



FLOPRO-SAMPLER

ZONE FLUIDICS SAMPLING AND DILUTION SYSTEM

USER MANUAL

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The FloPro-Sampler was developed by Global FIA in close consultation with researchers at Eli Lilly and is depicted in Figure 1. At its core, the device is equipped with a bi-directional positive displacement milligAT pump and two multi-position selection valves. These devices, collectively called the fluidics engine, provide a means of manipulating fluids in narrow bore tubing using Zone Fluidics. Zone Fluidics is an approach to flow-based automation that has been pioneered by Global FIA (Marshall, Wolcott, & Olson, 2003). In Zone Fluidics one or more unit operations are positioned around the fluidics engine. A Zone Fluidics sequence comprises of a series of fluid manipulation steps whereby small zones of fluids are sequentially presented to the various unit operations to process the sample or and in the case of an analyzer transform it into a detectable species, measure an analytical property and prepare the device for subsequent measurements. Zone Fluidics is increasingly being used to automate sample preparation steps.

In the FloPro-Sampler there are several unit operations that are used to extract a sample from a process reactor and dilute the sample and either archive it in a fraction collector for subsequent measurement or send it to a chromatograph. It is also possible to add a simple flow-through detector such as a UV spectrometer to measure properties of the diluted sample immediately after dilution.

Zone Fluidics sequences are developed and executed in a software package called FloZF installed on a laptop computer. Zone fluidics is discussed in more detail below.



Figure 1: FloPro-Sampler with Sample Probe

A unique characteristic (USP# 8,365, 616) (Wolcott & Marshall, 2012) of the FloPro-Sampler is its Sample Probe which allows for some interesting sample processing operations in close proximity to the sampling point. This feature is vital in allowing the FloPro-Sampler to be used to sample super-saturated solutions and heterogeneous samples. A separate manual (Marshall & Wolcott, 2013) describes the sample probe and how it can be used.

ZONE FLUIDICS

Zone Fluidics (ZF) is the precisely controlled physical, chemical, and fluid-dynamic manipulation of zones of miscible and immiscible fluids and suspended solids in narrow bore conduits to accomplish sample conditioning and chemical analysis.

A *zone* is a volume region within a flow conduit containing at least one unique characteristic.

From an operational point of view, ZF is an approach to sample handling where a zone or zones are shuttled between and within an assembly of one or more unit operations where different sample processing steps are performed. Fluid handling is accomplished in a fluidics manifold like the one depicted in the following schematic.

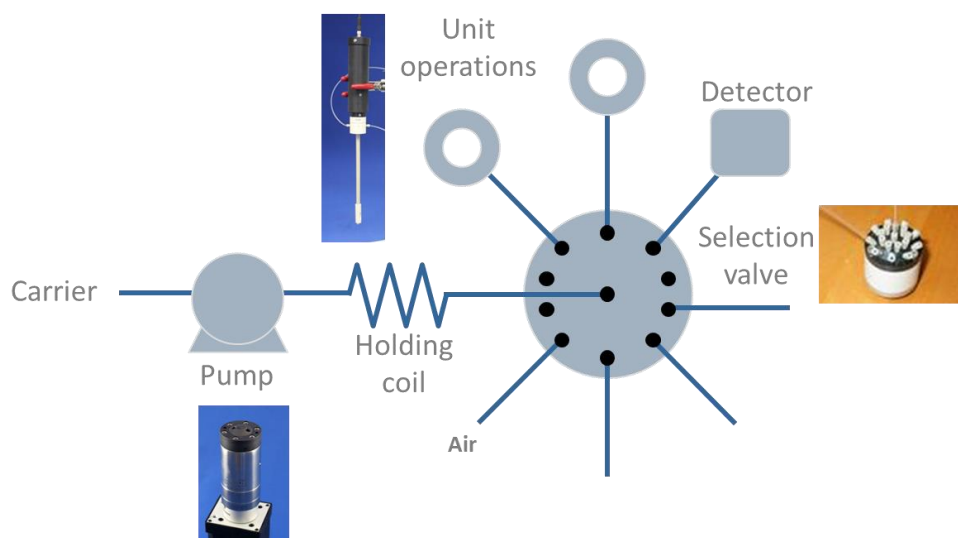


Figure 2: Zone fluidics as a versatile sampling interface

The pump and selection valve are key components in a ZF manifold. Zones of sample are aspirated into the holding coil. The selection valve port is then switched, allowing these zones to be transported via the pump to one or more unit operations.

Unit operations are hardware devices that transform the sample in one way or another and may include sample probes, heaters, separators, reactors, mixers, fraction collectors and detectors to name just a few. Unit operations may be employed for pre-analysis sample preparation or manipulation of the sample to form a detectable compound. The operation of a unit operation in a particular measurement sequence is controlled from the FloZF device control and data acquisition software.

Frequently, one port is left open to air so that air zones can also be included in the zone stack. These air zones can be used to prevent dispersion of one zone into another. Taylor flow, which results in thorough mixing, occurs when zones bracketed between two bubbles are pumped through tubing. Bubbles are also used to empty a tube of its contents.

FLOPRO-SAMPLER FLUIDICS MANIFOLD

The fluidics manifold for the FloPro-Sampler is given in Figure 3. The pump (P) moves fluids that are selected and controlled by the two multi-position selection valves (V1 and V2). The pump is protected from being overpressurized by the pressure relief valve (PRV). Primary dilution occurs between the Sample Probe (SP) and the 18-port selection valve (V1). Secondary dilution is carried out in the dilution coil (DC-3200). The diluted sample is deposited in the fraction collector FC. The carrier fluid (C) is usually distilled water or a suitable wash solution for flushing out lines between samples. Waste solutions are pumped to the waste reservoir (W). Several diluent solutions are plumbed to V1. One of the V1 ports is open to air to allow air zones to be drawn into the tubing manifold. The system is controlled by FloZF device control and data acquisition software installed on a laptop or PC equipped with the Windows OS.

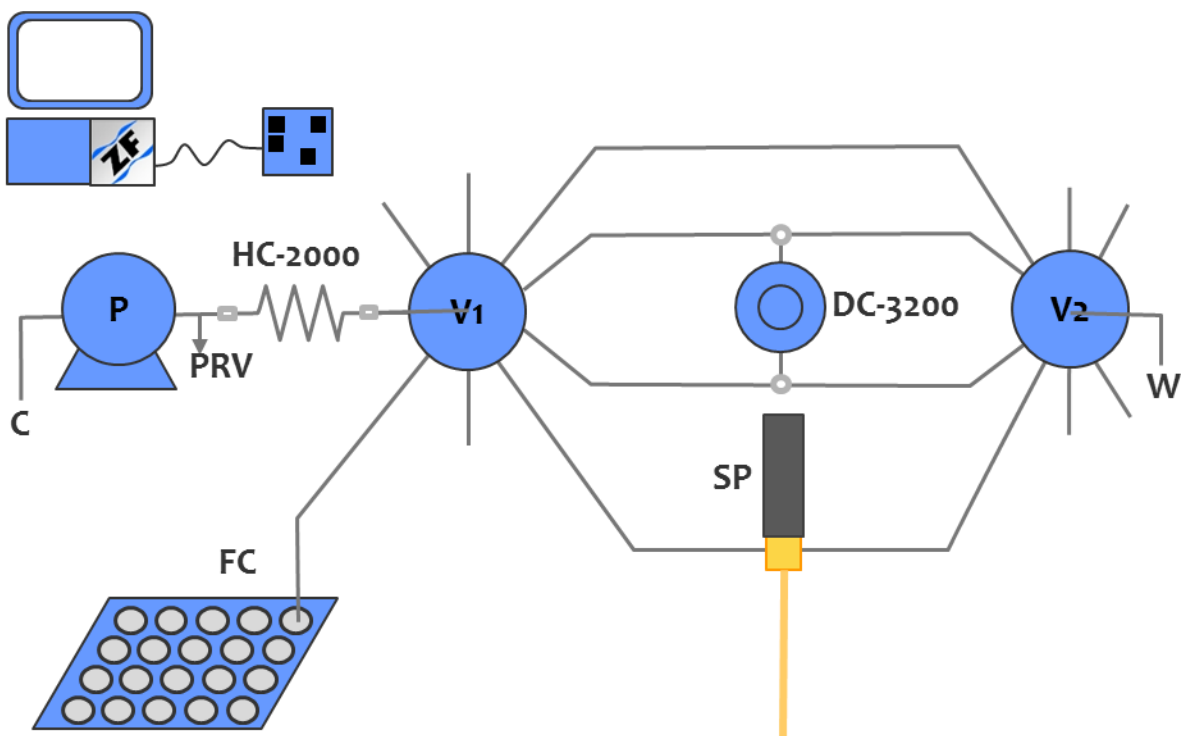


Figure 3: Fluidics manifold of the FloPro-Sampler

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 features that are of particular importance for the FloPro-Sampler include:

- Bi-directional smooth flow
- Flow range from 0.1 nL/sec – 167 μ L/sec (6 nL/min to 10 mL/min)
- Meters precise volumes from nL – mL.
- Excellent precision (\pm 12nL 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

The multi-position selection valve (see Figure 4) selects one of 10 or 18 streams arranged in a circle around a central common port, with the remainder dead-ended. These valves are coupled to the same motor with integrated controller used for the pump and an embedded algorithm controls port selection. This valve is able to withstand pressures up to 250 psi and has a chemically inert wetted flow path. The rotor flow path contains no unswept volumes and can be scaled to match channel dimensions. The red channel in Figure 5 (for an 8-port valve) shows the channel machined into the rotor of the valve.



Figure 4: 10-port selection valve (left), 18-port selection valve (center) and milliGAT pump (right)

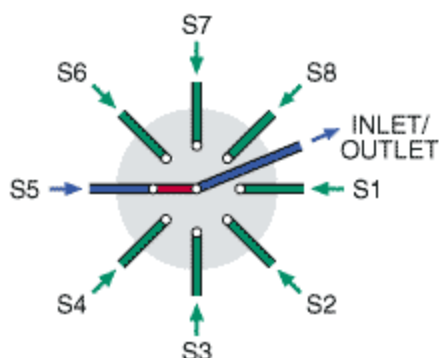


Figure 5: Flow path for the multi-position valve (only 8 ports are shown)

PRESSURE RELIEF VALVE

The FloPro-Sampler is equipped with a pressure relief valve immediately downstream of the pump. This 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 bars) this valve opens and the fluid is pumped to waste. To restore normal operation, the source of the blockage must be eliminated.

SAMPLE PROBE

The sample probe provides the means to extract sample from a process or process reactor. What distinguishes this probe from many others is that it includes a stream switching device. This device is either a two-position three-port valve or a two position four-port switching valve. This valve allows the design of fluid handling systems with certain sample manipulations carried out in close proximity to the sampling point. This feature is vital in the handling of heterogeneous samples or super-saturated solutions. It allows sample manipulation unit operations such as dilution, solvent modification, and enrichment which are described in the Sample Probe manual. The idea behind this probe is to transform the sample into a form that is easier to handle as close to the sampling point as possible. In this way it is possible to simplify many tedious and troublesome sampling projects and expand the degree of automation achievable for real-time at-line analysis.

The sample probe can be equipped with one of several probe tips. These tips allow sampling of homogenous and heterogeneous samples with or without filtration through a range of filter tips. The available tips are depicted in Figure 6. The simplest tip provides no sample conditioning and is a straightforward 0.03" ID inlet (A in Figure 6 **Error! Reference source not found.**). This tip can be equipped with a stainless steel 75 µm screen (B in Figure 6). Several filter elements have been developed. Each filter element is replaceable. The large volume filter element is equipped with a 2cm long 25µm porous Teflon filter (C in Figure 6). The small volume filter is equipped with a 7mm 2µm filter (D in Figure 6). The flat bottom filter tip allows sampling from shallow reactors (E in Figure 6) and has a filter surface area of 30 mm² and can be equipped with any filter disk. The sterile barrier filter is equipped with a 7mm 0.2µm filter (F in Figure 6). Syringe filters can be attached to the luer fitting tip (G in Figure 6).

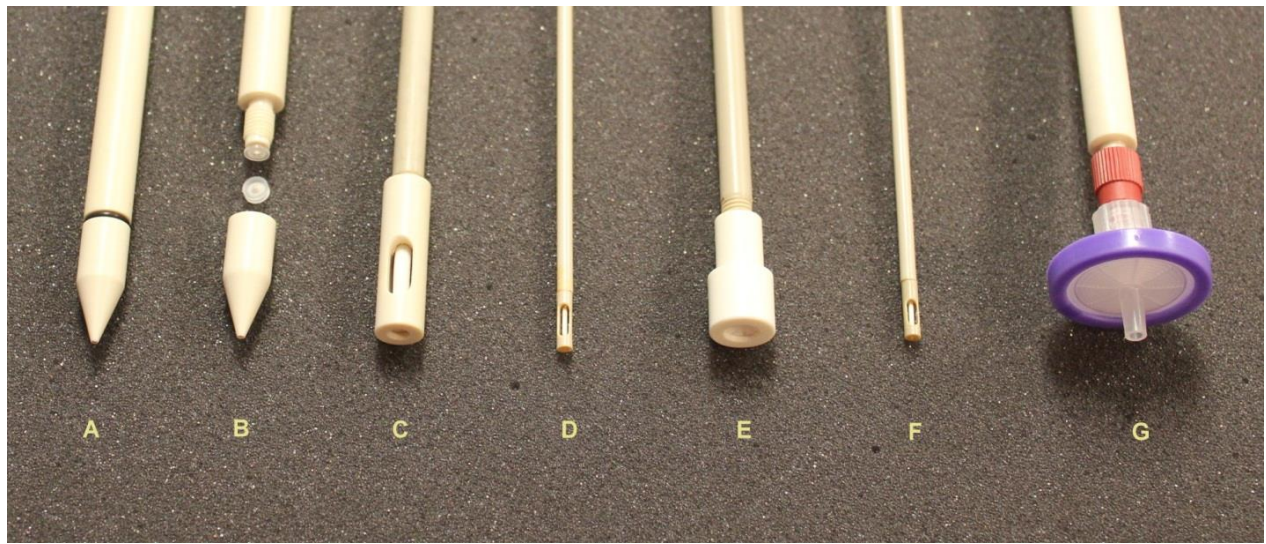


Figure 6: Batch sampling tips

The Sample Probe is depicted in Figure 7.

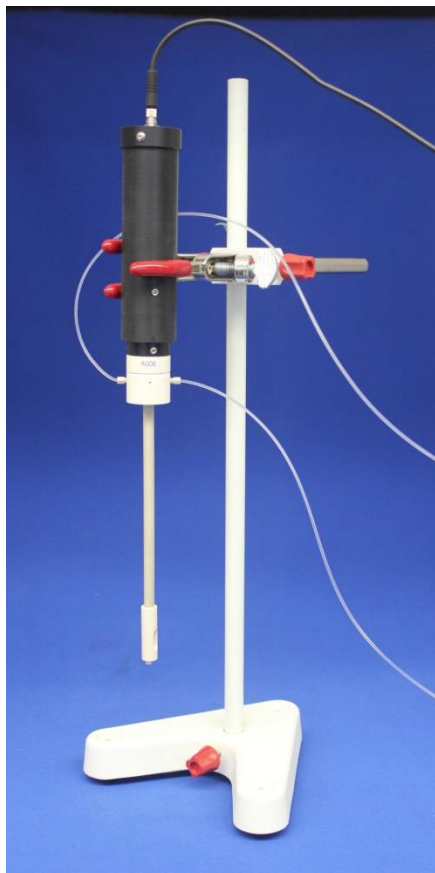


Figure 7: Sample Probe with 3-port switching valve

DILUTION COIL

The dilution coil (see Figure 8) is a long length of 0.062" ID tubing coiled and connected to two Tee pieces which are coupled to ports on the selection valve as shown in the fluidics manifold (see Figure 3). The sample is bracketed between two diluent zones and this zone stack is preceded and followed with two air bubbles. As this zone stack moves down the tubing, Taylor flow conditions prevail, and the diluent and samples are thoroughly mixed resulting in a diluted sample.



Figure 8: Holding coil (left) and dilution coil (right)

FRACTION COLLECTOR

The fraction collector is accessed via the x-y-z motion control axes. A needle is mounted on the z-axis and is positioned above the desired vial by the x and y-axes (see Figure 9).

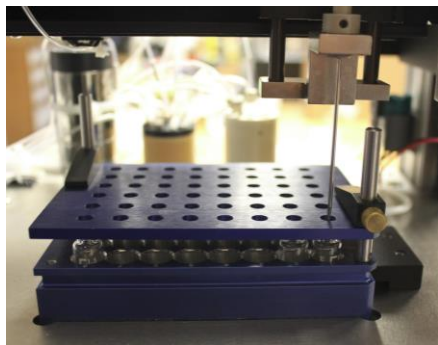


Figure 9: Needle and vial holder of fraction collector

Two vial holders have been used. The one, originally custom machined by Global FIA and modeled after the SYMYX Tools holder, has space for 48 vials (8x6) and also includes a cover to retain the vial in the vial holder as the needle was withdrawn from the vial. The second is a standard Agilent holder (P/N: G2255-68700) with space for 54 vials (9x6), but no cover. When this vial holder is used, a foot is mounted to the z axis positioned just above the height of the vials (see Figure 10).



Figure 10: Vial retention foot

This foot retains the vial in the holder as the needle is withdrawn. The needle is a needle-in-needle design that allows air to be displaced through the outer needle while diluted sample is injected via the inner needle. A wash station is provided where the needle can be flushed to waste between samples. This wash station is drained by a small diaphragm pump (see Figure 11).

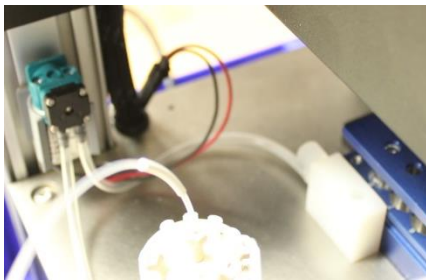


Figure 11: Wash station and drain pump

RESERVOIRS

Two large 125 mL Boston round clear glass reservoirs (P/N: 129-0250) serve as the carrier reservoir and waste reservoir. Five clear 22mL glass vials with PTFE lined caps by National Scientific (P/N: B7800-05) are used as diluent and wash solution reservoirs. Reservoir caps are mounted to vented vial /reservoir holders.

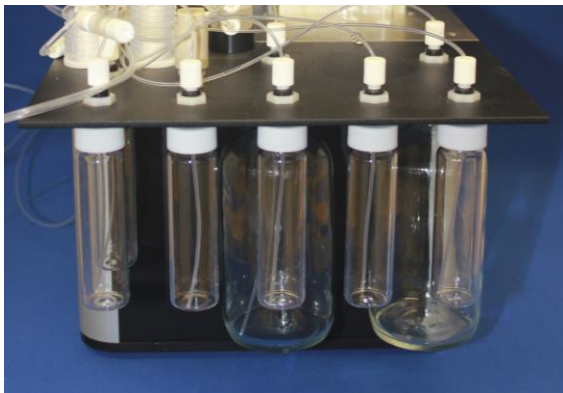


Figure 12: Reservoirs

POWER

The FloPro-Sampler is 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. The power socket is equipped with 0.5Amp slow-blow fuses.

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 fraction collector for command and control. Both the Fraction Collector and other devices make use of virtual COM ports on the USB hub. The driver for these ports can be downloaded from [FTDI](#)¹.

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.
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.
3. Connect the USB cable between the computer and the FloPro-Sampler USB type B connector shown in Figure 13. In some systems, a second USB cable will extend from one of the USB type A connectors on the USB hub to provide communication with the fraction collector. In other systems, this connection is made internally.

¹ <http://www.ftdichip.com/FTDrivers.htm>



Figure 13: FloPro-Sampler rear view

4. Connect the Sample Probe cable to the Green connectors as indicated in Table 2. The IO ports that are used for the sample probes are B1 – B4. Some systems only have 3 IO ports per device. If a fourth IO port is needed, you may use one of the A device ports. In this case it will be necessary to make an appropriate change to the device settings on the Setup tab of the device manual control in FloZF. The pin outs for the 2 green connectors on the back of the FloPro-Sampler are as shown in Table 1 – the connectors are also labeled to indicate which configuration is being used.

Table 1: Pin-outs of FloPro-Sampler Power and IO connector

Pin	Power (Config I)	Power (Config II)	IO (Config I)	IO (Config II)
1	N/C	N/C	A4	Gnd
2	N/C	N/C	A3	A3
3	24 VDC	24 VDC	A2	A2
4	Gnd	Gnd	A1	A1
5	24VDC	24VDC	B4	Gnd
6	Gnd	Gnd	B3	B3
7	5VDC	5VDC	B2	B2
8	Gnd	Gnd	B1	B1

5. Connect the Sample probe common tube to Valve A port 12 and the waste line to Valve B port 5. If there is more than one probe then connect the additional probes to sequential valve ports.

Table 2: Connection of Sample Probe

Description	Connector	Pin
Power Ground	Brown	P8
5VDC	Blue	P7
Dig IO B1	Black	IO8

6. Ensure that the power entry module is configured to match the voltage of the mains power supply.
7. Connect the power cable to the FloPro-Sampler power entry connector and switch on the FloPro-Sampler power switch. 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. Drivers for these converters from [here](#)². Two drivers will be downloaded for each of the two converters.
8. Fill the carrier bottle with a suitable carrier solution such as DI water or an appropriate wash solution.
9. Fill the diluent vials with the chosen diluent.
10. Familiarize yourself with FloZF by going through the FloZF Getting Started pamphlet.
11. Sequences to fully automate the sampling and dilution procedure have been provided. For preconfigured computers, these can be found in your **Documents** Folder under **FloZF Projects**. These sequences are described in detail in the sequence appendix.

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

² <http://www.ftdichip.com/Drivers/VCP.htm>

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. FloPro-Sampler procedures are stored as Projects. 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 FloPro-Sampler devices for the supplied methodology as well as basic measurement sequences has been included on the installation media and installed on the computer (if supplied). 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 14). 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 (see §0) and monitoring of the device, and a **Setup** tab for device configuration. 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.

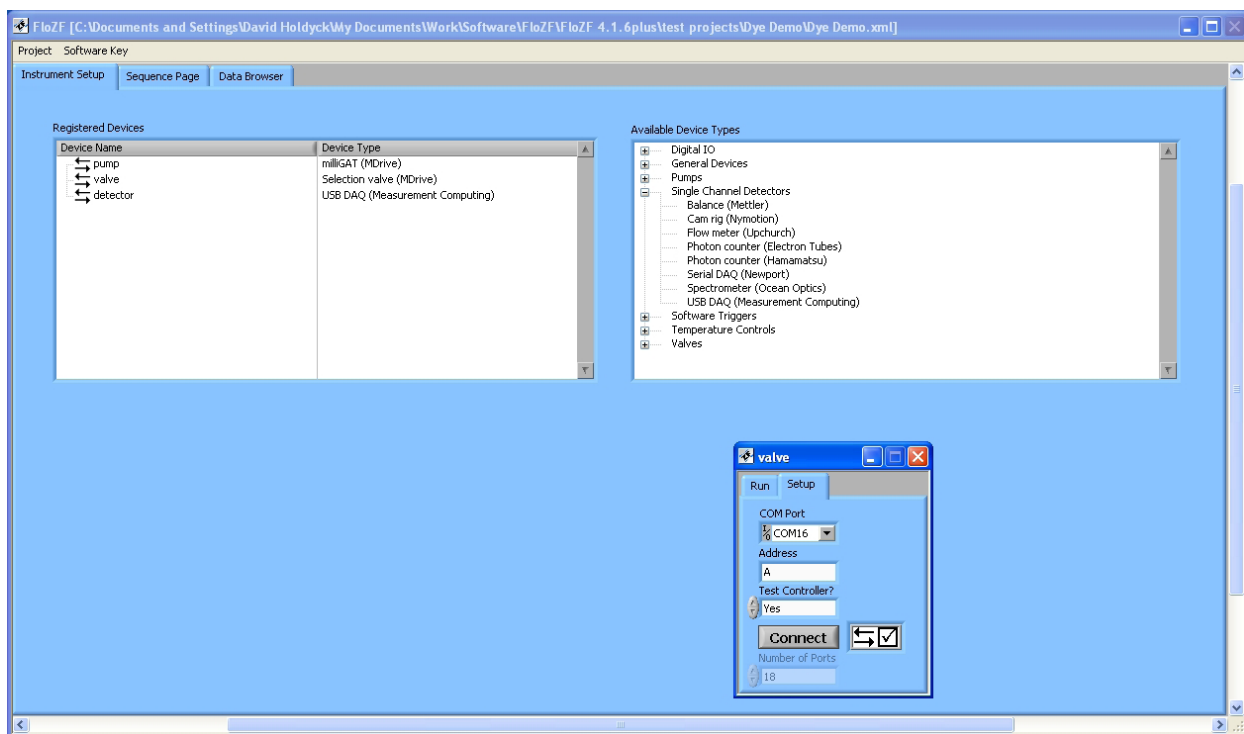


Figure 14: Instrument Setup Page in FloZF

BUILDING SEQUENCES

Sequences are created, edited and executed on the **Sequence Page** (see screen shot of the Sequence page in Figure 15). 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).

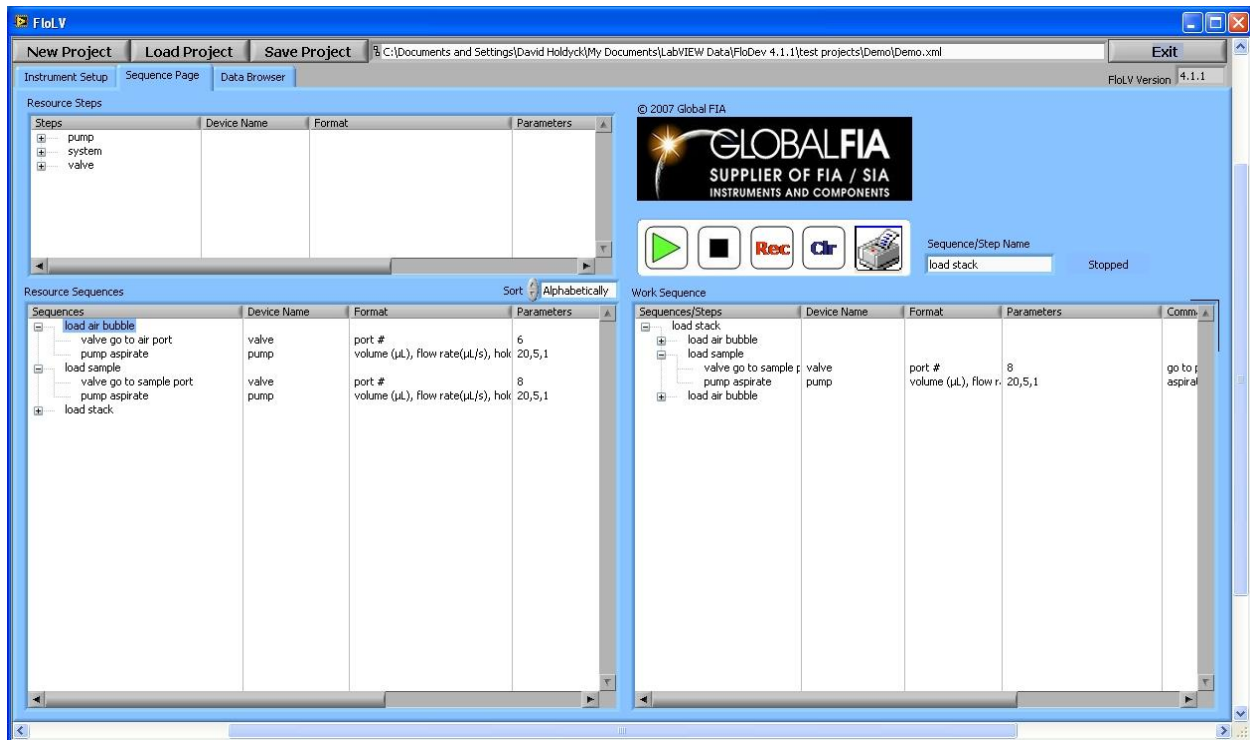


Figure 15: FloZF Develop Page Interface [obtain new screen shot](#)

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. Global FIA can provide basic FloZF sequences for interfacing to Excel.

Typically a Master Spreadsheet is provided. This spreadsheet contains data fields that define the work for the following run. This sheet is saved and when the main sequence is executed, a new spreadsheet is created this is a copy of the master spreadsheet. The file name of this spreadsheet contains the date. It is this spreadsheet that is used during the run and any data written back to this spreadsheet will be saved in the date marked file, leaving the Master spreadsheet for intact for future configuration definitions.

The spreadsheet contains a **Variables** section (with **Parameters** field), a **Biographical Data** section, and a **Results** section. The variables that may be manipulated are listed under the Variables heading, along with acceptable ranges or values where applicable. The variables are changed / updated by filling in the corresponding shaded Parameters field box. Only the cells that have been shaded in the Excel document are designated as fields that can be updated by the end user. The end user can also enter information into the Biographical Data section (User name, Date, and Project) in the designated boxes. It is important to remember to save the master file before executing the sequence as the sequence will make a copy of the saved master Excel spreadsheet.

As the sequence proceeds, certain data will be stored back to the spreadsheet providing an up-to-date record of what the FloPro-Sampler has done. Recorded information will include sample names, date stamps for the time the sample was taken, well numbers where samples have been archived in the fraction collector, and response data with hyperlinks to profiles if a detector is included in the manifold.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	
1															
2	VARIABLES:									Parameter	BIOGRAPHICAL DATA:				
3	Select Unit Operation (Enter 1: Detector, Enter 2: Fraction Collector, Enter 3: Injector)									1	User:	Rachel Steffenson			
4	Detector-Specific Variables:										Date:	26-Jan-10			
5	Wavelength									625	Project:	Universal Sequence			
6	Fraction Collector-Specific Variables:														
7	Starting Well Number (Acceptable Range: 1-96)									37					
8	Starting Well Plate (Acceptable Value: A or B)									A					
9	General Variables:														
10	Number of Diluents (Acceptable Range: 1-4)									2					
11	Number of Samples (Acceptable Range: 1-100)									2					
12	Wait Time Between Samples (sec)									10					
13	Dilution Variables:														
14	Primary Dilution (Sample Probe)														
15	Diluent Port (Acceptable Range: 2-5)									2					
16	Diluent Volume (Acceptable Range: 50-200uL)									90					
17	Probe Sample Volume (Acceptable Range: 10-50uL)									10					
18	Second-Stage Dilution (Detector)														
19	Diluent Port (Acceptable Range: 2-5)									2					
20	Diluent Volume									380					
21	Sample Volume (Sample taken from Primary Dilution)									20	CALCULATIONS:				
22	Second-Stage Dilution (Fraction Collector)										Aspirate Sample at Probe:	20			
23	Sample Volume (Sample taken from Primary Dilution)									50	Build Zone Stack (Diluent):	190			
24															
25															
26	RESULTS:														
27	Date Stamp		Sample Name		Peak/Plateau Height		Well # (Plate Sampler)		Reference/Hyperlink						
28	01-26-2010 11:31:24 AM		test		0.439263				profile						
29	01-26-2010 11:43:42 AM		test		0.470145				profile						

Figure 16: Sample Excel Spreadsheet for Running FloZF [\[Update\]](#)

The sequence will end by saving the spreadsheet to disk. The default location for the spreadsheet is in the **Active Data Folder** of the project folder (see Figure 17).

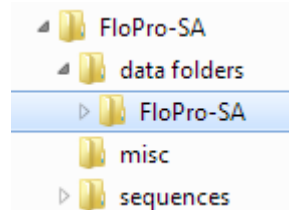


Figure 17: Active data folder where Excel spreadsheet is saved

You can create a new active data folder on the Store tab on the Measure page (see Figure 18). Once you have created the new data folder you will have to copy the Excel Master spreadsheet to the new folder. To swap from one active data folder to another, activate the desired folder by selecting it and then right-click to pop-up the **Activate folder** menu option.

Do not change the name of the Master spreadsheet because this is specifically referenced in the FloZF sequence.

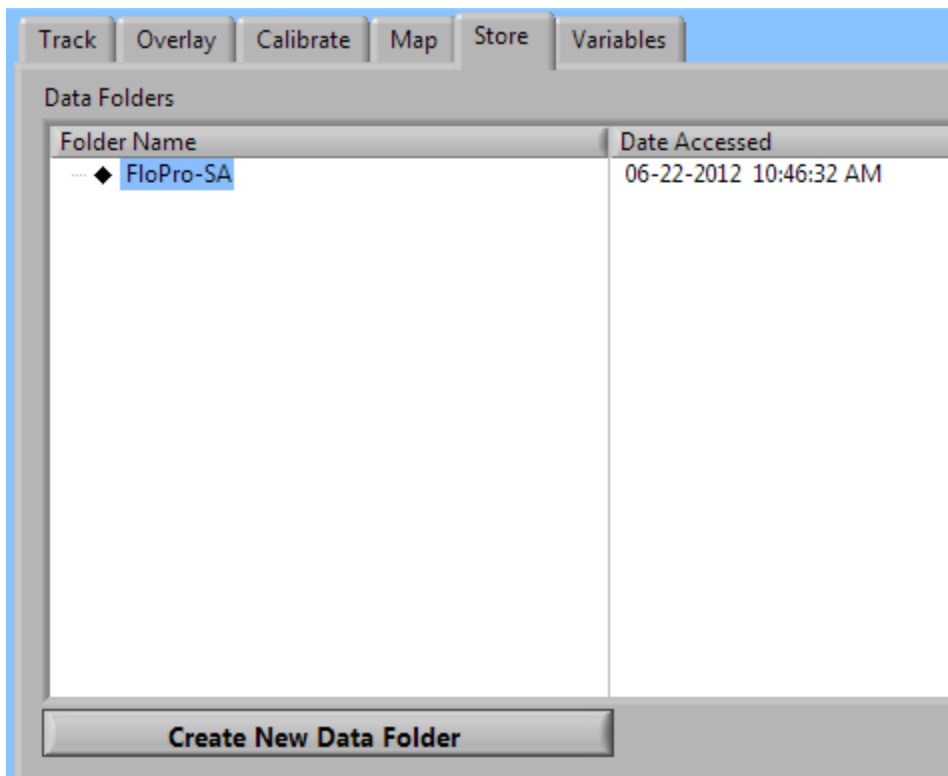


Figure 18: Store tab on the Measure page

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 19).

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 19: 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 20).

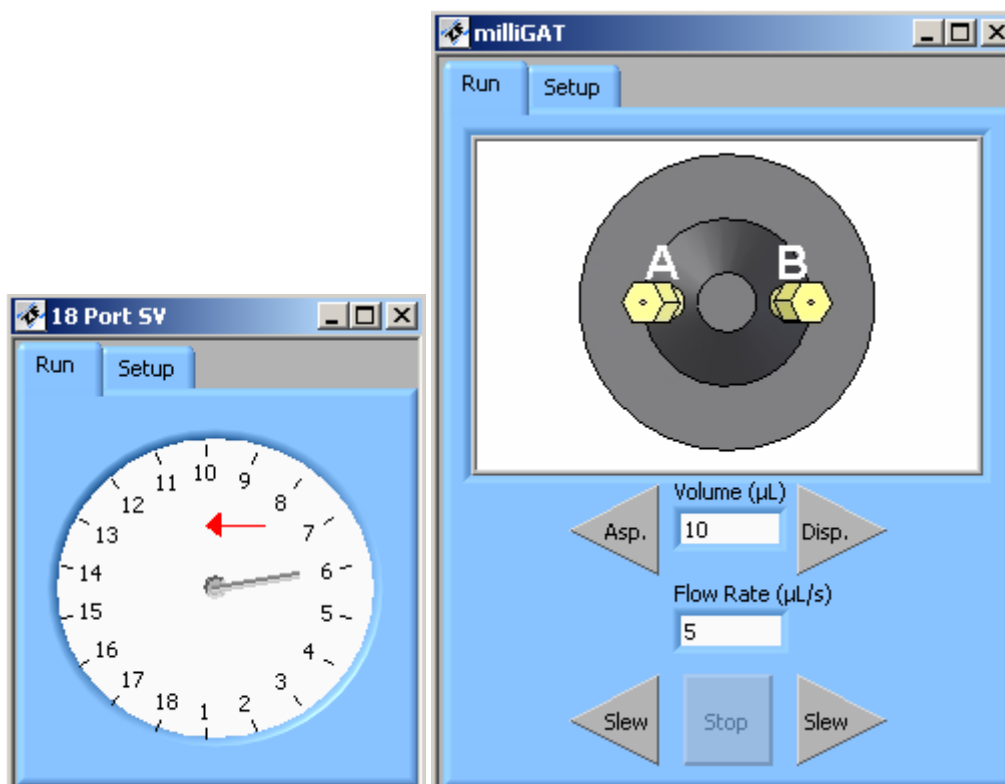


Figure 20: Instrument Panel for the 18 Port Selection Valve and the milliGAT Pump

The following two examples of manual operation are based on the above figure. The 18-port selection valve (above left) currently is showing port 6 selected. To manually select a different port, say port 10, simply move the cursor to the location indicated by the red arrow 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 as they 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 21) 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.

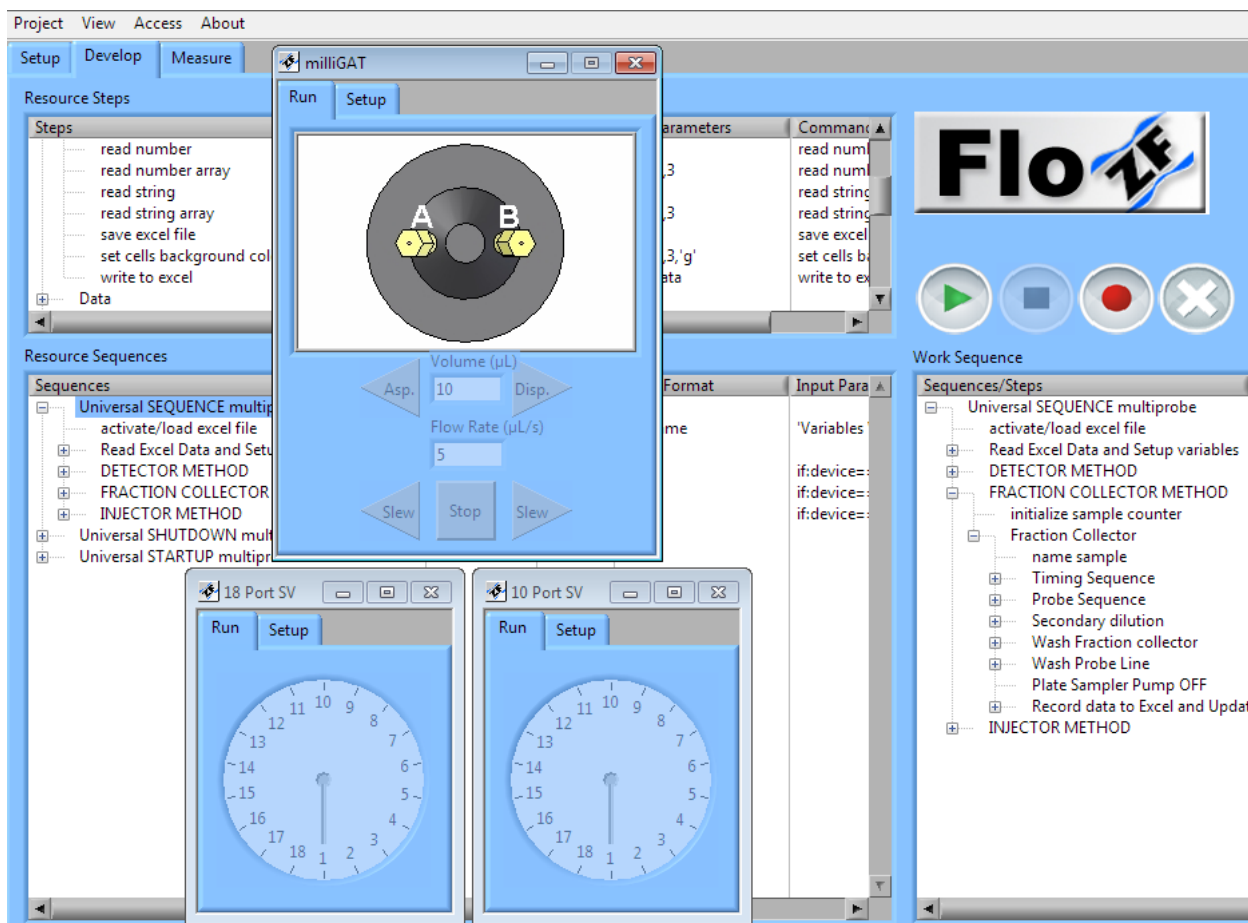


Figure 21: Manual Controls displayed in Instrument View

The **Setup** tab on the manual control is where various settings for each device are specified.

SEQUENCES

COMMON DEFAULT SEQUENCES

In addition to the Universal control sequence which is described in more detail in the appendices, there is also a Startup and Shutdown sequence. As their names imply, these 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:

1. **“UNIVERSAL 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, wash the sample probe lines, and prep each unit operation in use are contained within this sequence.
2. **“UNIVERSAL 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.

LISTING OF COMMON DEFAULT SEQUENCES

Still need to include these sequence listings

Step	Variable Name	Parameter
UNIVERSAL STARTUP		
Step	Variable Name	Parameter
UNIVERSAL SHUTDOWN		

Step	Variable Name	Parameter
UNIVERSAL SEQUENCE		

--	--	--

AUTOMATED MEASUREMENT

While it is possible to run a method from the **Develop** page, it is preferable to run from the **Measure** page (refer to Figure 22).

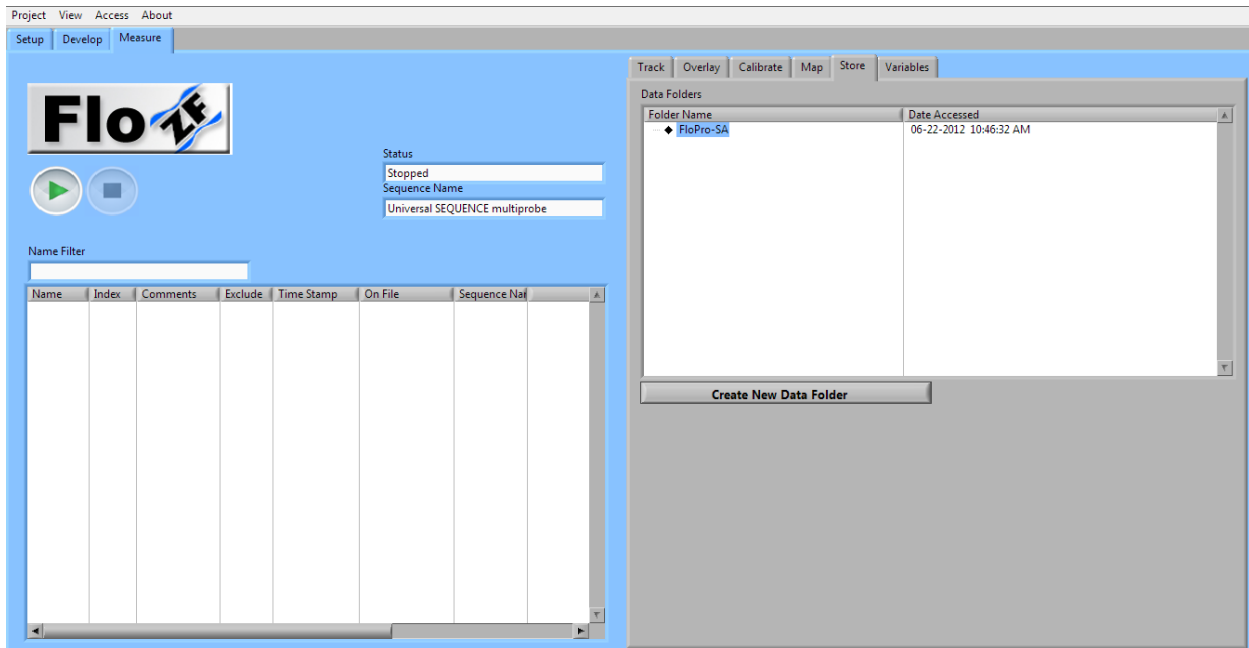


Figure 22: FloZF Measure page

The **Sequence Name** field specifies the sequence that will be executed when the green arrow **Go** button is pressed. You can add sequences from the **Resource Sequences** List by right clicking the Sequence name field to pop up a **Modify List** menu option

The **Name Filter** field allows you to just show samples in the data file whose sample name match the **Name Filter** mask.

The **Status** field shows the run status of the selected sequence.

A **Run** and **Stop** button are used to execute or stop a sequence. The **Run** button transforms into a **Pause** button while the sequence is running.



Figure 23: RUN STOP and PAUSE buttons

The **Create New Data Folder** button in the **Store** tab of the **Measure** page provides a means of creating new data folders.

The data folders for the current project are listed in the **Data** Folders field where it is possible to swap to a different data folder, sort data folders alphabetically or by date, or delete whole data folders by selecting a Data folder and pressing **Ctrl-Delete**. **Note** – you cannot recover a data folder once it has been deleted.

ROUTINE OPERATION

1.

ACCESSING DATA

RESULTS TABLE

EXCEL DATA

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, refer to section 0). 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 are frequently used to separate a zone stack from the carrier solution and prevent dispersion. For instance, bracketing a zone stack with bubbles for detection purposes with a spectrometer enables a higher degree of precision to be achieved. However, bubble fragmenting can negatively affect one's data and are therefore undesirable. Fragmented bubbles within the tubing can be explained by a few different possibilities, which are typically quite easily corrected. When flow rates are too high, bubbles tend to fragment. 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 fragmenting 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.

NO COMMUNICATION WITH DEVICES

If communication is lost with the devices, exit FloZF, power down the MobiChem. Unplug the USB cable. Wait 30 seconds. Plug it in again and power up MobiChem. Start up 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 #253-549-2223).

APPENDIX B: SPARE PARTS LIST

P/N	Description
HKS	Hex key set
B-W	Box Wrench
640W	Wrench for 6-40 fittings
F-345	Flushnut wrench
FC-AP	Flow cell alignment pin
FF-16-W	Flange-free fittings, 1/4-28, white
F-358X	Flushnut
P-200X	Flangeless Ferr 1/16 Blue/10pk
1032FN-16M	Zero dead volume nut, 10-32, medium
1032FF-16G	Zero dead volume ferrule, 1/16"
640FF-16	Fitting for 1/16" tubing, 6-40
P-1428N	Plug, 1/4-28
P-1032P	Plug, 10-32
640P	Plug, 6-40
16T-03-PFA	Tubing, PFA, 1/16" OD 0.030" ID

APPENDIC C: WETTED MATERIALS

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 and the sample probe make use of a PAEK-based composite. The green tubing between the reagent reservoirs and the selection valve 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%).

VALCON P

The pump rotor is made from Valcon P.

This composite, the majority of which is PTFE and carbon, has been used extensively in many analytical applications that use Valco valves. It is routinely used in other applications at 1000 psi, 75°C, and can also be used at temperatures approaching 200°C with decreased sealing tension.

VALCON-E3

The pump port end cap is made of a PAEK-based composite called Valcon-E3.

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%).

SAPPHIRE

The milliGAT[®] 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), Chemflour® PFA Tubing is widely used in the semiconductor, 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 bubble tolerant flow cell are made from quartz glass.

BOROSILICATE GLASS

The reactor in the MD cell is made from borosilicate glass (also called Pyrex™). Some reservoir contains are made from borosilicate glass.

LDPE

Carrier and waste reservoirs are made from either borosilicate glass or 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.

GUIDELINES

Specific reagent combinations should be tested to ensure compatibility with pump components. Since the least chemically inert component in these composites is PEEK, for optimum component life time, the following guidelines³ given for PEEK should be followed:

Acids and bases	No stronger than 1 M
H ₂ SO ₄	Resistant to 10%
HCl	Resistant to 20%
HF	Not Resistant to 70% (no data on lower conc.)
HNO ₃	Resistant to 20%
dibutylamine in toluene	Not Rated
TBAH	Not Rated (Dionex uses PEEK as column material at 1 mM)
NaOH	Resistant
H ₂ S	Resistant
CH ₃ SH	Not Rated
acetone	Resistant
methyl ethyl ketone	Resistant at Room Temp.
methyl alcohol	Resistant
toluene	Resistant at Room Temp.
xylene	Resistant
chloroform	Resistant
chlorobenzene	Resistant at Room Temp.
iodine/iodide in water	Not Rated
sodium hypochlorite (household bleach)	Resistant at Room Temp.
permanganate	Resistant at Room Temp.
hexane	Resistant at Room Temp.

³ Corrosion Resistance Tables, Schweitzer, Marcel Dekker, 1991

www.zeusinc.com/product_sheets/resins/peek/chemical_compatibility.html

APPENDIX E: FLOW RATE GUIDELINES

Table 3: Flow rate guidelines

Action	Suggested Flow Rate
Pushing diluent to sample probe/extracting sample from probe	2 μ L/s – 5 μ L/s
Aspirating sample or aspirating air/headspace gas	5 μ L/s – 10 μ L/s
Aspirating diluents	10 μ L/s – 20 μ L/s
Priming/cleaning diluent port tubing	10 μ L/s – 25 μ L/s
Priming/Cleaning sample probe	10 μ L/s – 25 μ L/s
Flow rates for flow cell use	10 μ L/s – 25 μ L/s ⁴
Dispensing diluent, zone stack, or sample through tubing	10 μ L/s – 25 μ L/s
Washing Plate Sampler line	25 μ L/s – 50 μ L/s
Sending dilution zone stack for collection via plate sampler	25 μ L/s – 50 μ L/s
Mixing dilution zone stacks (through Mixing Coil)	35 μ L/s – 55 μ L/s ⁵
Flush Holding Coil	50 μ L/s – 170 μ L/s
Flushing Mixing Coil	50 μ L/s – 170 μ L/s

⁴ When taking a measurement (spectrophotometrically), a slower flow rate (10-20 μ L/s) should be used. The upper limit (25 μ L/s) can be used for flushing the flow cell, but should not be exceeded, as leaking may result.

⁵ Higher flow rates have been shown to improve mixing within the zone stack. However, dispersing the zone stack at too high of a flow rate will result in bubble fragmenting, which could negatively impact results.