

TN3610: ACQUITY UPLC System Connection Guide for Wyatt Instrumentation

Summary

This document provides the basic guidelines for interfacing a Waters™ ACQUITY UPLC™ system and Wyatt Technology™ instruments. If you are planning to connect a Waters HPLC system to Wyatt components, please refer to Technical Note TN3601.

The first section details the electrical connections necessary to transfer the signal of an auxiliary concentration detector and the contact closure signal from an ACQUITY UPLC autosampler (to synchronize sample injection and data collection) into ASTRA™ software.

The second section describes the necessary software settings in both ASTRA software and Empower™ software.

Related References

M1000/M1006/M1111 ASTRA software User's Guides
M1510/M1520 microOptilab detector User's Guides
M3500/M3520 microDAWN MALS detector User's Guides
M1610/M1620 microViscoStar viscometer User's Guides
TN1007 ASTRA software Quick Guide
Any relevant ACQUITY UPLC system User's Guides

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Introduction

This guide describes the connections necessary to interface a Waters ACQUITY UPLC system setup with Wyatt instruments as shown in Figure 1. During installation, place the optical detectors of the UPLC system as close as possible to the Wyatt instruments: short tubing reduces delay volumes and maintains the chromatographic resolution. Note the arrangement of the components in Figure 1, with the chromatography system located on the left of and under the Wyatt Technology instrumentation, to minimize inter detector tubing lengths. If necessary, adjust the placement of the detectors within the Waters instrument stack to ensure the minimum length of tubing.



Figure 1: Example of an ACQUITY UPLC system with Wyatt instrumentation. Note that the Waters components, column oven, optical detectors, and Wyatt detectors are stacked and the Wyatt instruments are on the left of the ACQUITY UPLC system to help minimize the tubing length. Short tubing minimizes delay volumes and maintains the chromatographic resolution. No tubing connections are present in this image.

The Wyatt microDAWN™ multi-angle light scattering (MALS) detector and microOptilab™ refractive index detectors are designed to be used with UHPLC system applications (e.g., with Waters ACQUITY UPLC systems) to fully exploit the high resolution from UHPLC system.

Establishing the Connection

Transferring signals from Waters instruments to ASTRA software requires establishing a physical connection to a Wyatt instrument. Waters uses a screw terminal I/O signal connector on the rear panel of their equipment (Figure 2) for signal output. A RJ-12 cable with flying leads (Wyatt p/n P4045-19; Figure 2) transfers the signal to an in-port on the rear panel of a Wyatt instrument (Figure 3). The principle is the same for all Waters equipment, but the identity of the ports of the screw terminal vary between instruments.

After describing the general procedure in this section, some representative port assignments will be shown for a Waters detector in [Connecting to a ACQUITY UPLC UV Detector](#). Sending a contact closure signal from a Waters ACQUITY UPLC autosampler to ASTRA software is covered in [Connecting to an ACQUITY UPLC Autosampler](#). In case of any questions about your instrument's port assignment, please refer to the respective Waters manual.

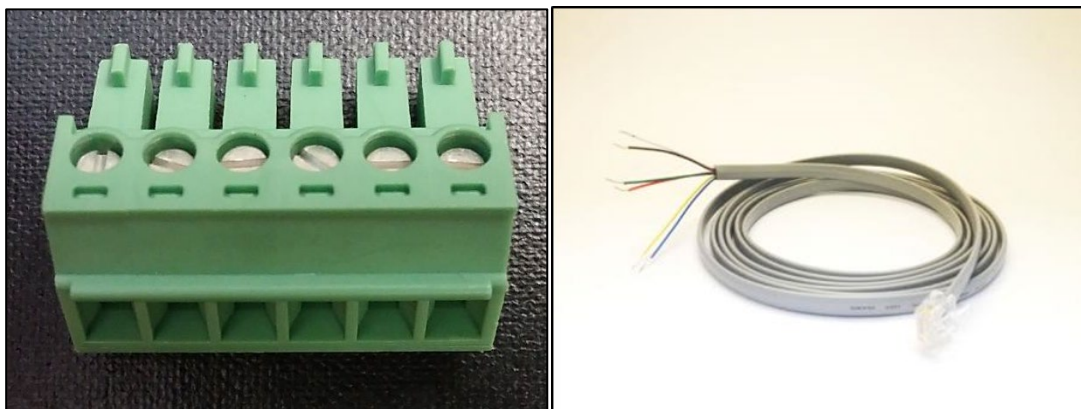
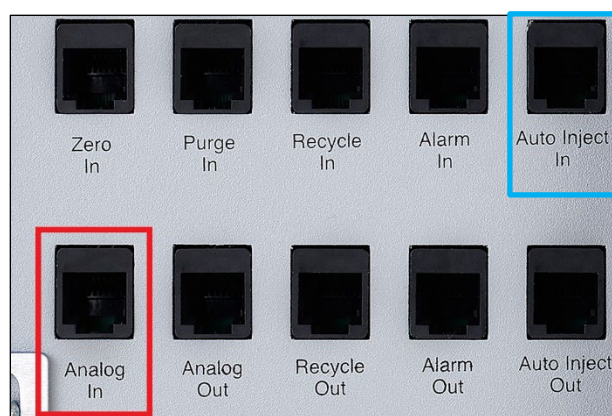


Figure 2: Screw terminal connector used on the rear panel of Waters instruments (left) and RJ-12 cable (right).

On the Wyatt instrument side, the RJ-12 cable has to be connected to an in-port. For analog detector signals, the receiving ports of the light scattering detectors are labelled as [Aux In 1 & 2](#) and, if there is, [Aux In 3 & 4](#) (Figure 3a). The port of an Optilab™ detector instrument is labelled as [Analog In](#) (Figure 3b). Contact closure signals (auto inject signals) that denote sample injections are received through the [Auto Inject In](#) port of all Wyatt instruments (Figure 3).



a) Auxiliary signal port (red) and auto inject signal port (blue) location on the rear panel of the microDAWN (NEON™) MALS detector. The older versions, i.e. μ DAWN™ MALS detector (serial number xxxx-UD) have two Aux In ports (Aux In 1&2 and Aux In 3&4).



b) Auxiliary signal port (red) and auto inject signal port (blue) location on the rear panel of the microOptilab detector. It is the same for the Optilab UT-rEX™ detector. The microViscoStar™ viscometer location is similar, but its rear panel has fewer ports.

Figure 3: In-ports of Wyatt Technology instruments.

The RJ-12 cable has six available leads to establish connections. The input channel assignment of an optical signal in the ASTRA software depends on which lead is connected to the analog out port of the Waters instrument. The possible combinations are detailed in [Table 1](#).

			BNC	
			red	black
DAWN MALS detector, miniDAWN MALS detector, microDAWN MALS detector	AUX IN 1 & 2	channel 1	White RJ-12	Black RJ-12
		channel 2	Red RJ-12	Green RJ-12
	AUX IN 3 & 4	channel 3	White RJ-12	Black RJ-12
		channel 4	Red RJ-12	Green RJ-12
Optilab detector, ViscoStar viscometer	Analog In		Red RJ-12 White RJ-12	Green RJ-12 Black RJ-12

Table 1: Wire color conventions for using an RJ-12 cable.

Crimping the white and black wires to the BNC adaptor and using **Aux In 1 & 2** as the signal in port transmits the signal to Aux channel 1. If **Aux In 3 & 4** is used as the in port, the transmission is to Aux channel 3. Alternatively, connecting the red and green wires to the BNC adaptor transmits the signal to Aux channel 2 or 4, depending on the Wyatt Aux In port the RJ-12 is plugged in to.

*The Wyatt Optilab T-rEX™ detector, Optilab UT-rEX detector, Optilab (NEON) detector and microOptilab detector or Wyatt ViscoStar II viscometer instruments have only one Analog Input port, labeled “**Analog IN**”. This port has only one active channel, which uses the green (-) and red (+) wires of the RJ-12 cable. When wiring to connect to either of these ports, one must connect the green wire from the RJ-12 cable to the black binding post of the BNC connector, and the red wire from the RJ-12 cable to the red binding post of the BNC connector. In contrary to miniDAWN™ TREOS™ I MALS detector and the DAWN™ HELEOS™ I/II MALS detectors and DAWN (NEON) MALS detectors with two Aux In ports (Aux In (1&2) and Aux In (3&4)), the miniDAWN TREOS II MALS detector/ miniDAWN (NEON) MALS detector and microDAWN (NEON) MALS detector have one **Aux In** (1&2) port where the active channel depends on the wiring in the above table. The ViscoStar™ III viscometer/ViscoStar (NEON) viscometer as well as microViscoStar viscometer (NEON) have one **Analog In** port where the active channel depends on the wiring and is different than the above table (2 channels with either white-black (Analog In 2) or red-green (Analog In 1) wiring pairs).

Note: The blue and yellow wires of the RJ-12 cable are ground wires and, therefore, are not necessary for any connections. We recommend to taping off any unused wires on the RJ-12 cable with electrical tape unless you observe excessive noise pickup.

Connecting to a ACQUITY UPLC UV Detector

The steps below describe how to connect a Waters ACQUITY UPLC UV detector and a Wyatt instrument in order to transfer the signal of the detector to the ASTRA software.

1. Locate a RJ-12 cable with flying leads.
2. Pull out the screw terminal connector on the back of the Waters detector (Figure 4).

- Attach two of the wires of the RJ-12 cable (see [Table 1](#) for wire colors) to screw terminals 5 and 6 of the I/O signals connector on the back panel of the ACQUITY UPLC UV detector (Figure 4) using a flat head screwdriver. Make sure the wires are secure and contact with the connector is achieved.

Waters port	description	RJ-12 lead
port 5	Analog out +	white or red
port 6	Analog out -	black or green

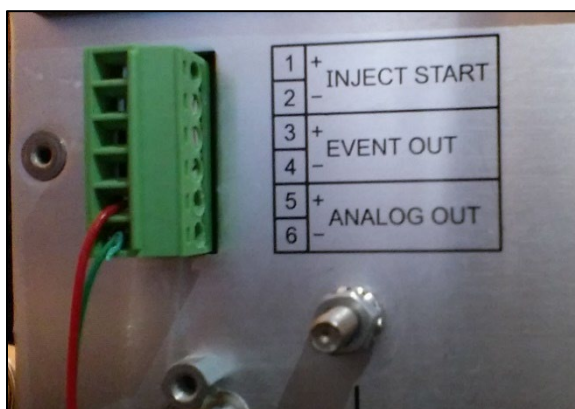


Figure 4: Auxiliary channel connection on the rear panel of the ACQUITY UPLC UV Detector. A RJ-12 cable is connected to the Analog out port.

- Plug the screw terminal connector back into the rear panel of the ACQUITY UPLC UV detector.
- Plug the RJ-12 cable into your Wyatt instrument. See Figure 3 for port locations and [Table 1](#) for channel assignments.
- The output range of this analog UV signal is 0 to 2.25 V. In the Empower software, the most common setting for the UV analog response is 1 AUFS. With this analog response, you will need to enter a UV response factor of 0.4444 AU/V into the ASTRA software configuration (see [Configuring the ASTRA Software to Accept ACQUITY UPLC UV Detector Signals](#) for details.). If the Empower software analog response is different, the response factor in the ASTRA software has to be changed accordingly (0.5 AUFS in Empower software results in 0.2222 AU/V in ASTRA software).

Note: The ACQUITY UPLC UV Detector only has one available analog output available on the rear panel (refer to Figure 4). An “ANALOG 2” port may be available depending on the type of ACQUITY UPLC system concentration detector being connected. For example, the *ACQUITY UPLC system Photodiode Array eλ detector* typically has two analog out ports. In this later case, the second set of flying leads (black & white or red & green) may be used to output a second signal to the microDAWN MALS detector instrument. Please follow the directions above to connect a second set of wires to the output ports on the green connector corresponding to the “ANALOG 2” ports to use the second AUX channel.

Connecting to an ACQUITY UPLC Autosampler

To synchronize sample injection and data collection in ASTRA software, the contact closure signal has to be transferred to the Wyatt detector.

- Locate a RJ-12 cable with flying leads.

- Remove the green screw terminal connector on the rear panel of the ACQUITY UPLC autosampler (Figure 5).
- Connect the green and red wires to screw terminals 1 and 2 of the I/O signals connector on the back panel of the ACQUITY UPLC autosampler (Figure 5) using a flat head screwdriver. Make sure the wires are secure and contact with the connector is achieved.

Table 2. Wire colors for outputting UV channels from Waters ports

Waters port	Description	RJ-12 lead
port 1	Inject Start Out	green
port 2	Inject Start Out	red

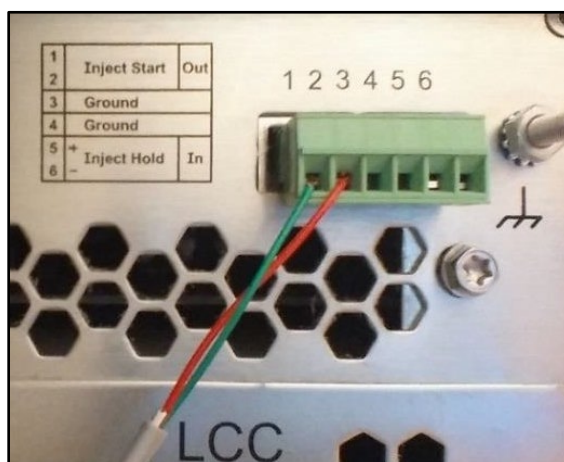


Figure 5: Inject Start Out port of an ACQUITY UPLC autosampler back panel with the green and red wires of a RJ-12 cable connected to ports 1 and 2.

- Plug the RJ-12 cable into the [Auto Inject In](#) port located on the rear panel of the Wyatt instrument (see Figure 3).
- The ACQUITY UPLC autosampler is probably already configured to send an adequate electrical pulse (contact closure) to the Wyatt detector upon injection; this pulse can be used to initiate ASTRA software data collection if ASTRA software is configured as described in [Configuring the ASTRA Software to Accept ACQUITY UPLC system Autoinject Signals](#).

Note: The ASTRA software cannot control the Waters ACQUITY UPLC system. There is no formal communication between the Waters ACQUITY UPLC system and the ASTRA software beyond this auto-injection contact closure signal.

Synchronization of the Data Traces

To optimize synchronization of the data traces recorded with two Wyatt detectors, we recommend sending the autoinject signal to both detectors. This is accomplished by connecting the two instruments through an autoinject crossover cable (Wyatt p/n 164250; Figure 16). Plug the end of the cable labeled [AUTO INJECT OUT](#) into the [Auto Inject Out](#) port on the rear panel of the detector that received the signal from the UHPLC. Plug the other end labeled [AUTO INJECT IN](#) into the [Auto Inject In](#) port on the rear panel of the second detector. (See Figure 3 for the location of the ports, Figure 6 for an illustration of the connection.)



Figure 6: An autoinject crossover cable connection between the microDAWN (NEON) MALS detector (top) and microOptilab detector or Optilab detector (NEON) (bottom) for optimized synchronization. The crossover cable transmits the autoinject signal received by the light scattering detector to the RI detector for optimized synchronization.

Configuring the ASTRA Software to Accept ACQUITY UPLC UV Detector Signals

In order for ASTRA software to process analog signals from external instruments connected through the auxiliary inputs of Wyatt instruments, the experiment **Configuration** has to contain a definition of the *instrument* that is connected and the *channel* used to transmit the signal. These steps are detailed here.

1. Select the appropriate ASTRA software method and instrument configuration. Refer to the *ASTRA software Quick Guide* and *ASTRA software User's Guide* for assistance with your particular detector configuration.
2. Expand the “**Configuration**” tree and double-click on **Generic UV Instrument (UV)**. This will expand the instrument profile, and allow you to enter the necessary instrument information (see Figure 7):
 - **Cell Length (cm)** – The length of the UV cell in cm (this value is often 1.0 cm).
 - **Channels** – Enable the number of channels that correspond to the number of analog out signals that were physically connected in the procedure detailed above. Note that unless your detector has two analog out ports, only one channel is being used.
 - **Wavelength** – The wavelength monitored in this channel (typically 280 nm for proteins).

- **UV response** – The UV calibration constant for the Aux channel which is used to convert the volts of the analog signal to absorbance units. For an ACQUITY UPLC UV detector with an analog output range of 0.25 V and an analog response of 1 AUFS, the value is 0.4444 AU/V.
- **Active Channel** – Select the channel that will be used to calculate sample concentration from UV absorption.

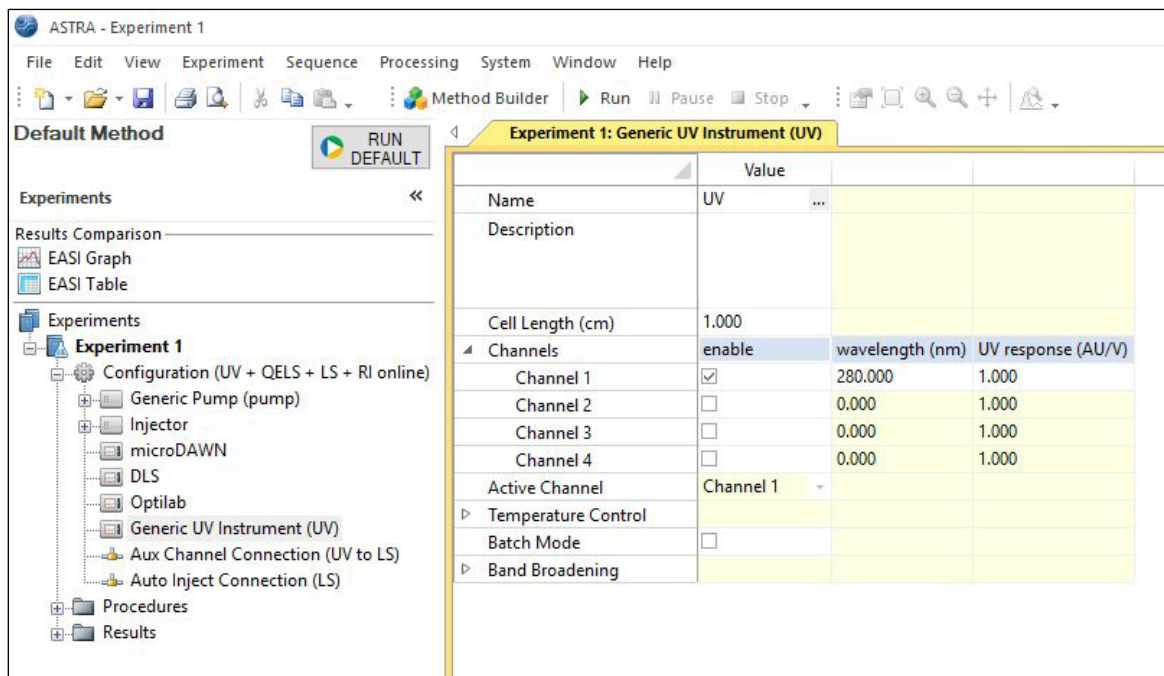


Figure 7: A screenshot of ASTRA software with the **Configuration** node expanded and **Generic UV Instrument (Generic UV)** selected.

3. Select **OK** to save changes and exit this window.
 4. Double-click on **Aux Channel Connection (UV to LS)** to see the settings for this configuration in the right-hand pane (see Figure 8). One **Aux Channel Connection** has to be defined in the experiment configuration for every physical analog input connection that has been established to enable ASTRA software to display and use the analog signal. In case multiple auxiliary input channels have been connected, multiple **Aux Channel Connections (UV to LS)** need to be defined. (Calculating the concentration of a sample from an auxiliary input is only possible for the **Active Channel** defined in step 2 above.)
- The parameters are:
 - **Source Device** – The instrument that sends analog data over this connection. The default setting here is 'Generic UV Instrument'.
 - **Source AUX Channel** – If multiple channels are enabled under the **Generic UV Instrument** configuration (step 2 above), the drop down menu will list all available channels. Select the channel for this connection.
 - **Destination Device** – The instrument that receives analog data over this connection. The default setting is the light scattering detector (e.g. 'microDAWN').
 - **Destination AUX Channel** – The destination channel is defined by the physical connection to the Wyatt instrument **AUX IN** port from the ACQUITY UPLC UV detector (refer to [Table 1.](#)). For example, a RJ-12 cable with its white and black cables connected to the analog out of a ACQUITY UPLC UV

detector and plugged into the **AUX IN 1 & 2** port of the microDAWN MALS detector instrument will be input AUX channel number 1.

Note: If the analog UV signal will be sent to a Wyatt Optilab UT-rEX detector or microOptilab detector that device should be set as the Destination Device (above) and the Destination Aux Channel must be set to AUX channel 2.

- **Calibration Constant** - This number is a correcting constant that can be used to adjust the value by which the AUX signal should be scaled. Under most circumstances, this value should be left at 1. If the calibration constant is changed to 2, then the calculated molar mass will be doubled. If this value is changed to 0.5, then the calculated molar mass will be halved, if the concentration source is chosen to be the UV signal.

Note: The calibration constant can be adjusted to scale the UV voltage signal to correct for small deviations of the ACQUITY UPLC UV detector and obtain the correct molar mass for a standard such as BSA. For example, the cell length of a Waters ACQUITY UPLC UV detector can range from approximately 1.02 to 0.98 cm. Additionally, there may be some UV backscattering that affects the length of the cell in the measurement. The calibration constant can be adjusted to correct for these. For more information about variations in your instrument, contact Waters.

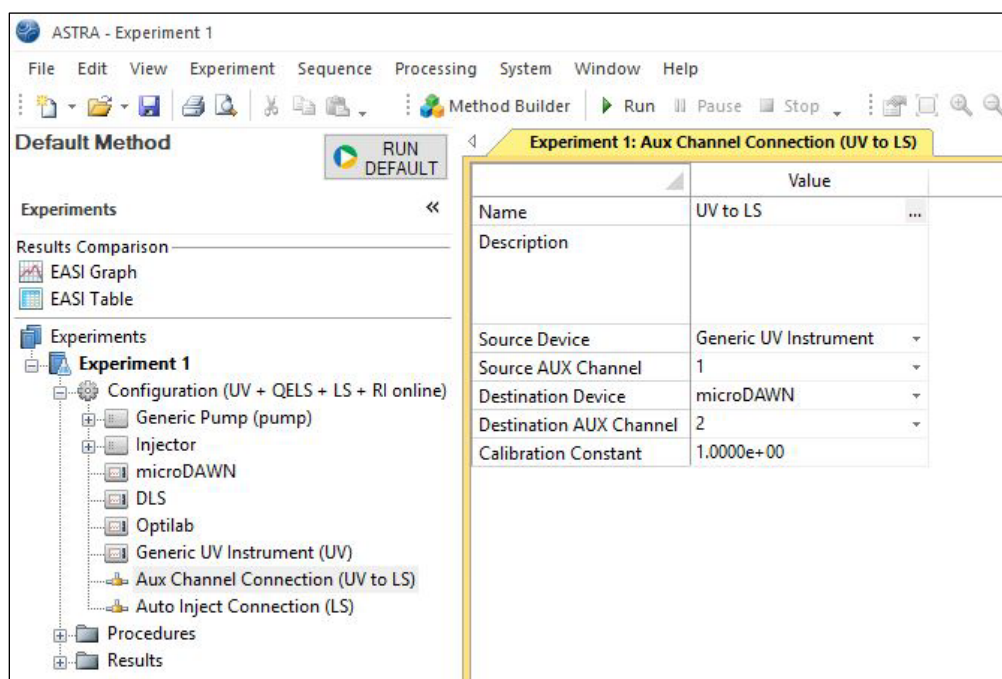


Figure 8: A screenshot of the ASTRA software with the **Configuration** node expanded and **Aux Channel Connection (UV to LS)** selected.

5. Select **OK** to save changes and exit this window.

This completes the ASTRA software setup necessary for the ACQUITY UPLC UV detector.

Configuring the ASTRA Software to Accept ACQUITY UPLC system Autoinject Signals

Follow the steps below to enable ASTRA software to accept an autoinject signal from your UPLC:

1. Expand the experiment [Configuration](#) and double-click on [Injector](#) to define the [Injected Volume \(μL\)](#) (refer to Figure 9):

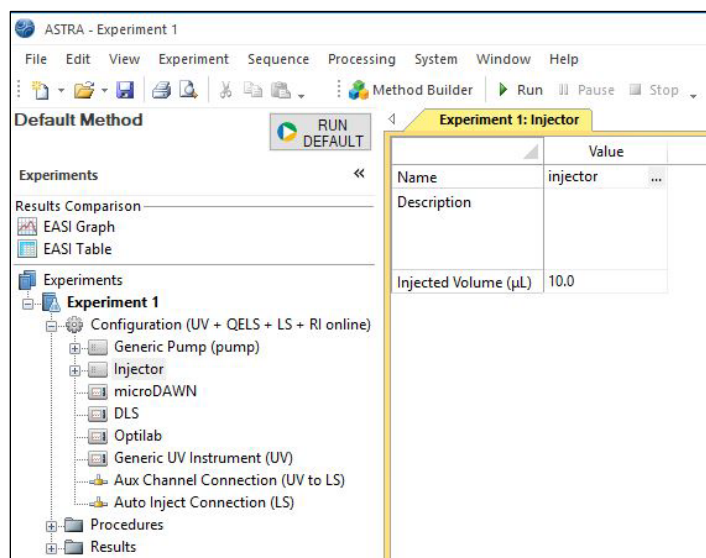


Figure 9: A screenshot of the ASTRA software with the [Configuration](#) node expanded and [Injector](#) selected.

Note: ASTRA software uses the injection volume to calculate the injected mass. The injected mass is the product of the injection volume and the sample concentration entered in the [Sample](#) profile (sub-category of the [Injector](#) node). Using these values, ASTRA software calculates the recovered masses, as well as associated mass recovery percentages. Entering the injection volume in ASTRA software does not control the injection volume that the ACQUITY UPLC autosampler will draw from the sample vial.

2. Select [OK](#) to exit the window.
3. Double-click on [Auto Inject Connection \(LS\)](#) to open that profile (see Figure 10). The parameters are:
 - [Source Device](#) – The instrument that sends the autoinject signal. The default setting is Injector.
 - [Destination Device](#) – The instrument that receives the autoinject signal. The default setting is the light scattering detector (e.g. microDAWN).
4. Select [OK](#) to save and close this window.

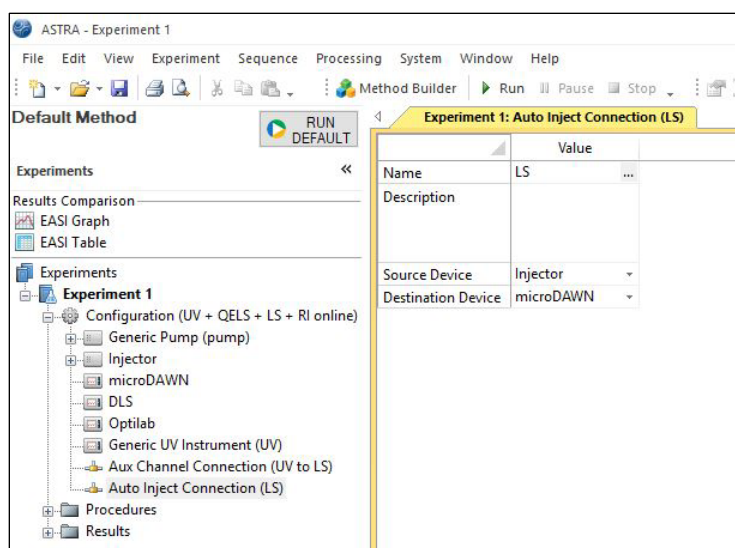


Figure 10: A screenshot of ASTRA software with the **Configuration** node expanded and **Auto-Inject Connection (LS)** selected.

- Expand the **Procedures** node in the ASTRA software workspace and double click on **Basic Collection** (see Figure 11).

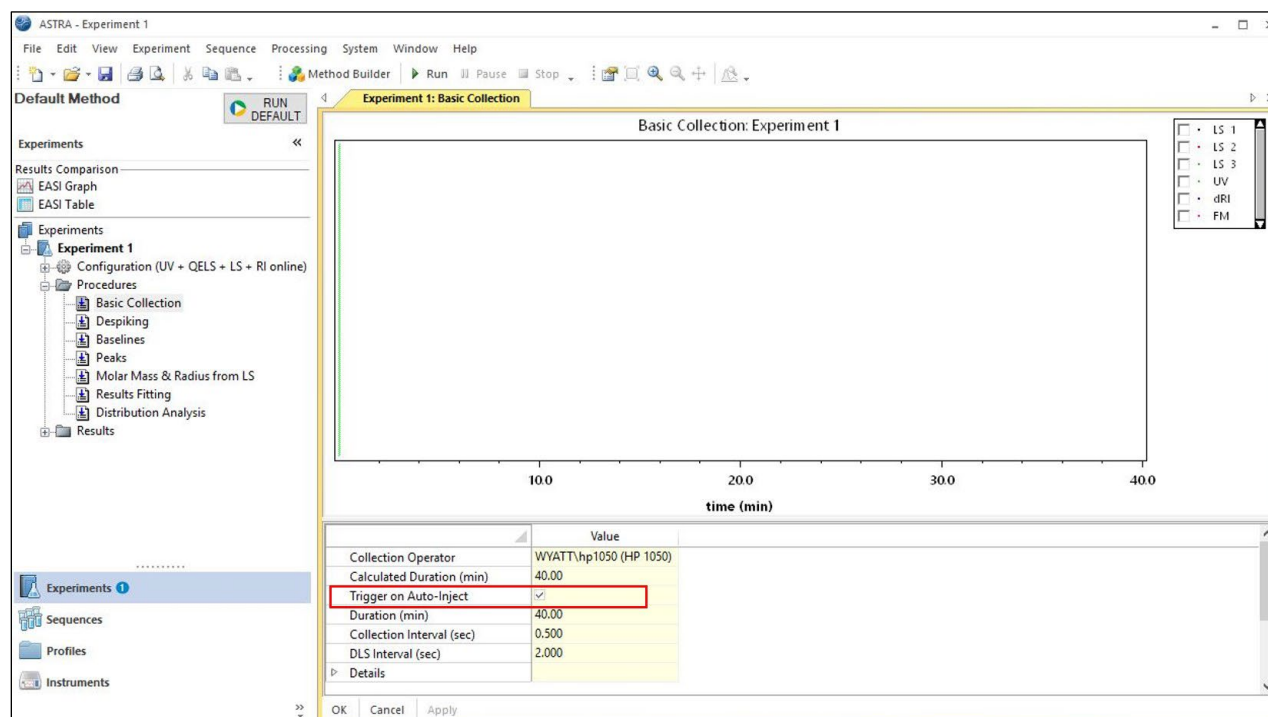


Figure 11: A screenshot of the ASTRA software screen with the **Procedures** node expanded and **Basic Collection** selected.

- Check the “**Trigger on Auto-Inject**” box to have the ACQUITY UPLC system injection signal initiate the start of the ASTRA software data collection.
- Set the **Collection Interval** to 0.050 sec and the **QELS interval (DLS)** to 0.500 sec.

8. Select [Apply](#) to save your settings. You are now ready to begin your collection. This experiment can also be saved as a method for use in a sequence run.

For details on how to set up sequences and process experiments please see *TN1007 – ASTRA Software Quick Guide*.

Configuring the Empower 3 Software to Export the UV Signal

Note: The following screenshots and procedures are specific to Empower 3 software controlling an ACQUITY UPLC system. If additional assistance is needed to configure with different versions of Empower software, please contact Wyatt Technology Support.

1. Launch the Waters Empower 3 software.
2. In the main workspace, select the method you wish to run and click [Edit](#). Refer to Figure 12.

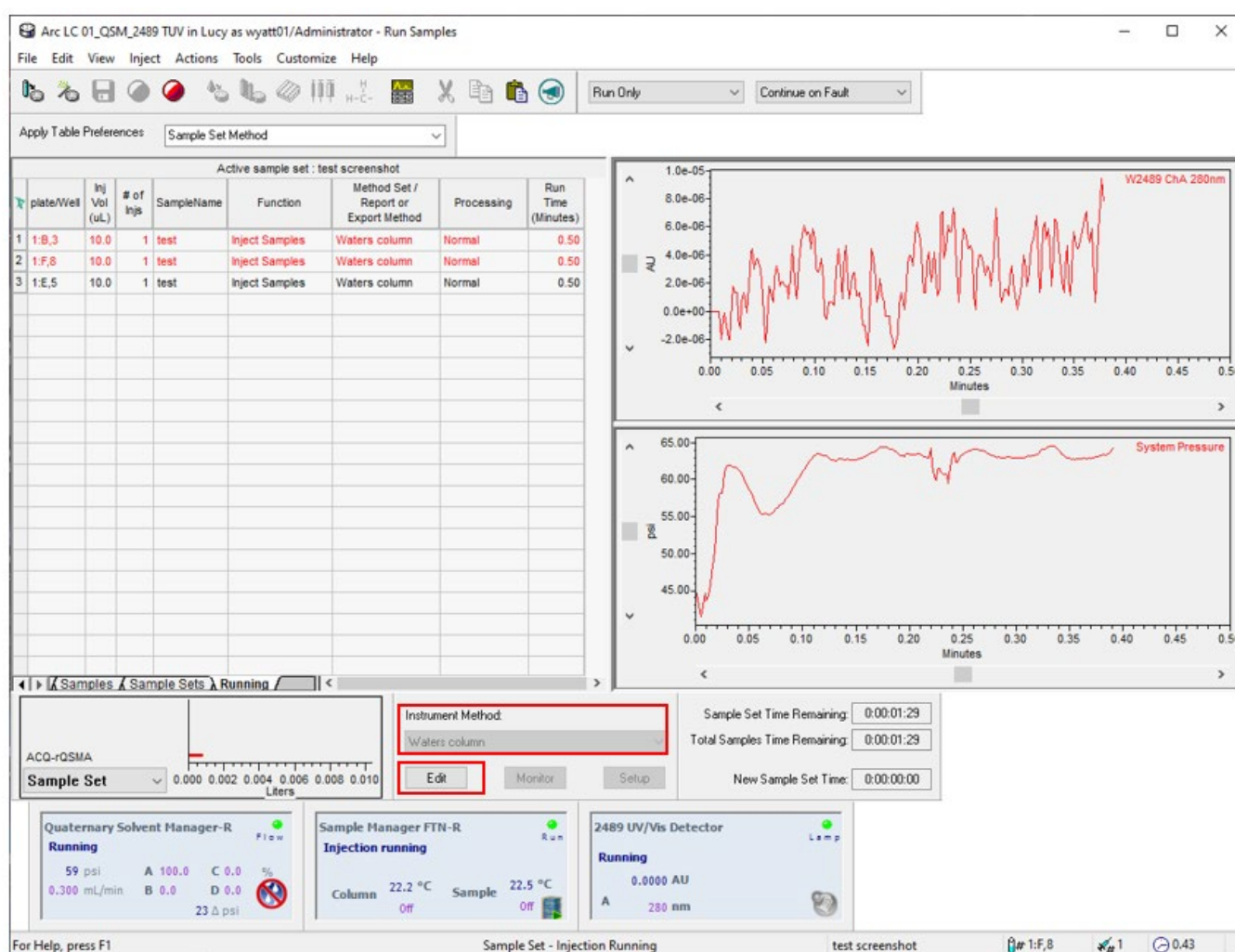


Figure 12: Screenshot of the Empower 3 software main screen.

3. Select the UV-detector module from the top of the method window. Refer to Figure 13.

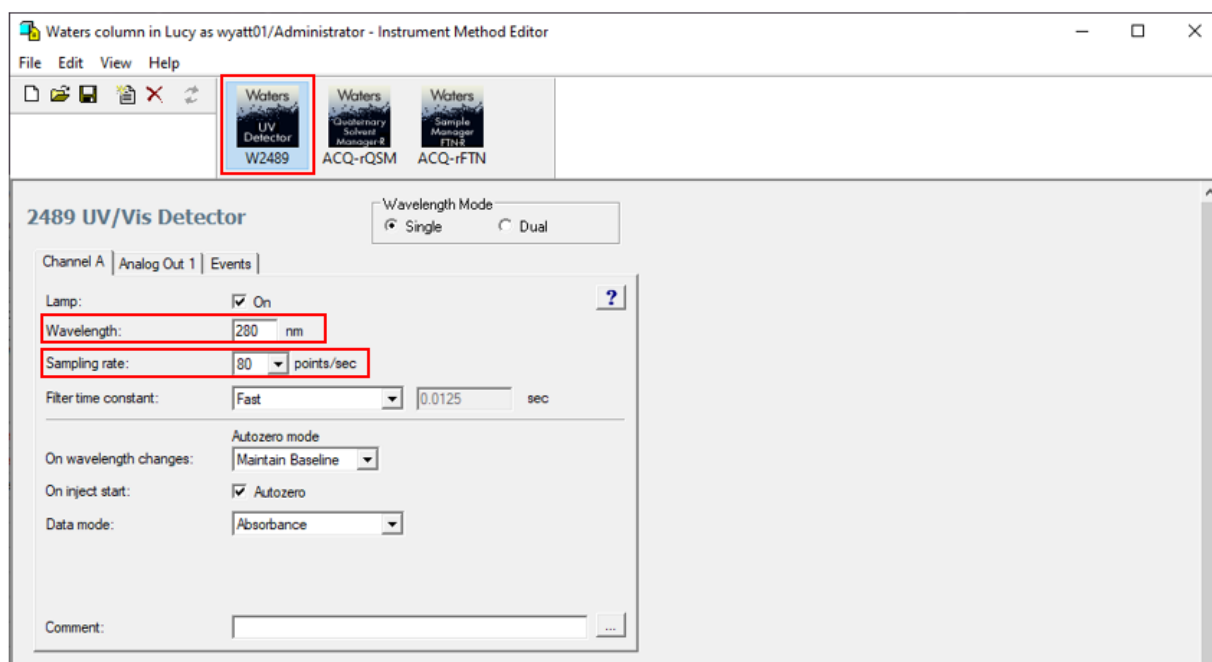


Figure 13: Screenshot of the Empower 3 software showing the method editing menu. The location of the **UV-detector** module is highlighted by the red square as well as the field for **Wavelength** and **Sampling Rate**.

4. Under the **Channel A** tab, enter your wavelength (typically 280 nm) and set the sampling rate to 80 points/sec.
5. Click on the **Analog Out** tab. This will open the menu shown in Figure 14. Verify that the **Sensitivity** is set to 1.00 AUFS and the **Voltage Offset** is set to 0 mV.

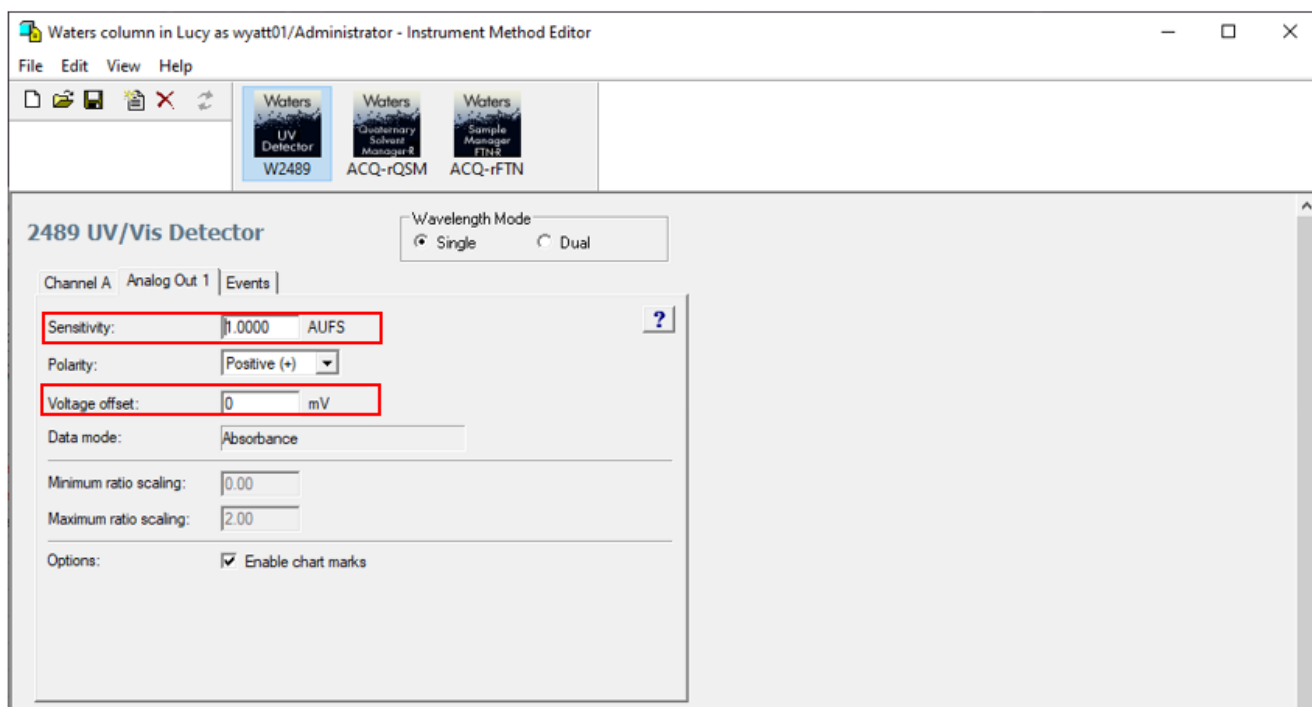


Figure 14: Screenshot of the Empower 3 software showing the **Analog Out** UV detector settings menu. The location of **Sensitivity** and **Voltage Offset** are highlighted by the red squares.

If you have any questions, please email support@wyatt.com or call Wyatt's support team at 805-681-9009, option 4 (within the US and Canada).

If you are one of our international customers, feel free to contact your local representative directly. You can find contact information for our global offices at www.wyatt.com/Distributors.

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Appendix

Tools and Hardware

The following tools and hardware are necessary for a successful installation:

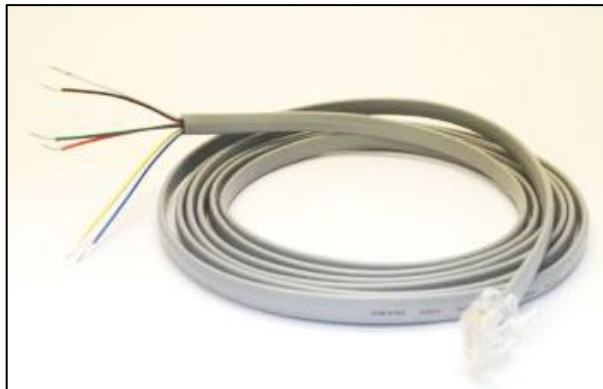


Figure 15: RJ-12 Cable with Flying Leads (Wyatt p/n P4045-19).

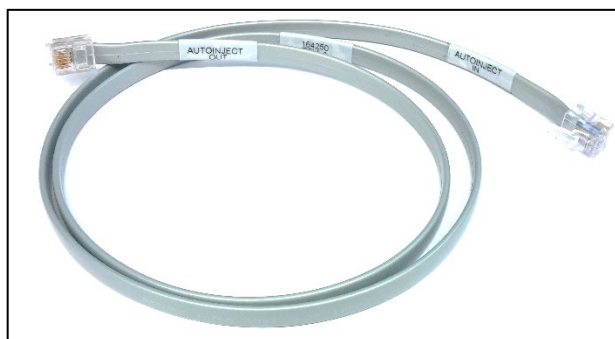


Figure 16: Autoinject crossover cable (Wyatt p/n 164250).

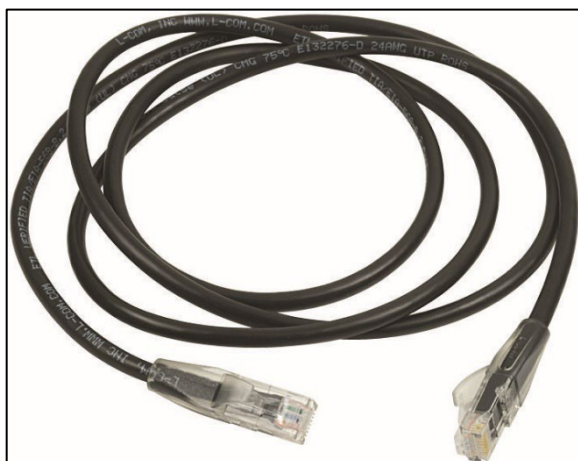


Figure 17: Six (6) foot Ethernet Cable (Wyatt p/n P4045-14).



Figure 18: Slotted Screwdriver 3/16" (Wyatt p/n P9016-A).



Figure 19: Instrument Power Cable. 120V120VAC US plug (Wyatt p/n P4100-120-A) or a universal power cord. International customers will be sent a power cord that meets their country's specifications.

UPLC System Tubing Connection Overview

The following figure summarizes the relevant fluid connections and recommended tubing sizes for a successful installation:

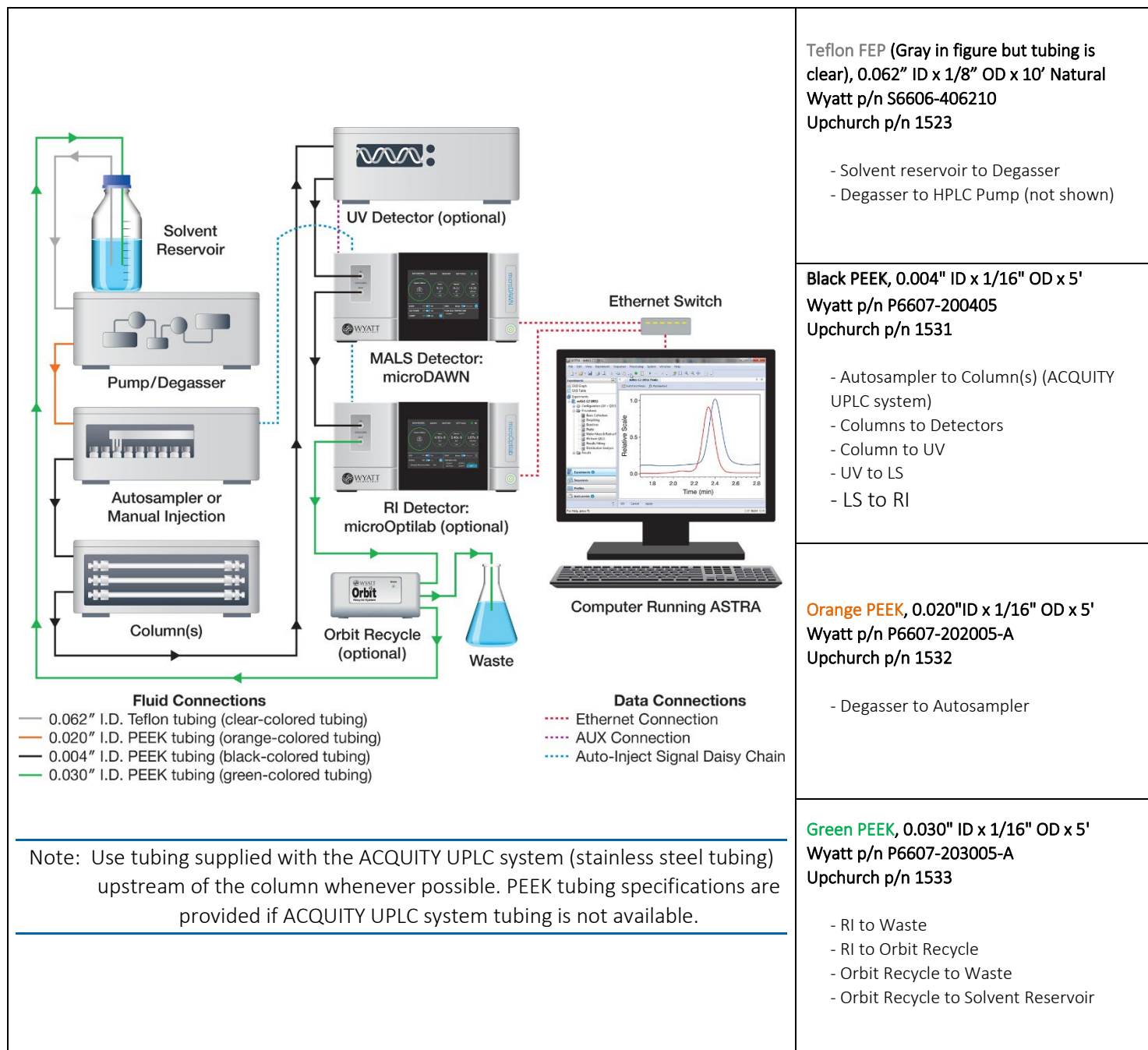


Figure 20: Overview of the recommended tubing connections between the UPLC System and instrumentation. Tubing distances are not drawn to scale, tubing lengths should be minimized to reduce delay volumes and maintain the chromatographic resolution. Refer to the tubing reference table above for tubing descriptions and relevant part numbers. Use standard Upchurch Scientific® PEEK finger-tight fittings (Wyatt p/n 6412-610; Upchurch p/n 55067-U). *Since sometimes 0.004" ID tubing can be blocked (it is more prone to clogging), it might be substituted with 0.005" ID tubing.