modules may exceed the recommended signal range 0 – 100 LU. Under certain circumstances this could lead to clipped peaks.
- Different G7121 FLD modules show different signal heights with identical methods. This is not a problem in general but could be inconvenient when operating more than one G7121 FLD in the lab.

Both scaling issues can be resolved. Refer to Optimize the PMT-Gain-Level on page 114.

Optimize the PMT-Gain-Level

Start the PMT-Gain-Test with your operating conditions (used method parameter, EX-/EM-wavelength, solvent, flow rate, …). The resulting PMT value will give you the best signal to noise performance with the maximum usable signal range for this method and this specific instrument. For another FLD this PMT level may vary (based on the individual PMT-Gain-Test).

The figure below demonstrates the impact of changing the PMT Gain.

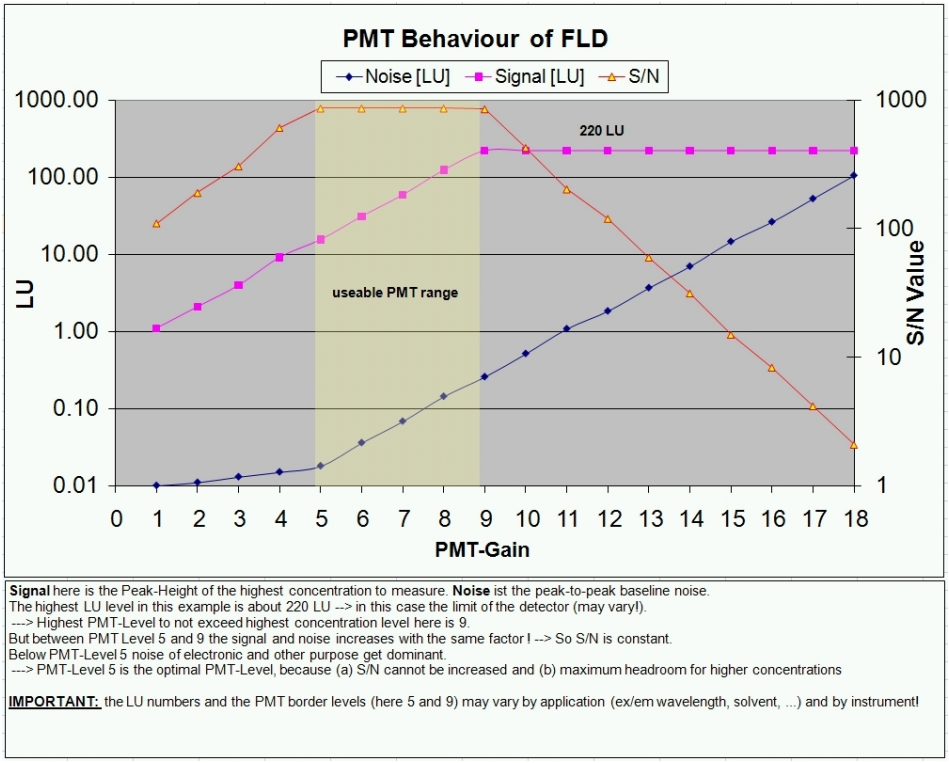


Figure 44: PMT Gain Behavior

In this example the maximum output is around 220 LU and further increase of the PMT (above 9) results in a signal overload (clipping) and drop of signal to noise value.

1 Set the PMT-Gain Level

Now check with your highest concentration amount, that your highest peak does not clip or overflow.

- If this check is ok, you finished the PMT-Gain-Level optimization. Continue with "Set your Luminescence Units in LU".
- If the check shows that the highest concentration doesn't fit to the selected range (e.g. by clipping), you may decrease the sensitivity of your FLD by gradually decreasing the PMT-Level by 1 to get roughly half the signal height by each step. Be aware that by that step you will lose sensitivity at low signal levels (LOD).

2 Set your Luminescence Units in LU.

If you are not satisfied with the LU output level of the detector or if you want to align the output of multiple instruments with different output levels you can scale each instrument output.

The recommended setting of the FLD is around 100 LU for the highest peak height to get optimum signal to noise and signal range. Lower LU values normally do not influence the performance of the instrument if PMT-Gain Test was executed fine.

For analog out less than 100 LU is optimum to get best analog signal performance with the default attenuation of 100 LU/ 1 V. Adapt your LU setting such that your maximum signal level under default attenuation is between 50 to 80 LU (analog output equivalent to 500 mV to 800 mV).

After correct PMT Setting you can scale any instrument to your favorable LU level. We recommend not exceeding around 100 LU. The parameter of choice is called 'Scale factor' and is applicable by the local controller, the Instant Pilot (B.02.07 or later).

In case older revisions are used, the 'Scale factor' can be entered using the command line of

- Agilent ChemStation: `PRINT SENDMODULE$(LFLD,"DMUL x.xx")`
- Instant Pilot: Service Mode – FLD, then type `DMUL x.xx` and press SEND.
- LAN/RS-232 Firmware Update Tool: via Send Instruction menu: `DMUL x.xx`
- Agilent Lab Advisor Software: via Instruction menu: `DMUL x.xx`

This setting is resident to the instrument even for firmware updates and is independent of the software environment.

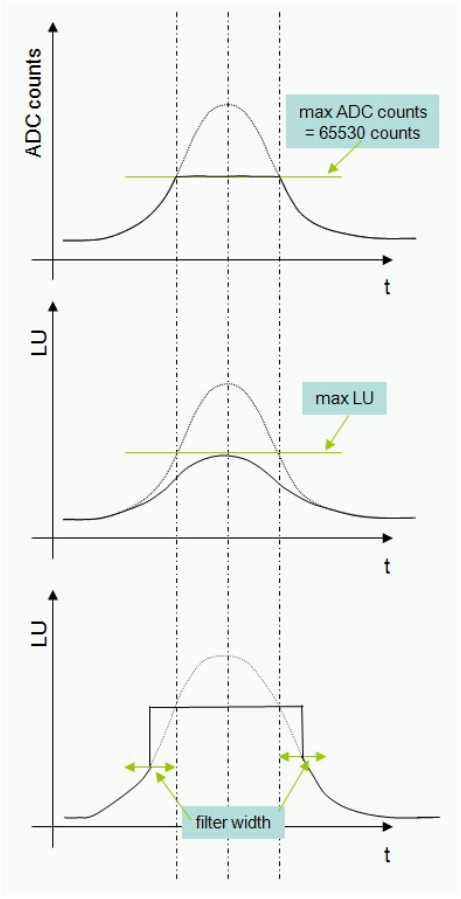
The level of LU is no measure of instrument sensitivity! At the lowest concentration limit (limit of detection), the signal to noise (for example by Raman S/N Test) is the only measure that can accurately be used to compare chromatograms and results and to confirm the performance of the instrument.

For low background and highest sensitivity keep the flow cell clean and always use fresh water to prevent biological background from native fluorescence by algae and bacteria.

Visualization of ADC Limits

Overflow could be concealed by smoothing of a filter and thus not visible for the user. In the Agilent ChemStation, the "ADC overflow" event was only shown in the logbook.

This problem did only occur if the Peakwidth (Responsetime) parameter has been set similar or larger than the real width of the chromatographic peak.



Raw ADC counts

The measured light intensity is limited by the max range of the ADC-converter.

A filter smoothes the peak making it not clearly visible that the max intensity is reached. Also peak area and peak height are distorted which leads to poor linearity performance.

Note that "max LU" is not a fix number but depends on the intensity of the reference channel!

New implementation (with firmware A.06.11 or above)

While any sample value within the filter width is in state "ADC overflow" the max possible LU is displayed in chromatogram.

Note that "max LU" is slightly dependent on lamp drift and lamp noise but strongly dependent on the excitation wavelength.

As a result, the "ADC overflow" is visible as a real flat peak in the chromatogram showing the user, that the setting of the detector parameter (PMT gain or the concentration of the solution) is set to high.

NOTE

The transfer of methods 1:1 from one FLD to another may result into the above "ADC overflow" problem. For details see FLD Scaling Range and Operating Conditions on page 114.

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

Best sensitivity can be expected with "**no economy**", see Figure 45 on page 119.

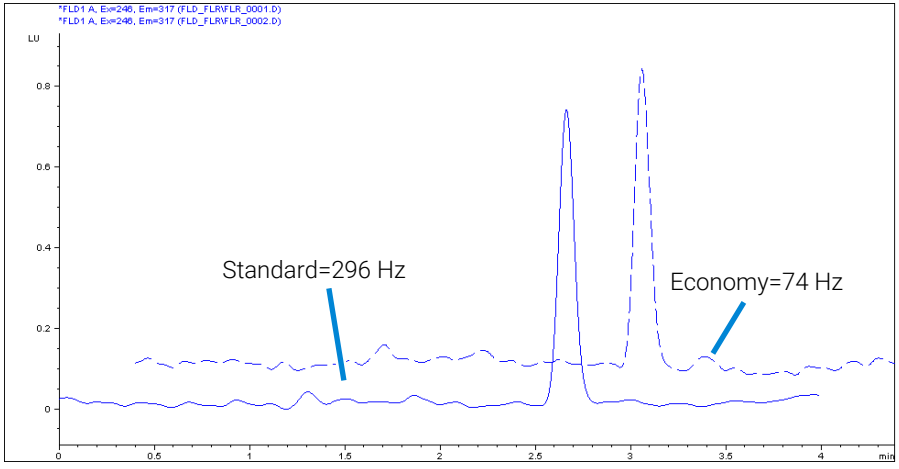


Figure 45: Xenon Flash Lamp Frequency

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 46 on page 121.

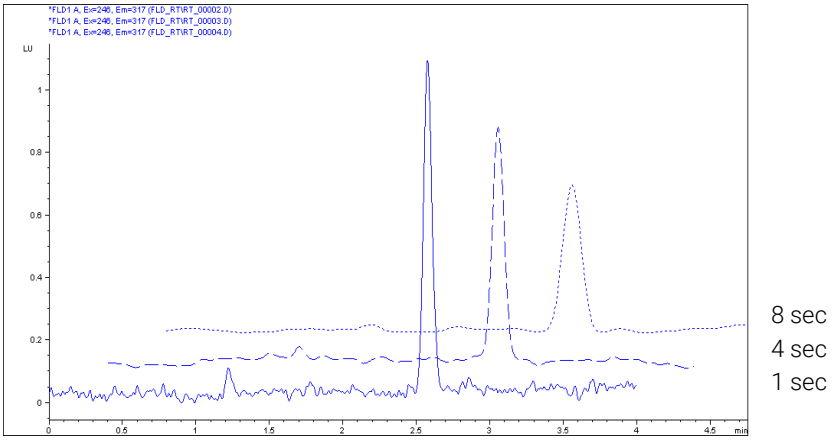


Figure 46: Finding Best Response Time

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

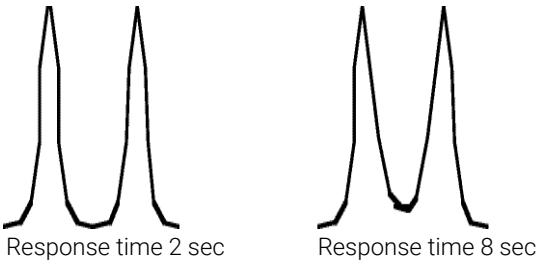


Figure 47: Separation of Peaks using Responsetime

Peakwidth Settings

NOTE Do not use peak width shorter than necessary.

Peakwidth enables you to select the peak width (response time) for your analysis. The peak width is defined as the width of a peak, in minutes, at half the peak height. Set the peak width to the narrowest expected peak in your chromatogram. The peak width sets the optimum response time for your detector. The peak detector ignores any peaks that are considerably narrower, or wider, than the peak width setting. The response time is the time between 10 % and 90 % of the output signal in response to an input step function.

Limits: When you set the peak width (in minutes), the corresponding response time is set automatically and the appropriate data rate for signal and spectra acquisition is selected as shown in the table below.

Table 19: Peakwidth Setting

Peak Width		Data Rate		
At half height [min]	**Response [sec]**	**Hz**	**ms**	
> 0.0016	0.016	148.15	6.9	G7121B
< 0.003	0.03	74.07	13.5	G7121A/B
> 0.003	0.06	37.04	27.0	
> 0.005	0.12	37.04	27.0	
> 0.01	0.25	37.04	27.0	
> 0.025	0.5	18.52	54.0	
> 0.05	1.0	9.26	108.0	
> 0.1	2.0	4.63	216.0	
> 0.2	4.0	2.31	432.0	
> 0.4	8.0	1.16	864.0	

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 48 on page 124).

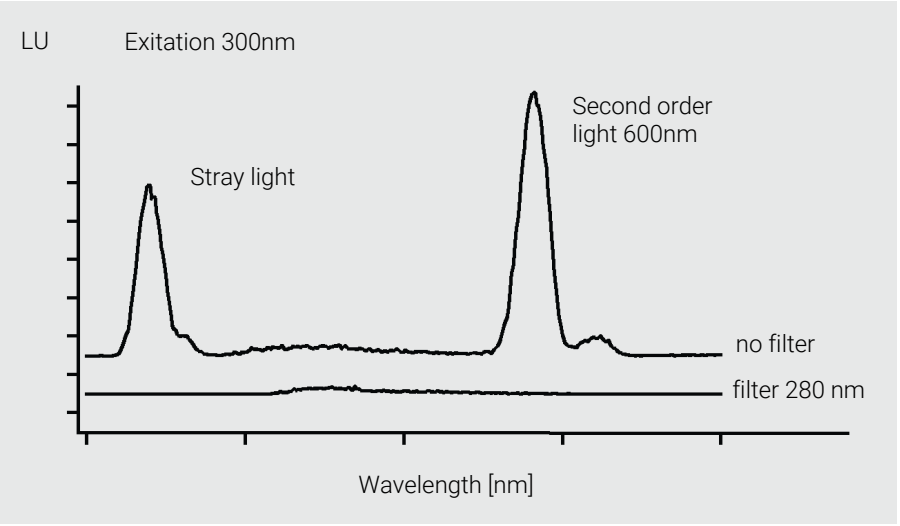


Figure 48: Reducing Stray Light

6 Diagnostics and Troubleshooting

This chapter gives an overview of the maintenance, troubleshooting, and diagnostic features available.

Diagnostic Features

This section gives an overview of the diagnostic features available.

User Interfaces

InfinityLab Assist

InfinityLab Assist provides you with assisted troubleshooting and maintenance at your instrument.

If the system in use supports the InfinityLab Assist, follow the instructions provided. Else, the preferred solution is to use Agilent Lab Advisor Software.

- Depending on the user interface, the available tests and the screens/reports may vary.
- The preferred tool for troubleshooting and diagnostics should be Agilent Lab Advisor Software, see Agilent Lab Advisor Software on page 164.
- Screenshots used within these procedures are based on the Agilent Lab Advisor Software.

Troubleshooting With HPLC Advisor

Baseline, Peak Shape, Pressure, Retention related issues, can be solved using the HPLC Advisor App. For more information, see Troubleshooting Reversed-Phase Chromatographic Techniques With HPLC Advisor.

If using an InfinityLab Assist, navigate to **Health** > **Troubleshooting** to help solve baseline, peak shape, pressure, and retention related issues.

Overview of Diagnostic Signals

The detector has several signals (internal temperatures, signal, reference signal) that can be used to diagnose problems. These can be

- baseline problems deriving from the Xenon flash lamp / trigger pack assembly
- wander / drift problems due to temperature changes

These signals can be used in addition to the normal baseline signal to determine if there is any correlation to the temperature or voltage/current of the lamp.

Baseline Problems Deriving from Xenon Flash Lamp/ Trigger Pack Assembly

Problem Description

With the low noise of the Detector, the Xenon flash lamp or the trigger pack assembly may cause periodic baseline instabilities at the end of lamp life.

Lamp instabilities come in many different forms. The period may vary from a few seconds to hours.

Problem Verification

Perform the following steps to diagnose whether the lamp is the cause of the baseline instability:

1 Ensure that the detector has been properly warmed up, see Overview of Diagnostic Signals on page 128.

2 Flush the flow cell with water and start runs without sample injections (blank runs).

3 Use a restriction capillary to remove any influence of the column.

4 If you see instabilities, stop the flow and compare.

   a If there are no instabilities, it's detector related and a replacement of the trigger pack assembly (first) and lamp (second) is required.

   b If there are still instabilities, check the system in front of the FLD (for example for air leaks).

Wander/Drift Problems Due to Temperature Changes

The most frequent cause of ambient temperature fluctuation are unstable laboratory air conditioning systems. Other causes include direct sunshine or drafts from open doors and windows. These temperature changes may cause baseline wander which can make reproducible integration of trace level peaks difficult or impossible.

Problem Verification

Use the diagnostic signals to find a correlation to ambient changes.

- Normal signal [LU]
- Reference signal [LU]
- Board Temperature (instrument curves)

Monitoring of Additional Signals

The detector has several signals (internal temperatures, Reference only) that can be used for diagnosing problems. These can be

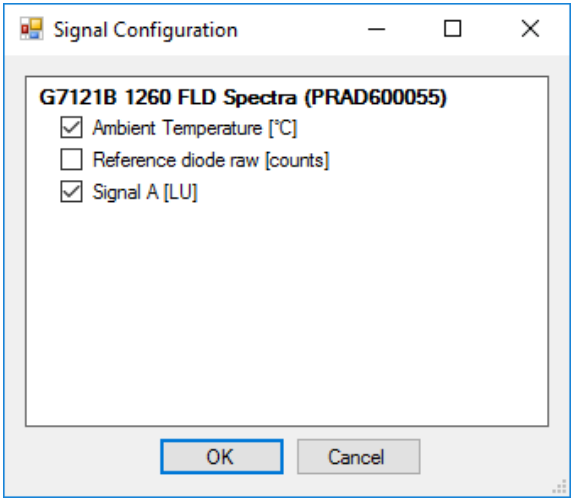
- baseline problems deriving from lamp,
- wander / drift problems due to temperature changes.

For intermittent baseline problems the board temperature should be monitored to see the impact of it to the signal.

NOTE Measurements should be taken over 60 – 120 min minimum to include long-term effects (e.g. cycle of air condition systems).

Agilent Lab Advisor

- Open Instrument Control.
- Select Main signal and board temperature.
- Scale the signals.



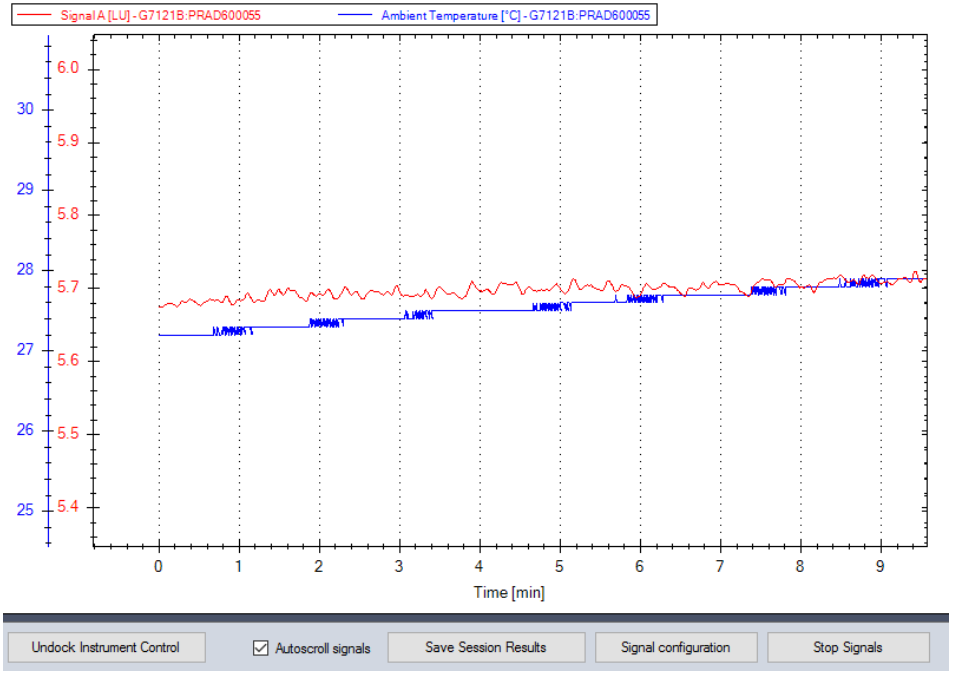


Figure 49: Signal plus board temperature (Agilent Lab Advisor)

The board temperature (short-/long term) is also stored in the module's memory and can be retrieved via the Module Info - Signals.

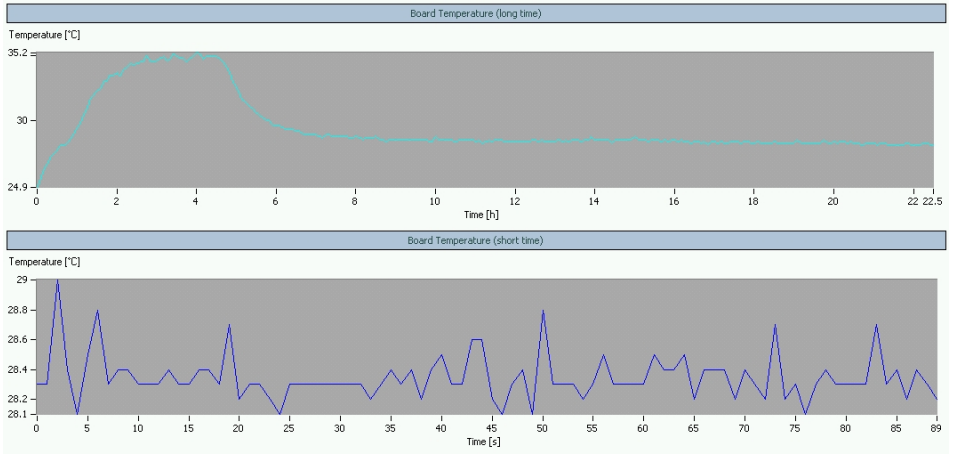


Figure 50: Internal board temperature (Agilent Lab Advisor - Module Info - Signals)

Overview of Available Tests and Tools

Tests and Calibrations in Agilent Lab Advisor

Use the tests and diagnostic features provided in the Agilent Lab Advisor software to check if your module is working correctly.

For further details, refer to the Agilent Lab Advisor software help files.

Maintenance and Troubleshooting Tools of the Module

This chapter describes the tests for the module.

Introduction

All tests are described based on the Agilent Lab Advisor Software B.02.08. Other user interfaces may not provide any test or just a few.

For details on the use of the interface refer to the interface documentation.

The Lab Advisor shows the available test under Service & Diagnostics.

Table 20: Interfaces and available test functions

Interface	Comment	Available Function
Agilent Lab Advisor	For functions, refer to Function Overview Lab Advisor • Table 21 on page 133	Available functions depend on Product Level (Basic - Advanced - FSE)
Agilent ChemStation	No tests available Adding of temperature/lamp signals to chromatographic signals possible	Temperature ambient
Agilent Instant Pilot	Some tests are available	• Intensity • WL Calibration • Spectra Scan (Tools) • Module Info (Tools) • Diagnostic

Table 21: Function Overview Lab Advisor Basic/Advanced (G7121A/B)

	Product Level	
Tests		
- D/A Converter Test	Basic	Advanced

	Product Level	
- Dark Current Test	Basic	Advanced
- Intensity Test	Basic	Advanced
- Raman ASTM Signal/Noise Test	Basic	Advanced
- Wavelength Accuracy Test	Basic	Advanced
Calibrations		
- Wavelength Calibration	Basic	Advanced
Tools		
- Diagnostic Buffers	Basic	Advanced
- Module Info	Basic	Advanced
- Test Chromatogram	Basic	Advanced
- Spectral Scan (G7121B only)	Basic	Advanced
Controls		
-Advanced Method Parameters		
- Analog Output 1 Offset [% Full Scale]		Advanced
- Configuration		
- Remote Pulse Duration [s] *	Basic	Advanced
- Analog Output 1 Range		Advanced
- Control		
- Lamp	Basic	Advanced
- Response Multiply Factor		Advanced
- Conversions		
- 7121A * (G7121B only)	Basic	Advanced
- Method Parameters		
- Set Flashlamp mode		Advanced
- Set PMT Gain		Advanced
- Set Exitation Wavelength		Advanced
- Set Emission Wavelength		Advanced
- Set Data Rate [Hz]		Advanced
- Module Information		
- Identify Module	Basic	Advanced

	Product Level	
- Special Commands		
- Detector Reset	Basic	Advanced
- Clear Error	Basic	Advanced
Statemachines		
- UV Lamp		Advanced
Signals		
- Signal A [LU] (G7121A allows just Signal A)		Advanced
- Signal B [LU]		Advanced
- Signal C [LU]		Advanced
- Signal D [LU]		Advanced
- Photomultiplier raw [counts]		Advanced
- Reference diode raw [counts]		Advanced
- Ambient Temperature [°C]		Advanced
EMF Counters		
- Flash Lamp Life Time	Basic	Advanced

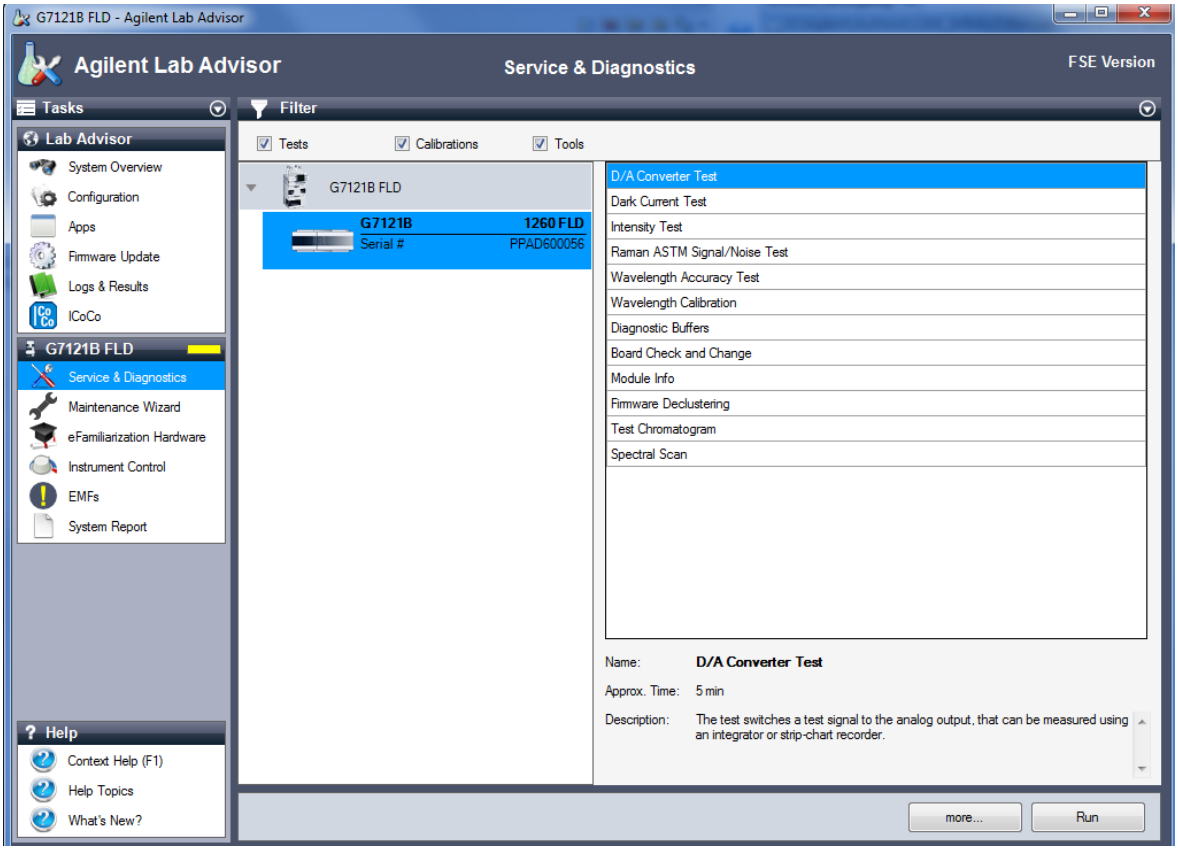


Figure 51: The Lab Advisor shows the available tests

Diagram of Light Path

The light path is shown in Figure 52 on page 137.

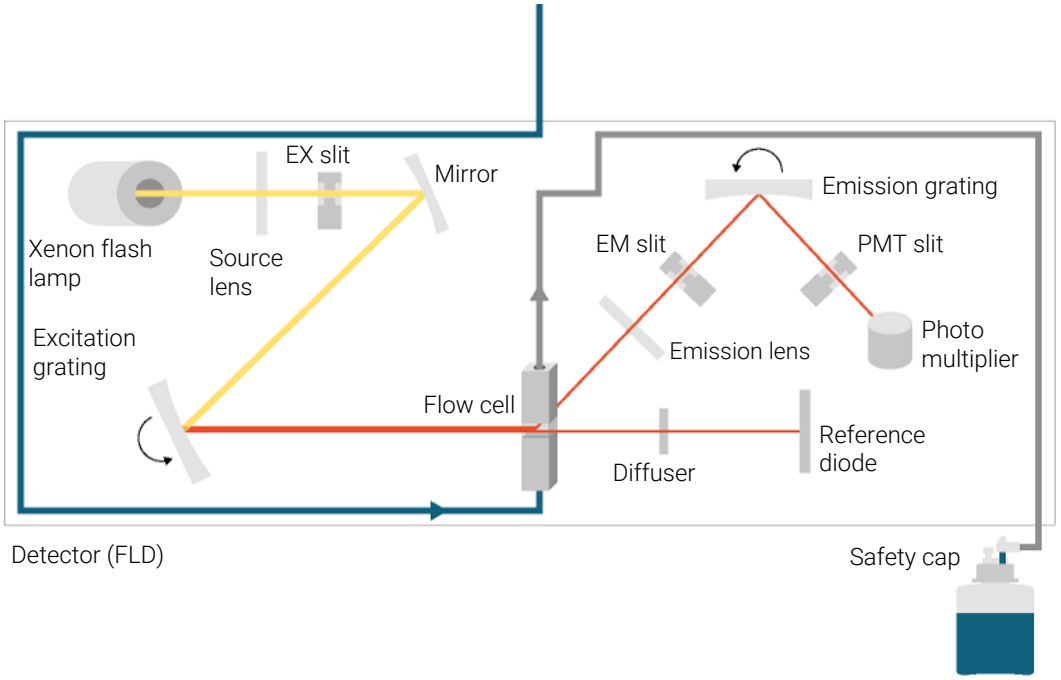


Figure 52: Schematic of the light path

Lamp Intensity Test

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

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

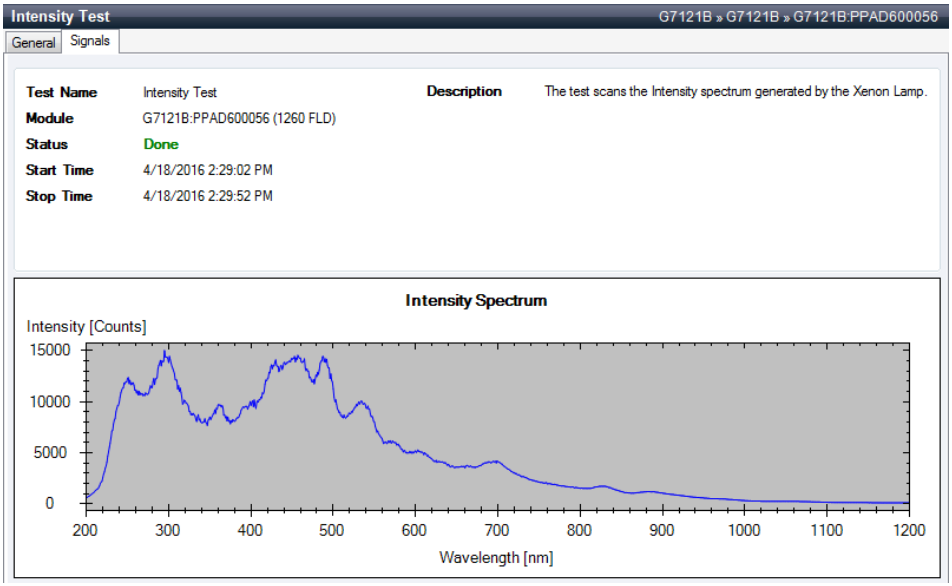


Figure 53: Lamp Intensity Test (Agilent Lab Advisor)

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.

Available tables:

Lamp Intensity History

Date	Reference Diode Counts at 250nm	Reference Diode Counts at 350nm	Reference Diode Counts at 450nm	Reference Diode Counts at 600nm
01/28/2013 14:15	2143	2994	7166	3150
12/17/2012 13:55	10	9	9	9
12/17/2012 13:55	9	9	11	10
12/17/2012 13:49	10	11	10	10
10/29/2012 16:48	388	2120	5776	2766
12/08/2011 10:39	88	1004	1227	935
12/06/2011 11:31	576	2155	5532	2679

Figure 54: Lamp Intensity History (Agilent Lab Advisor under **Module Info**)

Raman ASTM Signal-to-Noise Test

This test verifies the Raman ASTM signal-to-noise for the FLD detectors.

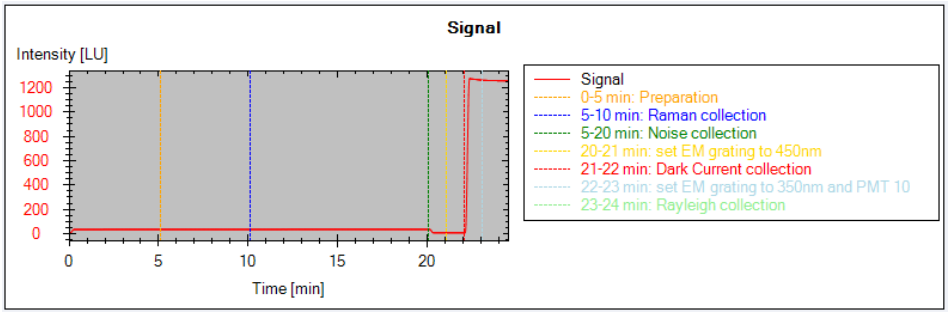


Figure 55: Raman ASTM Signal-to-Noise Test (Lab Advisor)

Depending on the version of the detector the specification has changed.

Table 22: Raman ASTM Signal-to-Noise Specification

Instrument	SNR Specification Raman / Dark	SNR Specification Dual WL
G7121A	500 / 3000	
G7121B	500 / 3000	300

Conditions: Standard flow cell (G1321-60005, G5615-60005), flow of 0.25 mL/min of water.

NOTE The **Dark** and **Dual WL** values are just additional specifications. Only the **Raman** value is used for the standard instrument checkout.

NOTE The specification single wavelength at signal can be measured with the Agilent Lab Advisor. All others (not used for standard checkout) have to be set up manually with the information from Table 25 on page 140 and Table 26 on page 141.

Table 23: Raman Signal-to-Noise Test Conditions

Duration	approximately 23 minutes
Standard Flow Cell	G1321-60005, G5615-60005
Solvent	LC grade water, degassed
Flow rate	0.25 mL/min
Specification (single wavelength at signal)	>500 (according to settings in Table 24 on page 140)
Specification (single wavelength at background)	>3000 (according to settings in Table 25 on page 140)
Specification (dual wavelength)	>300 (according to settings in Table 26 on page 141)

Table 24: Settings for Single Wavelength Specifications (at signal)

Time	EX	EM	PMT	Baseline
0	350	397	12	Free
20.30	350	450	12	Free

Table 25: Settings for Single Wavelength Specifications (at background)

Time	EX	EM	PMT	Baseline
0	350	450	14	Free
20.30	350	397	14	Free

Table 26: Settings for Dual Wavelength Specifications (Multi-EM Scan)

Time	EX	EM_A	EM_B	Spectra	From	To	Step	PMT	Baseline	Fit Spectra
00.00	350	397	450	None	280	450	10	12	Free	OFF
20.30	350	450	450	None	280	450	10	12	Free	OFF

Formulas for the Raman ASTM S/N value (see Figure 56 on page 141 for details):

$$\text{SNR\_Raman} = \frac{\text{mean\_raman}\,(ex = 350, em = 397) - \text{mean\_background}\,(ex = 350, em = 450)}{\text{noise\_raman}\,(ex = 350, em = 397)}$$

$$\text{SNR\_Dark} = \frac{\text{mean\_raman}\,(ex = 350, em = 397) - \text{mean\_background}\,(ex = 350, em = 450)}{\text{noise\_background}\,(ex = 350, em = 450)}$$

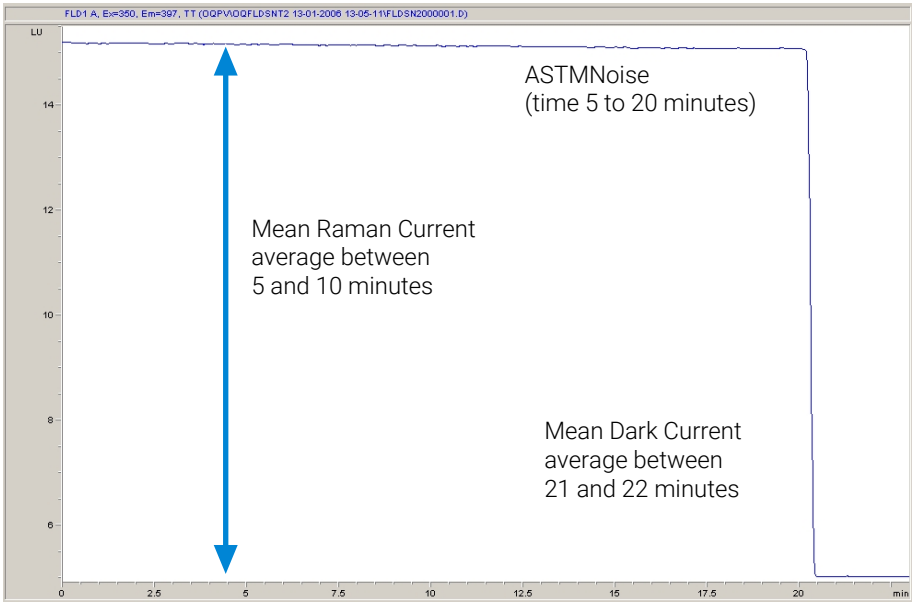


Figure 56: Raman ASTM signal/noise calculation

Using the Agilent Lab Advisor

1 Set up the HPLC system and the Lab Advisor.

2 Flush the flow cell with clean bi-distilled water.

3 Start the test in the Lab Advisor.

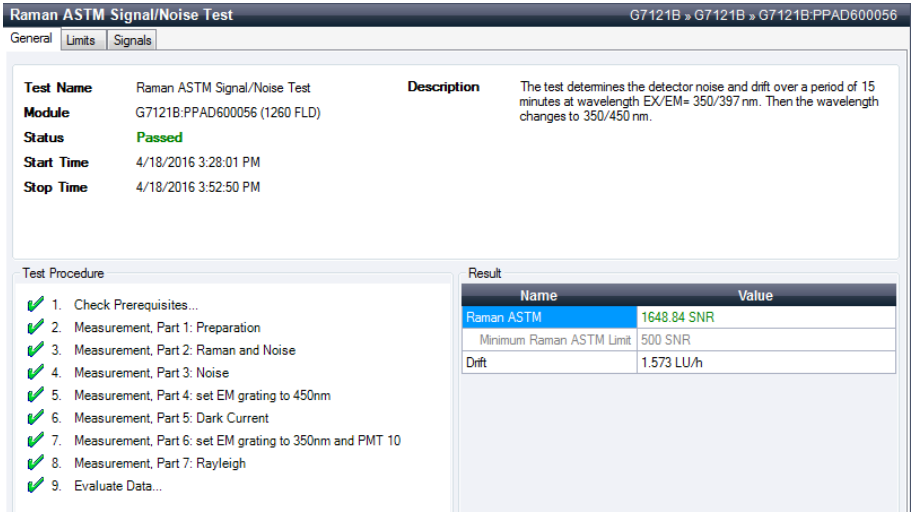
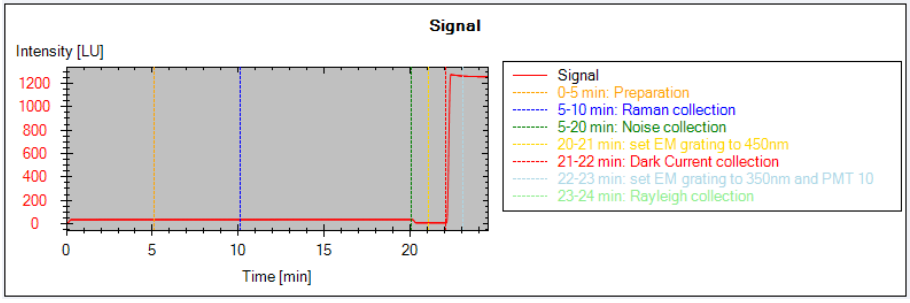


Figure 57: Raman ASTM Signal-to-Noise Test (Agilent Lab Advisor)



In case of failing this test (as shown above) see Interpretation of the Results on page 143.

Interpretation of the Results

If the test shows low Raman values, check for:

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

Wavelength Accuracy Test

Using the Agilent Lab Advisor

1 Set up the HPLC system and the Agilent Lab Advisor.

2 Flush the flow cell with clean bi-distilled water.

3 Turn on the FLD lamp.

4 Run the Wavelength Accuracy Test.

5 The FLD will change into the multi-excitation mode with emission wavelength at 397 nm and scan in the range of the expected maximum of 350 nm ± 20 nm.

As result, the maxima should be found at 350 nm ± 3 nm, see following figure.

The FLD will change into the multi-emission mode with excitation wavelength at 350 nm and scan in the range of the expected maximum of 397 nm ± 20 nm.

As result, the maxima should be found at 397 nm ± 3 nm, see following figure.

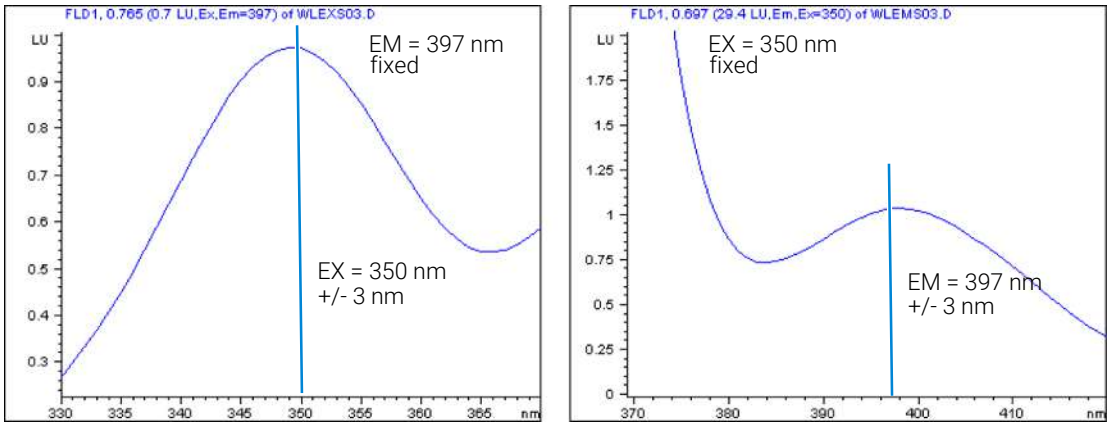


Figure 58: Excitation and Emission Spectrum (expected results)

NOTE

If the plots do not have a maximum around EM=397 nm and EX=350 nm (± 3 nm) the test fails. See Interpretation of the Results on page 146.

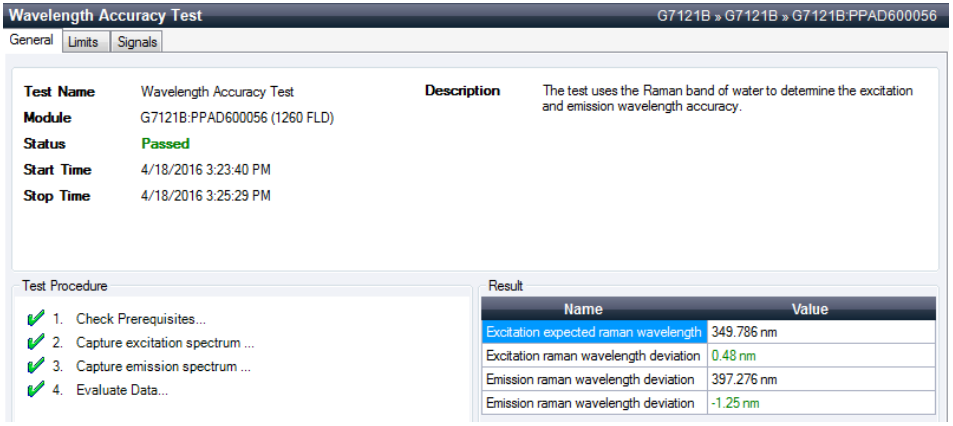


Figure 59: Wavelength Accuracy Test with Lab Advisor

If the test fails observe the maxima of the EX or EM side under the **Signals** tab.

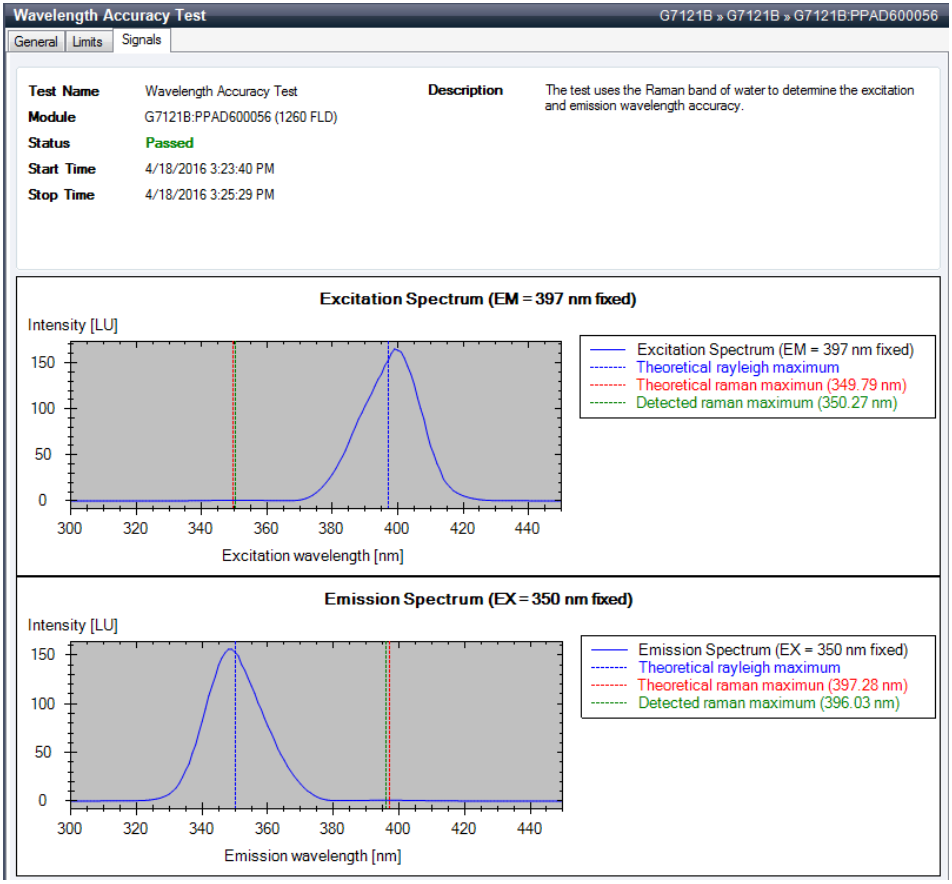


Figure 60: Example of good EX/EM maxima

If the plots do not have a maximum around EX=397 nm and EX=350 nm (±3 nm) the test fails, see figure below. Refer to Interpretation of the Results on page 146.

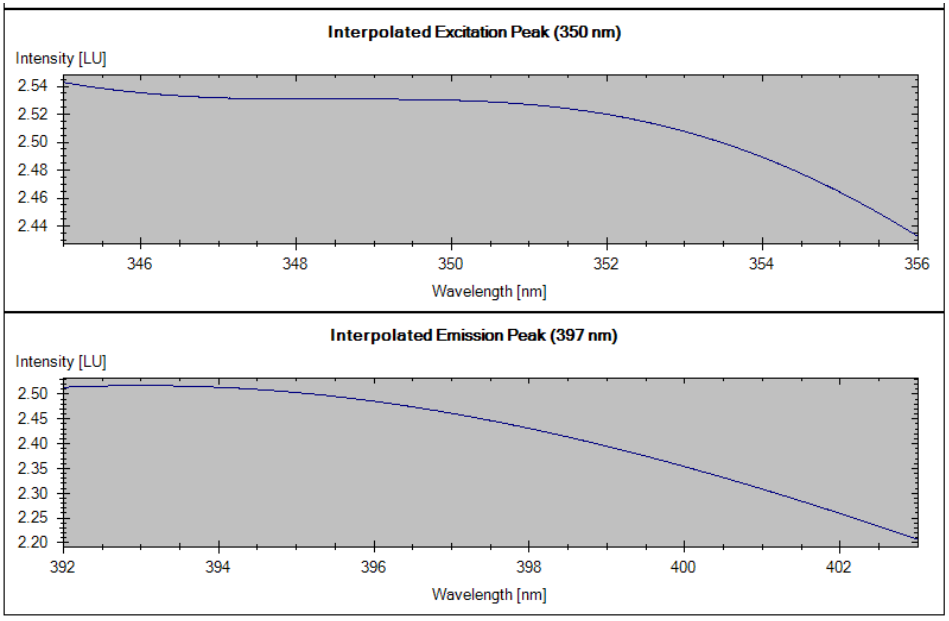


Figure 61: Example of bad EX/EM maxima (no maximum found)

Interpretation of the Results

If the test fails, check for:

- correctly positioned flow cell,
- clean flow cell (flush with isopropanol and clean bi-distilled water),
- no air bubble(s) (check via fluorescence scan or visual check of cell/cuvette),
- solvent inlet filter (may create air bubbles in flow cell).
- check optical path for contamination (service)
- check alignment of lamp / trigger pack assembly (service)
- perform a Wavelength Calibration

Wavelength Verification and Calibration

NOTE

New instruments are shipped with a flow cell installed and have a factory wavelength calibration.
If a flow cell is used other than the installed/shipped flow cell:
verify that the installed flow cell is fixed very tight,
perform a wavelength calibration using Glycogen (part of FLD Calibration Kit (G7121-68001)).

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

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

NOTE

A complete wavelength calibration is not always required. In most cases a quick wavelength accuracy verification is sufficient enough, see Table 27 on page 147.

Table 27: Reasons for doing a Verification or Calibration

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

Wavelength Calibration Process

NOTE Prior to a wavelength calibration, a wavelength accuracy verification should be performed, see Wavelength Accuracy Test. If the deviation is more than ±3 nm, the wavelength calibration should be done as described in Wavelength Calibration Procedure on page 149.

NOTE The duration of the wavelength calibration is about 20 minutes plus setup time for the calibration sample and system. Depending on the maximum intensity found during this scan, the PMT gain will be changed automatically and requires an additional 1 minute per scan.

Table 28 on page 149 shows the steps performed during the wavelength calibration.

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

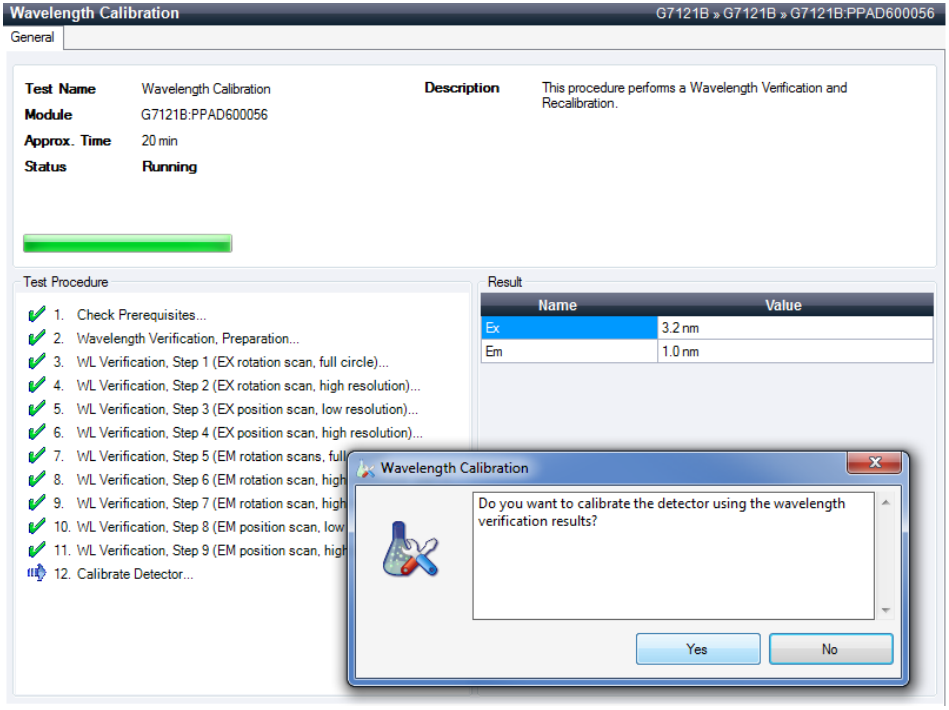


Figure 62: Wavelength Calibration (Agilent Lab Advisor)

Table 28: Wavelength Calibration Steps

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

NOTE

Variable times means that they could be a little bit longer.
When the lamp is off, the calibration process will stop within the first two steps with " Wavelength Calibration Failed" , see Wavelength Calibration Failed on page 189.

If you encounter calibration problems:

1. Check for air bubbles in the flow cell.
2. Flush the flow cell with isopropanol.
3. Change the water.

Wavelength Calibration Procedure

When

- If application requires, or see Table 28 on page 149.

Tools required

Qty.	p/n	Description
1		Laboratory balance

Parts required

Qty.	p/n	Description
1	G7121-68001	FLD Calibration Kit contains parts below
1	5063-6597	Calibration Sample, Glycogen
1	5190-1539	Syringe 5mL with Luer-Lock
1	9301-0407	Syringe, External Valve adapter, SST
1	5190-5111	Syringe filter, 0.45 µm, 100/pk
1	0100-1516	Finger-tight fitting PEEK, 2/pk

NOTE

New instruments are shipped with a flow cell installed and have a factory wavelength calibration.
If a flow cell is used other then the installed/shipped flow cell:
verify that the installed flow cell is fixed very tight,
perform a wavelength calibration using Glycogen (part of FLD Calibration Kit (G7121-68001)).

1 Preparation of the Glycogen Calibration Sample.

NOTE

The calibration solution needs to be prepared freshly. Once dissolved, Glycogen is only useable for 24 hours. Using water for calibration is not supported!

a Final concentration needs to be 1 mg/mL. To prepare 25 mL of the calibration solution, you have to use 25 mg of the Glycogen sample (a tolerance of ±20% is not critical).

b Weigh in the Glycogen into a volumetric flask.

c Fill 20 mL of distilled water (fresh, clean) into the volumetric flask and shake.

d Sonicate for 15 minutes, wait 5 minutes, fill up to 25 mL with water and shake again.

2 Preparation of the Flow Cell.

a Flush the flow cell with water.

b Remove the inlet capillary from the flow cell.

c Remove the outlet tubing from the flow cell and install a short (less than 5 cm) waste tubing instead. This helps avoiding unwanted syphoning effects.

d Take the syringe and fix the needle to the syringe adapter.

e Suck about 2.0 ml of the calibration sample into the syringe.

f Keep the syringe in a horizontal position.

g Remove the needle.

h Add the filter to the syringe and eject the calibration sample to waste (through the filter). This will ensure removing any particles from the filter.

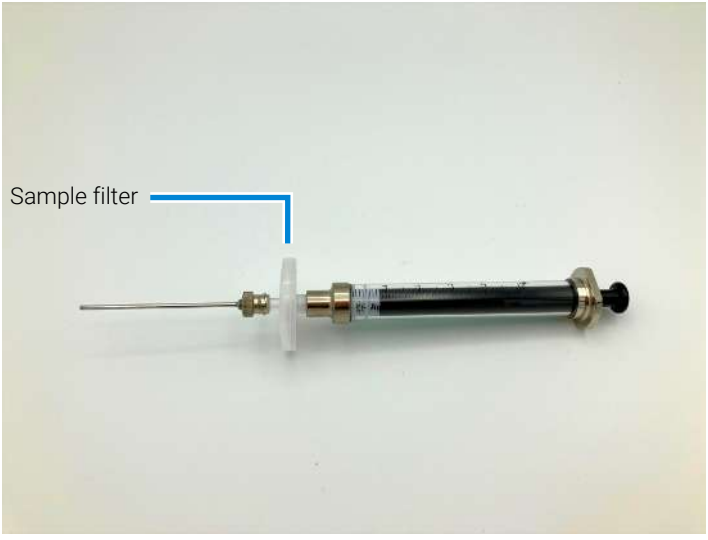


Figure 63: Syringe with Sample Filter

i Remove the filter.

j Take the syringe and fix the needle to the syringe.

k Suck about 4 mL of the calibration sample into the syringe.

l Keep the syringe in a horizontal position.

m Remove the needle.

n Add the filter to the syringe and fit the needle to the filter.

o Lift the needle tip and carefully eject approximately 0.5 ml to remove air out of the syringe.

p Add the PEEK fitting to the needle tip and fix both at the flow cell inlet. Avoid injecting any air bubble.

NOTE Do not inject the calibration sample without the sample filter.

q Slowly inject about 2 ml and wait for about 10 seconds to inject another 0.5 ml. This will assure that the cell is filled properly.

Additional hints for calibration:

- Do not forget to reset the grating calibration in LabAdvisor.
- Check that the cell has been fully inserted and fixed tightly.
- Prepare a little more calibration solution, so all glassware can be rinsed thoroughly prior to using.
- Never fill the syringe with the filter on.
- Never empty the syringe completely.
- With a 1mL glass syringe with Luer-lock, two to three injections of Glycogen might be required. Using a 5 mL glass syringe with Luer-Lock simplifies the procedure drastically lowers the risk of injecting air.
- After the final injection, leave the syringe in the in-port and keep it in a horizontal position.

3 Wavelength Calibration.

a From the user interface start the FLD wavelength calibration.

- Agilent Lab Advisor (preferred option): Under **Instrument Control** > **Special Commands** press the **Reset grating calibration** button, then go for **Service & Diagnostics** > **Wavelength Calibration**
- Instant Pilot (G4208A): **Maintenance** > **FLD** > **Calibration**

NOTE If the wavelength calibration process fails, refer to Wavelength Calibration Failed on page 189.

b If a deviation is displayed, press **Yes** to adjust to new values. The history table will be updated.

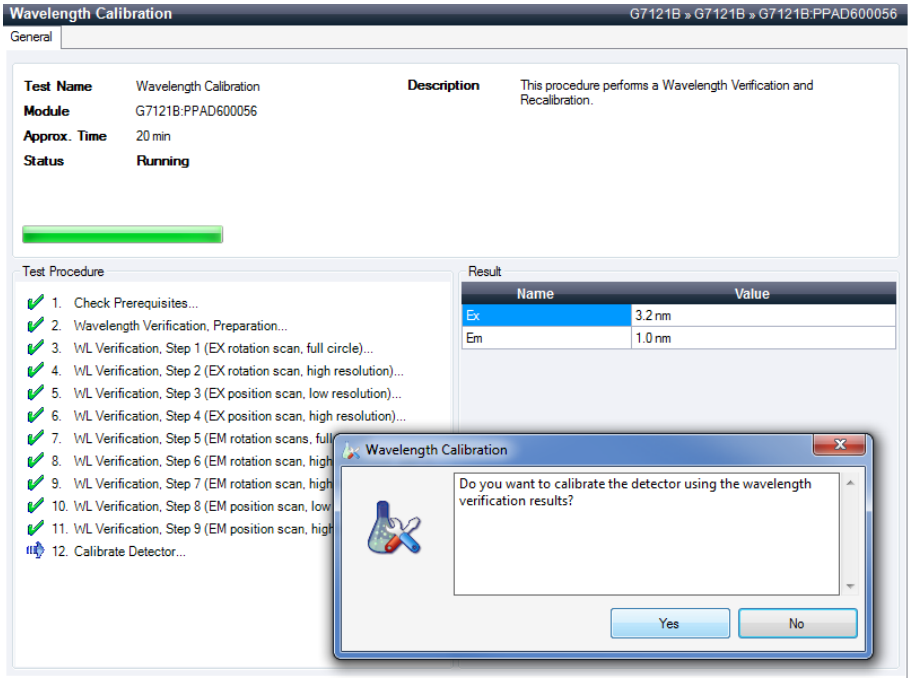


Figure 64: Wavelength Calibration (Agilent Lab Advisor)

WL Calibration History

Date	Deviation of Excitation	Deviation of Emission
02/11/2010 12:54	0.3	-1.6
02/09/2010 12:22	0.0	0.0
02/09/2010 11:48	13.2	12.5
10/20/2009 10:41	-2.2	0.5
07/21/2009 13:41	23.2	-1.1
07/21/2009 12:22	0.1	0.1
07/21/2009 11:31	-19.7	-6.6
08/25/2006 12:05	-0.2	0.2
01/09/2006 16:02	-0.2	-0.1
01/09/2006 15:30	0.6	0.8

Figure 65: Calibration History (Agilent Lab Advisor, under Module Info)

NOTE

Restore flow connections and flush with fresh water at 1.5 mL/min - 2 mL/min for at least 15-20 min. Prolonged flushing (e.g. overnight flushing) at low flow rates will not be sufficient.

4 Verification using the "Wavelength Accuracy Test".

a Refit the capillary to the flow cell.

b Follow the procedure "Wavelength Accuracy Test".

Excitation and Emission Grating Resistance History

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

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

The history data contains the data/time information and the number of turns. The data/plot can be retrieved via the diagnostics and provides turn data over a length of time.

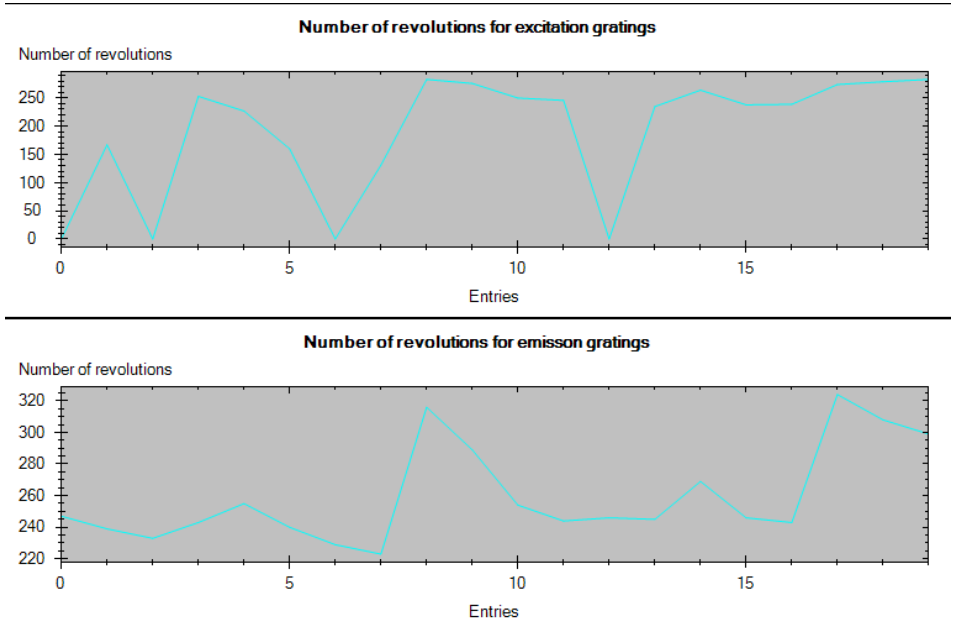


Figure 66: Resistance History (Agilent Lab Advisor under Module Info)

D/A Converter (DAC) Test

The detector provides analog output of chromatographic signals for use with integrators, chart recorders or data systems. The analog signal is converted from the digital format by the digital-analog-converter (DAC).

The DAC test is used to verify correct operation of the digital-analog-converter by applying a digital test signal to the DAC.

The DAC outputs an analog signal of approximately 50 mV (if the zero offset of the analog output is set to the default value of 5 %) which can be plotted on an integrator. A continuous square wave with an amplitude of 10 µV and a frequency of approximately 1 cycle/24 seconds is applied to the signal.

The amplitude of the square wave and the peak-to-peak noise are used to evaluate the DAC test.

When

- If the analog detector signal is noisy or missing.

Preparations

- Lamp must be on for at least 10 minutes. Connect integrator, chart recorder or data system to the detector analog output.

Running the test with Agilent Lab Advisor

1 Run the D/A Converter (DAC) Test (for further information see Online-Help of user interface).

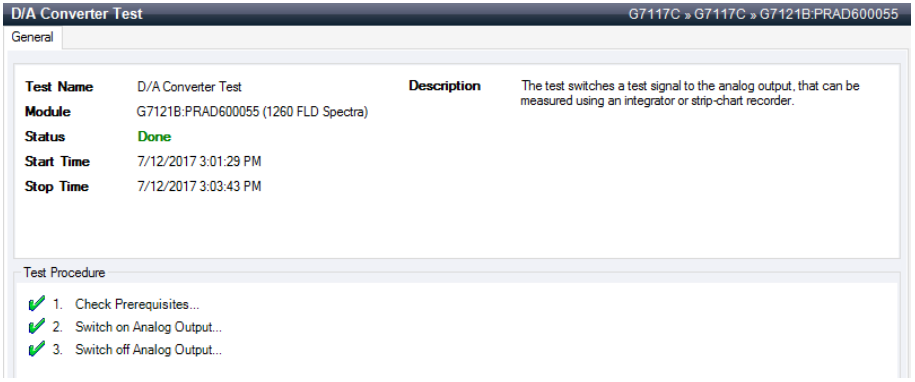


Figure 67: D/A Converter (DAC) Test

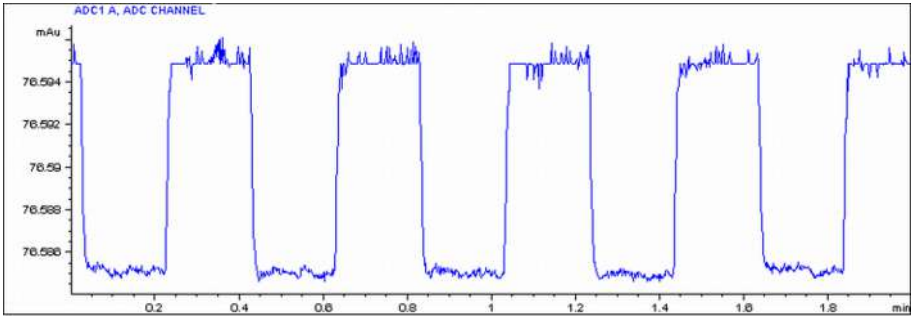


Figure 68: D/A Converter (DAC) Test – Example of Integrator Plot

Running the Test with Instant Pilot

The test can be started via the command line.

1 To start the test `TEST: DAC 1`

Reply: `RA 00000 TEST:DAC 1`

2 To stop the test `TEST:DAC 0`

Reply: `RA 00000 TEST:DAC 0`

D/A Converter Test failed

D/A Converter Test evaluation

The noise on the step should be less than 3 µV.

Probable cause		Suggested actions
1	Bad cable or grounding problem between detector and external device.	• Check or replace the cable.
2	Defective detector main board.	• Please contact your Agilent service representative.

Dark-Current Test

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

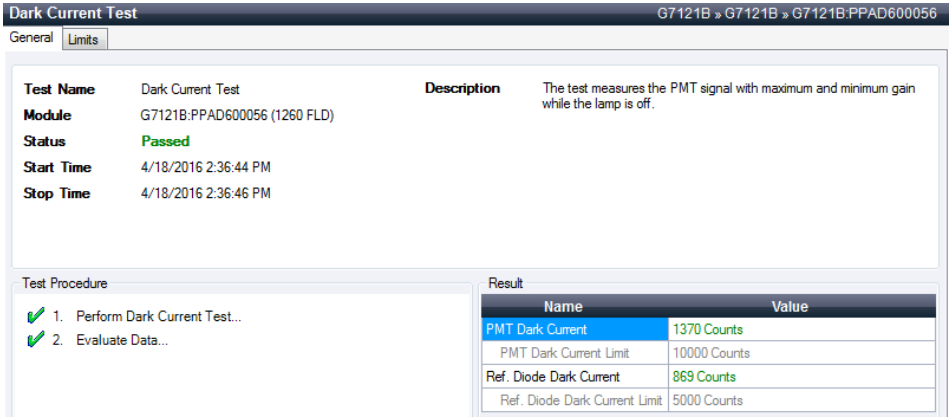


Figure 69: Dark-Current Test (Agilent Lab Advisor)

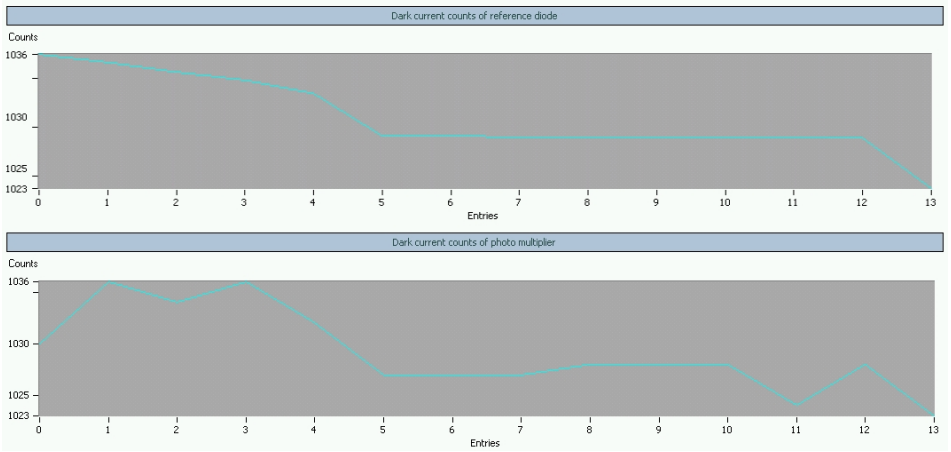


Figure 70: Dark Current History (Agilent Lab Advisor)

Dark-Current Test Failed

Probable cause		Suggested actions
1	Defective PMT.	• Exchange the PMT.
2	Defective reference diode or A/D converter.	• Exchange the FLF board.

Using the Built-In Test Chromatogram

This function is available from the Agilent ChemStation, Lab Advisor and Instant Pilot.

The built-in Test Chromatogram can be used to check the signal path from the detector to the data system and the data analysis or via the analog output to the integrator or data system. The chromatogram is continuously repeated until a stop is executed either by means of a stop time or manually.

NOTE

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

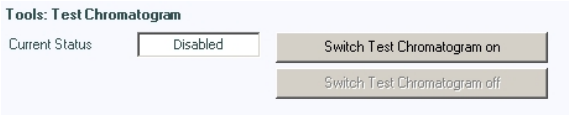
This procedure works for all Agilent 1200 Infinity detectors (DAD, MWD, VWD, FLD and RID). The example figure is from the RID detector.

Procedure using the Agilent Lab Advisor

1 Assure that the default LC method is loaded via the control software.

2 Start the Agilent Lab Advisor software (B.01.03 SP4 or later) and open the detector's **Tools** selection.

3 Open the test chromatogram screen



4 Turn the **Test Chromatogram** on.

5 Change to the detector's **Module Service Center** and add the detector signal to the Signal Plot window.

6 To start a test chromatogram enter in the command line: STRT

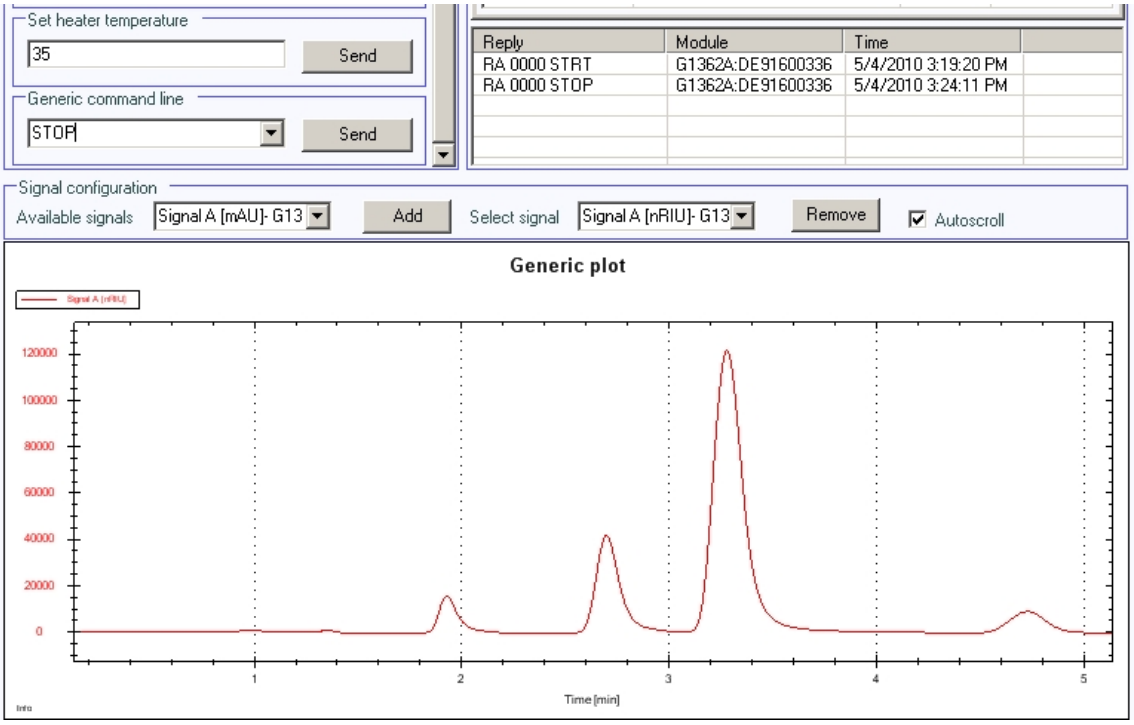


Figure 71: Test Chromatogram with Agilent Lab Advisor

7 To stop the test chromatogram enter in the command line: STOP

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

Available Tests vs User Interfaces

NOTE

Depending on the used interface, the available tests and the screens/reports may vary.
Preferred tool should be the Agilent Lab Advisor, see Agilent Lab Advisor Software on page 164.
Agilent Lab Advisor B.02.08 or later is required.
The Instant Pilot (G4208A) supports the G7121A/B with B.02.19 or later.

Screenshots used within these procedures are based on the Agilent Lab Advisor software.

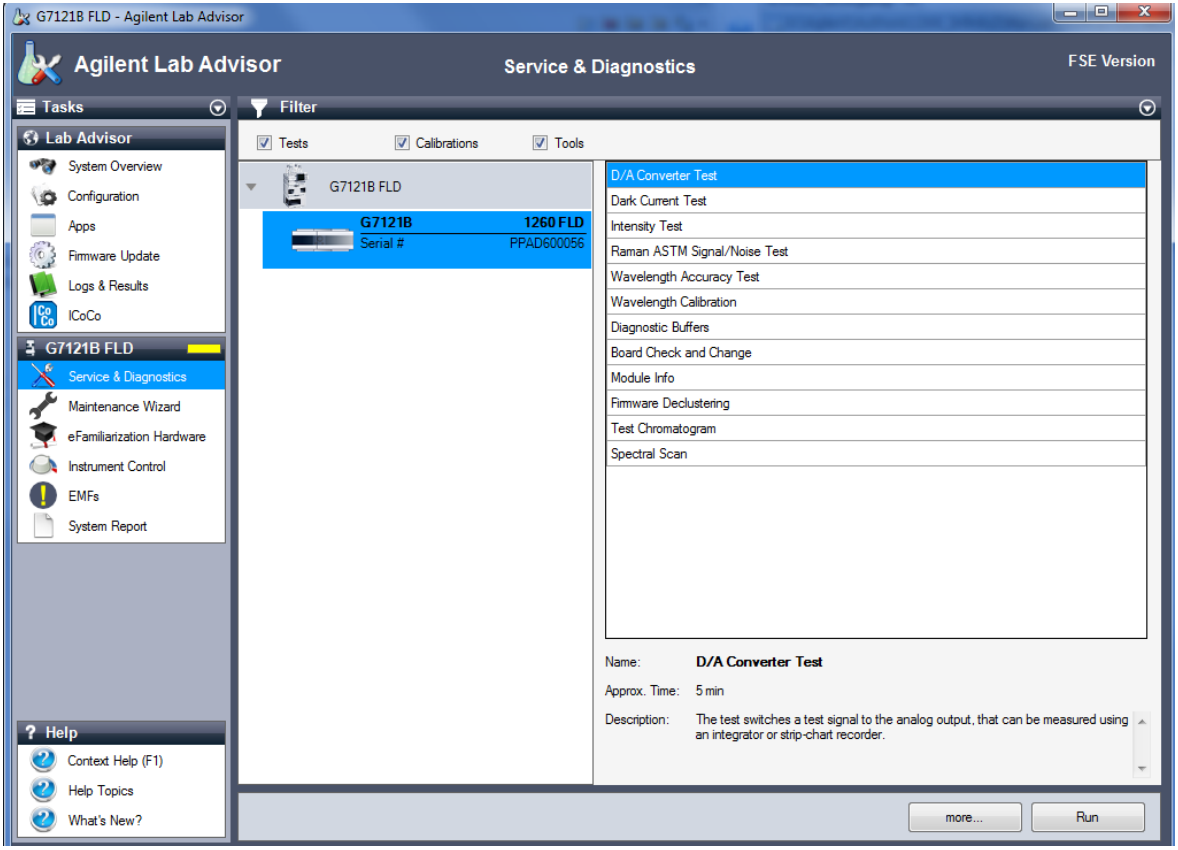


Figure 72: The Lab Advisor shows the available tests

Agilent Lab Advisor Software

The Agilent Lab Advisor Software (basic license, shipped with an Agilent LC pump) is a standalone product that can be used with or without a chromatographic data system. Agilent Lab Advisor helps to manage the lab for high-quality chromatographic results by providing a detailed system overview of all connected analytical instruments with instrument status, Early Maintenance Feedback counters (EMF), instrument configuration information, and diagnostic tests. With the push of a button, a detailed diagnostic report can be generated. Upon request, the user can send this report to Agilent for a significantly improved troubleshooting and repair process.

The Agilent Lab Advisor software is available in two versions:

- Lab Advisor Basic
- Lab Advisor Advanced

Lab Advisor Basic is included with every Agilent 1200 Infinity Series and Agilent InfinityLab LC Series instrument.

The Lab Advisor Advanced features can be unlocked by purchasing a license key, and include real-time monitoring of instrument actuals, all various instrument signals, and state machines. In addition, all diagnostic test results, calibration results, and acquired signal data can be uploaded to a shared network folder. The Review Client included in Lab Advisor Advanced makes it possible to load and examine the uploaded data no matter on which instrument it was generated. This makes Data Sharing an ideal tool for internal support groups and users who want to track the instrument history of their analytical systems.

The optional Agilent Maintenance Wizard Add-on provides an easy-to-use, step-by-step multimedia guide for performing preventive maintenance on Agilent 1200 Infinity LC Series instrument.

The tests and diagnostic features that are provided by the Agilent Lab Advisor software may differ from the descriptions in this manual. For details, refer to the Agilent Lab Advisor software help files.

Other Lab Advisor Functions

Instrument Control

The Instrument Control screen provides controls for each of the modules in the selected system. Module information and status are displayed; click to display the module's controls or to hide them.

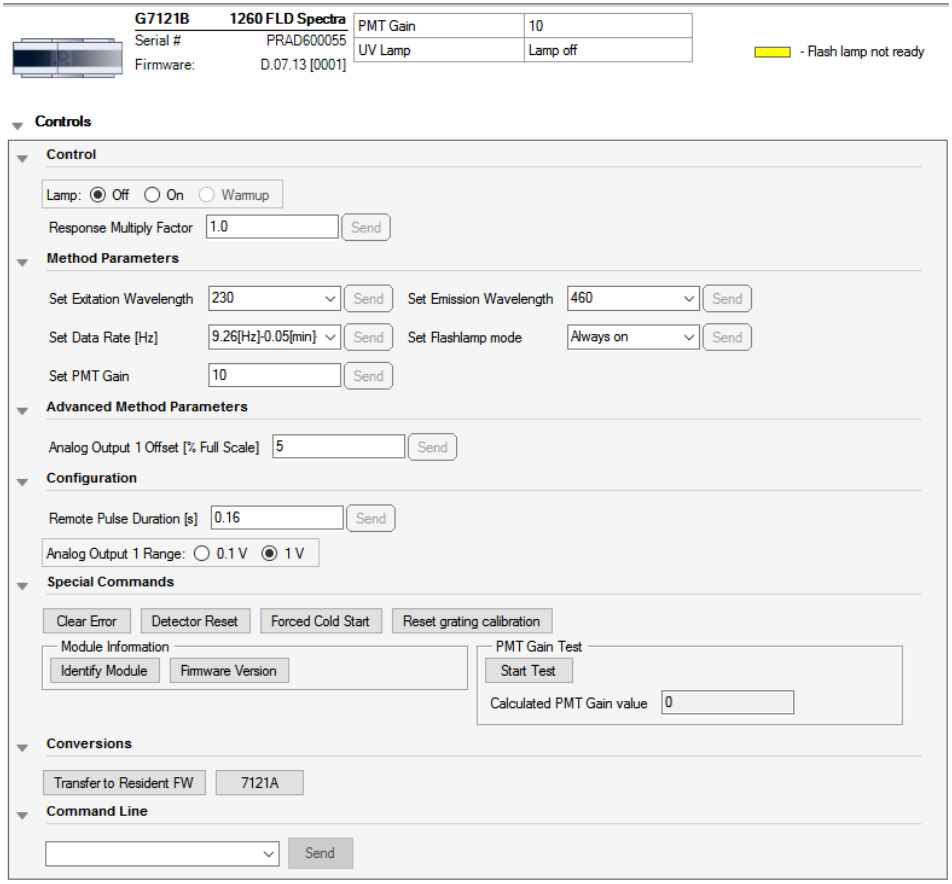
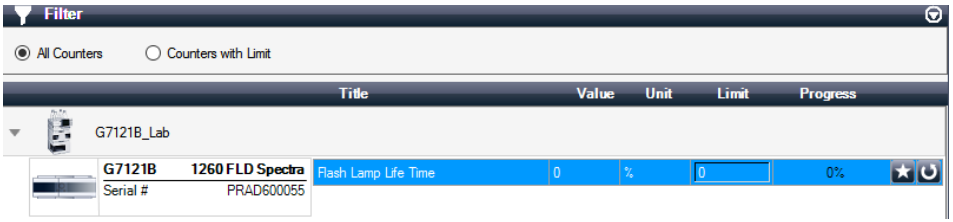


Figure 73: Instrument Control – Overview

EMF - Early Maintenance Feature

The EMFs screen allows you to view and manage the EMF counters for all modules in all systems.



Spectral Scan (G7121B only)

The Spectral Scan tool for the fluorescence detector (FLD) allows you to scan a spectrum over a specified wavelength range in a specified mode, and export the data to a csv (comma-separated values) file that can be used in other applications (for example, Microsoft Excel).

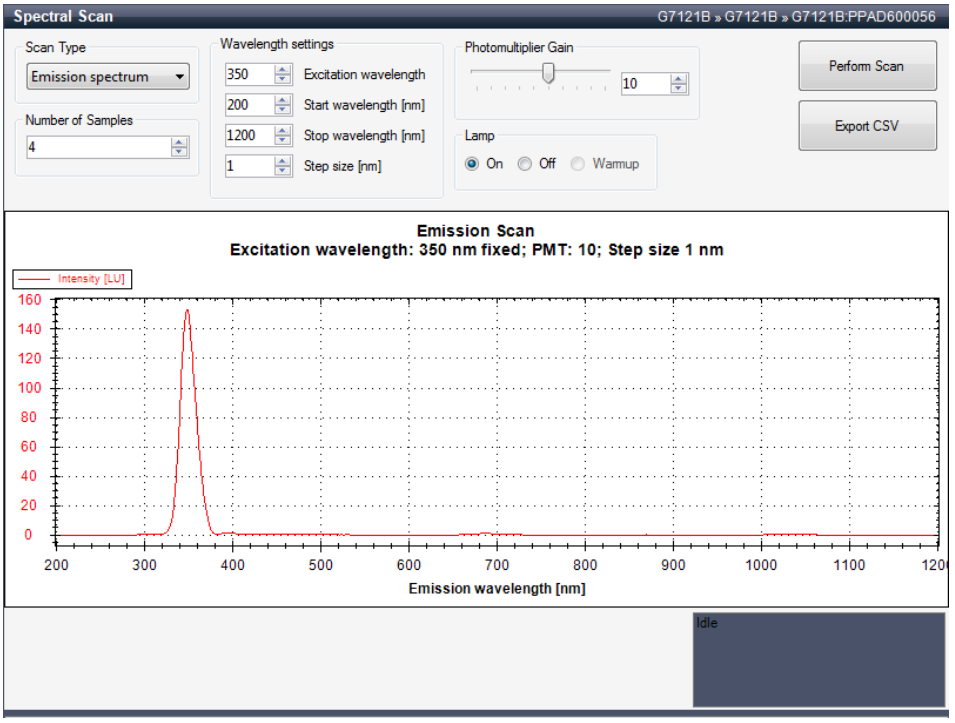


Figure 74: Spectral Scan

FLD Mode

- No multi WL: You specify an Excitation wavelength (Ex) and an Emission wavelength (Em). If you select No multi WL, you cannot set up a scan.
- Excitation Scan: You specify an Emission wavelength (Em) and the excitation scan range.
- Emission Scan: You specify an excitation wavelength (Ex) and the emission scan range.

Scan Parameters:

- Flash Lamp On: Switches on the FLD flash lamp.
- Sample Scan: Scans the sample spectrum over the specified wavelength range at the specified resolution and in the selected mode. You specify the wavelength range in the from and to fields, and the resolution in the step field. You select the mode in the FLD Mode section.
- Export Data Exports the selected data in csv format for use in other applications.

7 Error Information

This chapter describes the meaning of error messages, and provides information on probable causes and suggested actions how to recover from error conditions.

What Are Error Messages

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

If an error occurs outside a method run, other modules will not be informed about this error. If it occurs within a method run, all connected modules will get a notification, all LEDs get red and the run will be stopped. Depending on the module type, this stop is implemented differently. For example, for a pump, the flow will be stopped for safety reasons. For a detector, the lamp will stay on in order to avoid equilibration time. Depending on the error type, the next run can only be started if the error has been resolved, for example liquid from a leak has been dried. Errors for presumably single time events can be recovered by switching on the system in the user interface.

Special handling is done in case of a leak. As a leak is a potential safety issue and may have occurred at a different module from where it has been observed, a leak always causes a shutdown of all modules, even outside a method run.

In all cases, error propagation is done via the CAN bus or via an APG/ERI remote cable (see documentation for the APG/ERI interface).

If using the InfinityLab Assist, instrument errors will generate a notification. To view the probable causes and recommended actions for this error, click on Help button displayed on the notification.

General Error Messages

General error messages are generic to all Agilent series HPLC modules and may show up on other modules as well.

Timeout

Error ID: 62

The timeout threshold was exceeded.

Probable cause		Suggested actions
1	The analysis was completed successfully, and the timeout function switched off the module as requested.	• Check the logbook for the occurrence and source of a not-ready condition. Restart the analysis where required.
2	A not-ready condition was present during a sequence or multiple-injection run for a period longer than the timeout threshold.	• Check the logbook for the occurrence and source of a not-ready condition. Restart the analysis where required.

Shutdown

Error ID: 63

An external instrument has generated a shutdown signal on the remote line.

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

Probable cause		Suggested actions
1	Leak detected in another module with a CAN connection to the system.	• Fix the leak in the external instrument before restarting the module.
2	Leak detected in an external instrument with a remote connection to the system.	• Fix the leak in the external instrument before restarting the module.
3	Shut-down in an external instrument with a remote connection to the system.	• Check external instruments for a shut-down condition.
4	The degasser failed to generate sufficient vacuum for solvent degassing.	• Check the vacuum degasser for an error condition. Refer to the Service Manual for the degasser or the pump that has the degasser built-in. • Check the external vacuum degasser module (if installed) for an error condition. Refer to the *Service Manual* for the degasser or the pump that has the degasser built-in.

Remote Timeout

Error ID: 70

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

Probable cause		Suggested actions
1	Not-ready condition in one of the instruments connected to the remote line.	• Ensure the instrument showing the not-ready condition is installed correctly, and is set up correctly for analysis.
2	Defective remote cable.	• Exchange the remote cable.
3	Defective components in the instrument showing the not-ready condition.	• Check the instrument for defects (refer to the instrument's documentation).

Lost CAN Partner

Error ID: 71

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

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

Probable cause		Suggested actions
1	CAN cable disconnected.	• Ensure all the CAN cables are connected correctly. • Ensure all CAN cables are installed correctly.
2	Defective CAN cable.	• Exchange the CAN cable.
3	Defective mainboard in another module.	• Switch off the system. Restart the system, and determine which module or modules are not recognized by the system.

Leak

Error ID: 64

A leak was detected in the module.

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

Probable cause		Suggested actions
1	Loose fittings.	• Ensure all fittings are tight.
2	Broken capillary.	• Exchange defective capillaries.

Leak Sensor Open

Error ID: 83

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

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

Probable cause		Suggested actions
1	Leak sensor not connected to the on/off switch board.	• Please contact your Agilent service representative.
2	Defective leak sensor.	• Please contact your Agilent service representative.
3	Leak sensor incorrectly routed, being pinched by a metal component.	• Please contact your Agilent service representative.
4	On/Off switch assembly defective.	• Please contact your Agilent service representative.

Leak Sensor Short

Error ID: 82

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

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

Probable cause		Suggested actions
1	Defective leak sensor.	• Please contact your Agilent service representative.
2	Leak sensor incorrectly routed, being pinched by a metal component.	• Please contact your Agilent service representative.
3	On/Off switch assembly defective.	• Please contact your Agilent service representative.
4	Cable or contact problem.	• Please contact your Agilent service representative.

Compensation Sensor Open

Error ID: 81

The ambient-compensation sensor (NTC) on the power switch board in the module has failed (open circuit).

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

Probable cause		Suggested actions
1	Loose connection between the on/off switch board and the mainboard.	• Please contact your Agilent service representative.
2	Defective on/off switch assembly.	• Please contact your Agilent service representative.

Compensation Sensor Short

Error ID: 80

The ambient-compensation sensor (NTC) on the power switch board in the module has failed (open circuit).

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

Probable cause		Suggested actions
1	Defective on/off switch assembly.	• Please contact your Agilent service representative.
2	Loose connection between the on/off switch board and the mainboard.	• Please contact your Agilent service representative.

Fan Failed

Error ID: 68

Depending on the module, assemblies (e.g. the lamp in the detector) are turned off to assure that the module does not overheat inside.

Probable cause		Suggested actions
1	Fan cable disconnected.	• Please contact your Agilent service representative.
2	Defective fan.	• Please contact your Agilent service representative.
3	Defective mainboard.	• Please contact your Agilent service representative.

Open Cover

Error ID: 205

The top foam has been removed.

The sensor on the main board detects when the top foam is in place. If the foam is removed, the fan is switched off, and the error message is generated.

Probable cause		Suggested actions
1	The top foam was removed during operation.	• Please contact your Agilent service representative.
2	Foam not activating the sensor.	• Please contact your Agilent service representative.
3	Defective sensor or main board.	• Please contact your Agilent service representative.

Cover Violation

Error ID: 7461

The top foam has been removed.

The sensor on the main board detects when the top foam is in place. If the foam is removed while the lamps are on (or if an attempt is made to switch on for example the lamps with the foam removed), the lamps are switched off, and the error message is generated.

Probable cause		Suggested actions
1	The top foam was removed during operation.	• Please contact your Agilent service representative.
2	Foam not activating the sensor.	• Please contact your Agilent service representative.

ERI Messages

Error ID: 11120, 11121

The ERI (Enhanced Remote Interface) provides two error events related to over current situations on the +5 V and +24 V lines.

Probable cause		Suggested actions
1	The load on the ERI is too high.	• Reduce the load.

Fluorescence Detector Error Messages

Lamp Cover Open

Error ID: 6622, 6731

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

Probable cause		Suggested actions
1	Lamp cover removed.	• Please contact your Agilent service representative.

FLF Board Not Found

Error ID: 6620, 6730

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

Probable cause		Suggested actions
1	FLF board not connected to the FLM board.	• Please contact your Agilent service representative.
2	Defective FLF board.	• Please contact your Agilent service representative.
3	Defective FLM board.	• Please contact your Agilent service representative.

ADC Not Calibrated

Error ID: 6621, 6732

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

Probable cause		Suggested actions
1	Defective ADC or other FLF electronics.	• Please contact your Agilent service representative.

A/D Overflow

Error ID: 6618, 6619

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

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

1200 FLD 1 A/D overflow (RT is 0.32 min) 16:33:24 02/11/99

1200 FLD 1 A/D overflow finished (RT is 0.67 min)16:33:46 02/11/99

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

With firmware revision A.06.11 and above, the A/D overflow leads into a flat peak in the chromatogram. For details see Visualization of ADC Limits on page 117.

Probable cause		Suggested actions
1	PMT setting to high.	• Reduce PMT gain.
2	Wavelength setting wrong.	• Change wavelength setting.

Flash Lamp Current Overflow

Error ID: 6704

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

Probable cause		Suggested actions
1	Short-circuit of trigger pack assembly or defective FLL board.	• Please contact your Agilent service representative.
2	Short-circuit of flash lamp assembly.	• Please contact your Agilent service representative.

No Light at Reference Diode Despite Lamp Is On

Error ID: 6721

- Revision A/B/C Front End Board (FLF):
- There is no feedback mechanism that checks whether the lamp is ON! If no peaks are shown in the chromatogram, the user-interface shows the module still in **Ready**. Perform a "Lamp Intensity Test" (see Lamp Intensity Test on page 137) first. If flat use below steps.
- Revision D Front End Board (FLF):

  The flashing of the xenon flash lamp is monitored constantly. If the Lamp has not flashed for more than 100 times in series, an error is generated and the lamp is turned OFF.

Probable cause		Suggested actions
1	Defective Hardware.	• Please contact your Agilent service representative.

Flash Trigger Lost

Error ID: 6722

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

Probable cause		Suggested actions
1	Firmware problem.	• Reboot the detector (power cycle).
2	Multi Mode Off	• Please contact your Agilent service representative.
3	Defective encoder.	• Please contact your Agilent service representative.

Wavelength Calibration Failed

Error ID: 6703

This message may show up during a wavelength calibration.

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

Probable cause		Suggested actions
1	Flash lamp not ignited or position not correct.	• Please contact your Agilent service representative.
2	Cell position not correct.	• Check the cell position.
3	Solvent in the cell not clean or air bubble in the cell.	• Flush the flow cell.
4	Monochromator assembly position not correct (after replacement).	• Please contact your Agilent service representative.

Wavelength Calibration Lost

Error ID: 6691

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

Probable cause		Suggested actions
1	Reset of monochromator settings after exchange.	• Perform a wavelength calibration.
2	Replacement of FLM board.	• Perform a wavelength calibration.
3	Replacement of communication board	• Perform a wavelength calibration.

Flow Cell Removed

Error ID: 6616, 6702, 6760

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

Probable cause		Suggested actions
1	Flow cell has been removed during analysis.	• Insert flow cell and turn on the lamp.

Motor Errors

NOTE

Monochromator motor errors may show up duringthe *initialization* or during *operation* ofthe detector.There are individual messages for either the excitation or the emissionside.If an error occurs, do a lamp ignition. This will clear the errorand a re-initialization of the motors is performed.

Motor Error

Error ID: 6700, 6701

Probable cause		Suggested actions
1	Friction too high.	• Exchange the monochromator assembly.
2	Defective monochromator assembly.	• Exchange the monochromator assembly.

Motor or Encoder Not Found

Error ID: 6705, 6706

During initialization of the detector, the excitation and emission monochromator are activated.

Probable cause		Suggested actions
1	Encoder cables mixed on FLM board.	• Check encoder connections to FLM.
2	Monochromator assembly not connected.	• Check motor connections to FLF (Ex) and FLM (Em) and encoders to FLM.
3	Monochromator or encoder defective.	• Exchange the monochromator assembly.
4	Monochromator motor power driver defective.	• Defective FLF board (Ex) or FLM board (Em).

Encoder Index Not Found

Error ID: 6707, 6708

During initialization of the detector, the excitation- and emission monochromator are activated and the encoder should generate an index.

Probable cause		Suggested actions
1	Encoder defective.	• Exchange the monochromator assembly.
2	Encoder electric defective.	• Replace FLM board.
3	Monochromator defective or missing.	• Check for connection to FLF (Ex) and FLM (Em) or replace monochromator assembly.
4	One phase monochromator motor power driver defective.	• Defective FLF board (Ex) or FLM board (Em).

Motor Friction Too High

Error ID: 6709, 6710

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

Probable cause		Suggested actions
1	Friction too high.	• Exchange the monochromator assembly.
2	Defective monochromator assembly.	• Exchange the monochromator assembly.

Motor Position Not Found

Error ID: 6711, 6712

When the wavelength is changed the monochromator should move to the new position. The position could not be found.

Probable cause		Suggested actions
1	Defective monochromator assembly.	• Exchange the monochromator assembly.

Motor Position Lost

Error ID: 6713, 6714

A mechanical shock to the instrument during operation may cause a movement of the monochromator. The position is lost and the lamp will turn off.

Probable cause		Suggested actions
1	Short mechanical shock.	• re-ignite the lamp.
2	Message appears intermittently without mechanical shock.	• Exchange the monochromator assembly.

Motor Speed Too Low

Error ID: 6715, 6716

For proper operation the monochromator gratings must run at a certain constant revolution.

Probable cause		Suggested actions
1	Revolution too low.	• Exchange the monochromator assembly.

Motor Speed Unstable

Error ID: 6717, 6718

For proper operation the monochromator gratings must run at a certain constant revolution.

Probable cause		Suggested actions
1	Defective monochromator assembly.	• Exchange the monochromator assembly.

Motor Encoder Index Wrong

Error ID: 6719, 6720

The actual encoder pattern is checked against a known pattern.

Probable cause		Suggested actions
1	Encoder was replaced and has a different pattern or no reset of pattern was made.	• Reset pattern via user interface and recalibrate.
2	Encoder lost position completely.	• Exchange the monochromator assembly.

8 Maintenance

This chapter provides general information on maintenance of the module.

Introduction to Maintenance

The module is designed for easy maintenance. Maintenance can be done from the front with module in place in the system.

NOTE There are no serviceable parts inside.
Do not open the module.

Safety Information Related to Maintenance

WARNING Eye damage by detector light

Eye damage may result from directly viewing the UV-light produced by the lamp of the optical system used in this product.

— Always turn the lamp of the optical system off before removing it.

WARNING Fire and damage to the module

Wrong fuses

— Make sure that only fuses with the required rated current and of the specified type (super-fast, fast, time delay etc) are used for replacement.

— The use of repaired fuses and the short-circuiting of fuse-holders must be avoided.

WARNING Personal injury or damage to the product

Agilent is not responsible for any damages caused, in whole or in part, by improper use of the products, unauthorized alterations, adjustments or modifications to the products, failure to comply with procedures in Agilent product user guides, or use of the products in violation of applicable laws, rules or regulations.

— Use your Agilent products only in the manner described in the Agilent product user guides.

WARNING Electrical shock

Repair work at the module can lead to personal injuries, e.g. shock hazard, when the cover is opened.

— Do not remove the cover of the module.

— Only certified persons are authorized to carry out repairs inside the module.

WARNING Sharp metal edges

Sharp-edged parts of the equipment may cause injuries.

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

WARNING **Toxic, flammable and hazardous solvents, samples and reagents**

The handling of solvents, samples and reagents can hold health and safety risks.

- **When working with these substances observe appropriate safety procedures (for example by wearing goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet supplied by the vendor, and follow good laboratory practice.**
- **The volume of substances should be reduced to the minimum required for the analysis.**
- **Do not operate the instrument in an explosive atmosphere.**

CAUTION Safety standards for external equipment

- If you connect external equipment to the instrument, make sure that you only use accessory units tested and approved according to the safety standards appropriate for the type of external equipment.

Overview of Maintenance

The following pages describe maintenance (simple repairs) of the detector that can be carried out without opening the main cover.

Table 29: 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 147.
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.

Cleaning the Module

To keep the module case clean, use a soft cloth slightly dampened with water, or a solution of water and mild detergent. Avoid using organic solvents for cleaning purposes. They can cause damage to plastic parts.

WARNING **Liquid dripping into the electronic compartment of your module can cause shock hazard and damage the module**

- **Do not use an excessively damp cloth during cleaning.**
- **Drain all solvent lines before opening any connections in the flow path.**

NOTE A solution of 70 % isopropanol and 30 % water might be used if the surface of the module needs to be disinfected.

Remove and Install Doors

When

- The instrument doors or the hinges are broken.

Tools required

Qty.	p/n	Description
1	5023-3138	Reversible Screwdriver + Blade 1,0 x 5,5

Parts required (Infinity III)

Qty.	p/n	Description
	5004-3140	Door Kit Infinity III 140mm Latched

Parts required (Infinity II)

Qty.	p/n	Description
	5004-0140	Door Kit Infinity II 140mm Latched

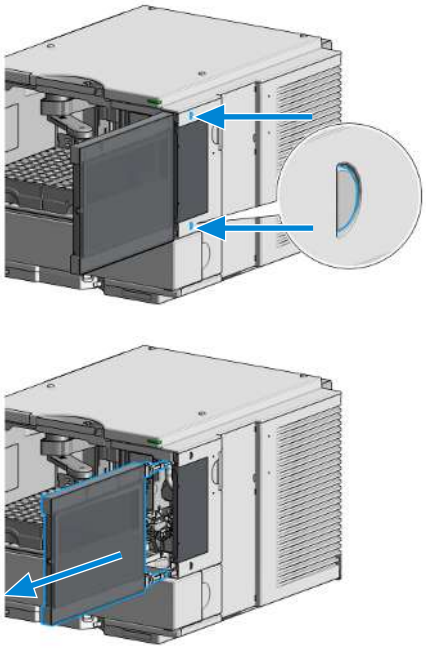
Preparations

- Finish any pending acquisition job.

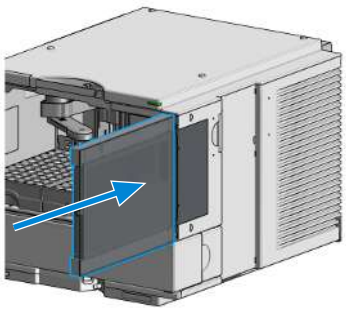
NOTE

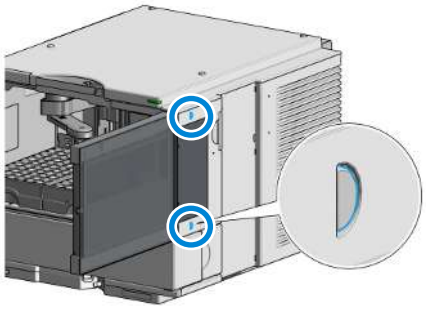
The figures shown in this procedure exemplarily show the Infinity III Vialsampler module. The principle of how to remove and/or install doors works in the same way for all Infinity III modules.

1 Press the release buttons and pull the front door out.



2 For the Installation of the front door, insert the hinges into their guides and push the door in until the release buttons click into their final position.





Exchange a Flow Cell

BIO INERT

For bio-inert modules use bio-inert parts only!

When

- If an application needs a different type of flow cell or the flow cell is defective (leaky).

Tools required

Qty.	p/n	Description
1		Wrench, 1/4 inch , **OR**
1	5043-0915	Fitting mounting tool

Parts required

Qty.	p/n	Description
1	G1321-60005	Flow cell, 8 µL, 20 bar
1	G1321-60015	Flow cell, 4 µL, 20 bar, requires a 0.12 mm i.d. capillary (e.g. p/n G1316-87318, 300 mm long), part of Capillary kit for 0.12 mm id (p/n G1316-68716)
1	G5615-60005	Bio-inert flow cell, 8 µL, 20 bar includes Capillary Kit Flow Cells BIO (G5615-68755) and PEEK fittings
1	G1321-60007	FLD Cuvette Kit, 8 µL, 20 bar

Preparations

- Turn off the flow.

CAUTION

Sample degradation and contamination of the instrument

Metal parts in the flow path can interact with the bio-molecules in the sample leading to sample degradation and contamination.

— For bio applications, always use dedicated bio parts, which can be identified by the bio-inert symbol or other markers described in this manual.

— Do not mix bio, and non-bio modules or parts in a bio system.

NOTE

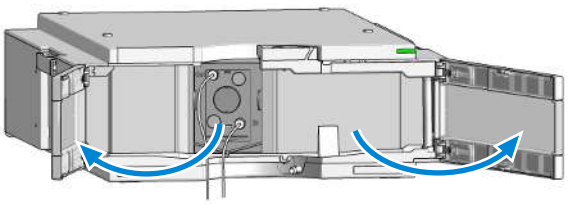
New instruments are shipped with a flow cell installed and have a factory wavelength calibration.
If a flow cell is used other than the installed/shipped flow cell:

- verify that the installed flow cell is fixed very tight,
- perform a wavelength calibration using Glycogen (part of G7121-68001 (FLD Calibration Kit)).

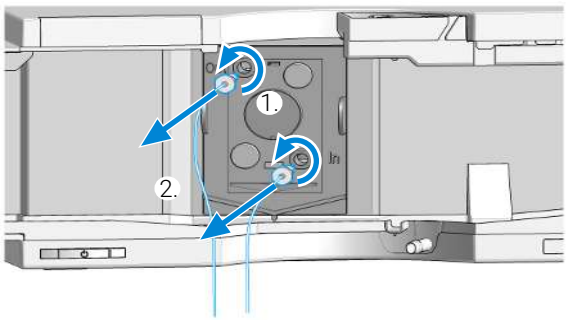
NOTE

Before storing the flow cell, flush it with iso-propanol and close it with 0100-1259 (Plug-Screw 1032- Fitting) .

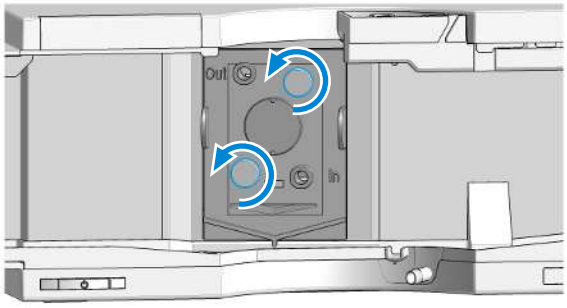
1 Open the doors of the module.



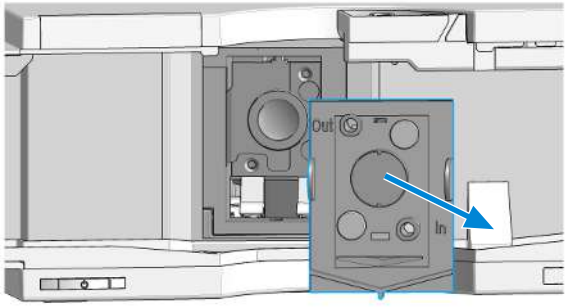
2 Disconnect the capillaries from the flow cell.



3 Unscrew the thumb screws of the flow cell.

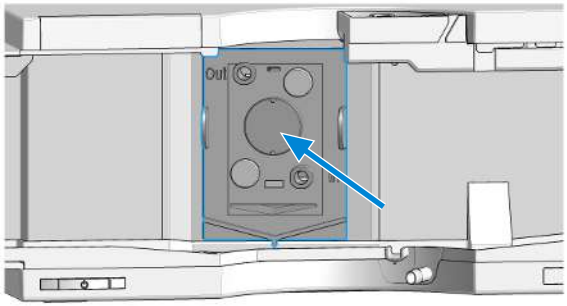


4 Remove the flow cell from the compartment.



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

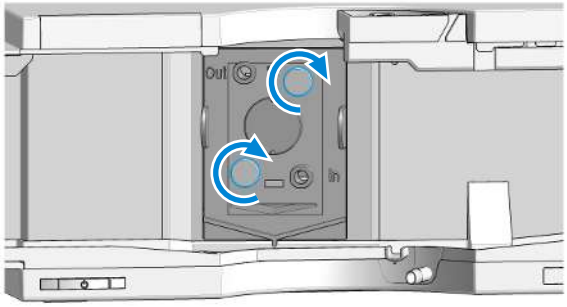
5 Insert the flow cell and tighten the thumb screws strongly.



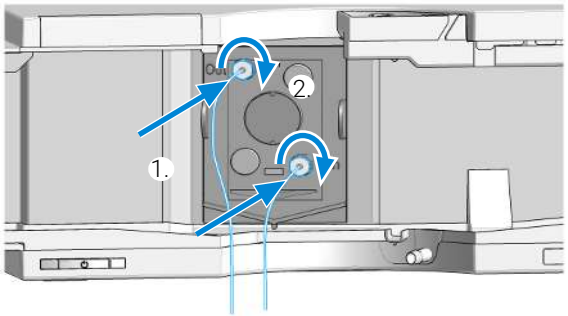
NOTE If the cell is not completely in its end position and tightened strongly this could result into a wavelength shift.

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.

6 Tighten the thumb screws of the flow cell.



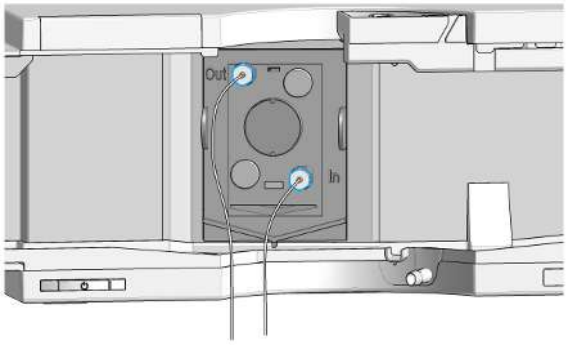
7 Reconnect the capillaries to the flow cell.



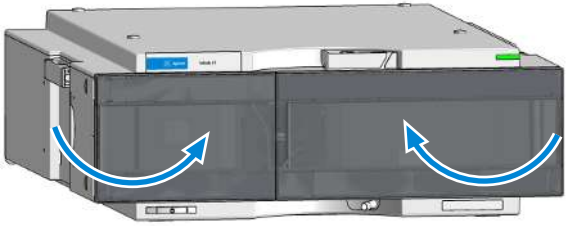
NOTE DO NOT install the inlet capillary to the outlet connection of the flow cell. This will result in poor performance or damage.

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 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.

8 Check for leaks: establish a flow and observe the flow cell (outside of the cell compartment) and all capillary connections.



9 Close the doors of the module.



10 Perform a Wavelength Calibration.

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 227) 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

- If flow cell is contaminated

Tools required

Qty.	p/n	Description
1		Glass syringe
1		Adapter

Parts required

Qty.	p/n	Description
1		Bidistilled water, nitric acid (65 %), tubings to waste

WARNING **Dangerous concentration of nitric acid**

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.

— **Give proper attention to safety.**

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 %).

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 thoroughly with bidistilled water at a higher flow rate, e.g. 1.5 mL/min.

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

NOTE Do not use the HPLC pump to pump nitric acid through the system.

Correcting Leaks

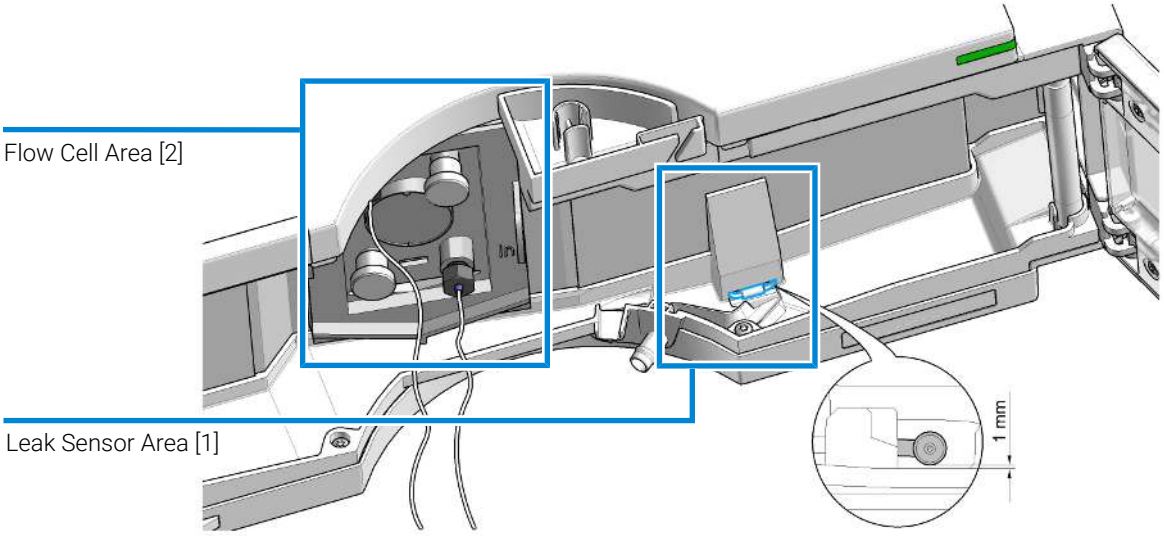


Figure 75: Correcting leaks

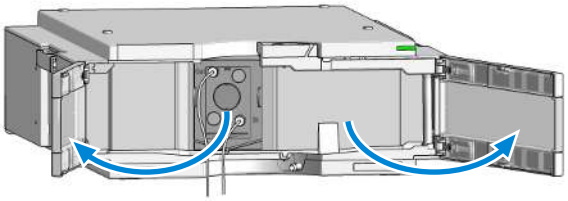
When

- If a leakage has occurred in the flow cell area or at the capillary connections

Tools required

Qty.	p/n	Description
1		Tissue
1		Wrench, 1/4 inch for capillary connections

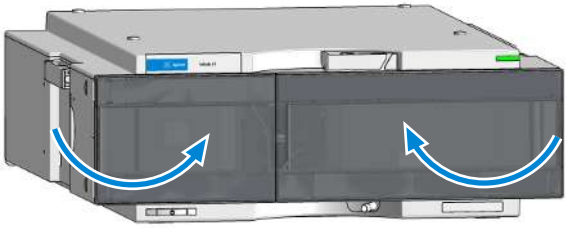
1 Open the doors.



2 Use tissue to dry the leak sensor area [1].

3 Observe the capillary connections and the flow cell area [2] for leaks and correct, if required.

4 Close the doors of the module.



Replace Leak Handling System Parts

Parts required

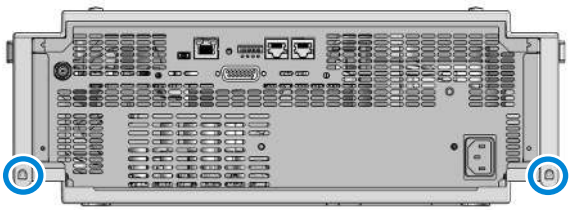
Qty.	p/n	Description
1	5043-0856	Leak Adapter
1	5063-6527	Tubing, Silicon Rubber, 1.2 m, ID/OD 6 mm/ 9 mm

Preparations

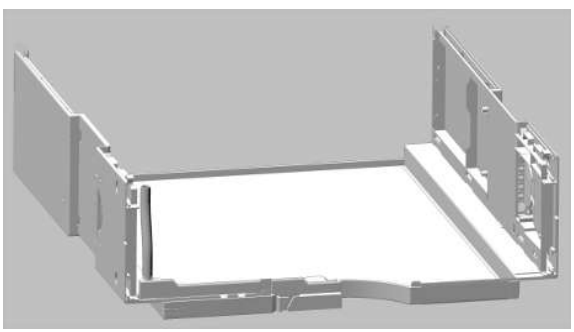
- Turn off the module.
- Disconnect the power cable.
- Disconnect the hydraulic connection from the flow.
- Remove the module from the stack and place it on the working bench.

1 Remove the doors, see Remove and Install Doors.

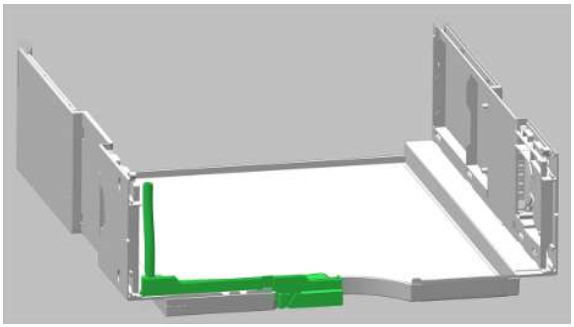
2 Lift the cover levers at the rear of the module until you can lift the cover.



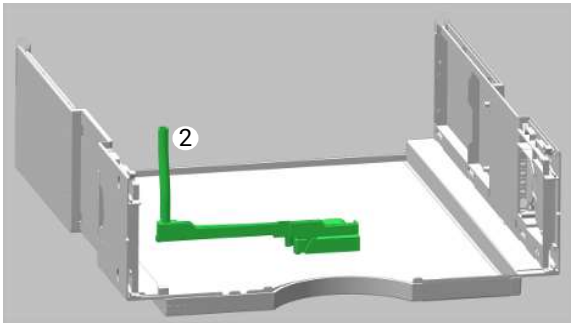
3 Remove the cover and place it on the bench.



4 Locate the Leak Interface Top.



5 Pull the Leak Adapter from the Top Cover and remove it together with the tubing.



6 Install the Leak Adapter by pressing it into the Main Cover.

7 Slide the Main Cover into its front position.

8 Press the main cover down at the rear until the levers click.

9 Insert the Tubing [2] (ca. 85 mm required for replacement) between Leak Adapter outlet and Leak Pan.

10 Install the Doors, see Remove and Install Doors.

Replace the Module Firmware

When

Install a newer firmware

- It fixes known problems of older versions, or
- It introduces new features, or
- It ensures keeping all systems at the same (validated) revision

When

Install an older firmware

- It ensures keeping all systems at the same (validated) revision, or
- It ensures compatibility after adding a new module to the system, or
- A third-party control software requires a special version

Software required

- Agilent Lab Advisor software

Parts required

Qty.	p/n	Description
1		Firmware, tools and documentation from Agilent web site

Preparations

- Read update documentation provided with the Firmware Update Tool.

To upgrade/downgrade the module's firmware carry out the following steps:

1 Download the required module firmware, the latest FW Update Tool and the documentation from the Agilent web.
https://www.agilent.com/en-us/firmwareDownload?whid=69761

2 For loading the firmware into the module follow the instructions in the documentation.

Module Specific Information

There is no specific information for this module.

Tests & Calibrations

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

- Lamp Intensity Test on page 137
- Wavelength Verification and Calibration on page 147

9 Parts and Materials for Maintenance

This chapter provides information on parts for maintenance.

Overview of Maintenance Parts

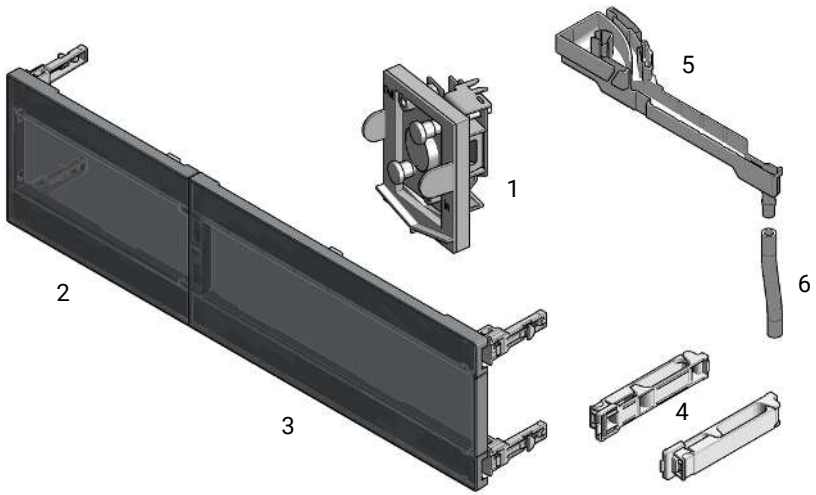


Figure 76: Overview of maintenance parts

#	p/n	Description
1	G1321-60005	Flow cell, 8 µL, 20 bar, OR
1	G1321-60015	Flow cell, 4 µL, 20 bar, requires a 0.12 mm i.d. capillary (e.g. p/n G1316-87318, 300 mm long), part of Capillary kit for 0.12 mm id (p/n G1316-68716), OR
1	G5615-60005	Bio-inert flow cell, 8 µL, 20 bar, includes Capillary Kit Flow Cells BIO (G5615-68755) and PEEK fittings, OR
1	G5615-68755	Capillary Kit Flow Cells BIO includes Capillary PK 0.18 mm x 1.5 m and PEEK Fittings 10/PK (p/n 5063-6591)
2	5360-0016	Door 140mm left
3	5360-0015	Door 140mm right
4	5043-1013	Tubing Clip IF-II
5	5043-0856	Leak Adapter
6	5063-6527	Tubing, Silicon Rubber, 1.2 m, ID/OD 6 mm/9 mm for Waste and Leak Adapter (ca. 85 mm required)

Kits

Accessory Kit

G7121-68755 (Detector Accessory Kit)

p/n	Description
5062-2462	Tube PTFE 0.7 mm x 5 m, 1.6 mm od re-order 5 m
0100-1516	Finger-tight fitting PEEK, 2/pk
G1315-87311	Capillary ST 0.17 mm x 380 mm S/S Column to detector (includes ST ferrule front, ST ferrule back and ST fitting)
5063-6527	Tubing, Silicon Rubber, 1.2 m, ID/OD 6 mm/9 mm see item 6 in Overview of Maintenance Parts on page 225
5181-1516	CAN cable, Agilent module to module, 0.5 m
5043-1013	Tubing Clip IF-II see item 4 in Overview of Maintenance Parts on page 225
5500-1155	Tube Connector, 90 degree, ID 6.4

Calibration Kit

G7121-68001 (FLD Calibration Kit)

p/n	Description
5063-6597	Calibration Sample, Glycogen
5190-1539	Syringe 5mL with Luer-Lock
9301-0407	Syringe, External Valve adapter, SST
5190-5111	Syringe filter, 0.45 µm, 100/pk
0100-1516	Finger-tight fitting PEEK, 2/pk

Capillary Kit Flow Cells BIO

G5615-68755 (Capillary Kit Flow Cells BIO includes Capillary PK 0.18 mm x 1.5 m and PEEK Fittings 10/PK (p/n 5063-6591)) includes:

p/n	Description
0890-1763	Capillary PEEK 0.18 mm x 1.5 m
5063-6591	PEEK Fittings 10/PK

Cuvette Kit

Qty.	p/n	Description
1	G1321-60007	FLD Cuvette Kit, 8 µL, 20 bar
1		Flow cell, 8 µL, 20 bar (pH 1 – 19.5)
1	5062-2462	Tube PTFE 0.7 mm x 5 m, 1.6 mm od
1	79814-22406	ST Fitting
1	0100-0043	ST front ferrule
1	0100-0044	ST back ferrule
1	0100-1516	Finger-tight fitting PEEK, 2/pk
1	9301-0407	Syringe, External Valve adapter, SST
1	9301-1446	Syringe

10 Identifying Cables

This chapter provides information on cables used with the modules.

Cable Overview

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

Analog cables

p/n	Description
35900-60750	Agilent 35900A A/D converter
01046-60105	Analog cable (BNC to general purpose, spade lugs)

Remote cables

p/n	Description
5188-8029	ERI to general purpose
5188-8044	Remote Cable ERI – ERI
5188-8045	Remote Cable APG – ERI
5188-8059	ERI-Extension-Cable 1.2 m
5061-3378	Remote Cable to 35900 A/D converter
01046-60201	Agilent module to general purpose
5188-8057	Fraction Collection ERI remote Y-cable

CAN cables

p/n	Description
5181-1516	CAN cable, Agilent module to module, 0.5 m
5181-1519	CAN cable, Agilent module to module, 1 m

LAN cables

p/n	Description
5023-0203	Cross-over network cable, shielded, 3 m (for point to point connection)
5023-0202	Twisted pair network cable, shielded, 7 m (for point to point connection)

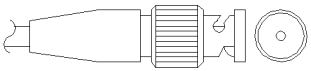
RS-232 cables

p/n	Description
RS232-61601	RS-232 cable, 2.5 m Instrument to PC, 9-to-9 pin (female). This cable has special pin-out, and is not compatible with connecting printers and plotters. It is also called "Null Modem Cable" with full handshaking where the wiring is made between pins 1-1, 2-3, 3-2, 4-6, 5-5, 6-4, 7-8, 8-7, 9-9.
5181-1561	RS-232 cable, 8 m

USB cables

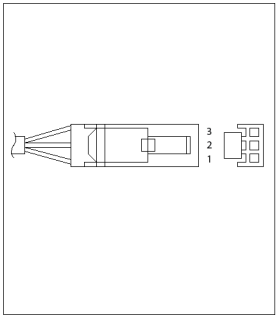
p/n	Description
5188-8050	USB A M-USB Mini B 3 m (PC-Module)
5188-8049	USB A F-USB Mini B M OTG (Module to Flash Drive)

Analog Cables

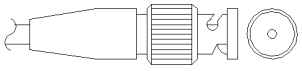


One end of these cables provides a BNC connector to be connected to Agilent modules. The other end depends on the instrument to which connection is being made.

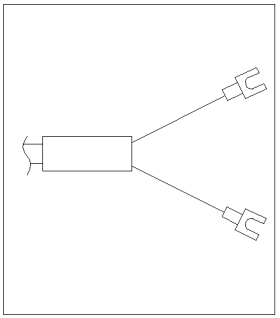
Agilent Module to 35900 A/D converters

p/n 35900-60750	35900	Pin Agilent module	Signal Name
	1		Not connected
	2	Shield	Analog -
	3	Center	Analog +

Agilent Module to BNC Connector

p/n 8120-1840	Pin BNC	Pin Agilent module	Signal Name
	Shield	Shield	Analog -
	Center	Center	Analog +

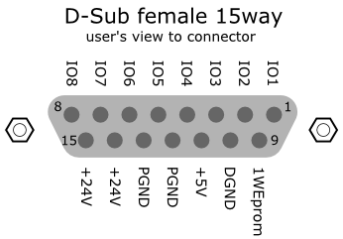
Agilent Module to General Purpose

p/n 01046-60105	Pin	Pin Agilent module	Signal Name
	1		Not connected
	2	Black	Analog -
	3	Red	Analog +

Remote Cables

ERI (Enhanced Remote Interface)

- 5188-8029 ERI to general purpose (D-Sub 15 pin male - open end)
- 5188-8044 ERI to ERI (D_Sub 15 pin male - male)
- 5188-8059 ERI-Extension-Cable 1.2 m (D-Sub15 pin male / female)



p/n 5188-8029	pin	Color code	Enhanced Remote	Classic Remote	Active (TTL)
	1	white	IO1	START REQUEST	Low
	2	brown	IO2	STOP	Low
	3	green	IO3	READY	High
	4	yellow	IO4	PEAK DETECT	Low
	5	grey	IO5	POWER ON	High
	6	pink	IO6	SHUT DOWN	Low
	7	blue	IO7	START	Low
	8	red	IO8	PREPARE	Low
	9	black	1wire DATA		
	10	violet	DGND		
	11	grey-pink	+5V ERI out		
	12	red-blue	PGND		
	13	white-green	PGND		
	14	brown-green	+24V ERI out		
	15	white-yellow	+24V ERI out		
	NC	yellow-brown			

NOTE Configuration is different with old firmware revisions.
The configuration for IO4 and IO5 is swapped for modules with firmware lower than D.07.10.

NOTE Peak Detection is used for LCMS systems connected with the Fraction Collection Remote Y-Cable (5188-8057).

- 5188-8045 ERI to APG (Connector D_Subminiature 15 pin (ERI), Connector D_Subminiature 9 pin (APG))

p/n 5188-8045	Pin (ERI)	Signal	Pin (APG)	Active (TTL)
	10	GND	1	
	1	Start Request	9	Low
	2	Stop	8	Low
	3	Ready	7	High
	5	Power on	6	High
	4	Future	5	
	6	Shut Down	4	Low
	7	Start	3	Low
	8	Prepare	2	Low
	Ground	Cable Shielding	NC	

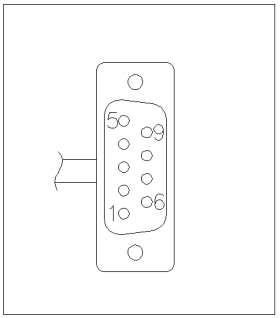
- 5188-8057 ERI to APG and RJ45 (Connector D_Subminiature 15 pin (ERI), Connector D_Subminiature 9 pin (APG), Connector plug Cat5e (RJ45))

Table 30: 5188-8057 ERI to APG and RJ45

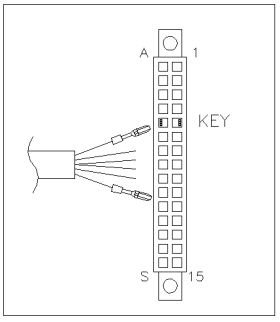
p/n 5188-8057	Pin (ERI)	Signal	Pin (APG)	Active (TTL)	Pin (RJ45)
					
	10	GND	1		5
	1	Start Request	9	High	
	2	Stop	8	High	
	3	Ready	7	High	
	4	Fraction Trigger	5	High	4
	5	Power on	6	High	
	6	Shut Down	4	High	
	7	Start	3	High	
	8	Prepare	2	High	
	Ground	Cable Shielding	NC		

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

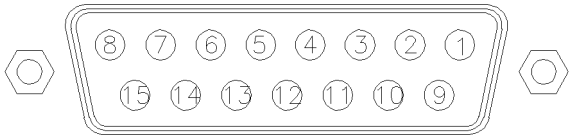
Agilent Module to Agilent 35900 A/D Converters

p/n 5061-3378	Pin 35900 A/D	Pin Agilent module	Signal Name	Active (TTL)
	1 - White	1 - White	Digital ground	
	2 - Brown	2 - Brown	Prepare run	Low
	3 - Gray	3 - Gray	Start	Low
	4 - Blue	4 - Blue	Shut down	Low
	5 - Pink	5 - Pink	Not connected	
	6 - Yellow	6 - Yellow	Power on	High
	7 - Red	7 - Red	Ready	High
	8 - Green	8 - Green	Stop	Low
	9 - Black	9 - Black	Start request	Low

Agilent Module to General Purpose

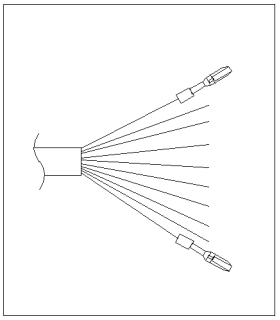
p/n 01046-60201	Wire Color	Pin Agilent module	Signal Name	Active (TTL)
	White	1	Digital ground	
	Brown	2	Prepare run	Low
	Gray	3	Start	Low
	Blue	4	Shut down	Low
	Pink	5	Not connected	
	Yellow	6	Power on	High
	Red	7	Ready	High
	Green	8	Stop	Low
	Black	9	Start request	Low

BCD Cables

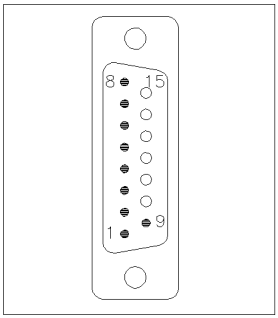


One end of these cables provides a 15-pin BCD connector to be connected to the Agilent modules. The other end depends on the instrument to be connected to

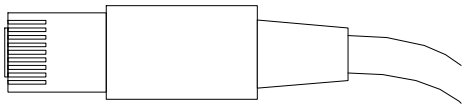
Agilent Module to General Purpose

p/n G1351-81600	Wire Color	Pin Agilent module	Signal Name	BCD Digit
	Green	1	BCD 5	20
	Violet	2	BCD 7	80
	Blue	3	BCD 6	40
	Yellow	4	BCD 4	10
	Black	5	BCD 0	1
	Orange	6	BCD 3	8
	Red	7	BCD 2	4
	Brown	8	BCD 1	2
	Gray	9	Digital ground	Gray
	Gray/pink	10	BCD 11	800
	Red/blue	11	BCD 10	400
	White/green	12	BCD 9	200
	Brown/green	13	BCD 8	100
	not connected	14		
	not connected	15	+ 5 V	Low

Agilent Module to 3396 Integrators

p/n 03396-60560	Pin 3396	Pin Agilent module	Signal Name	BCD Digit
	1	1	BCD 5	20
	2	2	BCD 7	80
	3	3	BCD 6	40
	4	4	BCD 4	10
	5	5	BCD0	1
	6	6	BCD 3	8
	7	7	BCD 2	4
	8	8	BCD 1	2
	9	9	Digital ground	
	NC	15	+ 5 V	Low

CAN/LAN Cables



Both ends of this cable provide a modular plug to be connected to Agilent modules CAN or LAN connectors.

Can Cables

p/n	Description
5181-1516	CAN cable, Agilent module to module, 0.5 m
5181-1519	CAN cable, Agilent module to module, 1 m

LAN Cables

p/n	Description
5023-0203	Cross-over network cable, shielded, 3 m (for point to point connection)
5023-0202	Twisted pair network cable, shielded, 7 m (for point to point connection)

RS-232 Cables

p/n	Description
RS232-61601	RS-232 cable, 2.5 m Instrument to PC, 9-to-9 pin (female). This cable has special pin-out, and is not compatible with connecting printers and plotters. It is also called "Null Modem Cable" with full handshaking where the wiring is made between pins 1-1, 2-3, 3-2, 4-6, 5-5, 6-4, 7-8, 8-7, 9-9.
5181-1561	RS-232 cable, 8 m

USB

To connect a USB Flash Drive use a USB OTG cable with Mini-B plug and A socket.

p/n	Description
5188-8050	USB A M-USB Mini B 3 m (PC-Module)
5188-8049	USB A F-USB Mini B M OTG (Module to Flash Drive)

11 Hardware Information

This chapter describes the module in more detail on hardware and electronics.

General Hardware Information

This section provides detailed hardware information on firmware that is valid for this module.

Firmware Description

The firmware of the instrument consists of two independent sections:

- a non-instrument specific section, called *resident system*
- an instrument specific section, called *main system*

Resident System

This resident section of the firmware is identical for all Agilent 1100/1200/1220/1260/1290 series modules. Its properties are:

- the complete communication capabilities (CAN, LAN, USB and RS- 232)
- memory management
- ability to update the firmware of the 'main system'

Main System

Its properties are:

- the complete communication capabilities (CAN, LAN, USB and RS- 232)
- memory management
- ability to update the firmware of the 'resident system'

In addition the main system comprises the instrument functions that are divided into common functions like

- run synchronization through APG/ERI remote,
- error handling,
- diagnostic functions,

- or module specific functions like
  - internal events such as lamp control, filter movements,
  - raw data collection and conversion to absorbance.

Firmware Updates

Firmware updates can be done with the Agilent Lab Advisor software with files on the hard disk (latest version should be used).

Required tools, firmware and documentation are available from the Agilent web: https://www.agilent.com/en-us/firmwareDownload?whid=69761

The file naming conventions are:

PPPP_RVVV_XXX.dlb, where

- PPPP is the product number, for example, 1315B for the G1315B DAD,
- R the firmware revision, for example, A for G1315B or B for the G1315C DAD,
- VVV is the revision number, for example 650 is revision 6.50,
- XXX is the build number of the firmware.

For instructions on firmware updates refer to section *Replacing Firmware* in chapter *Maintenance* or use the documentation provided with the *Firmware Update Tools*.

NOTE

Update of main system can be done in the resident system only. Update of the resident system can be done in the main system only.
Main and resident firmware must be from the same set.

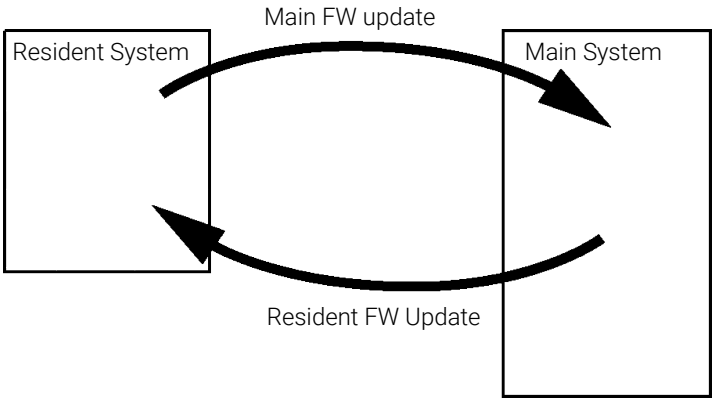


Figure 77: Firmware update mechanism

NOTE

Some modules are limited in downgrading due to their mainboard version or their initial firmware revision. For example, a G1315C DAD SL cannot be downgraded below firmware revision B.01.02 or to a A.xx.xx.
Some modules can be re-branded (e.g. G1314C to G1314B) to allow operation in specific control software environments. In this case, the feature set of the target type is used and the feature set of the original one is lost. After re-branding (e.g. from G1314B to G1314C), the original feature set is available again.
All this specific information is described in the documentation provided with the firmware update tools.

The firmware update tools, firmware and documentation are available from the Agilent web.

- https://www.agilent.com/en-us/firmwareDownload?whid=69761

Electrical Connections

- The CAN bus is a serial bus with high-speed data transfer. The two connectors for the CAN bus are used for internal module data transfer and synchronization.
- One analog output provides signals for integrators or data handling systems.
- The ERI 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 on.

- With the appropriate software, the LAN connector may be used to control the module from a computer through a LAN connection. This connector is activated and can be configured with the configuration switch.
- With the appropriate software, the USB connector may be used to control the module from a computer through a USB connection.
- The power input socket accepts a line voltage of 100 – 240 VAC ± 10 % with a line frequency of 50 or 60 Hz. Maximum power consumption varies by module. There is no voltage selector on your module because the power supply has wide-ranging capability. There are no externally accessible fuses because automatic electronic fuses are implemented in the power supply.

WARNING **Electric shock due to insufficient insulation of connected instruments**

Personal injury or damage to the instrument

— **Any other instruments connected to this instrument shall be approved to a suitable safety standard and must include reinforced insulation from the mains.**

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

Rear View of the Module

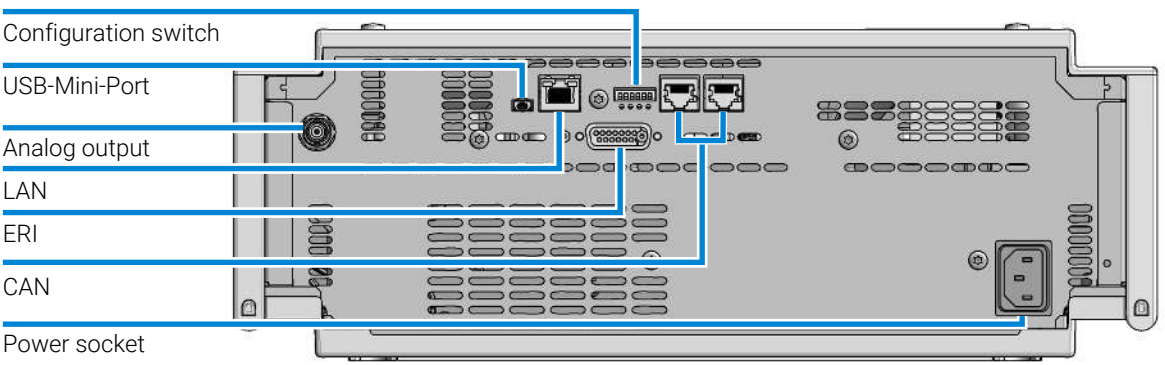


Figure 78: Rear view of detector (example shows a G7114A/B VWD) – electrical connections and label

Serial Number Information

The serial number information on the instrument labels provide the following information:

CCXZZ00000	Format
CC	Country of manufacturing • DE = Germany • JP = Japan • CN = China
X	Alphabetic character A-Z (used by manufacturing)
ZZ	Alpha-numeric code 0-9, A-Z, where each combination unambiguously denotes a module (there can be more than one code for the same module)
00000	Serial number

Interfaces

The Agilent InfinityLab LC Series modules provide the following interfaces:

Table 31: Agilent InfinityLab LC Series interfaces

Module	CAN	USB	LAN (on-board)	RS-232	Analog	APG (A) / ERI (E)	Special
Pumps							
G7104A/C	2	No	Yes	Yes	1	A	
G7110B	2	Yes	Yes	No	No	E	
G7111A/B, G5654A	2	Yes	Yes	No	No	E	
G7112B	2	Yes	Yes	No	No	E	
G7120A, G7132A	2	No	Yes	Yes	1	A	
G7161A/B	2	Yes	Yes	No	No	E	
Samplers							
G7129A/B/C	2	Yes	Yes	No	No	E	
G7167A/B/C, G7137A, G5668A, G3167A	2	Yes	Yes	No	No	E	

Module	CAN	USB	LAN (on-board)	RS-232	Analog	APG (A) / ERI (E)	Special
G7157A	2	Yes	Yes	No	No	E	
Detectors							
G7114A/B	2	Yes	Yes	No	1	E	
G7115A	2	Yes	Yes	No	1	E	
G7117A/B/C	2	Yes	Yes	No	1	E	
G7121A/B	2	Yes	Yes	No	1	E	
G7162A/B	2	Yes	Yes	No	1	E	
G7165A	2	Yes	Yes	No	1	E	
Fraction Collectors							
G7158B	2	Yes	Yes	No	No	E	
G7159B	2	Yes	Yes	No	No	E	
G7166A	2	No	No	No	No	No	Requires a host module with on-board LAN with minimum FW B.06.40 or C.06.40, or with additional G1369C LAN Card
G1364E/F, G5664B	2	Yes	Yes	No	No	E	THERMOSTAT for G1330B
Others							
G1170A	2	No	No	No	No	No	Requires a host module with on-board LAN or with additional G1369C LAN Card.
G7116A/B	2	No	No	No	No	No	Requires a host module with on-board LAN or with additional G1369C LAN Card.
G7122A	No	No	No	Yes	No	A	
G7170B	2	No	No	No	No	No	Requires a host module with on-board LAN with minimum FW B.06.40 or C.06.40, or with additional G1369C LAN Card

NOTE

LAN connection is made between at least one of the Agilent modules and the Control PC.

- If an Assist Hub is installed, connect the LAN to the Lab LAN port of this module.
- If an Assist Hub is NOT installed and a detector (DAD/MWD/FLD/VWD/RID) is installed, connect the LAN to this module.
- If an Assist Hub is NOT installed and there are multiple detectors with spectral capabilities, consider using additional LAN connections for each detector.
- If an Assist Hub is installed, connect additional LAN connections from the detectors and pumps to the Assist Hub.

- CAN connectors as interface to other modules
- LAN connector as interface to the control software
- RS-232C as interface to a computer
- USB (Universal Series Bus) as interface to a computer
- REMOTE connector as interface to other Agilent products
- Analog output connector for signal output

Overview Interfaces

CAN

The CAN is inter-module communication interface. It is a 2-wire serial bus system supporting high speed data communication and real-time requirement.

LAN

The modules have either an interface slot for a LAN card (e.g. Agilent G1369B/C LAN Interface) or they have an on-board LAN interface (e.g. detectors G1315C/D DAD and G1365C/D MWD). This interface allows the control of the module/system via a PC with the appropriate control software. Some modules have neither on-board LAN nor an interface slot for a LAN card (e.g. G1170A Valve Drive or G4227A Flexible Cube). These are hosted modules and require a Host module with firmware B.06.40 or later or with additional G1369C LAN Card.

NOTE

LAN connection is made between at least one of the Agilent modules and the Control PC.

- If an Assist Hub is installed, connect the LAN to the Lab LAN port of this module.
- If an Assist Hub is NOT installed and a detector (DAD/MWD/FLD/VWD/RID) is installed, connect the LAN to this module.
- If an Assist Hub is NOT installed and there are multiple detectors with spectral capabilities, consider using additional LAN connections for each detector.
- If an Assist Hub is installed, connect additional LAN connections from the detectors and pumps to the Assist Hub.

USB

The USB interface replaces the RS-232 Serial interface in new generation modules. For details on USB refer to USB (Universal Serial Bus) on page 254.

Analog Signal Output

The analog signal output can be distributed to a recording device. For details refer to the description of the module's mainboard.

Remote (ERI)

The ERI (Enhanced Remote Interface) connector may be used in combination with other analytical instruments from Agilent Technologies if you want to use features as common shut down, prepare, and so on.

It allows easy connection between single instruments or systems to ensure coordinated analysis with simple coupling requirements.

The subminiature D connector is used. The module provides one remote connector which is inputs/outputs (wired- or technique).

To provide maximum safety within a distributed analysis system, one line is dedicated to SHUT DOWN the system's critical parts in case any module detects a serious problem. To detect whether all participating modules are switched on or properly powered, one line is defined to summarize the POWER ON state of all connected modules. Control of analysis is maintained by signal readiness READY

for next analysis, followed by START of run and optional STOP of run triggered on the respective lines. In addition PREPARE and START REQUEST may be issued. The signal levels are defined as:

- standard TTL levels (0 V is logic true, + 5.0 V is false),
- fan-out is 10,
- input load is 2.2 kOhm against + 5.0 V, and
- output are open collector type, inputs/outputs (wired- or technique).

NOTE

All common TTL circuits operate with a 5 V power supply. A TTL signal is defined as "low" or L when between 0 V and 0.8 V and "high" or H when between 2.0 V and 5.0 V (with respect to the ground terminal).

Table 32: ERI signal distribution

Pin	Signal	Description
1	START REQUEST	(L) Request to start injection cycle (for example, by start key on any module). Receiver is the autosampler.
2	STOP	(L) Request to reach system ready state as soon as possible (for example, stop run, abort or finish and stop injection). Receiver is any module performing run-time controlled activities.
3	READY	(H) System is ready for next analysis. Receiver is any sequence controller.
4	POWER ON	(H) All modules connected to system are switched on. Receiver is any module relying on operation of others.
5		Not used
6	SHUT DOWN	(L) System has serious problem (for example, leak: stops pump). Receiver is any module capable to reduce safety risk.
7	START	(L) Request to start run / timetable. Receiver is any module performing run-time controlled activities.
8	PREPARE	(L) Request to prepare for analysis (for example, calibration, detector lamp on). Receiver is any module performing pre-analysis activities.

Special Interfaces

There is no special interface for this module.

ERI (Enhanced Remote Interface)

ERI replaces the AGP Remote Interface that is used in the HP 1090/1040/1050/1100 HPLC systems and Agilent 1100/1200/1200 Infinity HPLC modules. All new InfinityLab LC Series products using the communication board core electronics use ERI. This interface is already used in the Agilent Universal Interface Box 2 (UIB2)

ERI Description

The ERI interface contains eight individual programmable input/output pins. In addition, it provides 24 V power and 5 V power and a serial data line to detect and recognize further add-ons that could be connected to this interface. This way the interface can support various additional devices like sensors, triggers (in and out) and small controllers, etc.

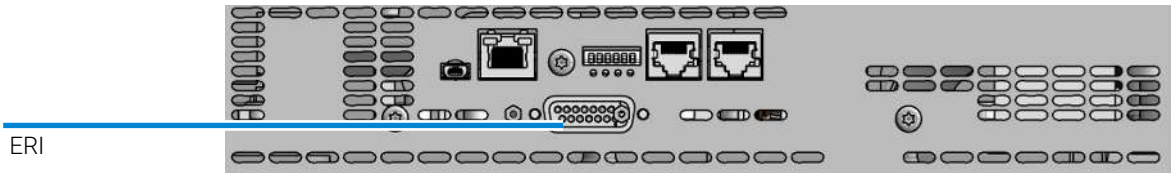
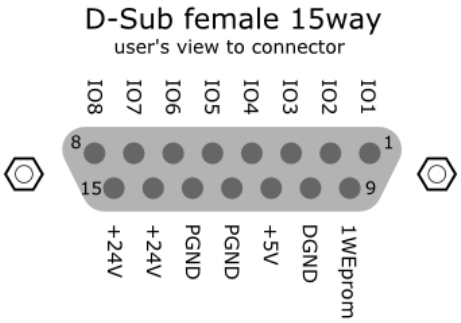


Figure 79: Location of the ERI interface



Pin	Enhanced Remote
1	IO 1 (START REQUEST)
2	IO 2 (STOP)
3	IO 3 (READY)
4	IO 4 (POWER ON)
5	IO 5 (NOT USED)
6	IO 6 (SHUT DOWN)
7	IO 7 (START)
8	IO 8 (PREPARE)
9	1 wire DATA
10	DGND
11	+5 V ERI out
12	PGND

	Pin	Enhanced Remote
	13	PGND
	14	+24 V ERI out
	15	+24 V ERI out

IO (Input/Output) Lines

- Eight generic bi-directional channels (input or output).
- Same as the APG Remote.
- Devices like valves, relays, ADCs, DACs, controllers can be supported/ controlled.

1-Wire Data (Future Use)

This serial line can be used to read out an EPROM or write into an EPROM of a connected ERI-device. The firmware can detect the connected type of device automatically and update information in the device (if required).

5V Distribution (Future Use)

- Available directly after turning on the hosting module (assures that the firmware can detect certain basic functionality of the device).
- For digital circuits or similar.
- Provides 500 mA maximum.
- Short-circuit proof with automatic switch off (by firmware).

24V Distribution (Future Use)

- Available by firmware command (defined turn on/off).
- For devices that need higher power
  - Class 0: 0.5 A maximum (12 W)
  - Class 1: 1.0 A maximum (24 W)
  - Class 2: 2.0 A maximum (48 W)
- Class depends on hosting module's internal power overhead.

- If a connected device requires more power the firmware detects this (overcurrent detection) and provides the information to the user interface.
- Fuse used for safety protection (on board).
- Short circuit will be detected through hardware.

USB (Universal Serial Bus)

USB (Universal Serial Bus) - replaces RS232, supports:

- a PC with control software (for example Agilent Lab Advisor)
- USB Flash Disk

Instrument Layout

The industrial design of the module 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 module 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 that are subject to wear or stress. Ideally, the frequency at which components are exchanged should be based on the intensity of use of the module and the analytical conditions, and not on a predefined time interval. The early maintenance feedback (EMF) feature monitors the use 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

EMF counters increment with use and can be assigned a maximum limit which provides visual feedback in the user interface when the limit is exceeded. Some counters can be reset to zero after the required maintenance procedure.

Using the EMF Counters

The user-settable EMF limits for the EMF Counters enable the early maintenance feedback to be adapted to specific user requirements. The useful maintenance cycle is dependent on the requirements for use. Therefore, the definition of the maximum limits needs 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 the default EMF limits should be set. When instrument performance indicates maintenance is necessary, take note of the values displayed by the EMF 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.

NOTE This function is only available via Agilent Lab Advisor or Instant Pilot.

The detector provides the following EMF counters:

- Flash Lamp Life Time

Module-Specific Hardware Information

Setting the 6-bit Configuration Switch

The 6-bit configuration switch is located at the rear of the module with communication board electronics. Switch settings provide configuration parameters for LAN and instrument specific initialization procedures.

All modules with communication board electronics:

- Default is ALL switches DOWN (best settings).
  - Default IP address for LAN 192.168.254.11
- For specific LAN modes switches 4-5 must be set as required.
- For boot resident/cold start modes switches 1+2 or 6 must be UP.

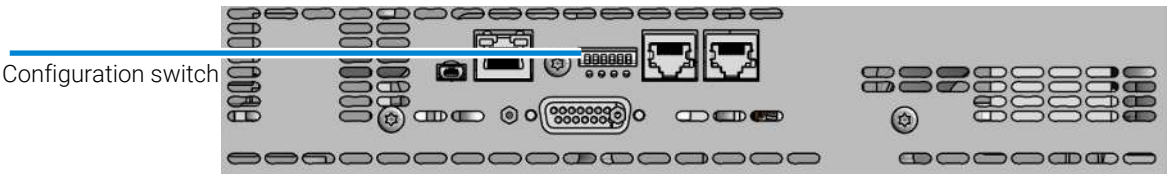


Figure 80: Location of configuration switch

Table 33: 6-bit configuration switch

SW1	SW2	SW3	SW4	SW5	SW6	Mode	Init Mode
0	0	0	0	0	0	COM	Use Default IP Address (192.168.254.11, Subnet mask: 255.255.255.0)
0	0	0	0	1	0	COM	Use Stored IP Address
0	0	0	1	0	0	COM	USE DHCP to request IP Address (Host name will be the MAC address)
1	0	0	0	0	0	Test	Boot Main System/Keep Data
1	1	0	0	0	0	Test	Boot Resident System/Keep Data

SW1	SW2	SW3	SW4	SW5	SW6	Mode	Init Mode
1	0	0	0	0	1	Test	Boot Main System/Revert to Default Data
1	1	0	0	0	1	Test	Boot Resident System/Revert to Default Data

Legend:

0 (switch down), 1 (switch up), SW (switch)

Special Settings

Boot-Resident/Main

Firmware update procedures may require this mode in case of firmware loading errors (main/resident firmware part).

If you use the following switch settings and power the instrument up again, the instrument firmware stays in the resident/main mode. In resident mode, it is not operable as a module. It only uses basic functions of the operating system for example, for communication. In this mode the main firmware can be loaded (using update utilities).

Forced Cold Start

A forced cold start can be used to bring the module into a defined mode with default parameter settings.

- Boot Main System / Revert to Default Data

  The instrument will boot to main mode and changes to the module's default parameter. May be also required to load resident firmware into the module.
- Boot Resident System / Revert to Default Data

  The instrument will boot to resident mode and changes to the module's default parameter. May be also required to load main firmware into the module.

CAUTION

Loss of data

Forced cold start erases all methods and data stored in the non-volatile memory. Exceptions are calibration settings, diagnosis and repair log books which will not be erased.

— Save your methods and data before executing a forced cold start.

12 Appendix

This chapter provides additional information on safety, legal and web.

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.

WARNING

Ensure the proper usage of the equipment.

The protection provided by the equipment may be impaired.

- **The operator of this instrument is advised to use the equipment in a manner as specified in this manual.**

Safety Standards

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

General

Do not use this product in any manner not specified by the manufacturer. The protective features of this product may be impaired if it is used in a manner not specified in the operation instructions.

Before Applying Power

WARNING Wrong voltage range, frequency or cabling

Personal injury or damage to the instrument

- Verify that the voltage range and frequency of your power distribution matches to the power specification of the individual instrument.
- Never use cables other than the ones supplied by Agilent Technologies to ensure proper functionality and compliance with safety or EMC regulations.
- Make all connections to the unit before applying power.

WARNING Use of unsupplied cables

Using cables not supplied by Agilent Technologies can lead to damage of the electronic components or personal injury.

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

NOTE Note the instrument's external markings described under Safety Symbols on page 265.

Ground the Instrument

WARNING Missing electrical ground

Electrical shock

- If your product is provided with a grounding type power plug, the instrument chassis and cover must be connected to an electrical ground to minimize shock hazard.
- The ground pin must be firmly connected to an electrical ground (safety ground) terminal at the power outlet. Any interruption of the protective (grounding) conductor or disconnection of the protective earth terminal will cause a potential shock hazard that could result in personal injury.

Do Not Operate in an Explosive Atmosphere

WARNING

Presence of flammable gases or fumes

Explosion hazard

- **Do not operate the instrument in the presence of flammable gases or fumes.**

Do Not Remove the Instrument Cover

WARNING

Instrument covers removed

Electrical shock

- **Do Not Remove the Instrument Cover**
- **Only Agilent authorized personnel are allowed to remove instrument covers. Always disconnect the power cables and any external circuits before removing the instrument cover.**

Do Not Modify the Instrument

Do not install substitute parts or perform any unauthorized modification to the product. Return the product to an Agilent Sales and Service Office for service and repair to ensure that safety features are maintained.

In Case of Damage

WARNING

Damage to the module

Personal injury (for example electrical shock, intoxication)

- **Instruments that appear damaged or defective should be made inoperative and secured against unintended operation until they can be repaired by qualified service personnel.**

Solvent Information

WARNING **Toxic, flammable and hazardous solvents, samples and reagents**

The handling of solvents, samples and reagents can hold health and safety risks.

- **When working with these substances observe appropriate safety procedures (for example by wearing goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet supplied by the vendor, and follow good laboratory practice.**
- **Do not use solvents with an auto-ignition temperature below 200 °C (392 °F). Do not use solvents with a boiling point below 56 °C (133 °F).**
- **Avoid high vapor concentrations. Keep the solvent temperature at least 40 °C (72 °F) below the boiling point of the solvent used. This includes the solvent temperature in the sample compartment. For the solvents methanol and ethanol keep the solvent temperature at least 25 °C (45 °F) below the boiling point.**
- **Do not operate the instrument in an explosive atmosphere.**
- **Do not use solvents of ignition Class IIC according IEC 60079-20-1 (for example, carbon disulfide).**
- **Reduce the volume of substances to the minimum required for the analysis.**
- **Never exceed the maximum permissible volume of solvents (8 L) in the solvent cabinet. Do not use bottles that exceed the maximum permissible volume as specified in the usage guideline for solvent cabinet.**
- **Ground the waste container.**
- **Regularly check the filling level of the waste container. The residual free volume in the waste container must be large enough to collect the waste liquid.**
- **To achieve maximal safety, regularly check the tubing for correct installation.**

NOTE For details, see the usage guideline for the solvent cabinet. A printed copy of the guideline has been shipped with the solvent cabinet, electronic copies are available in the Agilent Information Center or via the Internet.

Recommendations on the Use of Solvents

Observe the following recommendations on the use of solvents.

- Brown glass ware can avoid growth of algae.
- Follow the recommendations for avoiding the growth of algae, see the pump manuals.
- Small particles can permanently block capillaries and valves. Therefore, always filter solvents through 0.22 µm filters.
- Avoid or minimize the use of solvents that may corrode parts in the flow path. Consider specifications for the pH range given for different materials such as flow cells, valve materials etc. and recommendations in subsequent sections.
- 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 sulfuric acid and nitric acid, especially at higher temperatures (if your chromatography method allows, replace 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, diisopropyl ether) should be filtered through dry aluminium oxide which adsorbs the peroxides,
  - solvents containing strong complexing agents (e.g. EDTA),
  - mixtures of carbon tetrachloride with 2-propanol or THF.
- Avoid the use of dimethyl formamide (DMF). Polyvinylidene fluoride (PVDF), which is used in leak sensors, is not resistant to DMF.

Flow cell

To protect optimal functionality of your 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.

Magnets

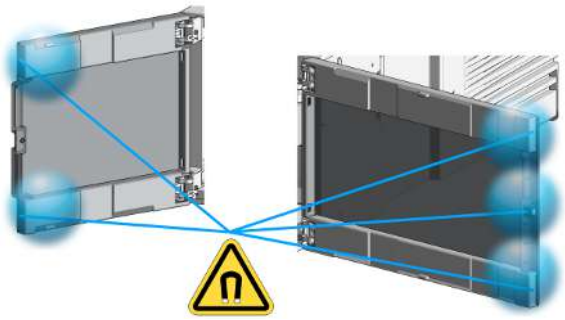
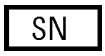


Figure 81: Magnets in doors of pumps, autosamplers, detectors, and fraction collectors

Safety Symbols

Table 34: Symbols

	The apparatus is marked with this symbol when the user shall refer to the instruction manual in order to protect risk of harm to the operator and to protect the apparatus against damage.
	Indicates dangerous voltages.
	Indicates a protected ground terminal.

	The apparatus is marked with this symbol when hot surfaces are available and the user should not touch it when heated up.
	Indicates flammable material used. Consult the Agilent Information Center / User Manual before attempting to install or service this equipment. Follow all safety precautions.
	Confirms that a manufactured product complies with all applicable European Community directives. The European Declaration of Conformity is available at: http://regulations.corporate.agilent.com/DoC/search.htm
	Manufacturing date.
	Product Number
	Serial Number
	Power symbol indicates On/Off. The apparatus is not completely disconnected from the mains supply when the on/off switch is in the Off position
	Pacemaker Magnets could affect the functioning of pacemakers and implanted heart defibrillators. A pacemaker could switch into test mode and cause illness. A heart defibrillator may stop working. If you wear these devices keep at least 55 mm distance to magnets. Warn others who wear these devices from getting too close to magnets.
	Magnetic field Magnets produce a far-reaching, strong magnetic field. They could damage TVs and laptops, computer hard drives, credit and ATM cards, data storage media, mechanical watches, hearing aids and speakers. Keep magnets at least 25 mm away from devices and objects that could be damaged by strong magnetic fields.
	Indicates a pinching or crushing hazard
	Indicates a piercing or cutting hazard.

WARNING

A WARNING

alerts you to situations that could cause physical injury or death.

- **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 loss of data, or damage of equipment.

- Do not proceed beyond a caution until you have fully understood and met the indicated conditions.

Material Information

This section provides detailed information about materials used in the HPLC system and general information about solvent/material compatibility.

Materials Used in the Bio-inert LC System

For the Bio-inert LC system, Agilent Technologies uses highest-quality materials in the flow path (also referred to as wetted parts), which are widely accepted by life science scientists, as they are known for optimum inertness to biological samples and ensure best compatibility with common samples and solvents over a wide pH range. Explicitly, the complete flow path is free of stainless steel and free of other alloys containing metals such as iron, nickel, cobalt, chromium, molybdenum, or copper, which can interfere with biological samples. The flow downstream of the sample introduction contains no metals whatsoever.

Table 35: Used bio-inert materials

Module	Materials
Agilent 1260 Infinity III Bio-inert Pump (G5654A)	Titanium, gold, platinum-iridium, ceramic, ruby, PTFE, PEEK
Agilent 1260 Infinity III Bio-inert Multisampler (G5668A)	Upstream of sample introduction: • Titanium, gold, PTFE, PEEK, ceramic Downstream of sample introduction: • PEEK, ceramic
Agilent 1260 Infinity III Bio-inert Manual Injector (G5628A)	PEEK, ceramic
Agilent 1260 Infinity III Bio-inert Analytical Fraction Collector (G5664B)	PEEK, ceramic, PTFE
Bio-inert Flow Cells:	
G5615-60022 (Standard flow cell bio-inert, 10 mm, 13 µL, 120 bar (12 MPa) for MWD/DAD, includes 0890-1763 – 0.18 x 1500 mm PEEK capillary and 5063-6591 – PEEK fittings) (*for Agilent 1260 Infinity III DAD G7115A, and MWD G7165A*)	PEEK, ceramic, sapphire, PTFE
G5615-60005 (Bio-inert flow cell, 8 µL, 20 bar) (*for Agilent 1260 Infinity III FLD G7121A/B*)	PEEK, fused silica, PTFE
Bio-inert Heat Exchangers, Valves and Capillaries:	
G7116-60041 (Quick Connect Heat Exchanger Bio-inert) (*for Agilent 1260 Infinity III Multicolumn Thermostat G7116A*)	PEEK (steel-cladded)
Bio-inert Valve heads (G4235A, G5631A, G5632A, G5639A)	PEEK, ceramic (Al_2O_3 based)
Bio-inert Connection capillaries	Upstream of sample introduction: • Titanium Downstream of sample introduction: • Agilent uses stainless-steel-cladded PEEK capillaries, which keep the flow path free of steel and provide pressure stability up to 600 bar.

NOTE To ensure optimum biocompatibility of your Bio-inert LC system, do not include non-inert standard modules or parts to the flow path. Do not use any parts that are not labeled as Agilent "Bio-inert". For solvent compatibility of these materials, see General Information About Solvent/Material Compatibility on page 270.

General Information About Solvent/Material Compatibility

Materials in the flow path are carefully selected based on Agilent's experiences in developing highest-quality instruments for HPLC analysis over several decades. These materials exhibit excellent robustness under typical HPLC conditions. For any special condition, please consult the material information section or contact Agilent.

Disclaimer

Subsequent data was collected from external resources and is meant as a reference. Agilent cannot guarantee the correctness and completeness of such information. Data is based on compatibility libraries, which are not specific for estimating the long-term life time under specific but highly variable conditions of UHPLC systems, solvents, solvent mixtures, and samples. Information also cannot be generalized due to catalytic effects of impurities like metal ions, complexing agents, oxygen etc. Apart from pure chemical corrosion, other effects like electro corrosion, electrostatic charging (especially for nonconductive organic solvents), swelling of polymer parts etc. need to be considered. Most data available refers to room temperature (typically 20 – 25 °C, 68 – 77 °F). If corrosion is possible, it usually accelerates at higher temperatures. If in doubt, please consult technical literature on chemical compatibility of materials.

MP35N

MP35N is a nonmagnetic, nickel-cobalt-chromium-molybdenum alloy demonstrating excellent corrosion resistance (for example, against nitric and sulfuric acids, sodium hydroxide, and seawater) over a wide range of concentrations and temperatures. In addition, this alloy shows exceptional resistance to high-temperature oxidation. Due to excellent chemical resistance and toughness, the alloy is used in diverse applications: dental products, medical devices, nonmagnetic electrical components, chemical and food processing equipment, marine equipment. Treatment of MP35N alloy samples with 10 % NaCl in HCl (pH 2.0) does not reveal any detectable corrosion. MP35N also demonstrates excellent corrosion resistance in a humid environment. Although the influence of a broad variety of solvents and conditions has been tested, users should keep in mind that multiple factors can affect corrosion rates, such as temperature, concentration, pH, impurities, stress, surface finish, and dissimilar metal contacts.

Polyphenylene Sulfide (PPS)

Polyphenylene sulfide has outstanding stability even at elevated temperatures. It is resistant to dilute solutions of most inorganic acids, but it can be attacked by some organic compounds and oxidizing reagents. Nonoxidizing inorganic acids, such as sulfuric acid and phosphoric acid, have little effect on polyphenylene sulfide, but at high concentrations and temperatures, they can still cause material damage. Nonoxidizing organic chemicals generally have little effect on polyphenylene sulfide stability, but amines, aromatic compounds, and halogenated compounds may cause some swelling and softening over extended periods of time at elevated temperatures. Strong oxidizing acids, such as nitric acid (> 0.1 %), hydrogen halides (> 0.1 %), peroxy acids (> 1 %), or chlorosulfuric acid degrade polyphenylene sulfide. It is not recommended to use polyphenylene sulfide with oxidizing material, such as sodium hypochlorite and hydrogen peroxide. However, under mild environmental conditions, at low concentrations and for short exposure times, polyphenylene sulfide can withstand these chemicals, for example, as ingredients of common disinfectant solutions.

PEEK

PEEK (Polyether-Ether Ketones) combines excellent properties regarding biocompatibility, chemical resistance, mechanical and thermal stability. PEEK is therefore the material of choice for UHPLC and biochemical instrumentation.

It is stable in the specified pH range (for the Bio-Inert LC system: pH 1 – 13 , see bio-inert module manuals for details), and inert to many common solvents.

There are still some known incompatibilities with chemicals such as chloroform, methylene chloride, THF, DMSO, strong acids (nitric acid > 10 %, sulfuric acid > 10 %, sulfonic acids, trichloroacetic acid), halogens or aqueous halogen solutions, phenol and derivatives (cresols, salicylic acid, and so on).

When used above room temperature, PEEK is sensitive to bases and various organic solvents, which can cause it to swell. Under such conditions, normal PEEK capillaries are sensitive to high pressure. Therefore, Agilent uses stainless steel clad PEEK capillaries in bio-inert systems. The use of stainless steel clad PEEK capillaries keeps the flow path free of steel and ensures pressure stability up to 600 bar. If in doubt, consult the available literature about the chemical compatibility of PEEK.

Polyimide

Agilent uses semi-crystalline polyimide for rotor seals in valves and needle seats in autosamplers. One supplier of polyimide is DuPont, which brands polyimide as Vespel, which is also used by Agilent.

Polyimide is stable in a pH range between 1 and 10 and in most organic solvents. It is incompatible with concentrated mineral acids (e.g. sulphuric acid), glacial acetic acid, DMSO and THF. It is also degraded by nucleophilic substances like ammonia (e.g. ammonium salts in basic conditions) or acetates.

Polyethylene (PE)

Agilent uses UHMW (ultra-high molecular weight)-PE/PTFE blends for yellow piston and wash seals, which are used in 1290 Infinity pumps, 1290 Infinity II/III pumps, the G7104C and for normal phase applications in 1260 Infinity pumps.

Polyethylene has a good stability for most common inorganic solvents including acids and bases in a pH range of 1 to 12.5 . It is compatible with many organic solvents used in chromatographic systems like methanol, acetonitrile and isopropanol. It has limited stability with aliphatic, aromatic and halogenated hydrocarbons, THF, phenol and derivatives, concentrated acids and bases. For normal phase applications, the maximum pressure should be limited to 200 bar.

Tantalum (Ta)

Tantalum is inert to most common HPLC solvents and almost all acids except fluoric acid and acids with free sulfur trioxide. It can be corroded by strong bases (e.g. hydroxide solutions > 10 %, diethylamine). It is not recommended for the use with fluoric acid and fluorides.

Stainless Steel (SST)

Stainless steel is inert against many common solvents. It is stable in the presence of acids and bases in a pH range of 1 to 12.5 . It can be corroded by acids below pH 2.3 . It can also corrode in following solvents:

- Solutions of alkali halides, their respective acids (for example, lithium iodide, potassium chloride) and aqueous solutions of halogens.
- High concentrations of inorganic acids like nitric acid, sulfuric acid, and organic solvents 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:

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

  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, diisopropyl ether). Such ethers should be filtered through dry aluminum 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, ethylenediaminetetraacetic acid).
- Mixtures of carbon tetrachloride with isopropanol or THF.

Titanium (Ti)

Titanium is highly resistant to oxidizing acids (for example, nitric, perchloric and hypochlorous acid) over a wide range of concentrations and temperatures. This is due to a thin oxide layer on the surface, which is stabilized by oxidizing compounds. Non-oxidizing acids (for example, hydrochloric, sulfuric and phosphoric acid) can cause slight corrosion, which increases with acid concentration and temperature. For example, the corrosion rate with 3 % HCl (about pH 0.1) at room temperature is about 13 µm/year. At room temperature, titanium is resistant to concentrations of about 5 % sulfuric acid (about pH 0.3). Addition of nitric acid to hydrochloric or sulfuric acids significantly reduces corrosion rates. Titanium is sensitive to acidic metal chlorides like $FeCl_3$ or $CuCl_2$. Titanium is subject to corrosion in anhydrous methanol, which can be avoided by adding a small amount of water (about 3 %). Slight corrosion is possible with ammonia > 10 %.

Diamond-Like Carbon (DLC)

Diamond-Like Carbon is inert to almost all common acids, bases, and solvents. There are no documented incompatibilities for HPLC applications.

Fused Silica and Quartz (SiO_2)

Fused silica is used in Max Light Cartridges. Quartz is used for classical flow cell windows. It is inert against all common solvents and acids except hydrofluoric acid and acidic solvents containing fluorides. It is corroded by strong bases and should not be used above pH 12 at room temperature. The corrosion of flow cell windows can negatively affect measurement results. For a pH greater than 12, the use of flow cells with sapphire windows is recommended.

Gold

Gold is inert to all common HPLC solvents, acids, and bases within the specified pH range. It can be corroded by complexing cyanides and concentrated acids like aqua regia.

Zirconium Oxide (ZrO_2)

Zirconium Oxide is inert to almost all common acids, bases, and solvents. There are no documented incompatibilities for HPLC applications.

Platinum/Iridium

Platinum/Iridium is inert to almost all common acids, bases, and solvents. There are no documented incompatibilities for HPLC applications.

Fluorinated Polymers (PTFE, PFA, FEP, FFKM, PVDF)

Fluorinated polymers like PTFE (polytetrafluorethylene), PFA (perfluoroalkoxy), and FEP (fluorinated ethylene propylene) are inert to almost all common acids, bases, and solvents. FFKM is perfluorinated rubber, which is also resistant to most chemicals. As an elastomer, it may swell in some organic solvents like halogenated hydrocarbons.

TFE/PDD copolymer tubings, which are used in all Agilent degassers except G1322A/G7122A, are not compatible with fluorinated solvents like Freon, Fluorinert, or Vertrel. They have limited life time in the presence of hexafluoroisopropanol (HFIP). To ensure the longest possible life with HFIP, it is best to dedicate a particular chamber to this solvent, not to switch solvents, and not to let dry out the chamber. For optimizing the life of the pressure sensor, do not leave HFIP in the chamber when the unit is off.

The tubing of the leak sensor is made of PVDF (polyvinylidene fluoride), which is incompatible with the solvent DMF (dimethylformamide).

Sapphire, Ruby, and Al_2O_3-Based Ceramics

Sapphire, ruby, and ceramics based on aluminum oxide Al_2O_3 are inert to almost all common acids, bases, and solvents. There are no documented incompatibilities for HPLC applications.

At-a-Glance Details About Agilent Capillaries

The following section provides useful information about Agilent capillaries and its characteristics.

Syntax for capillary description

Type - Material - Capillary dimensions - Fitting Left/Fitting right

Table 36: Example for a capillary description

Code provided with the part	Meaing of the code
Color code: 	Material of the product is MP35N, the inner diameter is 0.20 or 0.25 mm
Capillary	The part is a connection capillary
MP35N	Material of the part is MP35N
0.25 x 80 mm	The part has an inner diameter of 0.25 mm and a length of 80 mm
SI/SI	Left fitting: Swagelok + 1.6 mm Port id, Intermediate Right fitting: Swagelok + 1.6 mm Port id, Intermediate

To get an overview of the code in use, see

- Color: Table 37 on page 277
- Type: Table 38 on page 277
- Material: Table 39 on page 278
- Dimension: Table 40 on page 278
- Fittings: Table 41 on page 279

Color Coding Guide

Table 37: Color-coding key for Agilent capillary tubing

Internal diameter in mm		Color code
0.015		Orange
0.025		Yellow
0.05		Beige
0.075		Black
0.075	MP35N	Black with orange stripe
0.1		Purple
0.12		Red
0.12	MP35N	Red with orange stripe
0.17		Green
0.17	MP35N	Green with orange stripe
0.20 /0.25		Blue
0.20 /0.25	MP35N	Blue with orange stripe
0.3		Grey
0.50		Bone White

NOTE As you move to smaller-volume, high efficiency columns, you'll want to use narrow id tubing, as opposed to the wider id tubing used for conventional HPLC instruments.

Abbreviation Guide for Type

Table 38: Type (gives some indication on the primary function, like a loop or a connection capillary)

Key	Description
Capillary	Connection capillaries
Loop	Loop capillaries
Seat	Autosampler needle seats

Key	Description
Tube	Tubing
Heat exchanger	Heat exchanger

Abbreviation Guide for Material

Table 39: Material (indicates which raw material is used for the capillary)

Key	Description
ST	Stainless steel
Ti	Titanium
PK	PEEK
FS/PK	PEEK-coated fused silica [1]
PK/ST	Stainless steel-coated PEEK [2]
PFFE	PTFE
FS	Fused silica
MP35N	Nickel-cobalt-chromium-molybdenium alloy

Abbreviation Guide for Capillary Dimensions

Table 40: Capillary dimensions (indicates inner diameter (id), length, and volume of the capillary)

Description
id (mm) x Length (mm)
Volume (µL)

1 Fused silica in contact with solvent

2 Stainless steel-coated PEEK

Abbreviation Guide for Fitting Left/Fitting Right

Table 41: Fitting left/fitting right (indicates which fitting is used on both ends of the capillary)

Key	Description
W	Swagelok + 0.8 mm Port id
S	Swagelok + 1.6 mm Port id
M	Metric M4 + 0.8 mm Port id
E	Metric M3 + 1.6 mm Port id
U	Swagelok union
L	Long
X	Extra long
H	Long head
G	Small head SW 4
N	Small head SW 5
F	Finger-tight
V	1200 bar
B	Bio
P	PEEK
I	Intermediate

Waste Electrical and Electronic Equipment (WEEE) Directive

This product complies with the European WEEE Directive marking requirements. The affixed label indicates that you must not discard this electrical/electronic product in domestic household waste.

NOTE

Do not dispose of in domestic household waste
To return unwanted products, contact your local Agilent office, or see https://www.agilent.com for more information.

Radio Interference

Cables supplied by Agilent Technologies are screened to provide optimized protection against radio interference. All cables are in compliance with safety or EMC regulations.

Test and Measurement

If test and measurement equipment is operated with unscreened cables, 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

Sound Pressure

Sound pressure Lp < 70 db(A) according to DIN EN ISO 7779

Schalldruckpegel

Schalldruckpegel Lp < 70 db(A) nach DIN EN ISO 7779

Agilent Technologies on Internet

For the latest information on products and services visit our worldwide web site on the Internet at:

https://www.agilent.com

In This Book

This manual contains technical reference information about the Agilent 1260 Infinity III Fluorescence Detector (G7121A) and Agilent 1260 Infinity III Fluorescence Detector Spectra (G7121B).

The manual describes the following:

- introduction and specifications,
- using and optimizing,
- troubleshooting and diagnose,
- maintenance,
- parts identification,
- hardware information,
- safety and related information.

www.agilent.com

Edition: 10/2024

Document No: SD-29000222 Rev. D

Agilent