



MINISIA-2

MULTIPURPOSE FLOW ANALYZER FOR SIA AND FIA

USER MANUAL

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Date Released: 6/26/2015

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INTRODUCTION

The miniSIA-2 was developed by Global FIA in close consultation with Prof Jarda Ruzicka and is depicted in Figure 1. At its core, the device is equipped with a two bi-directional positive displacement milliGAT high flow (HF) pumps and a multi-position selection valves modified to accept a Chem-on-Valve™ (COV) flow manifold. These devices, collectively called the fluidics engine, provide a means of manipulating fluids in the channels of the COV and associated heated holding coils.

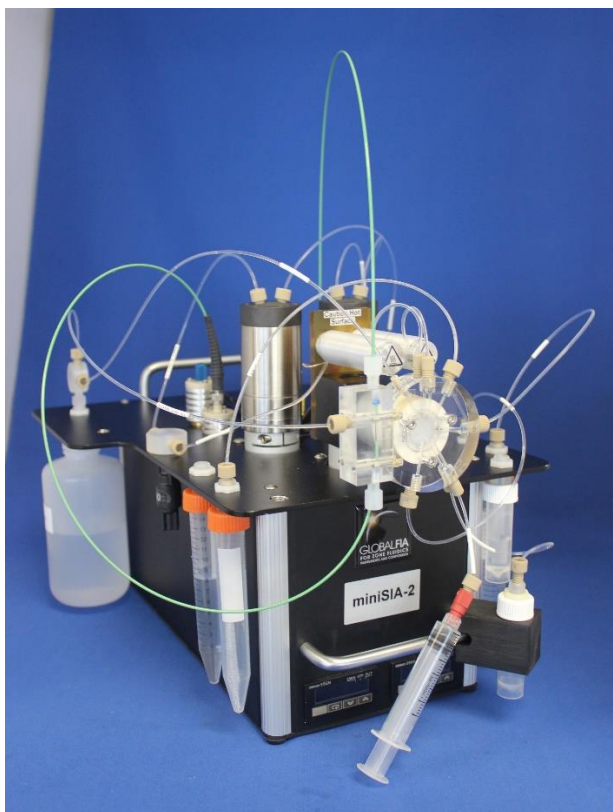


Figure 1: miniSIA-2 in two line configuration

The Global FIA miniSIA-2 combines the power of FIA and SIA in a single instrument without need for any hardware changes. The concept was first described by Ruzicka in the FIA Tutorial¹.

The system's two milliGAT pumps are coupled to twin heated holding coils. The tubing from these two holding coils merge at the center of a COV monolithic manifold. This configuration allows the simultaneous merging of two streams which is a characteristic feature of FIA and a necessity for many chemiluminescence reactions.

¹ <http://www.flowinjectiontutorial.com/> see § 2.3.3

An extra measure of modularity is built into the COV by making the detector flow cell a bolt-on piece. This means that the same COV manifold can be configured with a short or long path length absorbance cell, fluorescence cell, chemiluminescence cell, one of a host of different electrochemical cells, as well as cells that can be used together with bead detection or the increasingly powerful sequential injection chromatography (SICrom) modes of operation.

MiniSIA-2 Can be configured in two ways

- For Sequential Injection as a single line instrument (A)
- For Sequential and Flow Injection as a two line instrument (B)

Both configurations, depicted in the following figure which are equipped with a 10 cm light path absorbance flow cell using fiber optic (f.o.) cables to pipe light to and from the flow cell. A standard 1cm flow cell can also be mounted on COV module. A white LED and a W-Xe lamp are included in the package. The spectrometer is an Ocean Optics USB-4000.

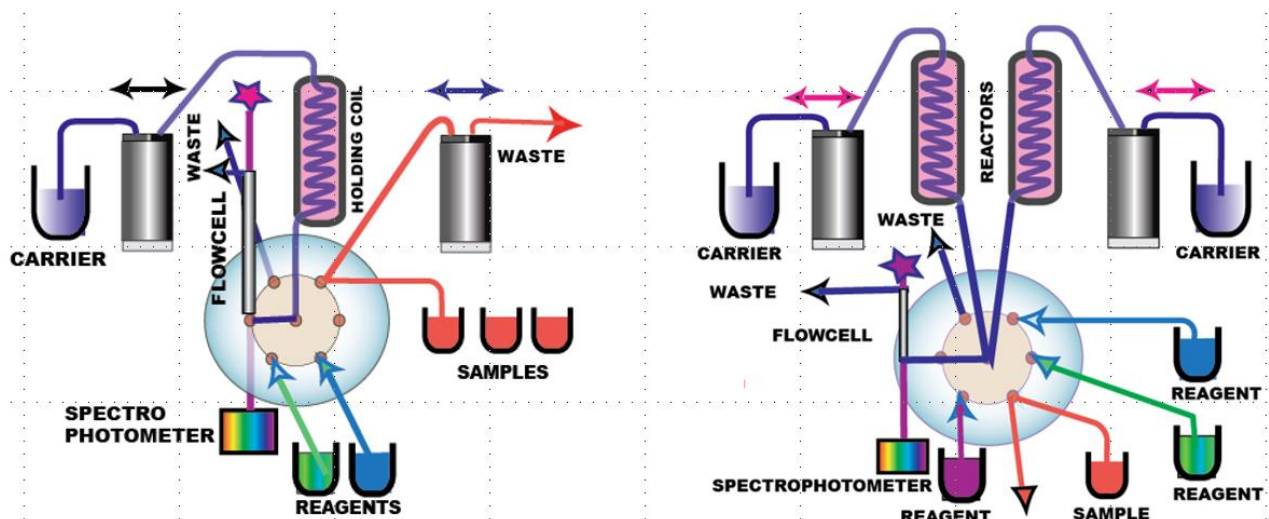


Figure 2: miniSIA-2 flow manifolds: left Single Line (A), right Two line (B) configuration

The sample port has an inlet and outlet which meet on the face of the COV rotor. Thus the sample can be primed to the valve without drawing sample into the rest of the manifold where it can cause carryover. The sample port can also be primed with the next sample while the present measurement is underway.

SIA IN SINGLE LINE CONFIGURATION

Operation of miniSIA-2 in single line mode is preferable, because the assay protocol and operation of instrument is simplified and duration of assay cycle is shorter than if two pumps are used to manipulate sample and reagent. The second pump can be used to aspirate sample into flow through port. The limitation of this approach is that the single line SIA configuration will support only one or two reagent

assays when DI water is used as a carrier. Examples of such assays can be reviewed in [section 2.2.13](#) of the Flow-injection Tutorial²:

When developing new assays use of the Stop Holding Coil method (SHC) is recommended as described in [section 2.1.3](#)³ of the Flow injection tutorial.

Note: Disconnect holding coil 2 and plug the corresponding inlet to central port while making sure that the blocked conduit does not contain air. Bypass the pressure relieve valve (see Figure 9) and connect the second milliGAT pump to sampling flow-through port.

SIA AND FIA IN TWO LINE CONFIGURATION

Merging of streams at the central port of Chem-on-Valve module (Figure 2 and Figure 3) provides an additional degree of freedom for sample/ reagent mixing.

In SIA mode it allows the use of three or more reagent by using a coil transfer technique and in FIA mode by using a merging stream technique.

The coil transfer technique is based on the following steps:

- Inject reagent into coil#1 followed by sample
- Inject reagent #2 into holding coil#2, followed by S+R1 transfer from coil #1
- Inject reagent #3 into holding coil #1 followed by S+R1+R2 transfer from coil #2
- Transport contents of holding coil #1 into flow cell
- Flush holding coils and flow cell by using both pumps

Note the during transfer step the valve should be in the blocked ports position.

In SIA mode, the second pump will facilitate sample splitting for two channel SIA assays. Two pump system will also allow automation of on-column separations, gas diffusion and other complex flow schemes. For more details refer to the FIA Tutorial. The second pump will also facilitate automation of chemiluminescence assays where sample/reagent mixing takes place close to the detector.

In FIA mode, sample (red) (see Figure 3) is aspirated into holding coil 1 followed by a small volume of reagent (blue) which is aspirated into holding coil 2. Next Pump 1 dispenses at a slow flow rate while pump 2 continues to aspirate reagent at a flow rate greater than pump 1 which is dispensing the

2

<http://www.flowinjectiontutorial.com/Methods%202.2.13.%20Assay%20Protocols,%20Fast%20and%20High%20Sensitivity%20SI.html>

³ <http://www.flowinjectiontutorial.com/Methods%202.1.3.%20Selecting%20Assay%20Protocol.html>

sample. This results in sample being merged with reagent, forming reaction product (yellow). Finally, flow is reversed, sending reacted sample product to detector.

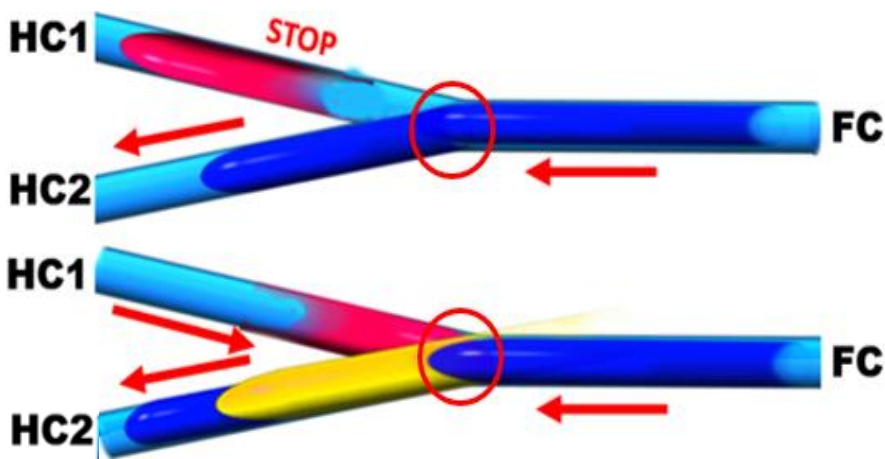


Figure 3: Two line configuration on the miniSIA-2 for FIA mode.

DEVICES AND UNIT OPERATIONS

MILLIGAT PUMP

The microprocessor-controlled milliGAT™ pump (Wolcott & Marshall, 1996) is a positive displacement linear flow pump that consists of the pump head / motor / gear assembly, and an integrated micro-electric controller.

The milliGAT HF features that are of particular importance for the miniSIA-2 include:

- Bi-directional smooth flow
- Flow range from 0.040 – 500 $\mu\text{L}/\text{sec}$ (2.4 $\mu\text{L}/\text{min}$ – 30 mL/min)
- Meters precise volumes from nL – mL.
- Excellent precision ($\pm 12\text{nL}$ for 250 nL dispenses)
- No pulse-dampeners
- No check valves
- No syringes to re-fill or exchange
- Chemically inert
- Self-priming

MULTI-POSITION SELECTION VALVE EQUIPED WITH COV

The multi-position selection valve is mounted atop a right angle drive which orientates the COV in its preferred conformation. The stator of the valve has been replaced with a monolithic flow manifold called the Chem-on-Valve (COV) with certain unique flow paths.

The common port is connected to two flow channels which are coupled via the heated holding coils to the two milliGAT HF pumps as indicated in the port detail rendered in Figure 4. This arrangement provides a merging point where with innovative flow programming, several interesting sample manipulation strategies may be implemented.

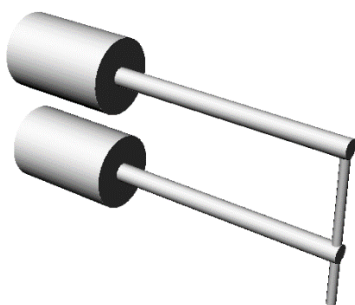


Figure 4: Common port channel detail

The sample port also provides a means of priming the sample to the face of the valve without drawing sample into the flow manifold. The sample port detail is rendered in Figure 5. This channel layout allows the sample port to be primed independently of the rest of the system as long as the sample port is not selected. Priming can be carried out by means of a simple syringe coupled directly the COV using P/N FL-1428M (be careful not to damage the port by applying a side force with the syringe) or via a length of tubing using PN: FL-1428F. The other port is coupled to the sample vial, or in single line configuration by milliGAT pump as shown in Fig.2.A. Alternatively, the systems may be furnished with a diaphragm pump which can be used to pump the new sample to the valve face from a sample vial or auto sampler.

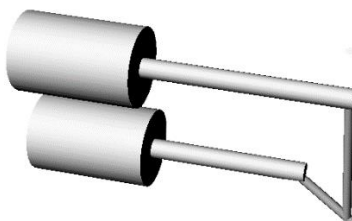


Figure 5: Sample port channel detail

The valve is coupled to the same motor type with integrated controller used for the pump and an embedded algorithm controls port selection (see Appendix D: Valve control program for a listing of the

Valve control program). This embedded program ensures that if the variable BL has a value greater than the backlash of the valve drive chain, port switches always approach the port from the same direction irrespective of whether it is a clockwise or counter-clockwise move. In the one case, the rotor passes the port by a small rotation and then reverses direction. This is important to eliminate misalignment to the ports of the COV caused by backlash. The extent of this backlash move is software selectable and is determined by the variable BL and is expressed in micro steps. The default is BL=25. Table 1 provides the commands that can be used to change the value of BL using the Terminal device.

Another variable (OS) controls the offset from the 12 o'clock position that corresponds to port number 1. When OS=0 port 1 is located at the 12 o'clock position of the valve and lines up with the indexing hole that aligns the valve on the motor clamp ring. When port 1 is at some other position as is the case for the mini-SIA, OS is used to specify the offset from the 12 o'clock position. OS is also measured in micro steps. Going in a counter clockwise direction, OS is a negative number between 0 and -1440. Each degree of offset is therefore 4 micro steps. Once the approximate offset has been calculated, the value of OS can be fine-tuned to ensure perfect alignment of the groove in the rotor with port 1. For the miniSIA-2 the value of OS will be about 120 but could vary by as much as 20 micro steps in either direction. In the appendix listing it is set at OS=-105. Table 1 provides the commands to change the value of OS.

Table 1: Commands to change valve control variables

Command	Description
CPR BL<CR>	Display present value for BL <CR> is carriage return
CBL n<CR>	Set BL equal to n (0 < n < 144)
CPR OS<CR>	Display present value for OS <CR> carriage return
COS n<CR>	Set OS equal to n (0 > n > -1440)
CS<CR>	Save new value to non-volatile RAM

The port assignments of the ports on the COV are given in Figure 6 and are detailed in Table 2.

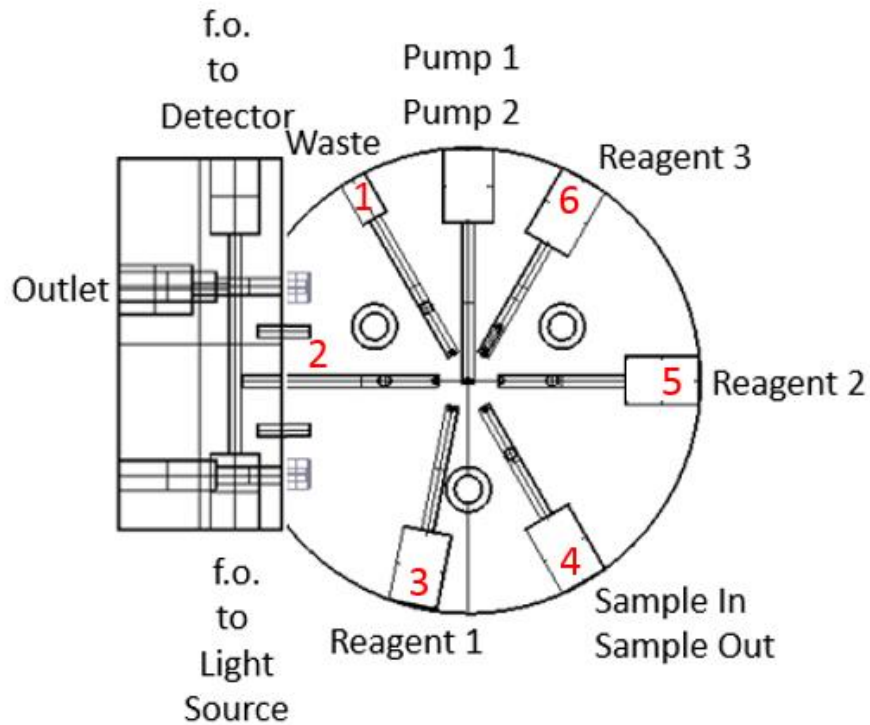


Figure 6: COV port assignments

Table 2: Port description

Port Number	Description
1	Waste
2	Detector
3	Reagent 1 / Standard / Wash solution
4A	Sample in
4B	Sample out
5	Reagent 2 / Standard / Wash solution
6	Reagent 3 / Standard / Wash solution
Common	Pump 1
Common	Pump 2

The COV is mounted with the common ports at the 12 o'clock position (see Figure 7). If for any reason a different rotational orientation is required, an offset of the position of port 1 from which all other port positions are calculated can be selected in using commands described above.

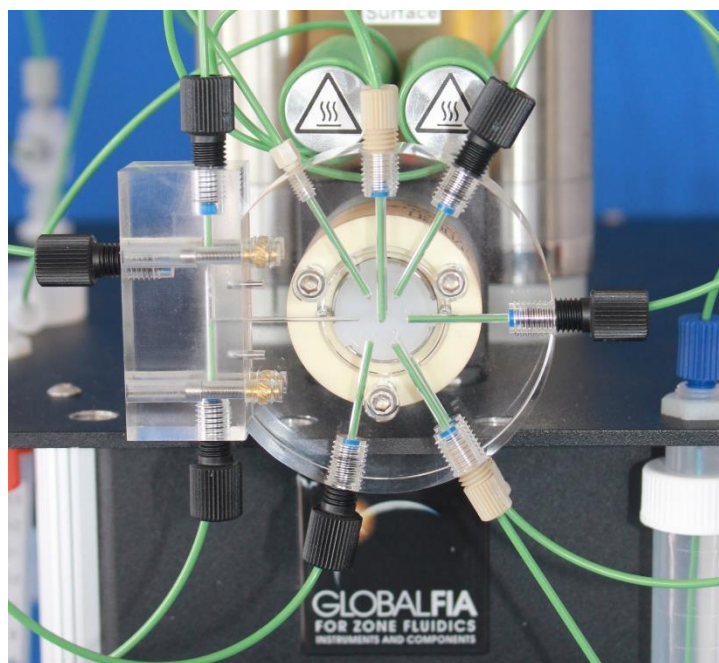


Figure 7: COV equipped with the short path length UV-vis absorbance cell

A blocked position is sometimes needed where no port is selected. This position corresponds to the 12 o'clock position on the COV and is selectable in the FloZF software or by issuing the serial command **CEX AP<CR>**, <CR> is carriage return or the Enter key.

The rotor flow path contains no un-swept volumes and has a channel width of 0.03 in (0.7 mm).

MILLIGAT PUMP

The microprocessor-controlled milliGAT pump⁴ (see Figure 8) is a positive displacement linear flow pump that consists of a pump head / motor / gear assembly, and an integrated micro-electric controller.

The central component in the pump is a cylindrical rotor that is fitted with four equally spaced pistons (two are shown in figure below). This rotor seals against the stator end cap which is equipped with an inlet and outlet port. These ports are coupled to an inlet channel in the shape of an arc and a similar outlet channel in the stator cap. As the rotor turns, a cam governs the back and forth displacement of the spring-loaded pistons with a piston stroke of 1/8" (3mm). The diameter of the pistons of HF (high flow) pump is larger than the pistons of the LF (low flow) pump. As the motor turns the rotor, one piston is dispensing into the outlet channel, one piston is filling from the inlet channel and the other two are

⁴ US Pat No. 6,079,313 issued to Global FIA, Inc.

moving between the inlet and outlet channels. The cam has been engineered to ensure a smooth transition when one piston hands off to the next.

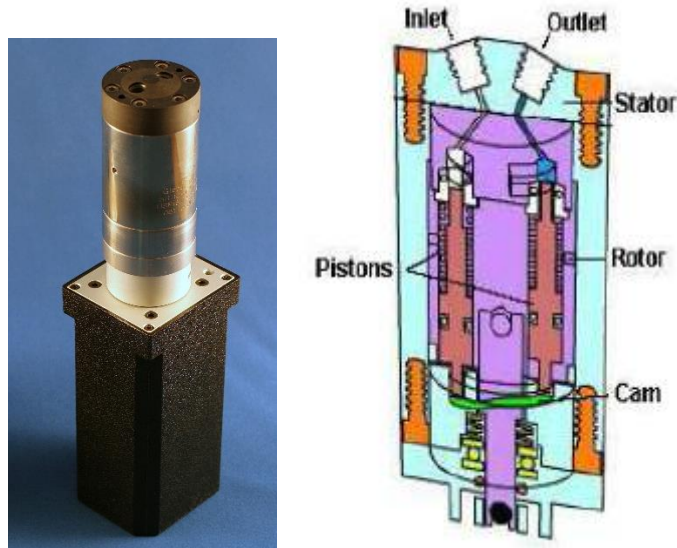


Figure 8: milliGAT pump - a) external showing fluid ports, b) schematic showing internal structure

A stepper motor governs rotation rate and direction. Flow direction is determined by the direction of rotation. Flow rate is determined by the rate of rotation. The metering capability of the pump is determined by the accuracy of the stepper motor working in micro-step mode. In addition to various levels of gearing, the stepper motor is operated in a mode where every stepper motor step is divided into 256 micro-steps. For the LF pump, one revolution of the pump displaces 100 μL and for the HF pump, one revolution displaces 630 μL . The volume per micro-step is dependent on motor, gearing, and pump used and can be calculated using a formula provided in the Pump Operating Manual.

In the event of a deadhead blockage, the backpressure overcomes the spring that holds the rotor against the stator. The rotor of the pump moves away from the stator and fluid leaks into the pump internals and eventually from one of the pump's leak ports. If this occurs, the leakage fluid must be well flushed from the pump using distilled water and isopropyl alcohol to prevent permanent damage. A pressure relief valve is plumbed between the pump and holding coil to relieve pressure and prevent leakage into the pump internals.

While care has been taken to ensure a smooth transition from one piston to the next, some users with sensors that are extremely sensitive to flow may notice a small perturbation as one piston hands off to the next.

The pistons are Teflon™ tipped and the Teflon tip is longer than the piston stroke thereby ensuring that the wetted surface of the piston chamber is not exposed to air. This has a positive implication for wear life of the pistons as crystals of dried dissolved solids cannot form and scratch the seal surface.

The milliGAT pump is a self-priming positive displacement pump and can tolerate a small volume of gas in the fluid lines. When a bubble is trapped in the pump though it can lead to pulsation in the flow. To sweep the bubble from the pump, flush for 10 seconds at 200 $\mu\text{L}/\text{sec}$ for the HF pump and 30 $\mu\text{L}/\text{sec}$ for 10 seconds for the LF pump.

Smooth Flow milliGAT features that are of particular importance for the miniSIA include:

- Bi-directional smooth flow
- Flow range covers six order of magnitude
- Meter precise volumes from nL – mL.
- No pulse-dampers
- No check valves
- No syringes to re-fill or exchange
- Chemically inert
- Self-priming

PRESSURE RELIEF VALVE

Pressure relief valve are plumbed into the stubbing line immediately downstream of the pumps. These valve provides protection for the pump in the event of a blockage or sequence programming error. If the pressure in the system exceeds 100 psi (~ 7 bar) this valve opens and the fluid is pumped to waste. To restore normal operation, the source of the blockage must be eliminated. Schematically. The pressure relief valve is deployed as indicated in the following sketch.

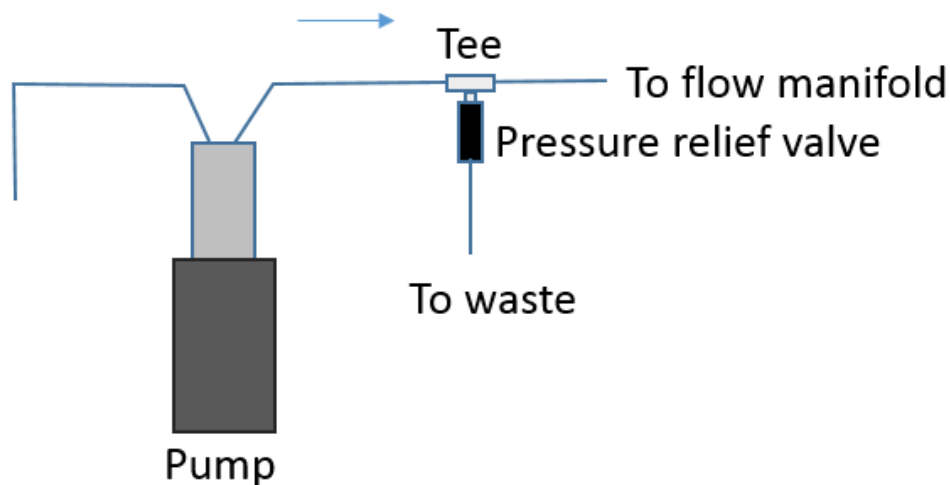


Figure 9: Deployment of pressure relief valve

HEATED HOLDING COILS

The system is equipped with two 1mL internal volume heated holding coils. These holding coils are wound onto aluminum heating rods that are resistively heated and under PID control by the Omron [E5GN temperature controllers](#)⁵. The desired set point can be set and the current temperature read in FloZF. The temperature is measured using a type K thermocouple which is positioned between the heating rod and the tubing.

Note: Care must be taken not to touch these heated rods when temperatures above 50 °C are used.

LIGHT SOURCES

The miniSIA-2 is equipped with two light sources and these are coupled to the flow cell by means of a single core silica optical fiber sheathed in a 1/16" OD PEEK conduit. The fiber is manually swapped from one light source to the other. The first is a high intensity white LED. The emission spectrum of this light source is given in Figure 10A.

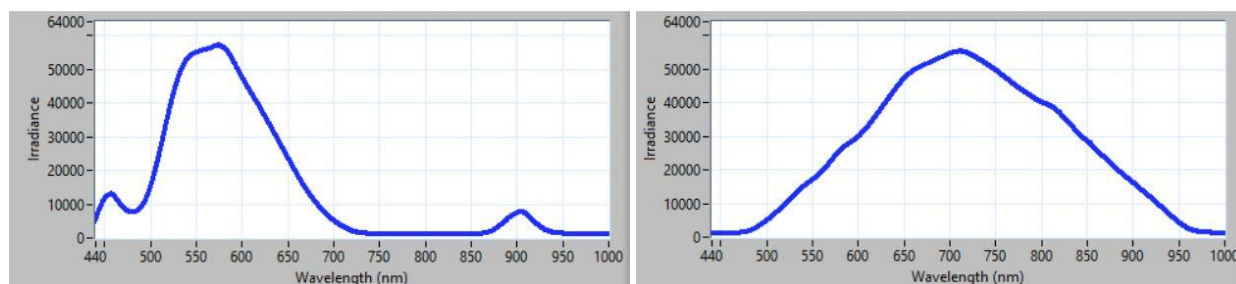


Figure 10: (A) White LED emission spectrum (B) W-Xe lamp spectrum

The second is a compact tungsten-xenon lamp. Its emission profile is given in Figure 10B.

When this lamp fails, power down the system. Replace the lamp by unscrewing the cylindrical heat sink (Figure 11A) and pulling out the old lamp and fitting a new lamp (P/N: W-XE available from Global FIA) which is held in place by a friction fit to the Teflon lamp holder (Figure 11B). When replacing the heat sink, care must be taken to ensure good thermal contact between the heat sink and the miniSIA-2 chassis through the brass mounting collar to dissipate the heat generated by the lamp.

⁵ <https://www.omron-ap.com/products/family/1434/download/manual.html>

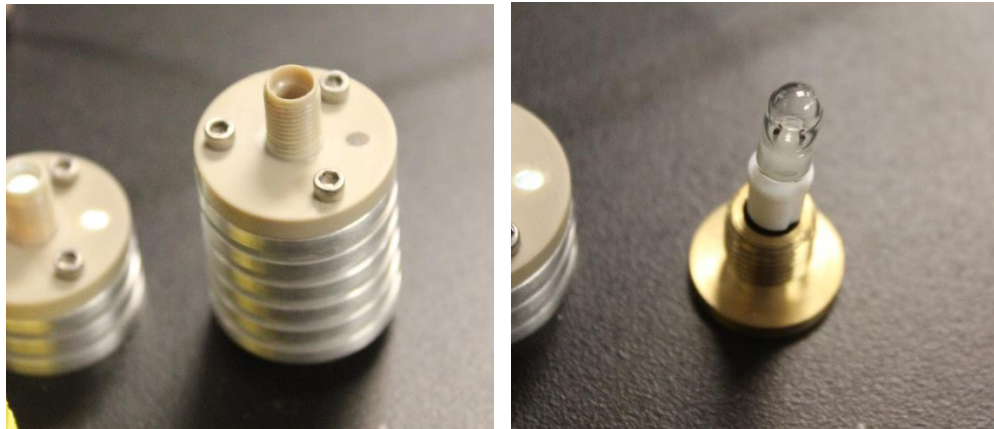


Figure 11: (A) Tungsten xenon (W-Xe) lamp holder with cylindrical aluminum heat sink (B) Exposed W-Xe lamp in Teflon lamp mounting

Manual controls in FloZF allow you to switch the lamps on and off. The intensity of the light from the lamps can be adjusted on the lamp power supply which is buried inside the miniSIA-2 enclosure.

It should not be necessary under normal operation to change the intensity of the lamp. But if for some reason it is desirable to change the lamp intensity, there is a potentiostat on the lamp power supply that controls the current to the lamp (see Figure 12). **Ensure that the power to the system has been removed.** Open the enclosure by loosening and removing the four rubber feet. This will allow you to gently shift the aluminum corner extrusions of the enclosure just enough to slide out the side panels. Identify the appropriate lamp power supply by the label on its side. Then using a small flat screw driver adjust the potentiostat. To increase the light output, turn the potentiostat counter clockwise or decrease the light output with a clockwise rotation. It is possible to overpower the light source and burn out the lamp so care should be taken when increasing the intensity.



Figure 12: Lamp power supply showing potentiostat

The signal obtained from the lamps is controlled from the spectrometer manual control (see Figure 13). The integration time determines how long the signal is integrated for each spectrum acquisition. Increasing the integration time increases the signal. Ideally the maximum signal from the lamp should be between 50 000 and 60 000 counts when getting the Reference Spectrum. Pressing the Auto Tune button will search for an integration time that will give an appropriate maximum signal. Ensure that the lamp is on before pressing Auto Tune. The Scans to Average helps smooth the signal by averaging a number of acquisitions. The number of Scans to Average multiplied by the Integration Time largely determines the frequency with which you can acquire data points. There is also some data transfer time overhead so Integration Time x Scans to Average should be less than about ¾ of the time allowed for each data point. This time is set as the parameter in the Start Acquire step in the sequence and is typically between 0.2 and 0.5 sec. If the actual acquisition time is greater than the requested acquisition time then the Sampling Period on the Spectrometer Run tab will be greater than the Requested Period and the timing of the profile will be adjusted to accommodate the longer actual acquisition period. To remedy this, reduce the Scans to Average or Integration Time. The Boxcar Width specifies how many pixels will be bunched together in a running smoothing of the spectrum. We often use a Scans to Average of 20 and a Boxcar of 50. The Spect Time defines the time that will be used to obtain the reference spectrum. This is usually set to zero and then corresponds to the Integration Time x Scans to Average.

The signal measured is specified by the quantity field. You can measure Absorbance (A), Fluorescence (F) and Irradiance (I) (see definition in Table 3). The number following the letter specifies the wavelength measured. You can also include simple calculations in the Quantity field e.g. A640-A555 would give the Absorbance at 640nm – the Absorbance at 555nm. The other two editable fields are information fields used to label axes and profiles.

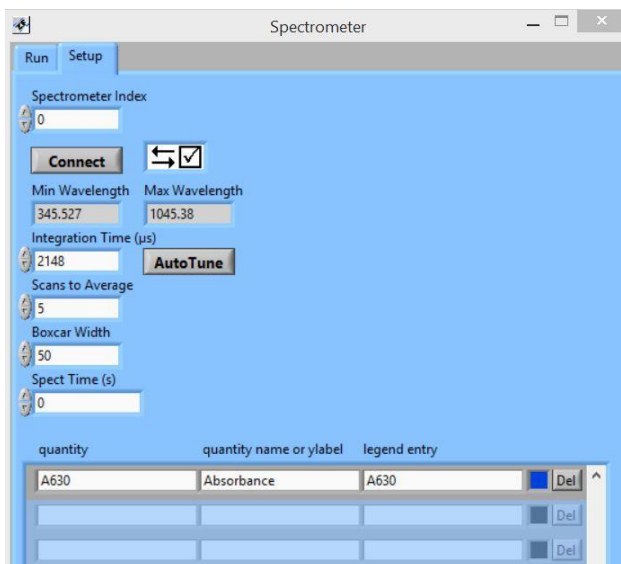


Figure 13: Spectrometer manual control

Table 3: Measurable quantities with Spectrometer detection

Quantity	Definition
Irradiance	Signal obtained over integration period averaged over specified Scans to Average
Absorbance	$-\log_{10}(\text{Current irradiance} / \text{Reference irradiance})$ at the specified wavelength
Fluorescence	Current irradiance – Reference irradiance at the specified wavelength

DETECTOR FLOW CELL

The detector flow cell is a bolt-on module that allows convenient swapping from one detection system to another. Locating pins assist in aligning the flow cell module to the .03 in (0.7 mm) outlet from the COV. Various flow cells have been developed or are envisaged including:

- a) A short path length (12mm) uv-vis absorbance cell.
- b) A long path length absorbance cell where the path length is defined by the interconnecting tubing between the two flow cell end-pieces which are arranged as a Garth cell with a .03 in (0.8mm) ID PEEK liner. This arrangement will also accommodate a liquid core waveguide. This 10 cm Garth flow cell is shown in Figure 14.
- c) A fluorescence flow cell with close-coupled Hamamatsu photon counter.
- d) Electrochemical flow cells, e.g. a flow through potentiometric cell, amperometric cell, etc.
- e) A chemiluminescence cell with close coupled photon counter that images an imprinted spiral flow channel depicted in Figure 15.
- f) A standard coupling to other larger detection system, e.g. ICP-MS, LC, IC, LC-MS, or GC.



Figure 14: Long path length uv-vis Garth cell



Figure 15: Spiral flow channel of chemiluminescence cell

The mounting pattern for the detector flow cell is given in Figure 16. The $\varnothing.157$ in holes are equipped with ISB-440 heat staking inserts. The $\varnothing.062$ in holes are fitted with precision stainless steel alignment pins that protrude less than $\frac{1}{8}$ in. The fluid channel has an ID of .03 in (0.8mm).

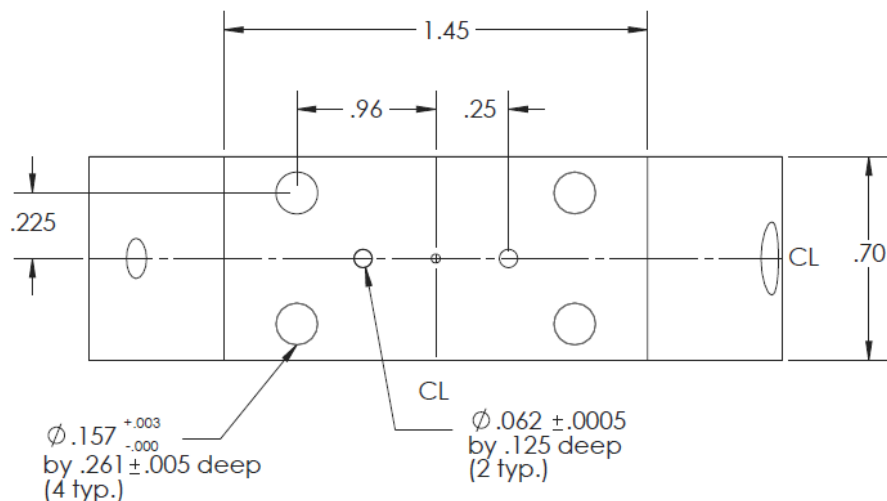


Figure 16: Whole pattern on COV where bolt-on flow cells are mounted.

DETECTOR

An Ocean Optics USB-4000 spectrometer is mounted in the miniSIA-2 and is coupled to the flow cell by means of a single core silica optical fiber sheathed in a $\frac{1}{16}$ " OD PEEK conduit. FloZF provides extensive support for the USB-4000 (refer to the FloZF software manual).

Other detectors can be added to suit the needs of the application deployed on the miniSIA-2.

VIALS AND RESERVOIRS

The miniSIA-2 is equipped with several vials and reservoirs. All are available from Global FIA as spares. Table 4 provides details of the part number and manufacturers of the vials and reservoirs.

Table 4: Reservoir and vial purchasing details

P/N	Function	Description	MPM	Manufacturer
RES-LDPE-4	Carrier and waste reservoir	Round plastic bottle, 4 oz, LDPE	2103-0004	Nalgene
62.554.205	Reagent / standard vial	Reagent Tube, 15 mL, PP	62.554.205	Sarstedt
3206	Sample vial	Transport vial, 5 mL, PP	3206	Stockwell Scientific

POWER

The miniSIA-2 makes use of a switch-mode power supply to provide 24VDC and 5VDC power for the various devices. This means that it can be powered by AC line voltage in the range 100-250VAC.

USB PORT

Communication with the FloPro-Sampler is via a USB 2.0 port. The device is also equipped with a USB hub. The hub is used to provide additional USB ports for peripheral devices. One of these ports is used by the USB-4000. The devices are controlled on a RS-422 loop that makes use of virtual COM ports on the USB hub. The driver for these ports can be downloaded from [FTDI](http://www.ftdichip.com/FTDrivers.htm)⁶.

COMPUTER AND FLOZF DEVICE CONTROL AND DATA ACQUISITION SOFTWARE

It is advisable to allow Global FIA to supply the computer to ensure compatibility with all system functionality and correct configuration and installation of appropriate drivers. Where the user provides their own computer, the computer should have the following minimum specification.

Windows XP, Vista 32 Bit, or Windows 7 32 Bit (English versions) or Windows 8 32 Bit or 64 Bit (English version)

- 512 MB (Windows XP) or 2 GB (Vista and Windows 7 and 8) of memory
- Screen resolution 1280×768 or greater (smaller will be inconvenient)
- 1 x USB type 2.0 ports
- 5 GB disk space (200 MB for program)

FloZF is Global FIA's device control and data acquisition package. It is described in more detail below and in the FloZF User Manual.

SYSTEM SETUP

UNPACKING

1. Unpack the shipping containers and check for shipping damage. If there is any shipping damage, do not discard the packaging and contact Global FIA. Compare the contents with packing list and notify Global FIA immediately of any shortages.

⁶ <http://www.ftdichip.com/FTDrivers.htm>

2. If a computer is not included in your order, install the FloZF software onto your computer. Refer to the FloZF Getting Started pamphlet for complete instructions. If a computer is provided, FloZF will be pre-installed and configured with a few demonstration sequences.
3. Connect the USB cable between the computer and the miniSIA-2 USB type B connector shown in Figure 17 . In some systems, a second USB cable will extend from one of the USB type A connectors on the USB hub to provide communication to an optional Auto Sampler. Where a photon counter is used, plug the USB to RS-232 cable into one of the USB type A connectors to provide the link between the miniSIA-2 and the photon counter.



Figure 17: miniSIA-2 rear view

4. The heaters are already connected to the appropriate terminals on the green power and IO connectors.

Note: The power to the heater is 120-250VAC. Beware of electrical hazards.

The remaining terminals may be used for interfacing external devices associated with the miniSIA-2. The pin outs for the 2 green connectors on the back of the miniSIA-2 are as shown in Table 5.

Table 5: Pin-outs of FloPro-Sampler Power and IO connector

Pin	Power (Config I)	IO (Config I)
1	Htr L (1)	24VDC
2	Htr N (1)	A1
3	Htr L (2)	A2
4	Htr N (2)	A3
5	5VDC	Gnd
6	Gnd	NC
7	24VDC	NC
8	Gnd	NC

5. Connect the power cable to the miniSIA-2 power entry connector. If this is the first time the device is connected to the computer, drivers for the USB to RS-422 converters will be installed. If needed you can download the virtual COMM port drivers from [FTDI](http://www.ftdichip.com/Drivers/VCP.htm)⁷.
6. Fill the carrier reservoirs with a suitable carrier solution such as DI water or an appropriate wash solution. [CAPSTONE FS-31](http://www.dupont.com/Capstone/en_US/assets/downloads/DuPont_Capstone_FS-31_Tech_Info_K-23712-3.pdf)⁸ is an excellent fluorocarbon surfactant for use with the miniSIA-2. Small 2 mL vials of this surfactant are available for Global FIA (P/N: CAPSTONE). A 0.01% v/v solution of CAPSTONE FS-31 in DI water will help lower the surface energy of the hydrophobic surfaces and help to ensure smooth flow and facilitate flushing bubbles from the flow channels.
7. Connect up the Waste reservoir and reagent vials.
8. The sample vial can be mounted on the chassis (A) or inserted into the vial holder that is mounted on the front handle (B) (see Figure 18).
9. If the COV is shipped separately, mount it on the valve and connect all tubing lines according to the port assignments given in Table 2.
10. Select the light source to be used and screw the fiber optic cable into the appropriate light source.
11. Mount the desired detector flow cell on the COV and couple up the remaining fiber optic cable to the spectrometer. The fiber optical cable for the spectrometer has an expanded fasting nut to allow easy coupling to the embedded spectrometer.
12. Familiarize yourself with FloZF by going through the FloZF Getting Started pamphlet.

⁷ <http://www.ftdichip.com/Drivers/VCP.htm>

⁸ http://www2.dupont.com/Capstone/en_US/assets/downloads/DuPont_Capstone_FS-31_Tech_Info_K-23712-3.pdf

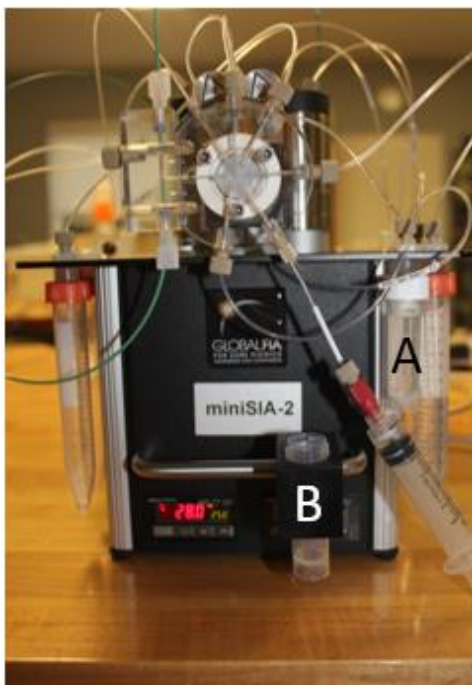


Figure 18: Sample vial positions

FLOZF SOFTWARE

FloZF is a device control, data acquisition, and data processing package designed for the development and operation of fluidic systems such as the FloPro-Sampler. In particular, it is well suited to exploring the power of FIA, SIA and Zone Fluidics. In FloZF, the user builds and manages **Projects**, which consist of hardware **Device** configuration information, the **Sequences** to control the devices, and data acquired from any detector devices in the Project. Full details of the software are available in the FloZF User Manual.

PROCEDURE DEVELOPMENT AND OPTIMIZATION

The universal sampling and dilution sequence provided provides a good starting point for most sampling and dilution applications. This universal sequence works in conjunction with an Excel configuration file. Certain sequence parameters are specified in the Excel spreadsheet and sampling information is written back to the spreadsheet for record keeping purposes. FloZF is a powerful method development software package and some users may wish to develop their own sampling and sample manipulation sequences.

DEVICE CONFIGURATION

A project file that includes the miniSIA-2 devices as well as a few basic housekeeping sequences have been included or are available from Global FIA. If additional devices are to be added then Device configuration takes place from the **Instrument Setup** page (see screen shot of the Instrument Setup page in Figure 19). A device is added to the Project from a list of **Available Device Types**. For information on adding a device, see the FloZF User Manual. The device is then configured and tested from inside its own **Instrument Panel**, which is accessed by double clicking on a device in the **Registered Devices** window. The Instrument Panel consists of a **Run** tab, which provides for “manual” operation and monitoring of the device, and a **Setup** tab for device configuration. Some devices have an additional Advanced Tab for more advanced parameter setup and device diagnostic information. On the Setup tab, fill in the correct COM Port and Address in the designated fields. Next, establish connection by pressing the **Connect** button. Information on connecting to a device is provided in the FloZF User Manual.

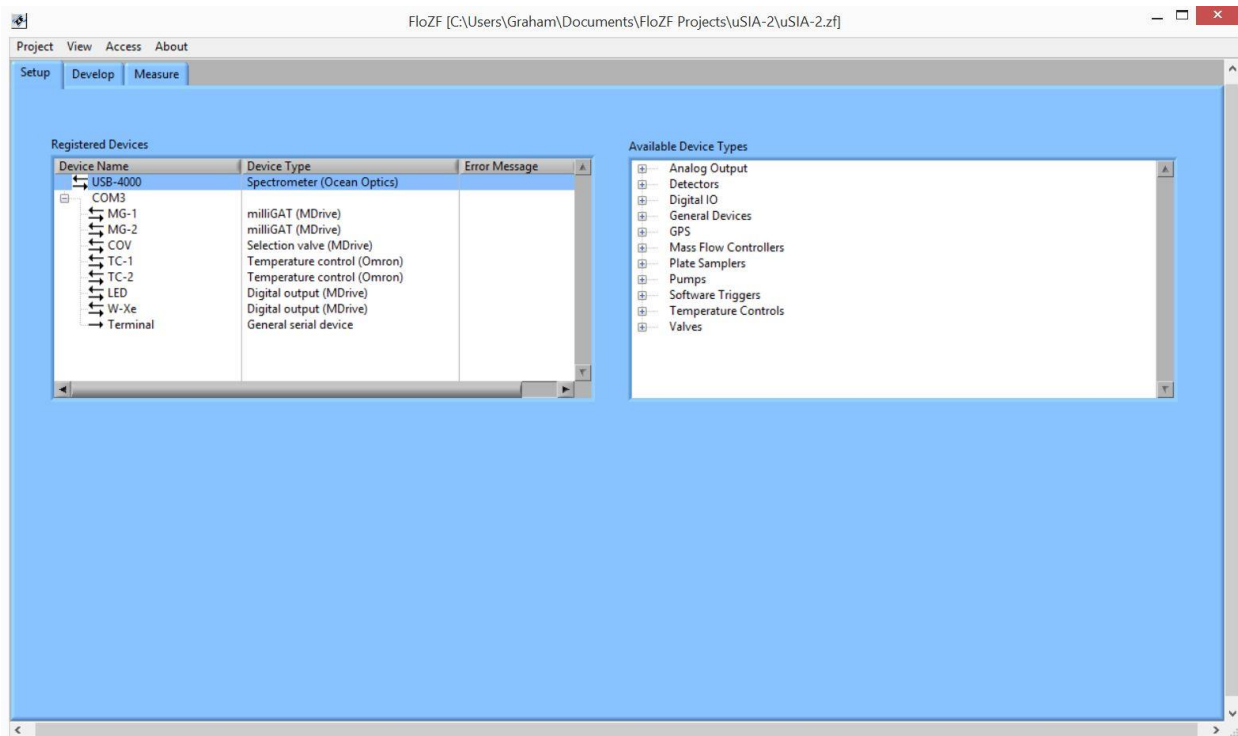


Figure 19: Instrument Setup Page in FloZF

BUILDING SEQUENCES

Sequences are created, edited and executed on the **Sequence Page** (see screen shot of the Sequence page in Figure 20). There are three panels on the Sequence Page as well as several buttons. The **Resource Steps** panel (at the top left) contains the building blocks of Sequences: device-specific Steps,

system control Steps, and data manipulation Steps. These Steps can be dragged and dropped into the **Work Sequence** panel (on the right) to generate method Sequences. From here, the Steps can be modified, grouped, saved, and executed. Groups of Steps can be assembled into hierarchical arrangements of Sequences, which are listed in the **Resource Sequences** panel (at the bottom left).

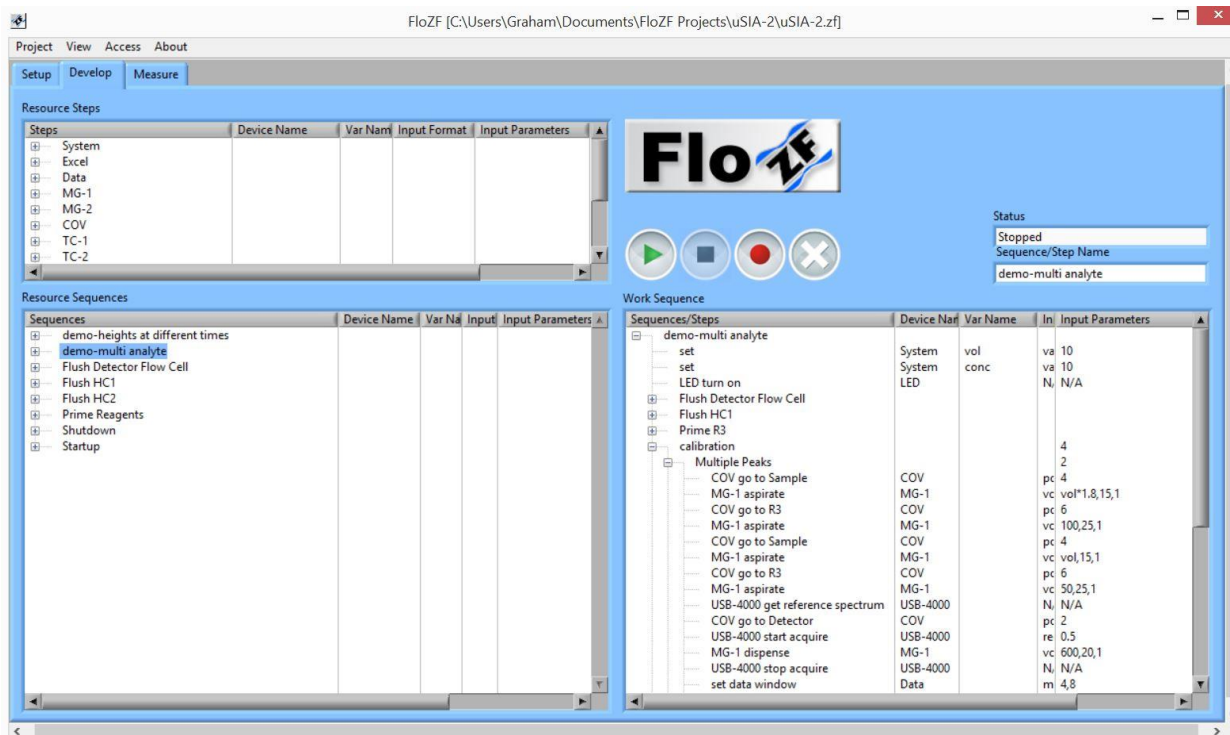


Figure 20: FloZF Develop Page Interface

INTERFACING TO MICROSOFT EXCEL

An interface exists between FloZF and Microsoft Excel to provide additional data manipulation and control functionality. This together with FloZF's use of variables provides a powerful tool for customizing the sequence to execute the desired fluid handling task or designing system experiments. Global FIA can provide basic FloZF sequences for interfacing to Excel. There is also a pamphlet available showing how FloZF and Excel may be used together for design of experiment.

In the Resource steps panel on the Develop page, there is an **Excel** device, with several device steps that allow configuration and communication between FloZF and Excel (see Figure 21).

Resource Steps				
Steps	Device Name	Var Name	Input Format	Input Parameters
Excel				
activate/load excel file	Excel		file name	'temp data.xls'
add hyperlink	Excel		sheet #, start	1,1,1, 'temp plot.xls', 'my
close excel file reference	Excel		N/A	N/A
copy excel file	Excel		original file n	'temp 1.xls', 'temp 2.xls'
create & activate excel file	Excel		file name	'temp data.xls'
read number	Excel	number	sheet #, row	1,1,1
read number array	Excel	number array	sheet #, start	1,1,1,10,3
read string	Excel	string	sheet #, row	1,1,1
read string array	Excel	string array	sheet #, start	1,1,1,10,3
save excel file	Excel		N/A	N/A
set cells background color	Excel		sheet #, start	1,1,1,10,3,'g'
write to excel	Excel		sheet #, start	1,1,1, data
Data				

Figure 21: Excel device in Resource Steps panel on Develop page

A full description of the Excel device is given in the FloZF manual.

MANUAL OPERATION

The Instrument Panel associated with each hardware device can be accessed from the Instrument Setup page, by double clicking on the name of the device in the Registered Devices window. Manual operation of a given device can be carried out from the **Run** tab of the Instrument Panel (see Figure 22).

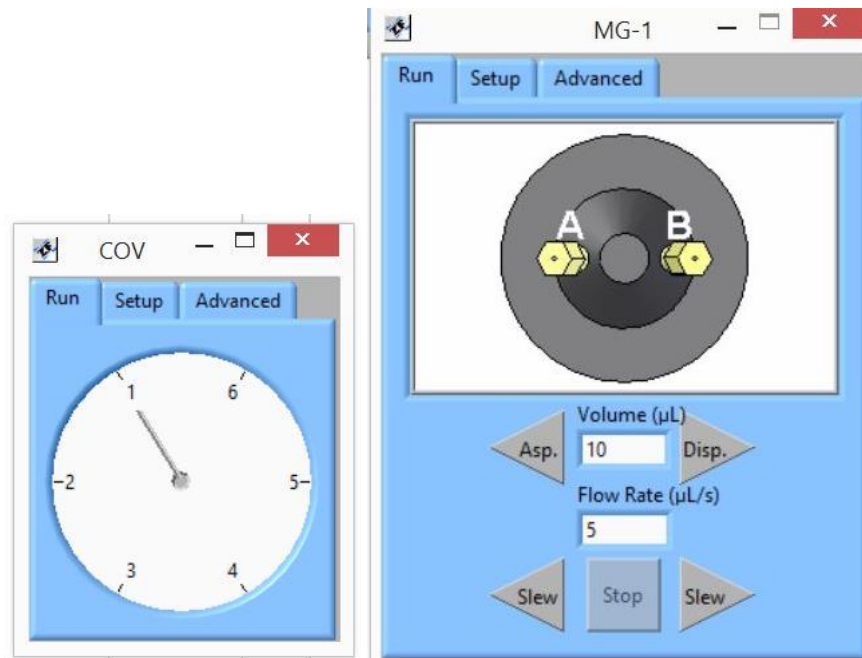


Figure 22: Instrument Panel for the COV and one of the milliGAT Pumps

The following two examples of manual operation are based on the above figure. The COV (above left) currently is showing port 1 selected. To manually select a different port, say port 2, simply move the cursor to a position inside of the port number and click the mouse one time. To manually run the milligAT pump, enter a volume and flow rate into the designated fields and push the “Asp” or “Disp” Button, to select the direction of fluid movement. Clicking the “Slew” button, in either direction will cause the pump to flow continuously at the specified flow rate until the “Stop” button is pushed. The actual volume pumped during a slew step will be recorded in the volume field.

All devices can be manually operated in this manner. Data acquired directly from the Instrument Panel (rather than a sequence) can only be viewed. It cannot be saved.

It is often useful to have the manual tabs open during a Run to provide a real-time view of what the device is doing such as the current selected port and pump flow direction.

Once a desired layout of Device Manual Controls on the screen has been selected (for example, Figure 23) you can save this layout by selecting **Record Instrument View** in the View Menu or press Ctrl-R. To show the Controls you can simply select **Display Instrument View** (Ctrl-I) or **Hide Instrument View** (Ctrl-H) from the **View** menu.

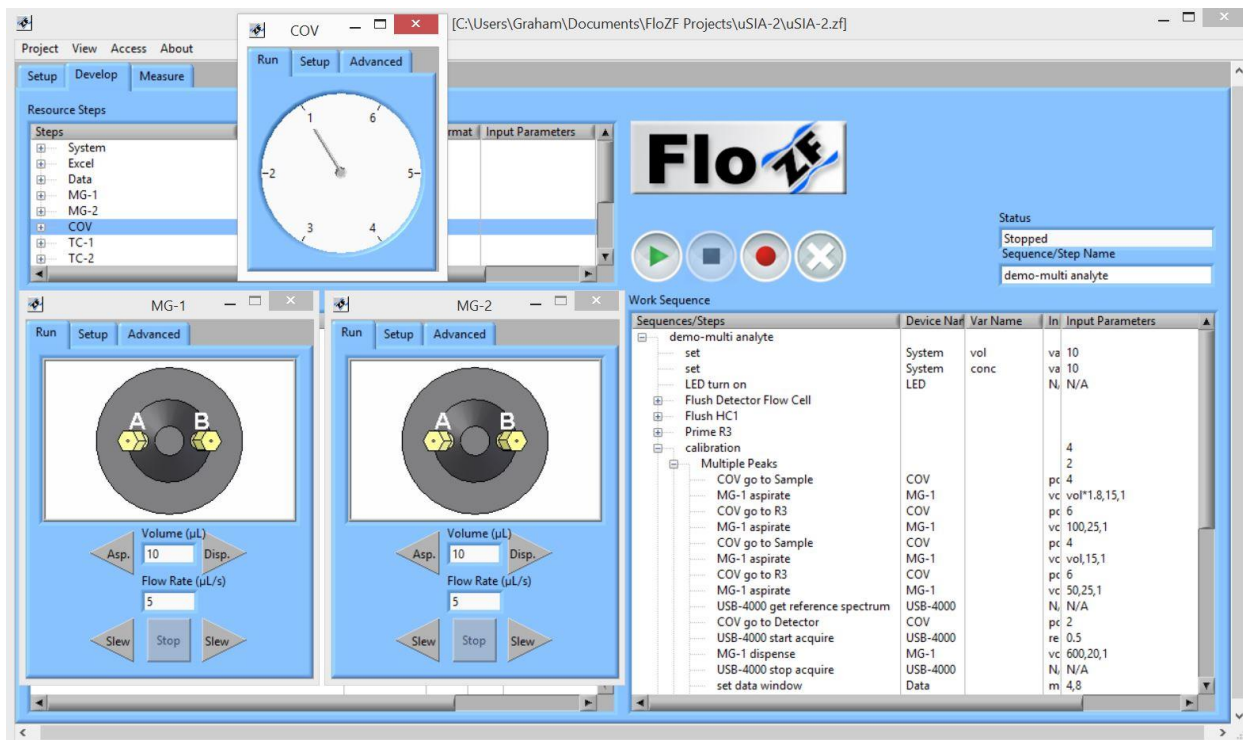


Figure 23: Manual Controls displayed in Instrument View

SEQUENCES

STARTUP, SHUTDOWN, AND VARIOUS HOUSEKEEPING SEQUENCES

As their names imply, the Startup and Shutdown sequences are used when starting up and shutting down the instrument. All sequences are executed directly (and easily) from the **Measure Page**. The sequences are also available in the **Develop Page** of FloZF if modification is needed, and can be executed from this page by dragging the sequence from the **Resource Sequences** panel to the **Work Sequence** panel and pressing the “play” button.

The default sequences are explained in below:

STARTUP – This sequence carries out a variety of steps in order to prepare the system so that samples can be measured. Steps to prime reagent lines, flush the holding coil and flow cell, and prep each unit operation (such as the heaters) in use are contained within this sequence.

SHUTDOWN – This sequence carries out a series of steps to wash out any remaining reagents or sample from the tubing lines and fill these lines with clean carrier. The shutdown sequence should be executed after all necessary samples have been measured. This sequence also turns off the heaters and lamps.

APPENDIX A: TROUBLESHOOTING TIPS

CLEARING BLOCKAGES

Occasionally blockages occur within the system. If there is a blockage in the system, carrier fluid will drip from the pressure relief valve (open to the bench top). After identifying the location of the blockage, disconnect the fitting to this port as well as the tubing routed into the selection valve common port. Manually select the blocked port number from the instrument setup tab in FloZF (for information on manual operation, see description above). Attach a short line of tubing (equipped with the correct fitting) to a syringe and connect the syringe to the common port of the selection valve via this line. The syringe should be filled with distilled water. Pushing distilled water through the blocked port via the common port should effectively clear the blockage.

BUBBLES FRAGMENTING

Bubbles inevitably get into the system. These bubbles if they are not swept out can negatively affect one's data. Bubbles sticking in the flow manifold can be explained by a few different conditions, which are typically quite easily corrected. When flow rates are too high, bubbles tend to fragment and leave smaller bubbles in the system. If bubble fragmenting is observed while running at a high flow rate, simply decrease the flow rate for the particular step that is resulting in bubble fragmenting and observe whether the problem persists. Bubble adhesion also occurs when the tubing is not properly wetted. Washing the tubing line with IPA followed by Micro-90 will properly wet out the line. Ensure that the line is clean by washing several times with carrier following the IPA/Micro-90 treatment. Increasing the surfactant concentration of the carrier solution is also beneficial in reducing the frequency of bubble fragmenting. It is also a good practice to leave the carrier with surfactant in the lines overnight.

DEGASSING

In order to prevent outgassing of reagents resulting in micro-bubble formation that may interfere with mixing and detection, especially when operating temperatures are $>20^{\circ}\text{C}$, it is recommended to degas the carrier stream using one of the following methods:

- Place distilled/deionized water under a strong vacuum for 15–20 minutes. Magnetic stirring or sonication aids in the degassing process.
- Purge distilled/deionized water with helium or nitrogen gas through a glass frit for 5 min.
- Boil distilled/deionized water in an Erlenmeyer flask for 15–20 minutes. Remove the flask from the heat source, cover it with an inverted beaker, and allow it to cool to room temperature.

DI water used to prepare standards and reagents should be allowed to de-gas for 24 hours.

NO COMMUNICATION WITH DEVICES

If communication is lost with the devices, exit FloZF, power down the miniSIA-2. Unplug the USB cable. Wait 30 seconds. Plug it in again and power up miniSIA-2. Startup FloZF.

DEBUGGING SEQUENCES

When a bug occurs within a complex sequence, it is often easiest to break up the sequence into its simplest component parts and run each portion separately. On the sequence page, expand the sequence by clicking once on the “+” sign to the left of the sequence in the Resource Sequences window. Drag the smaller sub-sequence to the Work Sequence panel and press the play button. Carefully observe the system as each sub-sequence is executed to identify the location of the bug. The sequence may simply need to be changed/re-written at that particular location. If the bug pertains to the software itself, contact David Holdych FloZF Software Manager, for software support (david@globalfia.com or #253-549-2226).

APPENDIX B: CONSUMABLES AND SPARE PARTS LIST

CONSUMABLES

P/N	Description
3206	Sample vial
430790	Reagent vials, 15mL, Corning
RES-LDPE-4	Carrier reservoir, 4oz (2103-0004)
RES-LDPE-8	Carrier reservoir, 8oz (2103-0008)
SS-1	Storage Satchel
CAPSTONE	Capstone FS-31, surfactant, 2mL
16T-030-PFA	Tubing, PFA 1/16 in OD, 0.030 ID, 3m
XP-235X	Flangeless Fitting Short, PEEK, 1/4-28 Flat-Bottom for 1/16" OD, 10 pack
P-283X	Headless Nut, 1/16 IN, Short, 10 pk
P-200X	Ferrule, flangeless, 1/16 in, Tefzel (ETFE), blue (10 pk)
640FF-16	6-40 Fitting for 1/16" tubing
640W	Wrench for 6-40 fittings
SC-440-05-S	Mounting Screws, 4-40 x .5 in, shaved to .155 in for COV flow cells (4 pack)
SC-632	Mounting Screws, 6-32 x 1 in, for COV (3 pack)
FC-W	COV flow cell wrench
COV-W	COV wrench
W-XE	Lamp, tungsten-xenon

SPARES

P/N	Description
COV	Chem-on-Valve manifold
C15-310M	Rotor, M material
MG-2NM	milliGAT HF pump head only
COV-SPFC	COV short path length photometric flow cell
COV-LPFC	COV long path length photometric flow cell (included 10cm extension)
COV-LPFC5	COV long path length photometric flow cell, 5 cm extension
COV-LPFC10	COV long path length photometric flow cell, 10 cm extension
COV-FOC	COV fiber optic cable (specify of detector end)
COV-TUB	COV tubing kit (13 piece)
LED-WHITE	LED, white

APPENDIC C: WETTED MATERIALS

The miniSIA-2 has been constructed using chemically robust material. The following notes provide some broad guidelines but the final arbiter is a physical soak test with the specific solvents or chemicals used.

PTFE

The piston tips used in the pump are made from PTFE.

Polytetrafluoroethylene is the generic name for the class of materials such as Teflon®. It offers superior chemical resistance and lends itself to good sealing characteristics. Because it's so easy to handle, it is often used in low pressure devices such as the pump. Volatile compounds of low molecular weight can permeate PTFE. Fumes of strong acids such as hydrochloric acid can permeate the PTFE and should be constantly purged from the internals of the device

PAEK

The pump port end cap and some valve components make use of a PAEK-based composite.

Polyaryletherketone is the generic name for the family of polyketone compounds. PAEK includes PEK, PEEK, PEKK, and PEKEKK, which differ in physical properties and, to a lesser degree, in inertness. This composite resists all common HPLC solvents and dilute acids and bases. However, concentrated or prolonged use of halogenated solvents may cause the polymer to swell. Avoid concentrated sulfuric or nitric acids (over 10%).

PEEK

The green tubing is made from PEEK.

Polyaryletherketone is the generic name for the family of polyketone compounds. PAEK includes PEK, PEEK, PEKK, and PEKEKK, which differ in physical properties and, to a lesser degree, in inertness. This composite resists all common HPLC solvents and dilute acids and bases. However, concentrated or prolonged use of halogenated solvents may cause the polymer to swell. Avoid concentrated sulfuric or nitric acids (over 10%).

CERAMIC

The milliGAT HF pump rotor is made from Ceramic.

This material is highly inert and has been used extensively in many analytical applications that use Valco devices. It is routinely used in other applications at 100 psi, 75°C, and can also be used at temperatures approaching 200°C with decreased sealing tension.

VALCON-M

The valve rotor is made of a Valcon-M. This material, basically a hydrocarbon in structure, is the most impermeable to light gases of all the rotor materials currently available, with wide acceptance in low-temperature (50°C maximum) trace gas applications. Avoid use with aromatic hydrocarbons.

SAPPHIRE

The milliGAT® LF piston chambers are made of sapphire and are sealed in place with a Viton® o-ring in the low flow pump.

Sapphire has a unique set of properties including high strength, hardness, surface smoothness, and excellent chemical compatibility. It is commonly used in applications where a combination of exceptional mechanical and chemical properties is essential.

VITON®

Viton® O-rings are used to seal the piston chambers in the pump rotor in the low flow pump. Besides its excellent mechanical properties, Viton® provides the best proven fluid and chemical resistance of the commercial non-fluorinated elastomers.

Kalrez has been found to be superior in certain instances and a special pump can be constructed with Kalrez o-rings.

PFA

PFA Tubing is made from standard PFA resin (perfluoroalkoxy), Chemfluor® PFA Tubing is widely used in the semi-conductor, laboratory, environmental and pharmaceutical industries where ultrapure chemicals (including water) require precise quality control. Volatile compounds of low molecular weight can permeate PTFE.

QUARTZ GLASS

The optical conduits in contact with the fluid in the flow cell are made from quartz glass.

LDPE

Carrier and waste reservoirs are made from low density polyethylene (LDPE). Chemically LDPE is unreactive at room temperature although it is slowly attacked by strong oxidizing agents and some solvents will cause softening or swelling. It may be used at temperatures up to 95° Celsius for short periods and at 80° Celsius continuously.

PP

Reagent and sample vials are made from polypropylene. This is widely used polymer for it is attacked by strong oxidizers, aromatic and chlorinated hydrocarbons.

ACRYLIC

The COV is constructed from Acrylic also called Plexiglas. These manifolds have been used extensively with aqueous solutions even including strong acids. Exposure to some organic solvents will damage the COV. A full list of chemicals and how they react with acrylic is given [here](#)⁹.

ULTEM

The COV can also be constructed from Ultem if greater resistance to certain solvents is needed. A full list of chemicals and how they react with acrylic is given [here](#)¹⁰.

⁹ http://www.eplastics.com/Plastic/Plastics_Library/Chemical-Resistance-of-Plexiglass-Acrylic

¹⁰ <https://www.haywardflowcontrol.com/images/flowcontrol/PDF/ChemChart.pdf>

APPENDIX D: VALVE CONTROL PROGRAM

```

VA OP          LB G1
VA FP          FP 1
VA M1         BR R0
VA PP         LB G2
VA HS         FP 2
VA OS=-105    'Valve offset      BR R0
VA BL=25      'Backlash         LB G3
VA P0=-1320   'blocked / air port FP 3
VA HN         'Valve head serial# BR R0
VA SD         'Service Date      LB G4
VA LS=0       'Life valve switches FP 4
VA LC=0       'Life cycles or revs BR R0
              LB G5
PG 1          FP 5
LB SU         BR R0
OE EH        LB G6
OP 1         FP 6
FP 1         BR R0
HI 1         LB G7
H            FP 7
P 0         BR R0
PP OS        LB G8
MA PP        FP 8
H            BR R0
P 0         LB G9
BR DO        FP 9
              BR R0
LB IZ        LB G0
R1 6         FP 10
EE 1         BR R0
EL 76
PM 1         LB R0
SM 1         BR DO, FP=OP          '////
SF 25        HS R1/2
VI 200       M1 FP-OP
VM 1000      BR DL, M1>HS
RC 70        PP FP-1
HC 10        PP PP*1440
HT 100       PP PP/R1
A 3000       M1 OP-FP
D 3000       CL DR, M1>HS
EM 0         BR MO
DN "A"
PY 1         LB DR
S1 16,0,0    PP PP+1440
S2 16,0,0    RT
S3 16,0,0
S4 16,1,0    LB DL
BR DO        PP R1-FP
              PP PP+1

```

PP 0-PP	H
PP PP*1440	BR SE, I6=0
PP PP/R1	PR P
BR MO	BR SU
LB MO	LB EH
PP 0-PP	BR BA, ST=0
PP PP-BL	ST 0
MA PP	ER 0
H	LB BA
PP PP+BL	RT
MA PP	
H	LB AP
P FP-1	PP P0-BL
P P*1440	MA PP
P P/R1	H
P 0-P	PP PP+BL
OP FP	MA PP
BR DO	H
	P P0
LB EC	OP R1+1
HI 1	BR DO
H	
P 0	LB DO
BR SE	E
	PG
LB SE	
MR 1	

APPENDIX E: PUMP CONTROL PROGRAM

```

VA BE
VA BL
VA EU
VA PD
VA VL
VA VS
VA HN 'Pump head serial#
VA SD 'Service Date
VA LV 'Total volume pumped
VA LD 'Total direction changes
VA LP 'Total pump moves

'VER 4.0 6-23-15
'milliGAT(TM) on MDrive
PG 1
LB SU
OE EH 'Set up error handler
VS -5*EU
BR PP

'LF 2432 HF(4.75) 389 HF(9.7)797
HF(14.8)1214 LF(22.6) 11552
LB IZ
BE 1
EE 0
EL 76
PM 0
SM 0
RC 70
HC 10
HT 100
EU 389
A 500*EU
D 500*EU
VM 20*EU 'Initialize move
speed
VI EU/100
EM 0 'EM=1 RS-485 EM=0
RS-422
DN "C"
PY 1
S1 16,0,0
S2 16,0,0
S3 16,0,0
S4 16,1,0
BR D0

LB FL
VS VL*EU

LB FS
BR PP, BE=0
BR PP, PD*VL>=0
BR NS, VL<0
VS VS+BL
BR PP

LB NS
VS VS-BL
BR PP

LB PP
MR VS
PD VS
BR D0

LB EC 'Encoder Check
EE 1
PM 1
SM 1
SF 25
HI 1 'Initialize to
port 1
H
P 0
BR SE
LB SE
MR 1
H
BR SE, I6=0
PR P
EE 0
PM 0
SM 0
BR SU

LB EH 'Error handler
BR BA, ST=0 'If not stall
error leave error code
ST 0 'Clear stall flag
ER 0 'Clear error flag

LB BA
RT

LB D0
E
PG

```