Profilers Samplers Flotation

mclanelabs.com

Robotic Cartridge Sampling Instrument (RoCSI)

User Manual

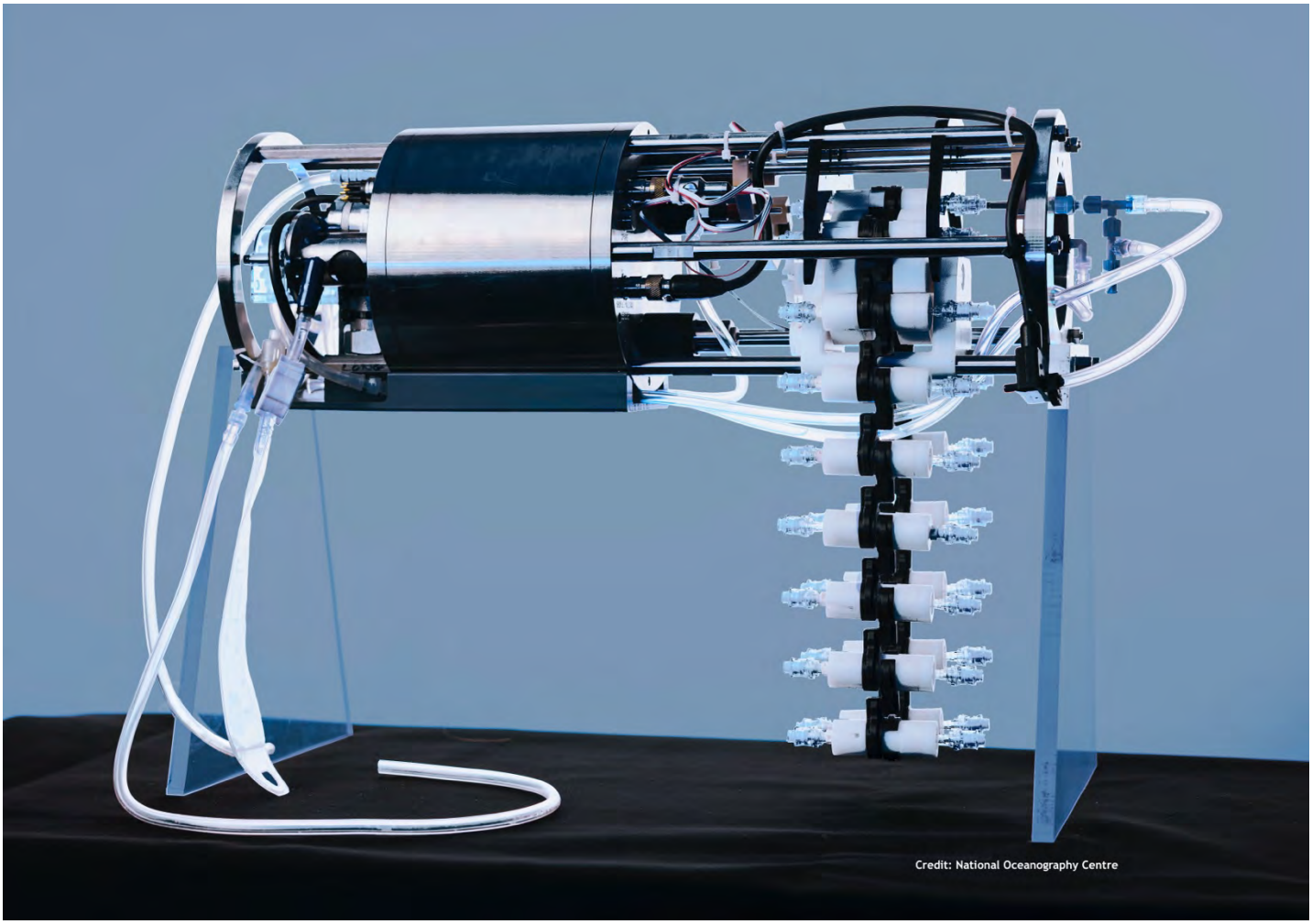

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Table of contents

Included with the RoCSI

A new Robotic Cartridge Sampling Instrument (RoCSI) includes a USB drive that contains the User Manual and Benchmaps interface software.

Each RoCSI also includes a toolkit. The toolkit and contents are referred to in this User Guide, and should remain with the instrument at all times.

The toolkit pictured is only an example.

Actual toolkit contents may vary and are subject to change without notice.

Contact McLane

TELEPHONE SUPPORT	+1 508.495.4000
FAX	+1 508.495.3333
SKYPE	MCLANE_RESEARCH
EMAIL	MCLANE@MCLANELABS.COM
WEBSITE	www.mclanelabs.com
Mailing Address	McLANE RESEARCH LABS 121 Bernard E. Saint Jean Drive, East Falmouth, MA 02536 USA

When contacting McLane for technical support, please provide the following:

- Firmware version, GUI version (Benchmaps) and instrument serial number.
- Problem description.

Contact mclane@mclanelabs.com with questions about retrieving files.

McLane Research Laboratories is on the Web at http://www.mclanelabs.com or via email at mclane@mclanelabs.com.

Printable User Manual

Check the RoCSI User Manual page on the McLane website for updates and a downloadable RoCSI User Manual.

Serial Number

A McLane instrument serial number begins with 'ML' followed by five numbers and a dash (-) with two more numbers.

Example: ML12345-01

This information is located on a label attached to the controller housing:

RoCSI General Information

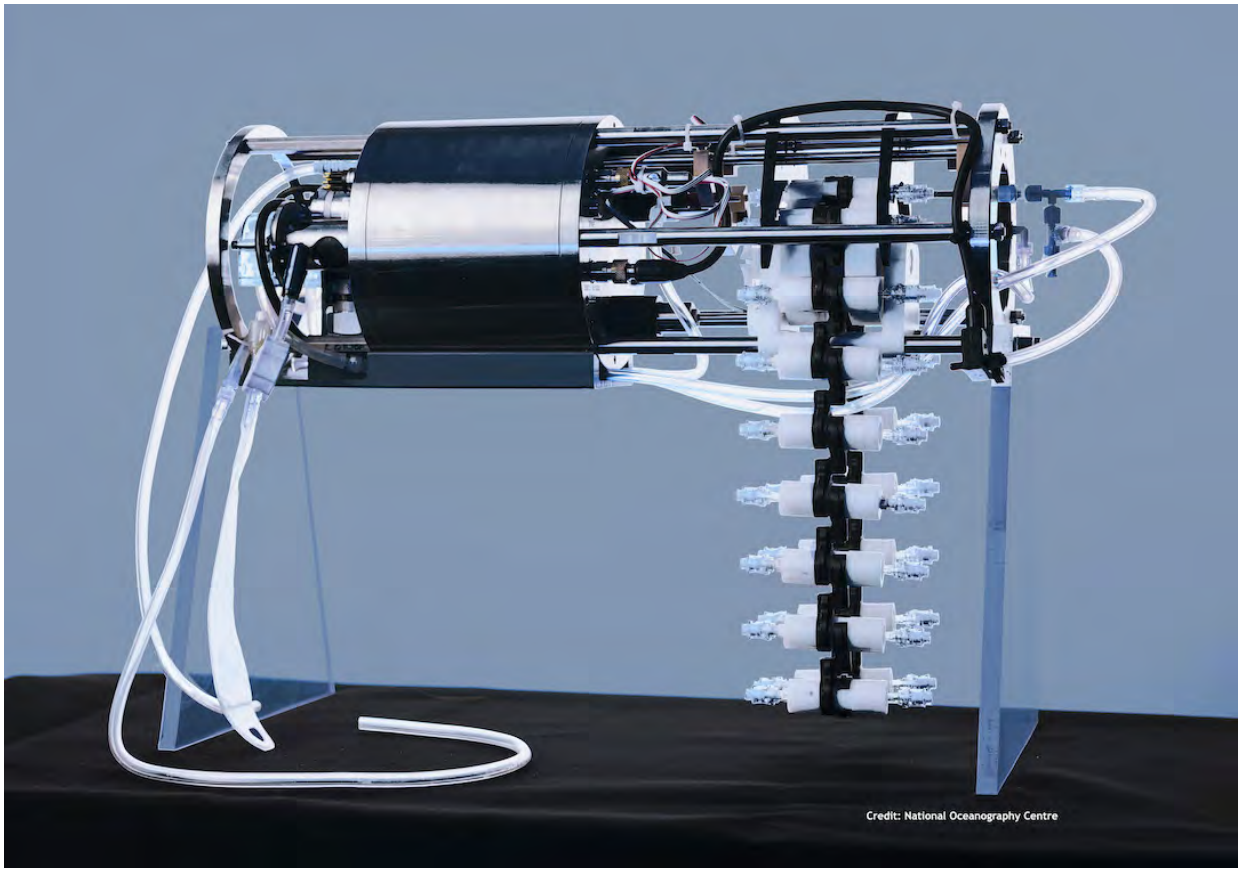
General Information includes a short description of the RoCSI. A more detailed specification sheet and selected references are available on the McLane website,RoCSI product page.

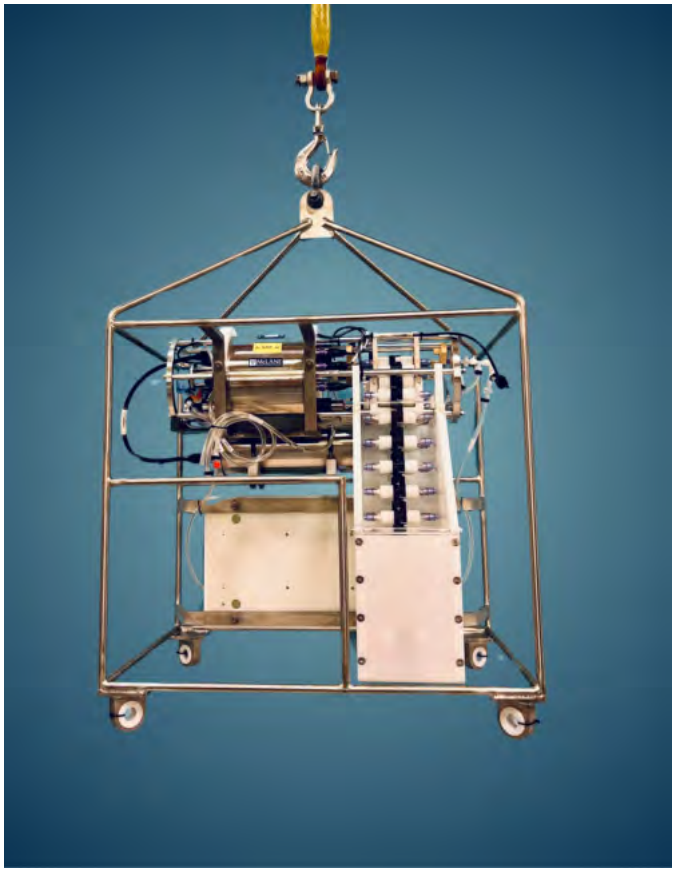
The Robotic Cartridge Sampling Instrument (RoCSI) is a field-proven, in situ, autonomous oceanographic sampler that collects and preserves water samples into industry standard 0.22 and 0.45 micron Sterivex™ filter cartridges for later eDNA analysis. RoCSI has high sample count capabilities in a compact instrument that is depth rated to 6,000 m.

- RoCSI Sterivex™ filter cartridge chains are available in 12, 24, or 48 samples.
- RoCSI uses common industry liquid preservatives such as RNAlater™.

RoCSI is also available as a fully self-contained, moored system including reagent bag storage, external battery housing and cartridge containment (hopper), mounted on a frame.

Please note that deployment duration is typically limited by the efficiency of preservative over time. Typical deployments may not exceed one month, if using RNAlater, as longer deployments may not return viable samples due to longevity/effectiveness of the preservative. Other preservatives may offer longer term sample viability, but should be tested and confirmed by the user.


Credit: National Oceanography Centre



Options

- Lithium primary battery
- Moored frame
- Cartridge containment hopper

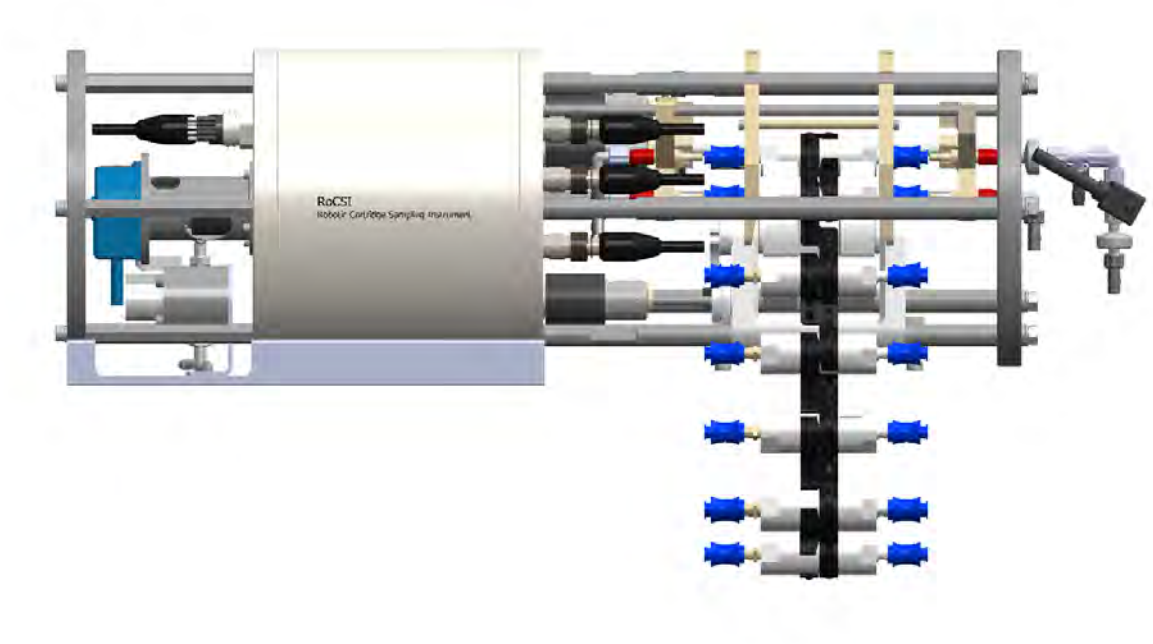
RoCSI Specifications

This section provides mechanical and electrical specifications for RoCSI.

Physical Specifications

Physical

For physical specifications, see the RoCSI data sheet here.



Electrical Specifications

Electrical

- Nominal: +12 Vdc (10-25 Vdc) / 2 A
- Absolute Max: 25 Vdc
- Typical Pumping Current (at 12 Vdc): 0.4-0.7 A

Communication Cable Specifications

Communication Cable

If you want to make your own lead, you will need a cable terminated with a Subconn MCIL8F plug (to mate with the Subconn MCBH8M bulkhead connector on the sampler's end-cap).

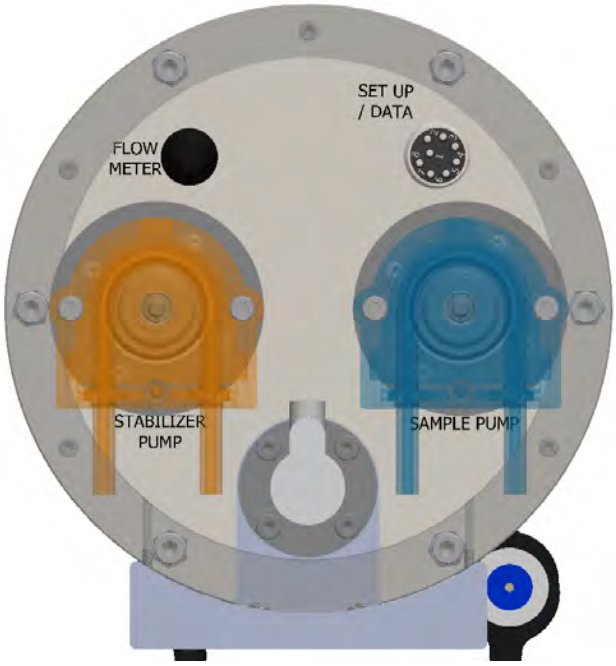
The pin-out of the connector is as follows:

- PIN 1 (black) 12 Vdc
- PIN 2 (white) GND
- PIN 3 (red) USB VBUS
- PIN 4 (green) USB GND
- PIN 5 (orange) USB DP
- PIN 6 (blue) USB DM
- PIN 7 (white & black) RS232 SAMPLER RX
- PIN 8 (red & black) RS232 SAMPLER TX

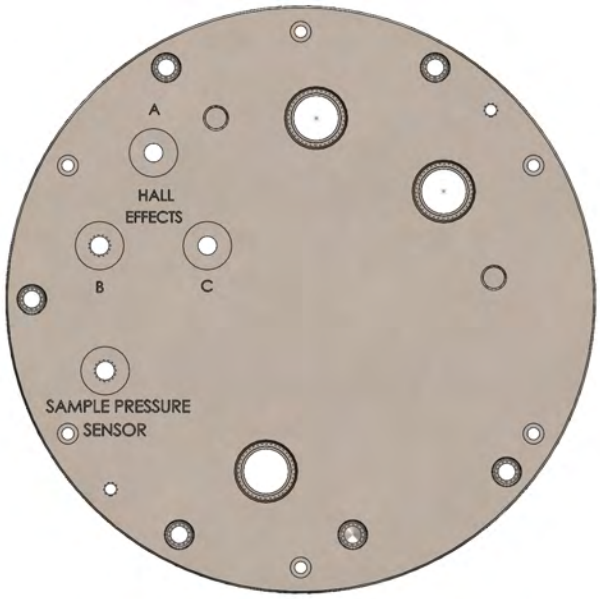
The deck lead provided with the unit has power, USB, and RS232 connections. A USB deck lead should not exceed 2m. An RS232 graphical user interface (GUI) is available with the same functionality as the USB GUI but you will need an RS232 port (or RS232-USB adapter) on your PC to use it. There is also an RS232 vehicle interface available to allow a vehicle to start/stop the sampler autonomously. An RS232 deck lead should work up to at least 7m.

Controller Housing End Cap Diagrams

Front End Cap:



Rear End Cap:



Bulkhead Connectors

Bulkhead Connectors

Front End Cap:

Set Up / Data - Communication and Power Port (+12 Vdc / USB / RS232)

Flow Meter - Flow Meter Sensor

Rear End Cap:

Hall Effects A - Sample Injector Position Sensor

Hall Effects B - Stabilizer Injector Position Sensor

Hall Effects C - Cartridge Load Position Sensor

Sample Pressure Sensor - Differential Pressure Sensor

Sterivex™ Filter Cartridge Specifications

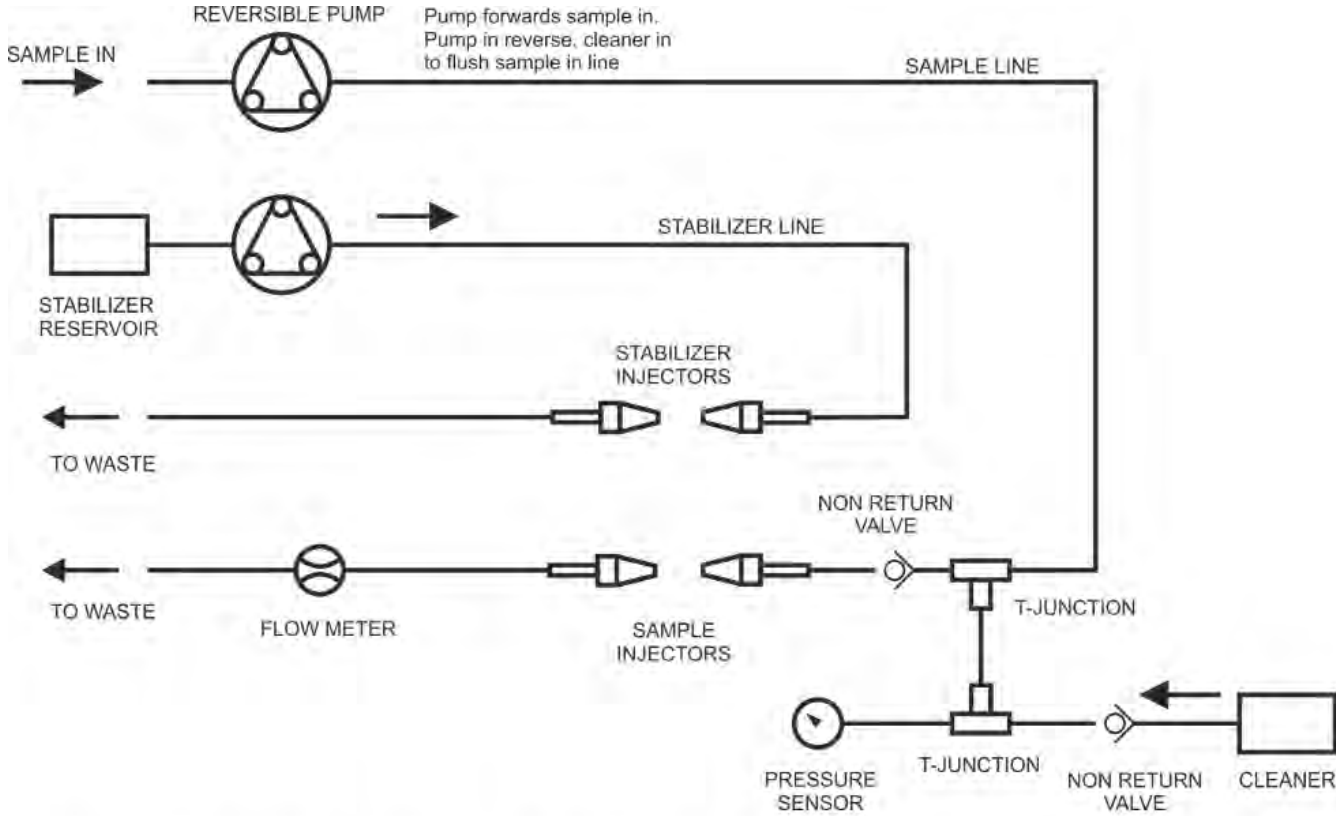
RoCSI Sterivex™ filter cartridge chains are available in 12, 24, or 48 samples. Each new sample belt includes one set of the specified number of cartridge filters (for example, the 12 sample chain includes 12 Sterivex™ filter cartridges), the 24 sample belt includes 24 cartridges, and so on.

Filter cartridges are Merk Millipore Sterivex™. Additional filter cartridges can be purchased from Merk or Sigma.

Sterivex GP – 0.22um PES membrane, p/n SVGP010 (pictured below)

Sterivex HV – 0.45um PVDF membrane, p/n SVHV010

Fluidic System Schematic



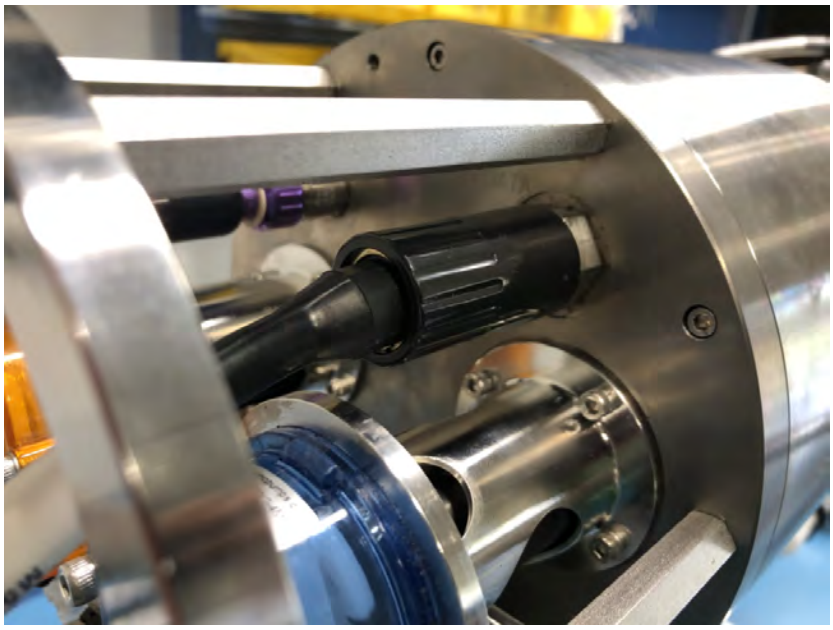
SCHEMATIC OF RoCSI FLUIDIC SYSTEM

Connecting to RoCSI

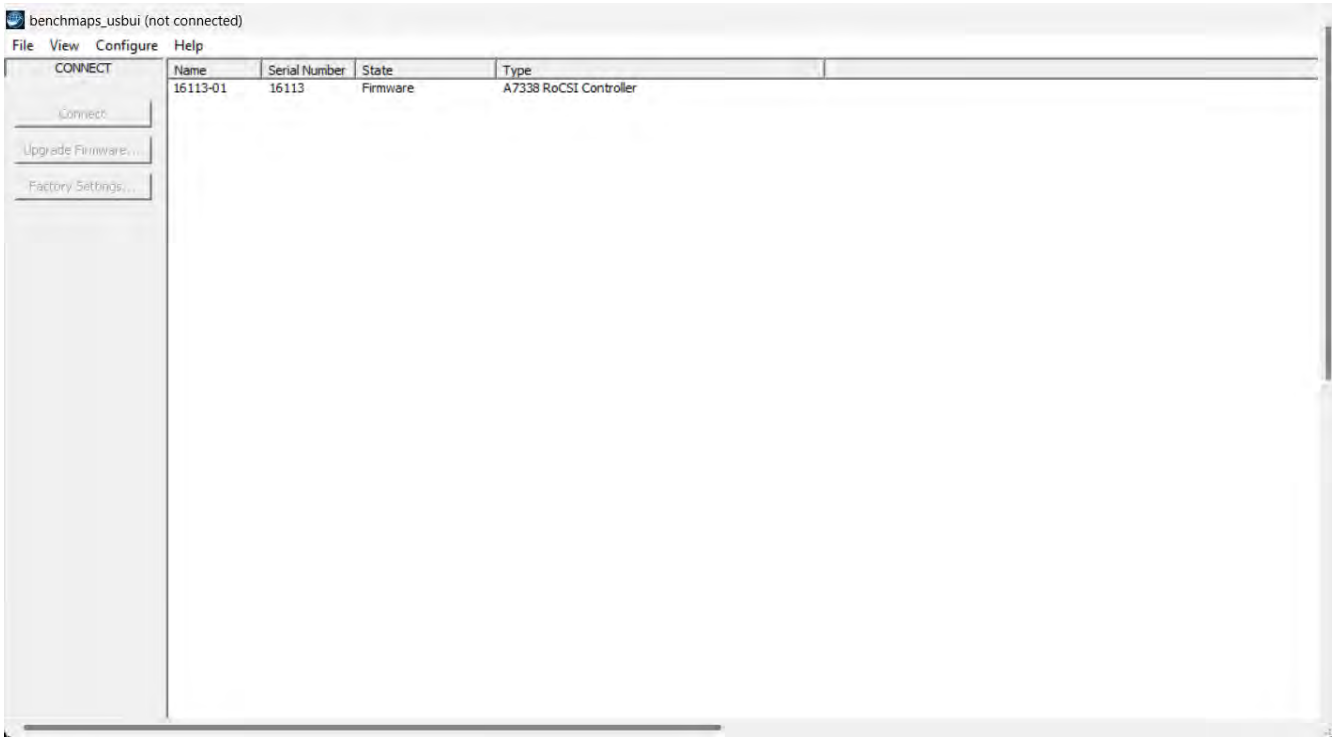
RoCSI is able to communicate using either a USB connection or an RS232 serial connection. The graphical user interface program (GUI) used to communicate with the RoCSI is called **Benchmaps**. **Benchmaps_usbui.exe** is used to communicate via USB and **benchmaps_rs232ui.exe** is used when connected via a serial port. Note that, at this time, benchmaps will only run using a Windows operating system on the host computer.

RoCSI is supplied with a communication cable that contains both USB and serial connectors. Be sure to always plug the communication cable into the RoCSI prior to turning on power and/or connecting the cable to the host computer.

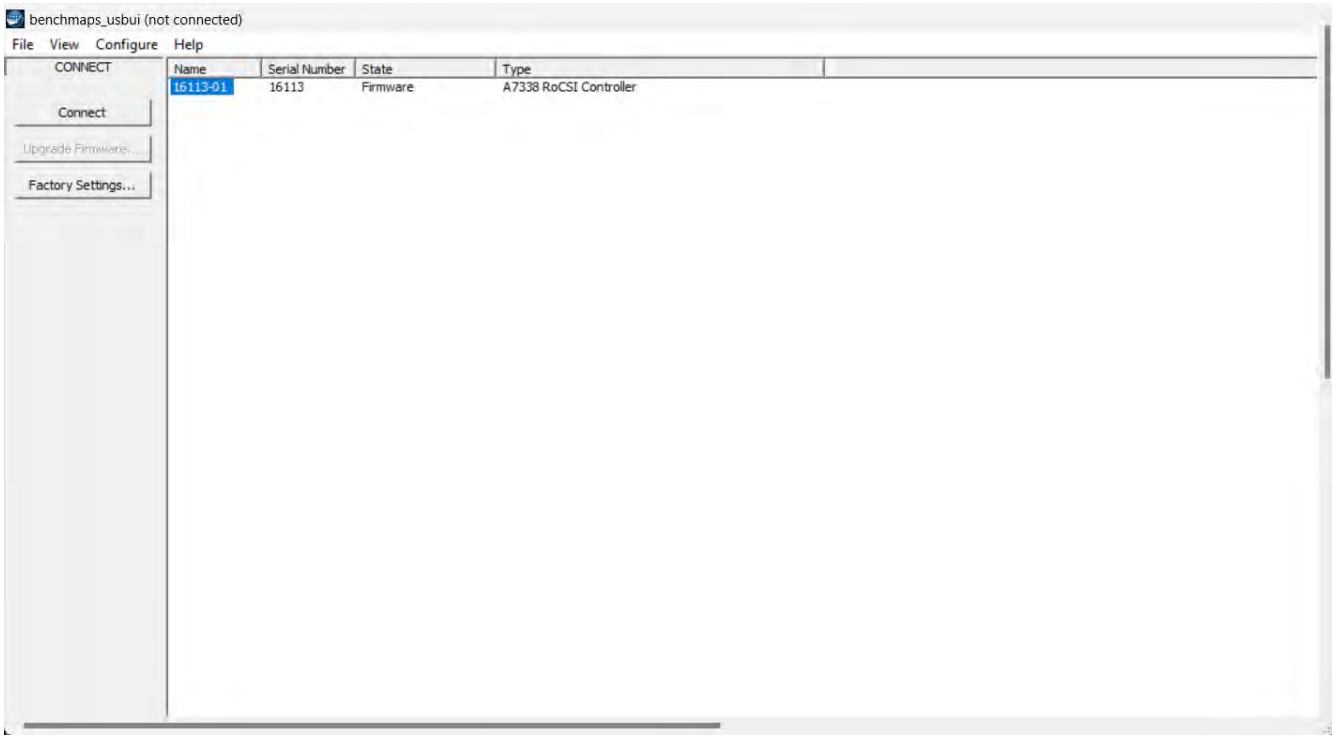
1. First, plug the communication cable into the Setup / Data port on the RoCSI.



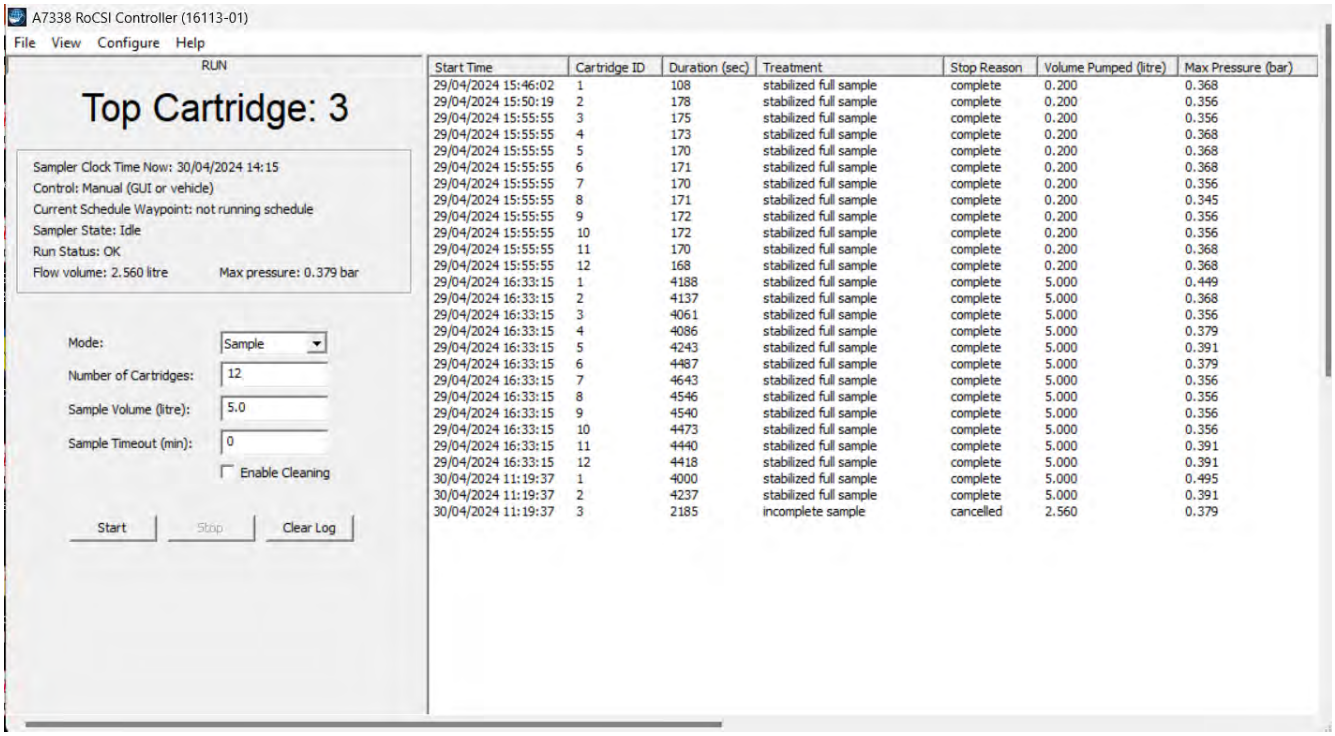
2. Next, plug in the USB connection to the host PC running windows. The computer should automatically find drivers in order to connect with RoCSI; this may take a few seconds to complete.
3. Launch **benchmaps_usbui.exe** on the host computer. There should be a list of at least one RoCSI device available to connect.



4. Click on the target device to select it, then click the **Connect** button.



5. Benchmaps will connect to the RoCSI and display the **RUN** view, which contains the sample log and any current information about the device.



6. Benchmaps will display the current cartridge as "unpowered" until the 12 Vdc power supply is connected.
7. Plug in the bench power supply and then connect the two banana plugs to the RoCSI communication cable to power the RoCSI.

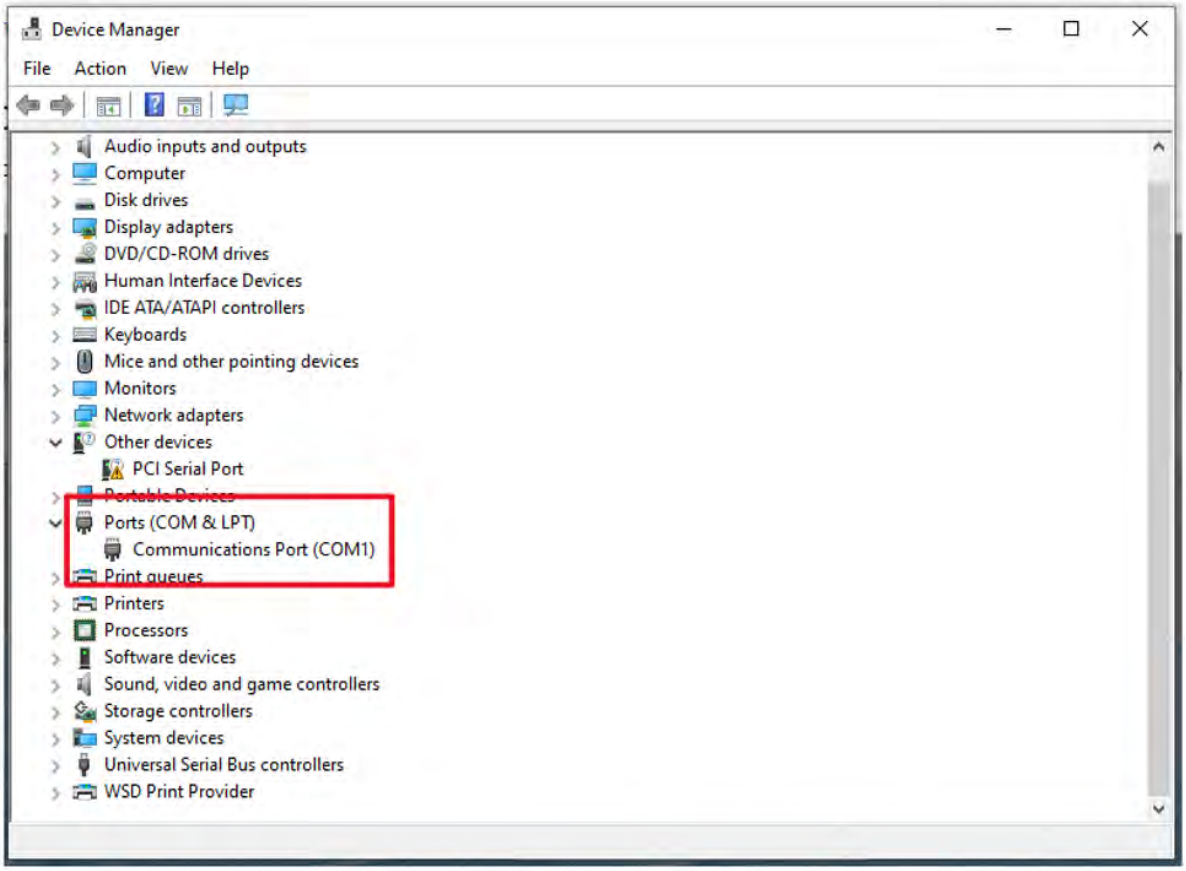
8. The RoCSI is now ready for operation on the bench top..

Communication Troubleshooting

When trying to connect to the RoCSI via RS232, there may be one or more reasons that the host computer fails to connect. Typically, RS232 is connected to the host computer via an USB-to-RS232 adapter. Ensure that the adapter is recognized in the Device Manager within the Windows OS.

If no communication ports are responding, or none are listed, check the available COM ports in the Windows Device Manager. Device Manager can be accessed by clicking the Windows start menu and typing “device manager”. Ensure that any drivers supporting the USB adapter are installed and functioning correctly.

Device Manager lists the available COM ports under "Ports." Look for "Communications Port" or "USB Serial Port" in the Device Manager tree.



When plugging and unplugging the USB adapter to/from the computer, a port should appear and/or disappear from the Device Manager list of ports. This indicates the COM port that should be selected in benchmaps and that the device should communicate on.

In rare cases, some computers may have difficulty reliably recognizing the USB COM port and a different computer may be necessary to successfully communicate with the device.

Note that when communicating to the RoCSI via RS232, the main power supply must be turned on and supplying ~12 Vdc to the RoCSI in order to communicate. Alternately, if communicating via USB, the sampler should respond using just USB power, but will be unable to run any peripherals in this state.

Updating RoCSI Firmware

RoCSI firmware can be updated using the USB deck lead and **benchmaps_usbui.exe** application only (not the RS232).

A firmware file will be called **benchmaps.bin**.

1. To apply a firmware update, first **turn off the 12 Vdc supply and disconnecting the USB**, then re-connect the USB only, do not connect the 12 Vdc supply.
2. In the **Connect View** of the user interface the sampler is listed with **State** set to **Bootloader**.

3. Select (but do not double-click) the sampler and click on the **Firmware Update** button.
4. Browse for the new **benchmaps.bin** file on the PC and click OK. The update will take several minutes. If it is interrupted for some reason just start again.

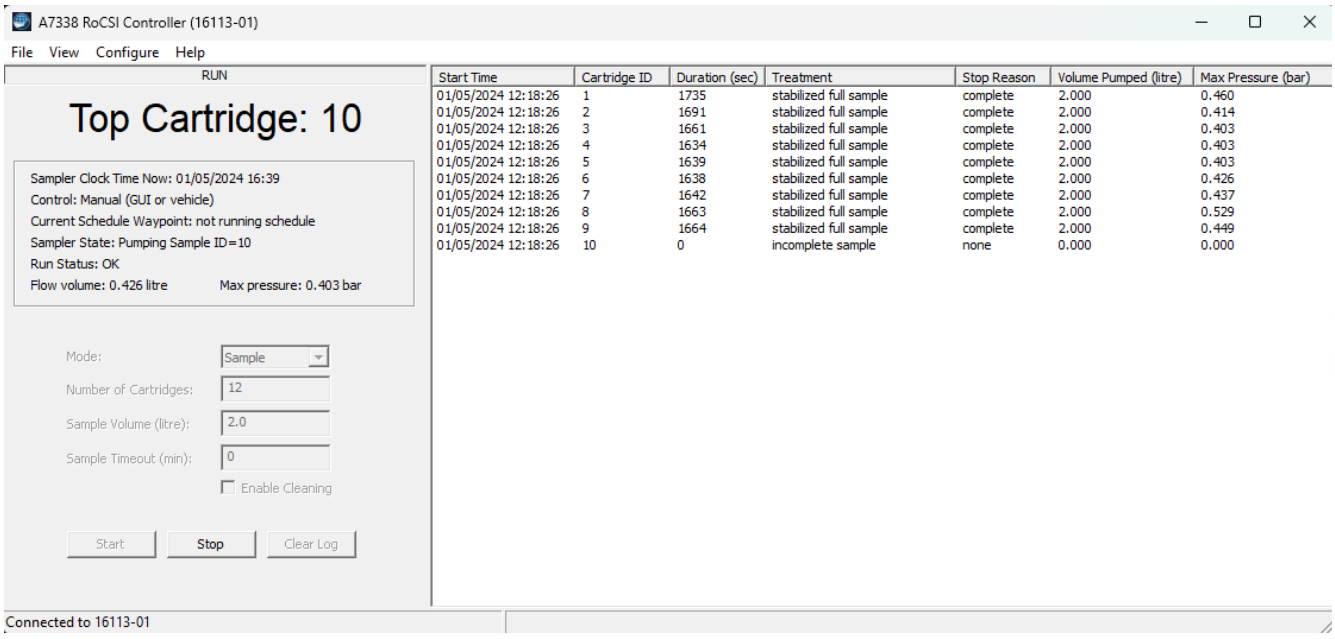
Contact McLane (mclane@mclanelabs.com) for firmware update questions.

RoCSI Operations

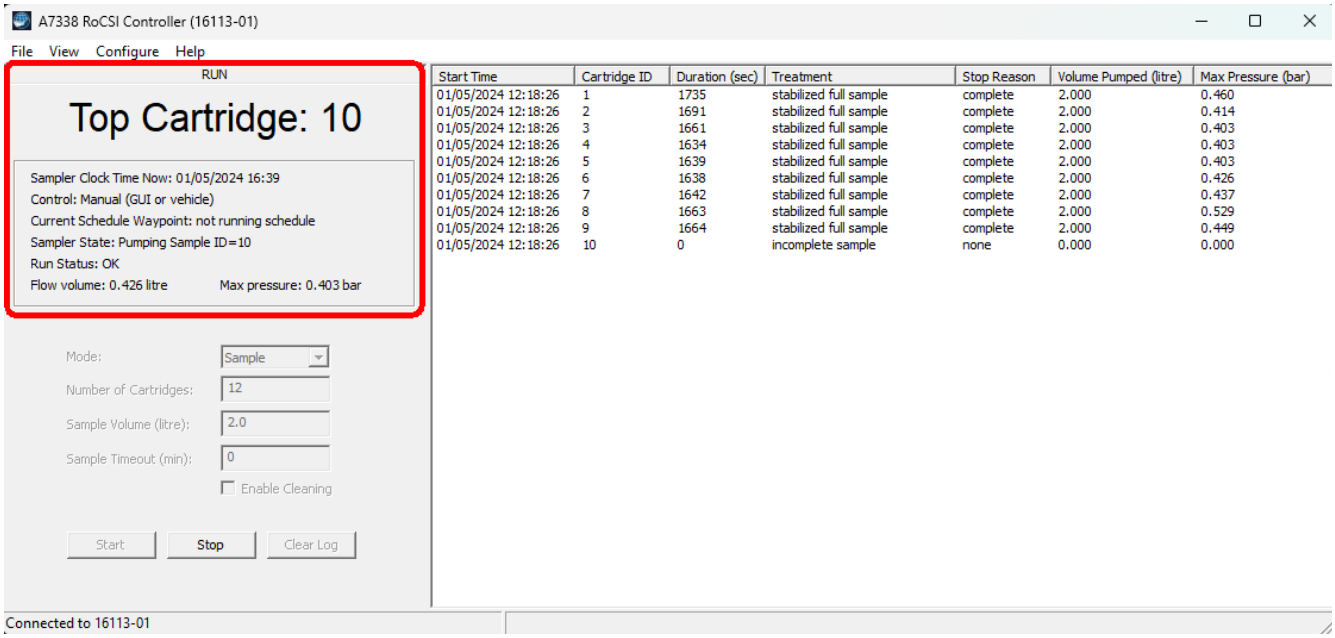
This section provides guidance for operating the RoCSI.

RUN View

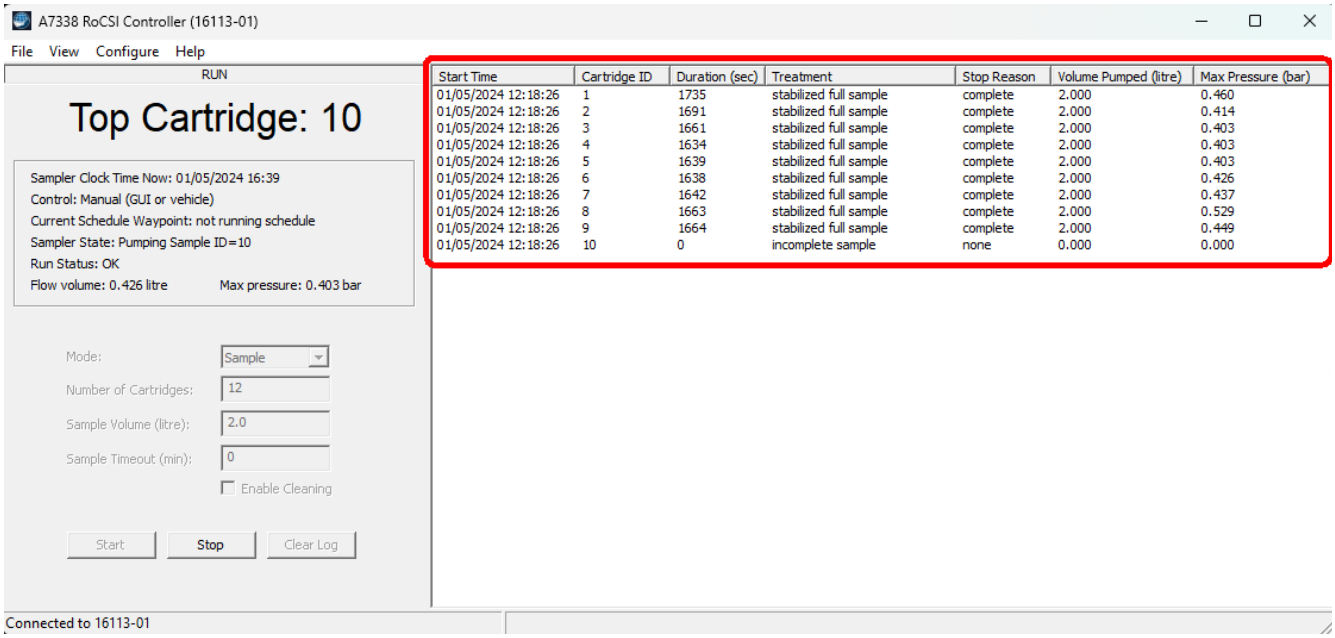
RUN View is the main interface view for RoCSI operation.



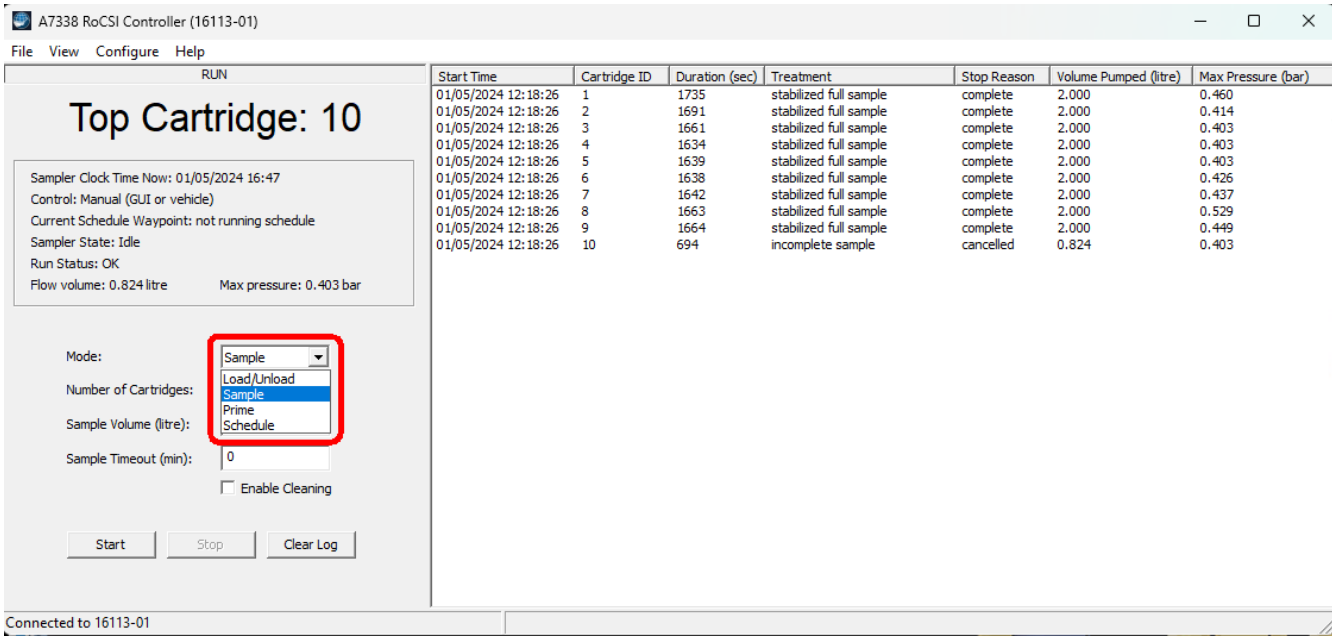
Real-time status of the RoCSI is displayed in the top left corner.



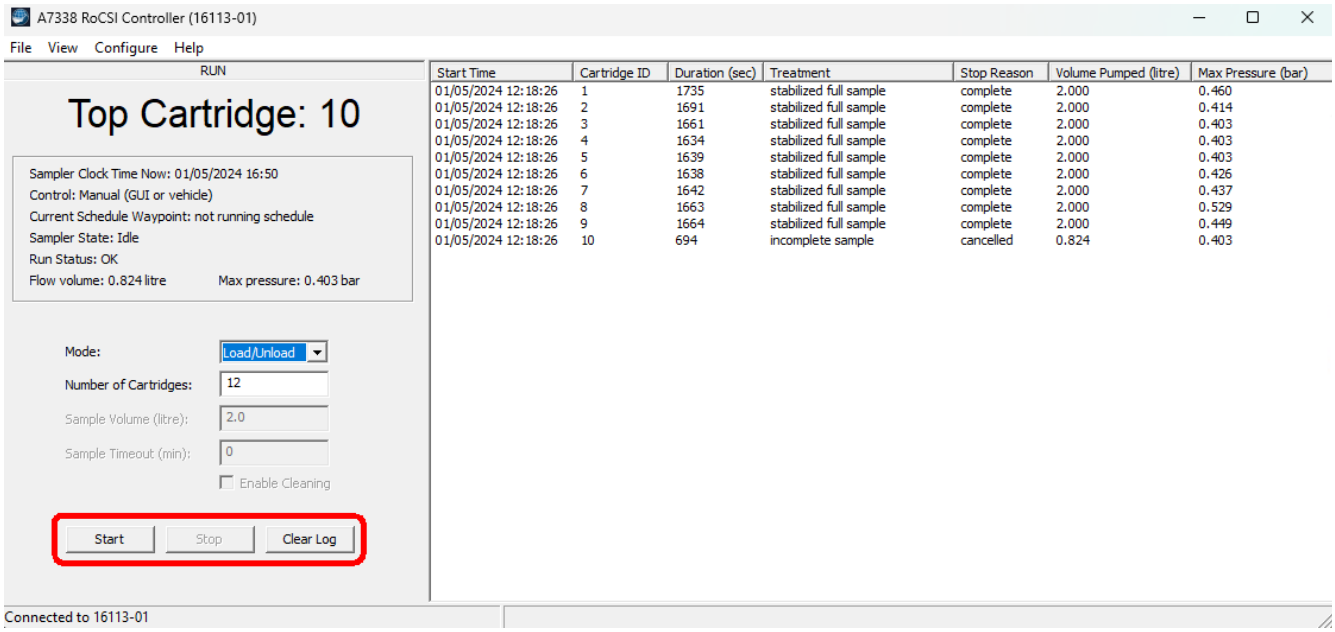
The right hand panel displays the sample log.



The sampler mode is set via the **Mode** drop-down box.

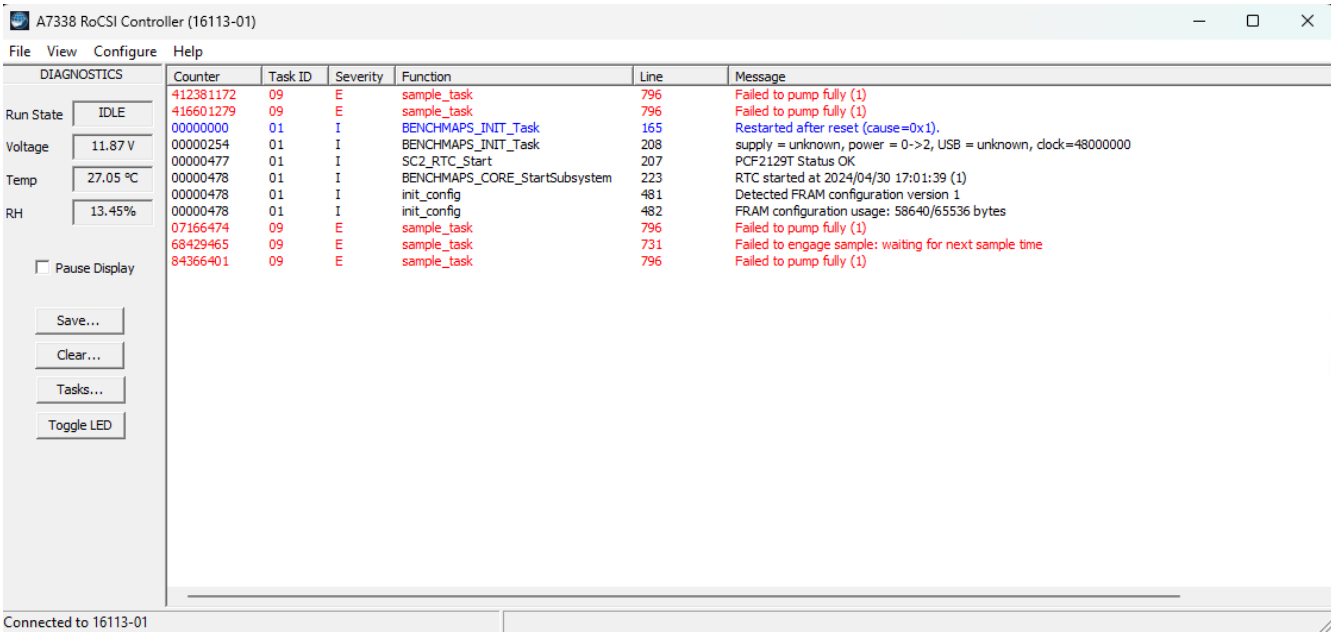


Loading and unloading, sampling, priming and scheduled sampling (modes) are initiated using the **Start** button. There is also a **Stop** button to terminate the current procedure and a **Clear Log** button to clear the sampler log and **Top Cartridge** ID.

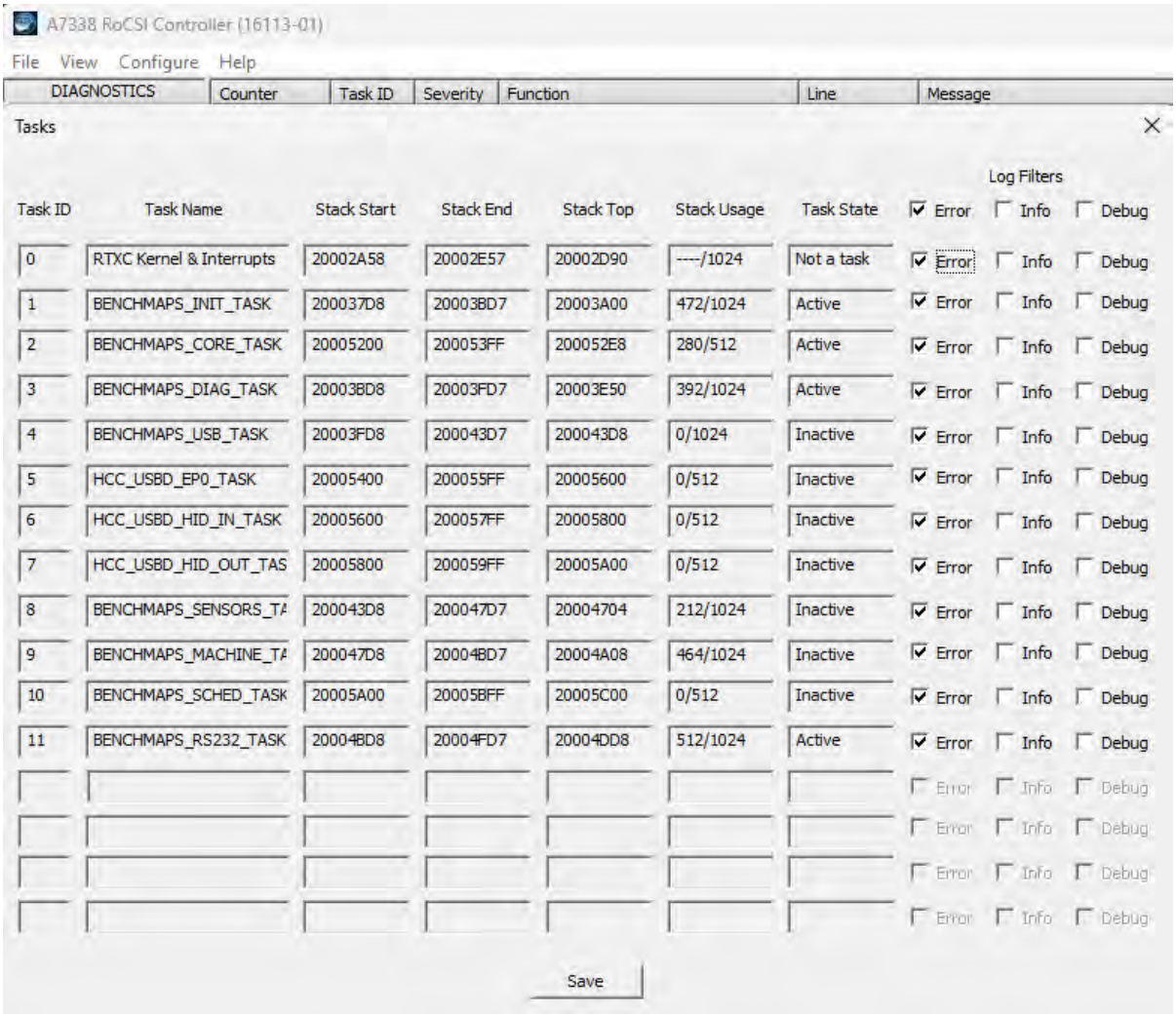


DIAGNOSTICS View

DIAGNOSTICS View is primarily used for troubleshooting; the **DIAGNOSTICS View** offers a configurable task log. Internal temperature, relative humidity and system voltage are displayed in real-time under the **DIAGNOSTICS View**.



Clicking the **Tasks** button allows the user to configure the DIAGNOSTICS logging parameters. Messages may be turned on or off depending on the Log Filters (Error, Info and Debug). Typically, during regular operation only Errors are logged.



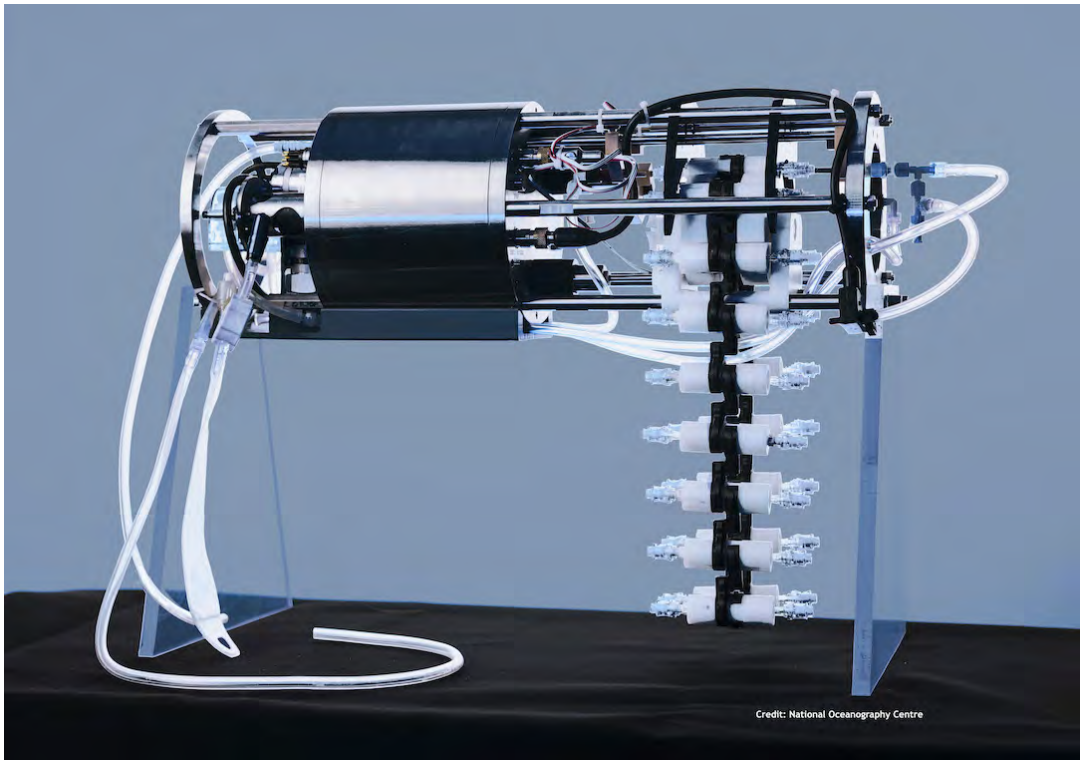
Mounting the Sampler

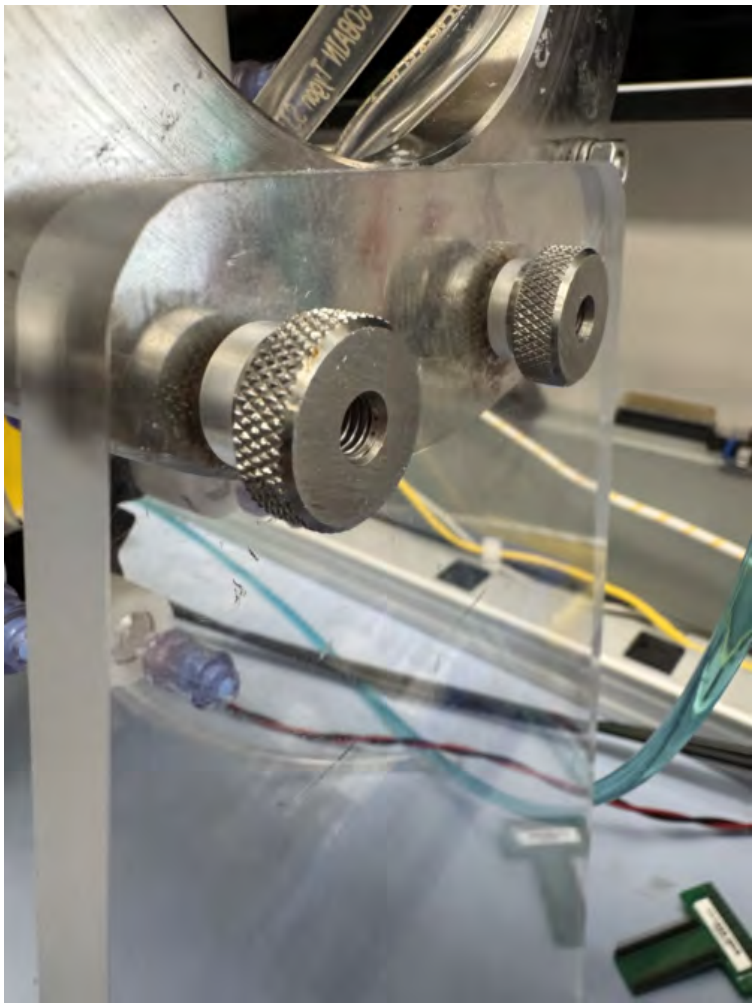
When operating the RoCSI on the bench-top, allow the cartridge chain to hang freely over the end of the bench, with gravity aiding the loading of the instrument. The main housing will need to be secured to the bench or other mounting apparatus prior to installing the sample chain.

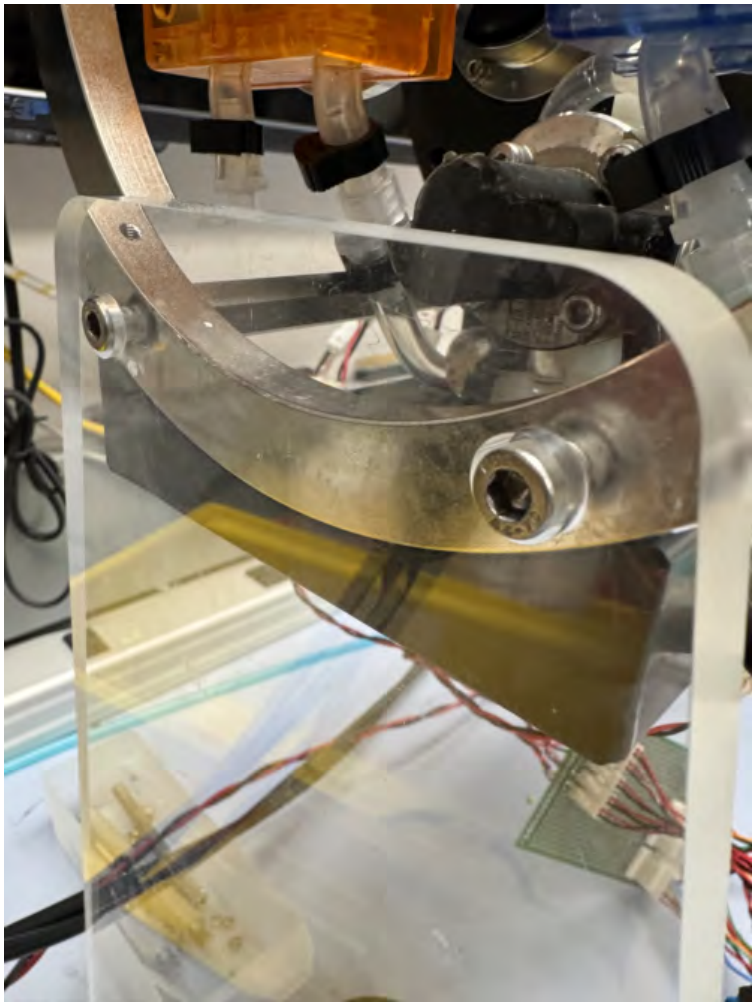
A bench stand is also supplied for bench top operation. See thenext section for instruction on mounting the stand.

Installing the Bench Stand

An acrylic bench stand is included with the RoCSI. Extra hardware is provided in the toolkit to mount the RoCSI to the stand. Two thumb screws are used to attach the stand closest to the cartridge mechanics and two long M5 screws are used to mount the stand on the pump end. Installation is straightforward, but is easiest achieved by laying the RoCSI on its side and attaching each stand before standing the RoCSI up.


Credit: National Oceanography Centre



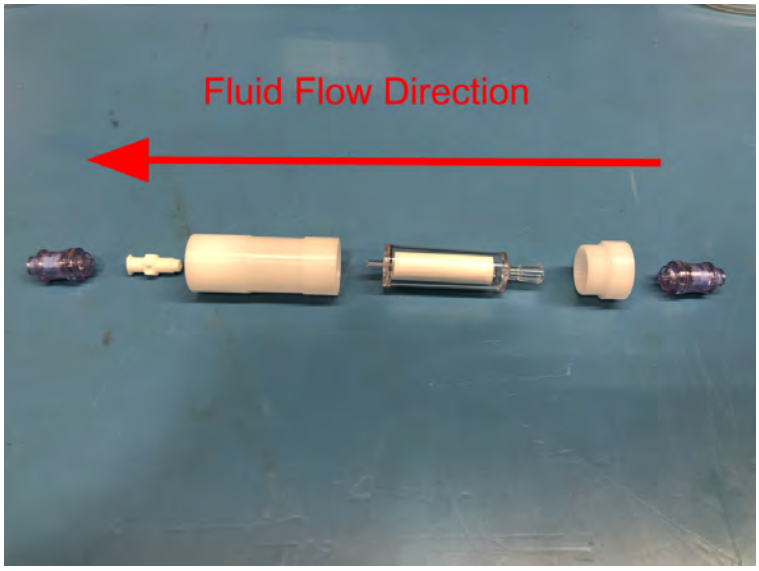


Assembling New Filter Cartridges

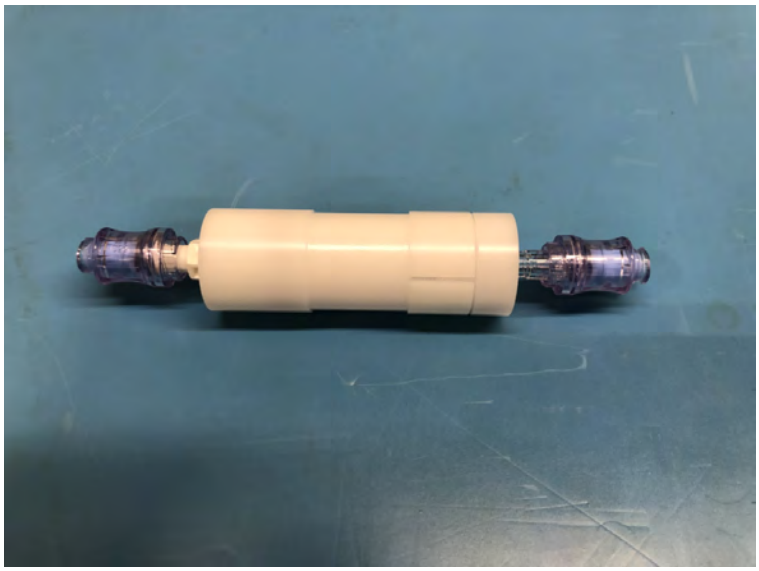
Follow the steps in this section to assemble the filter cartridges and sample chain. If cartridges are to be reused, follow the instructions for cleaning prior to filter assembly.

Be sure to follow best practices with regard to wearing gloves, cleanliness of the work area, and general clean handling protocols in order to reduce contamination of the filter components during assembly.

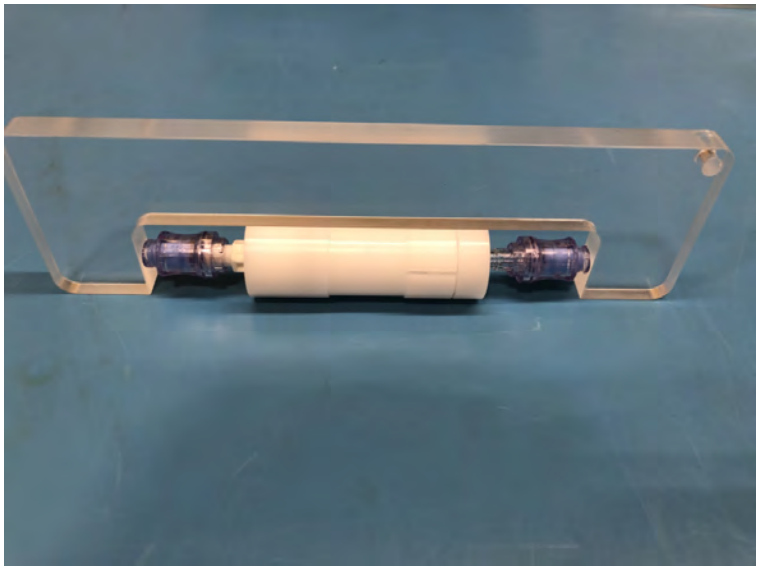
Please note that the direction of fluid flow will be from right to left as shown below. When mounting cartridges onto the chain and loading into the RoCSI, pay careful attention to direction of flow.

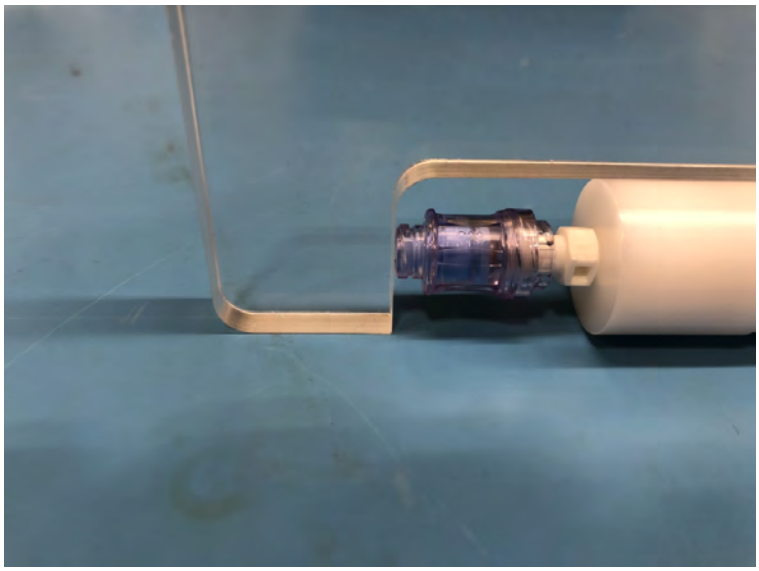


1. Noting the orientation of the parts shown above, start by screwing the Luer lock male to female connector into the sample holder using an 8 mm wrench.
2. Using (gloved) fingers, screw the needle free valve onto the Luer Lock female connection at the outlet of the sample cartridge holder.
3. Push a Sterivex™ filter cartridge firmly into the sample holder (it will self-center), then screw on the sample holder cap.
4. Screw the remaining needle free valve onto the cartridge inlet.

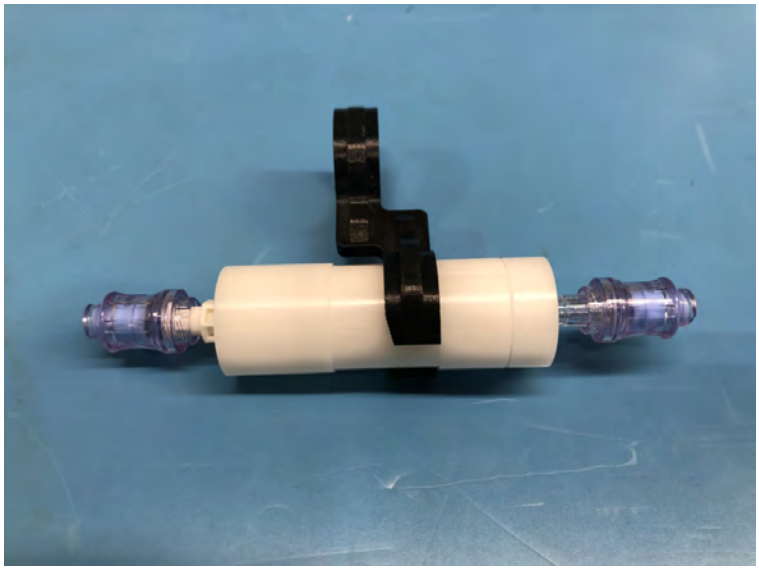


5. Use the gauge provided in the toolkit to ensure the cartridge is the correct length. The cartridge should gently slide into the gap and remain in position without falling out.

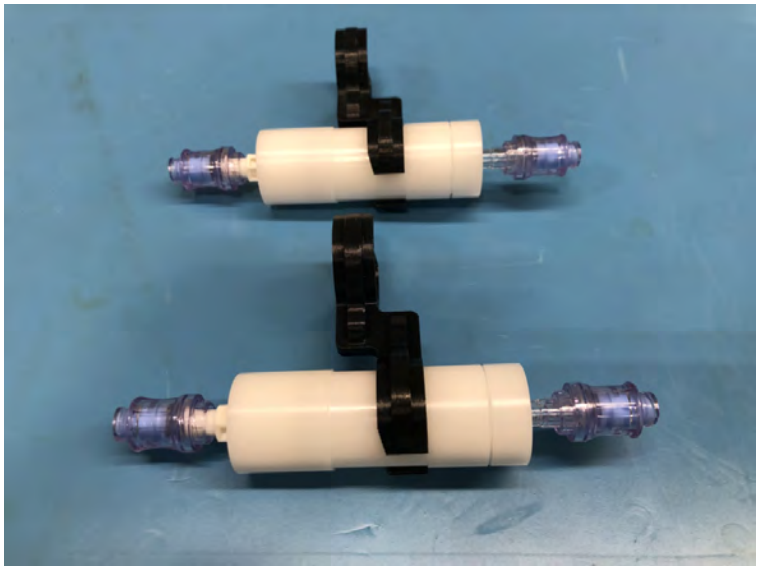


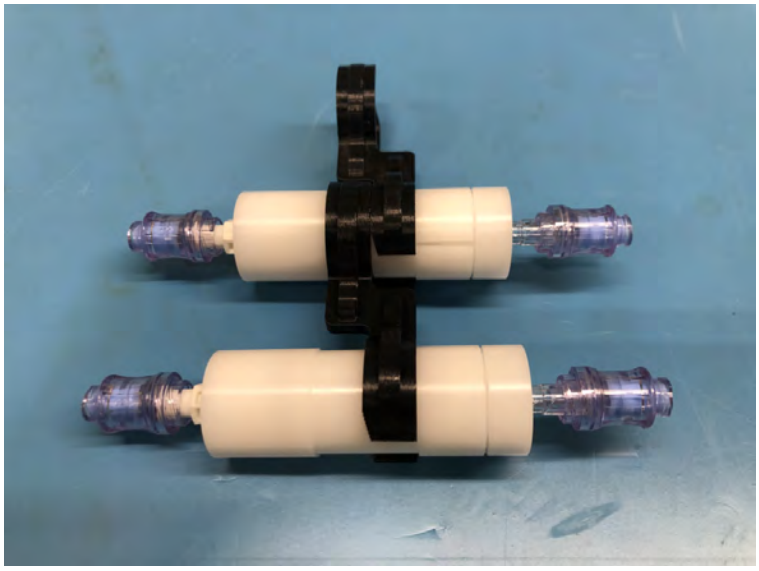


6. To begin creating a sample chain, install a chain link onto the cartridge as shown. Please note that in the orientation shown, water flow will be right to left.



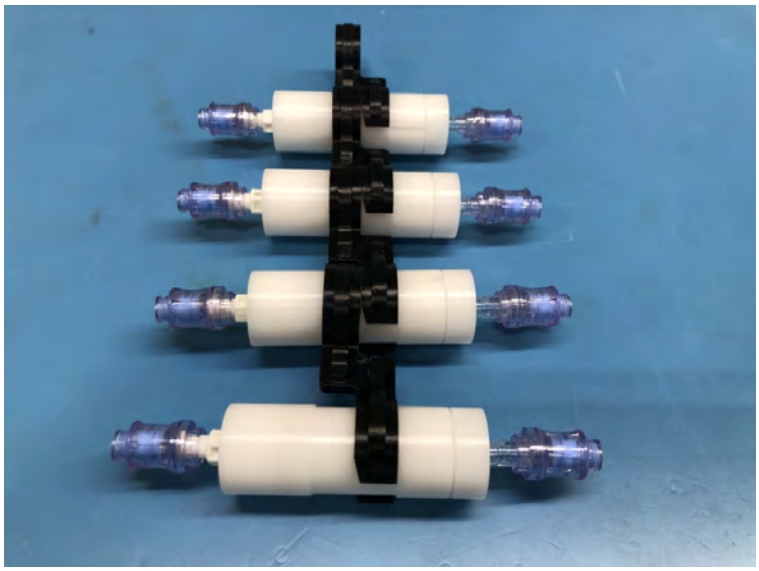
7. To begin creating a sample chain, install a chain link onto the cartridge as shown. The link is installed by firmly pushing it onto the reduced diameter section of the cartridge holder.
8. Prepare another sample cartridge as shown above and install another clip. Then clip the two assemblies together as shown.





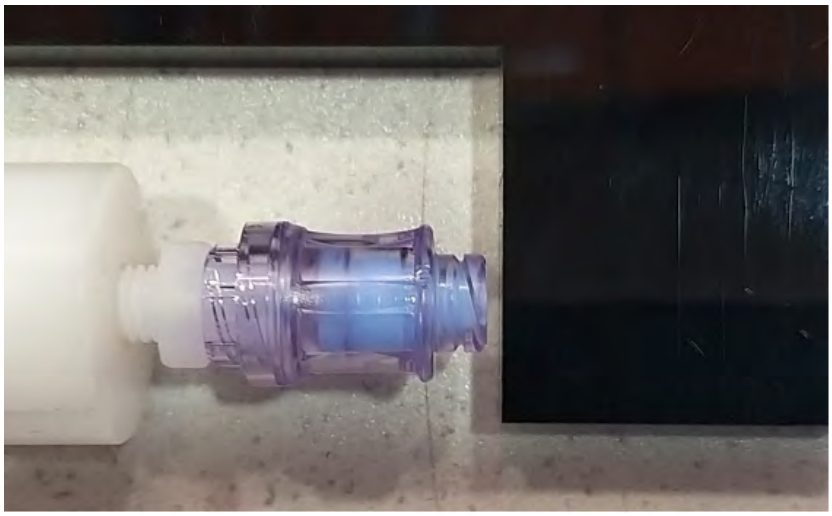
9. Continue adding cartridges until the desired sample chain length is achieved.

Note that RoCSI requires a minimum of 10 cartridges to load correctly.



Incorrect Filter Cartridge Assembly

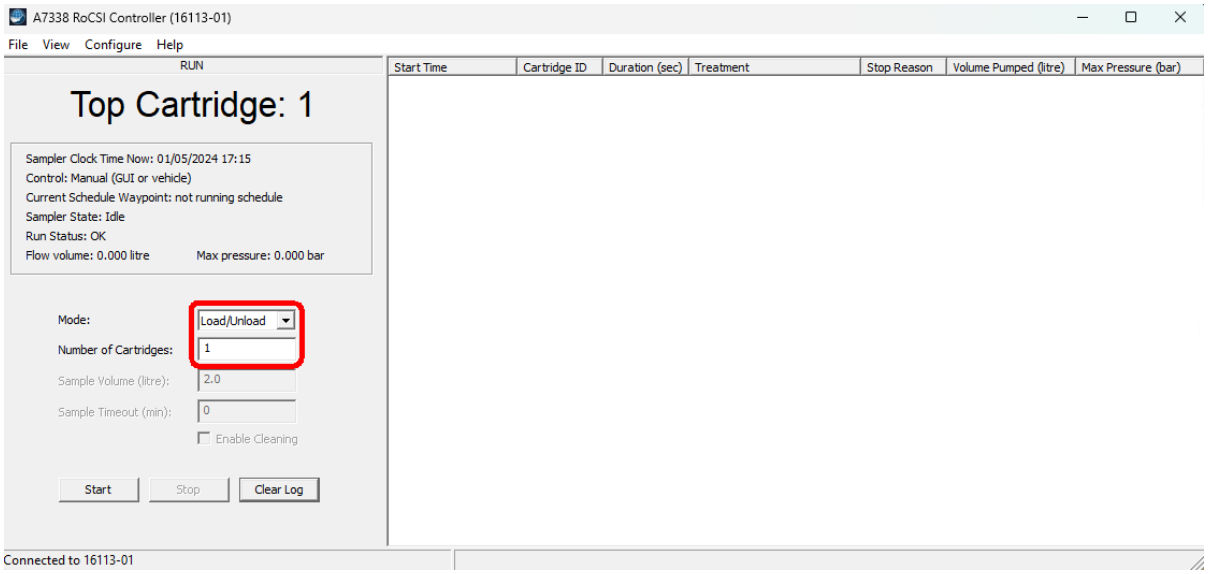
The image shown next illustrates a cartridge that is incorrectly assembled (there is a small gap between the needle free valve and the end of the gauge). If gaps are present when measuring the assembled length, the cartridge assembly will need to be rebuilt, or a different sample holder will need to be assembled. Cartridge assemblies that are too long or too short will not be loaded correctly by RoCSI.



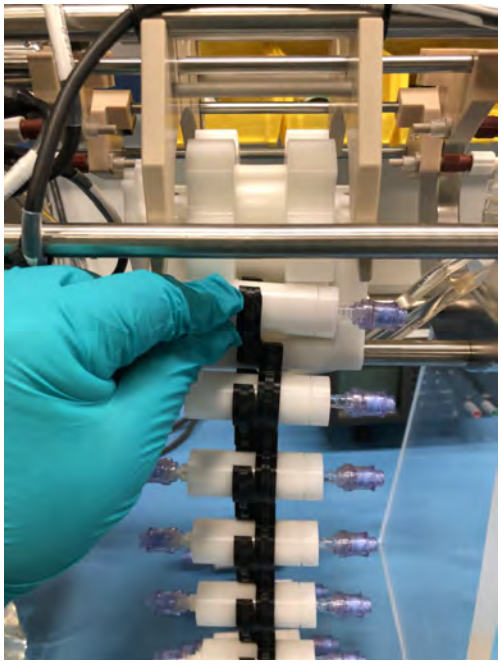
Loading the Cartridge Chain

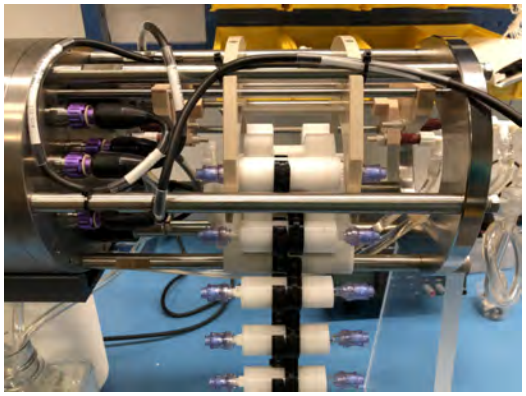
The Sterivex™ filter units are loaded into the sampler on a **cartridge chain** as shown next.

1. Ensure the 12 Vdc supply is turned on and the software is running. The software application should show the **RUN** view once you have connected to the sampler.
2. The **Sampler State** should be **Idle**. If it says **Unpowered** you have not connected the 12 Vdc supply correctly.
3. Change the **Mode** option to **Load/Unload**, and set the number of cartridges to 1.

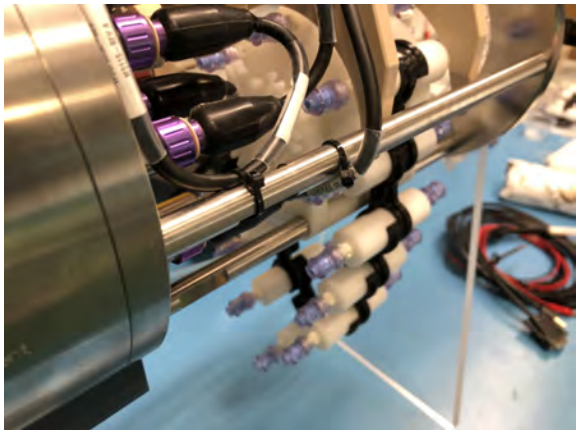


4. Gently hold the *first* cartridge (with no leading clip) against the loader wheel in the lowest accessible cartridge slot and click the **Start** button in the software. The cartridge should be pulled into position automatically.



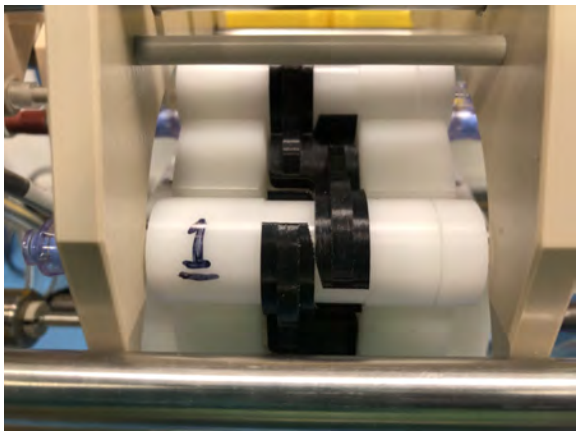


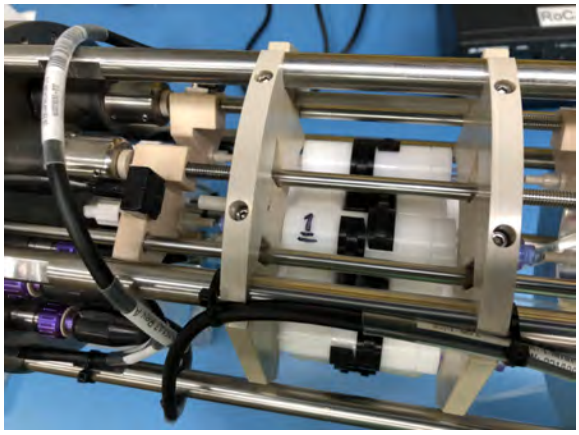
5. Continue to advance the cartridge chain (using **Load/Unload** mode) until there are an even amount of cartridges hanging below the RoCSI. This allows the chain to be completed by linking the first and last cartridges together.

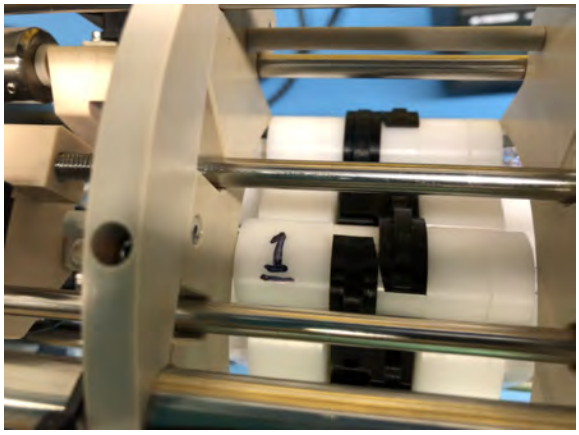


6. If not done so already, mark the first cartridge to be sampled with the number 1. Then

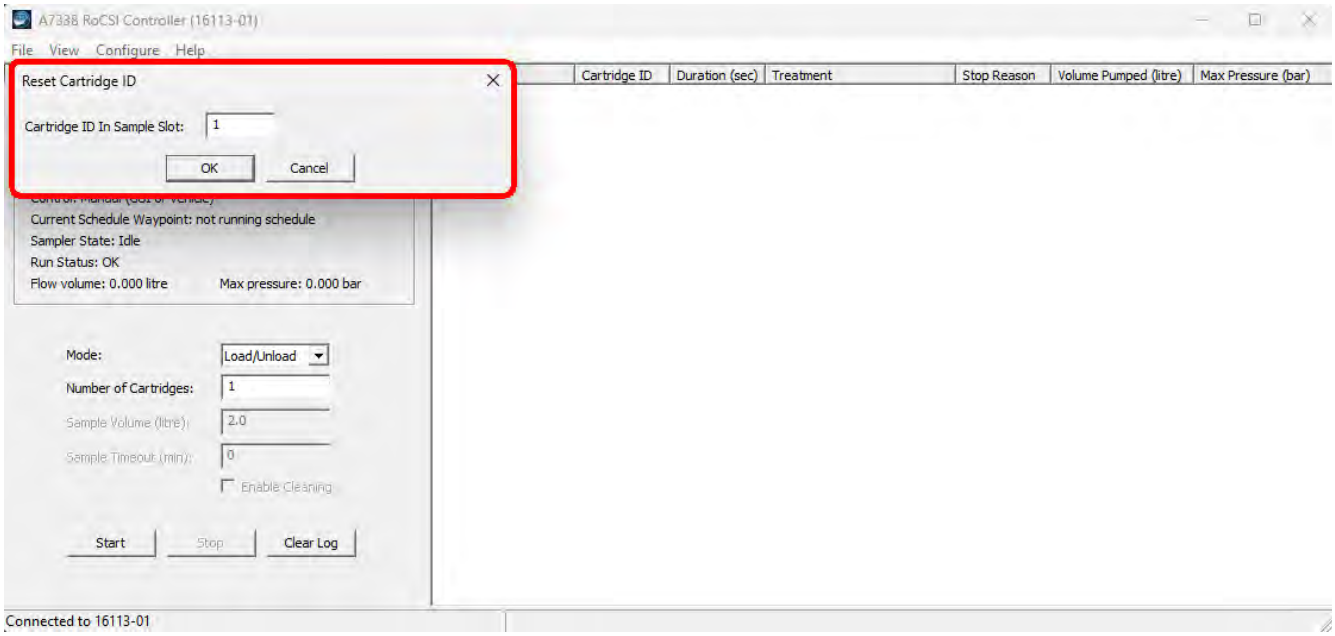
advance the chain until this cartridge is in the top position (in-line with the sample injectors).



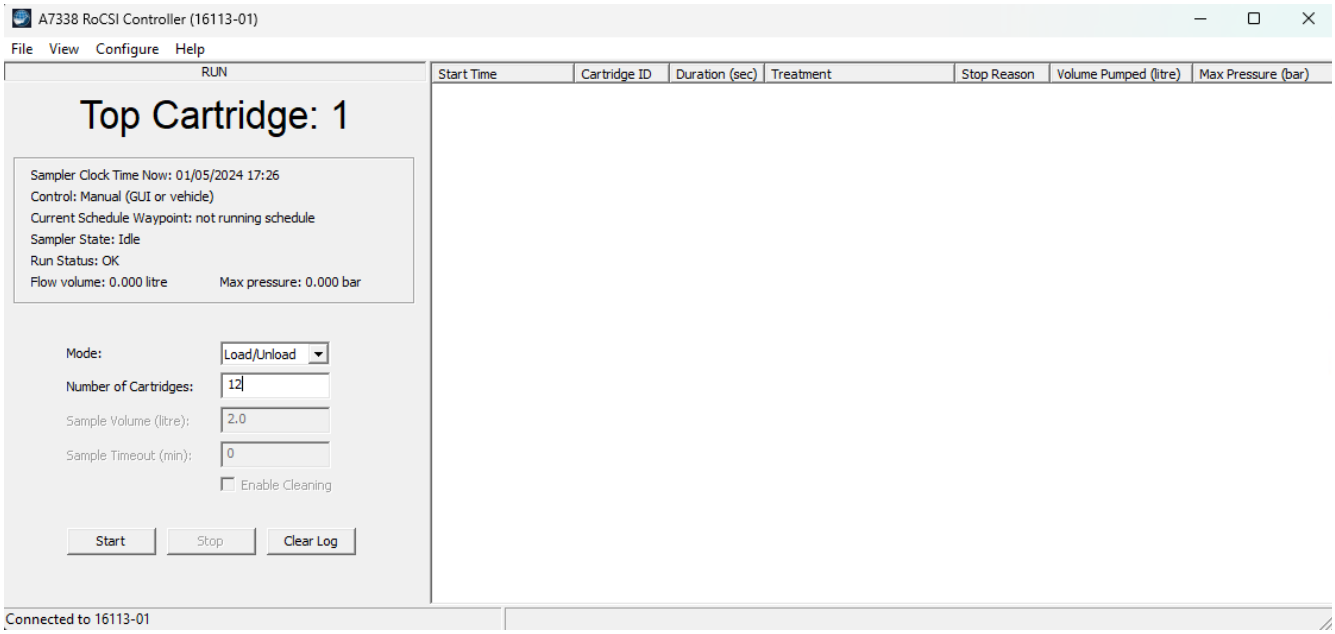




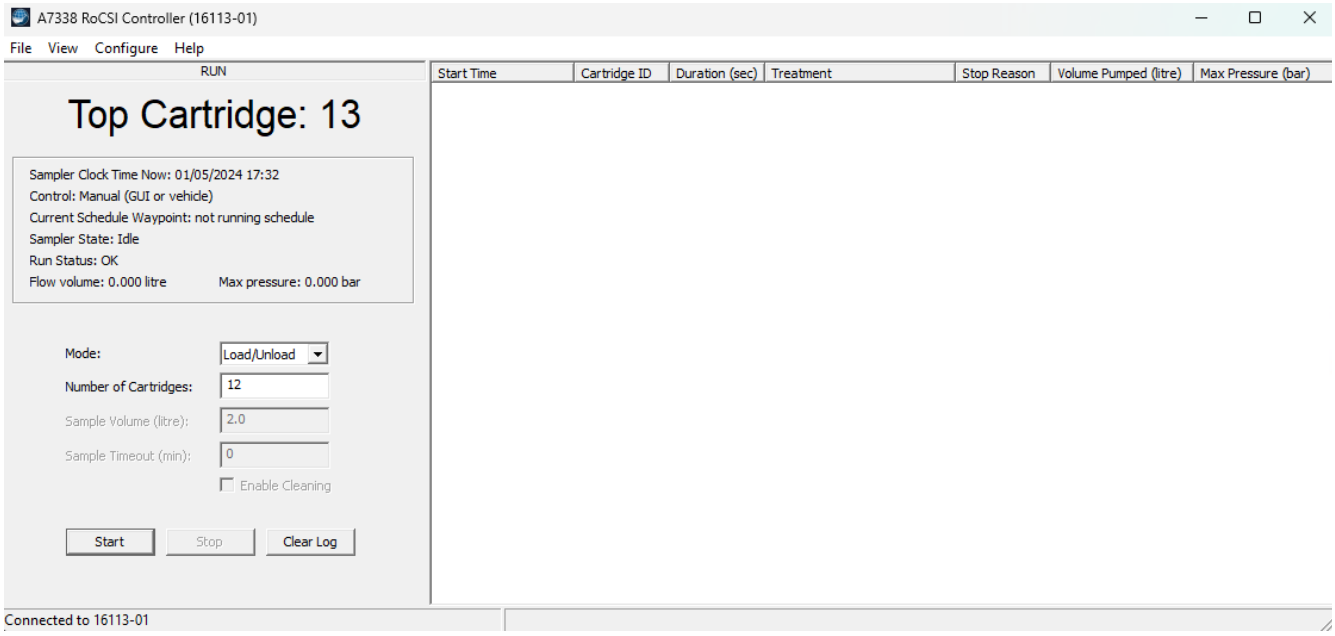
7. To change the Top Cartridge ID to match what is written on the cartridge that was just loaded, double-click **Top Cartridge**
8. Enter your cartridge number in the dialog box and click **OK**.



9. After the cartridge number is entered, RoCSI will keep track of cartridge numbers as long as they are consecutively numbered.
10. Before a deployment, cycle the entire cartridge chain through the sampler (without sampling) to check the cartridges are all aligned properly and do not cause any loading problems. You can do this by using the **Load/Unload** mode.
11. In this example, the sample chain is 12 cartridges long, so 12 will be entered in the Number of Cartridges box. Set the number of cartridges and click **Start** to cycle through the entire sample chain.



12. When the cycle is complete, the **Top Cartridge** is indicated as number 13. However, for this 12-sample chain, the top cartridge has returned to number 1. Reset the cartridge number by clicking on **Top Cartridge** and setting it back to 1.



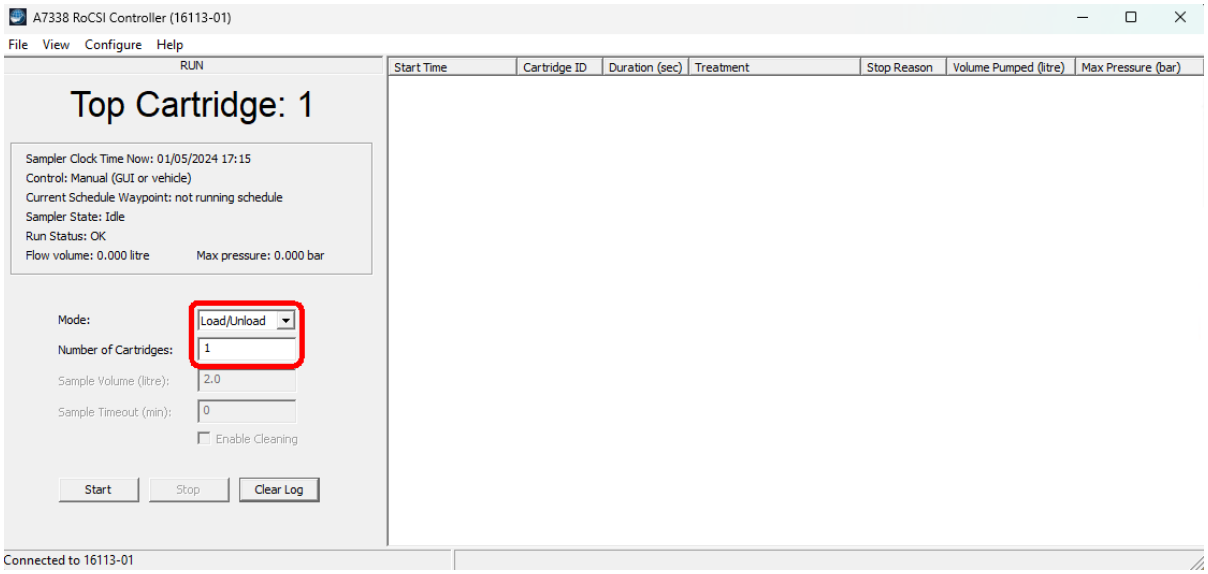
Run **two** complete cycles of the cartridge chain using **Load/Unload** mode to ensure trouble free loading of the sampler.

Unloading the Cartridge Chain

Unloading the Sterivex™ filter units from the **cartridge chain** uses the same functions as loading the chain. Use the **Load/Unload** software mode to ensure trouble free loading of the sampler.

The cartridge chain must be supported by hand when unloading.

1. Ensure the 12 Vdc supply is turned on and the software is running. The software application should show the **RUN** view once you have connected to the sampler.
2. The **Sampler State** should be **Idle**. If it says **Unpowered** you have not connected the 12 Vdc supply correctly.
3. Change the **Mode** option to **Load/Unload**, and set the number of cartridges to 1.



4. Disconnect the link from between the two lowest cartridges in the chain.

6. The magazine loader will be advanced until the sample chain is free of the mechanism. Please note that the weight of the chain must be supported while advancing the loading magazine. If not supported, there is a chance the drive mechanism may become jammed.
7. While supporting the weight of the chain as it is unloaded, advance the loader in single steps / cartridges until the chain is removed from the loader mechanism.

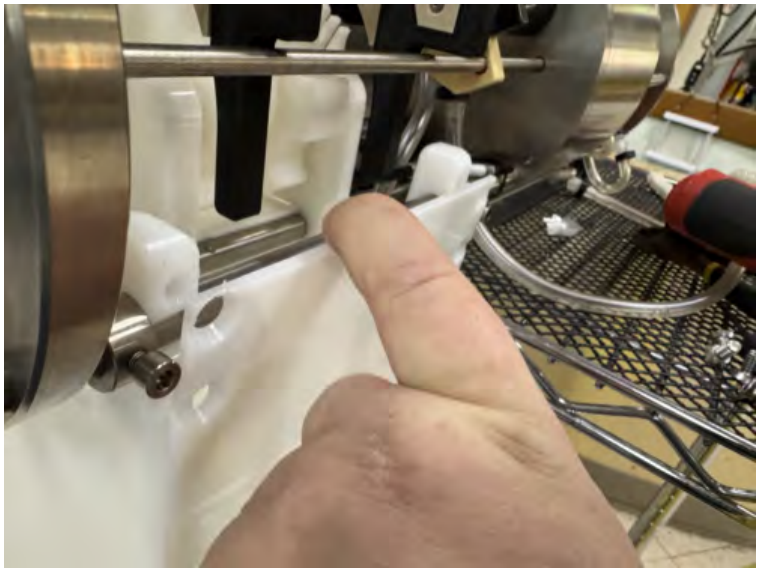
Hopper

If a hopper is provided, it can optionally be fitted to house the cartridge chain. The cartridges do not need to be specially arranged in the hopper.

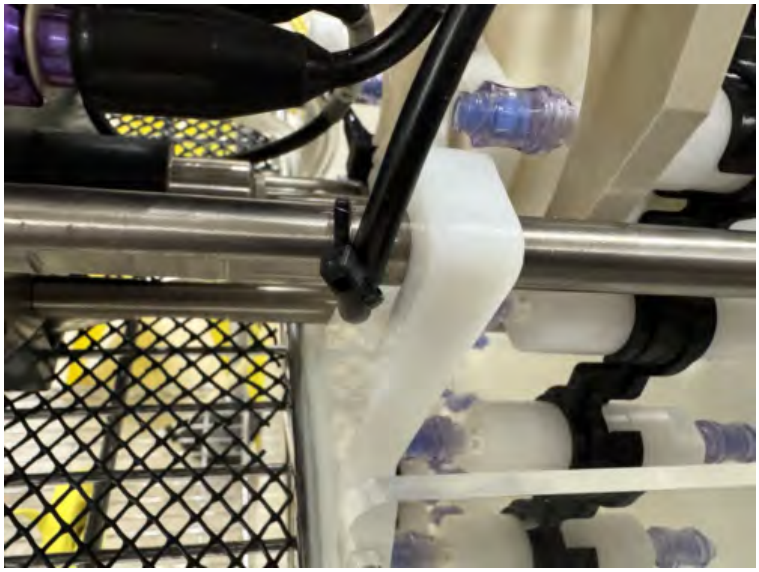
Please note that the maximum number of cartridges that can be used in the large hopper is 40 if operating in air, and 48 if operating submerged.

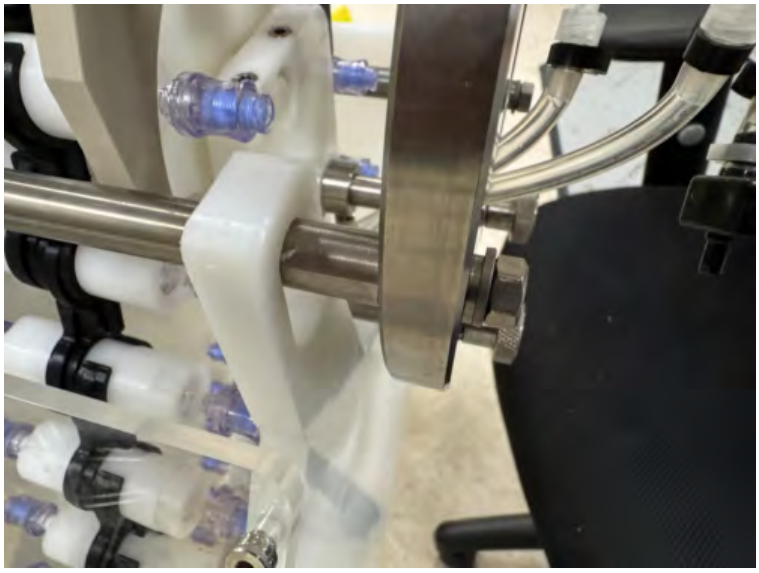
Installing the Hopper

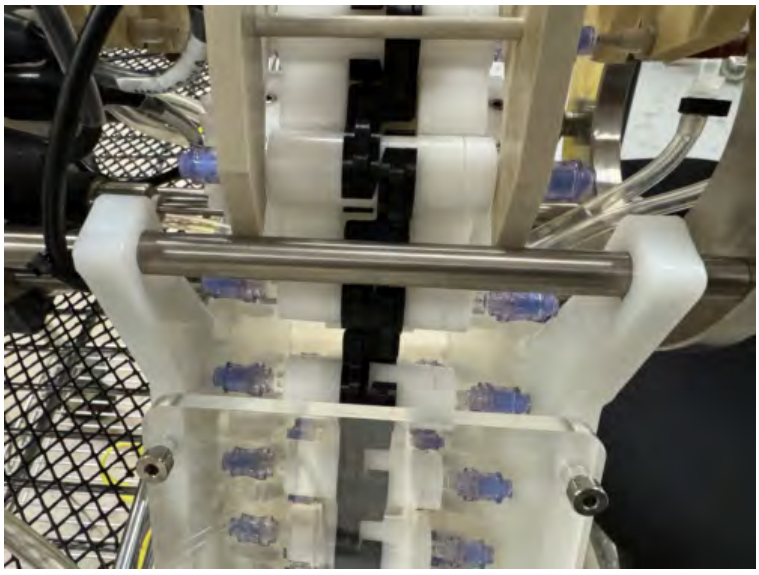
1. First, remove the 4 screws on the top of the back side of the hopper.
2. Gently push back the plastic panel and slide the hopper up onto the cross bar as shown.



3. In this position, it is then possible to rotate the hopper up and hook the front hangers onto the opposite cross bar.







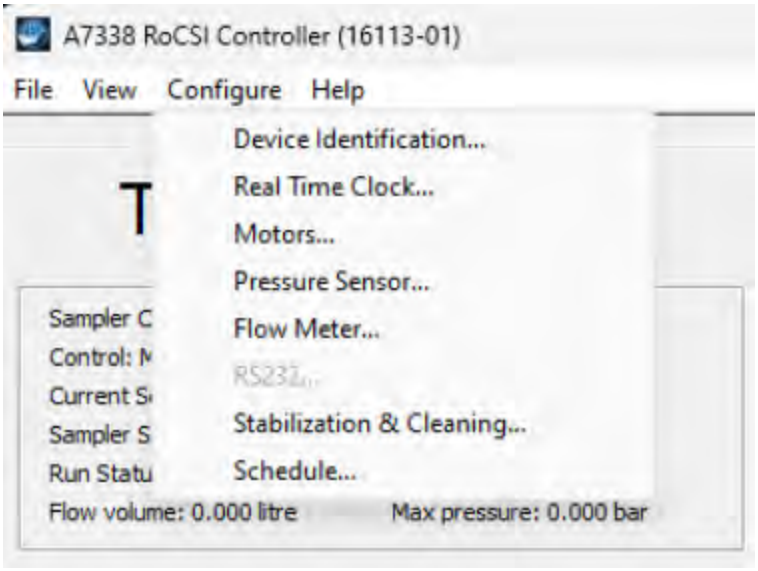
4. When the hopper is properly hung, reinstall the 4 screws on the back side to secure the hopper in place.

Priming the Pumps

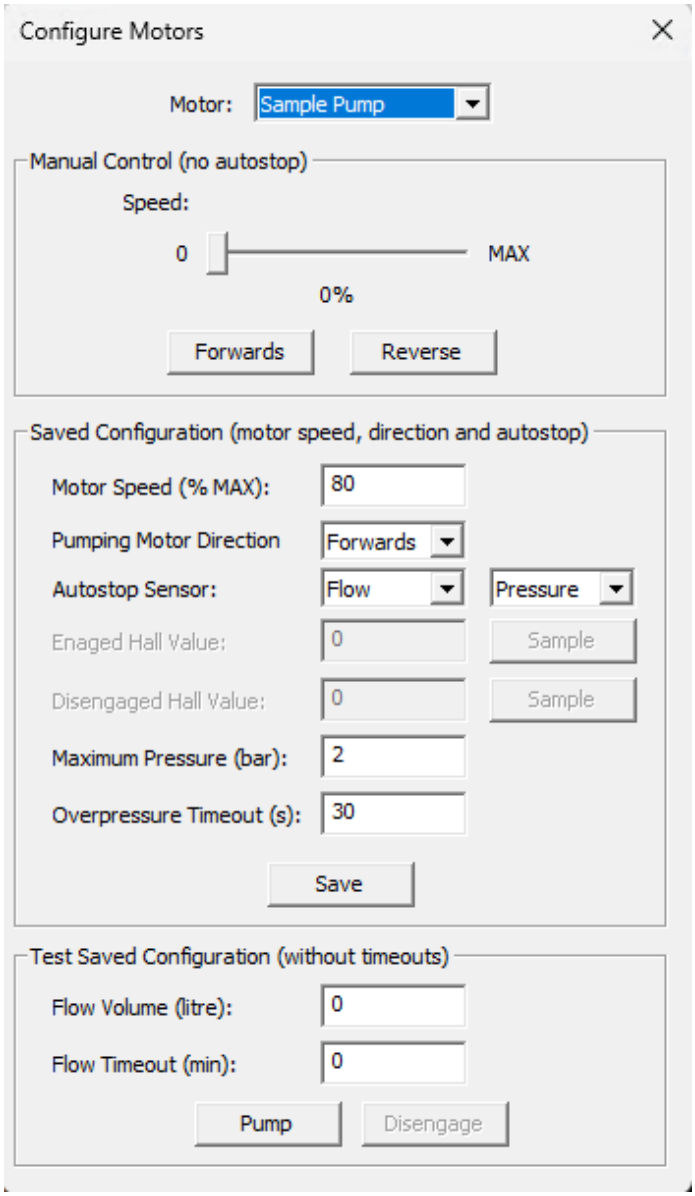
Inherent to the design of peristaltic pumps is an issue whereas the pump tubing becomes sticky and the pinched section of tubing may not initially separate to allow pumping. When the tubing is stuck together, the pumps will produce a "popping" sound while running and will not effectively move water. To remedy this issue, the pumps must be primed with clean water to allow the peristaltic tubing to separate.

Priming the sample pump:

1. Navigate to the Configure/Motors... menu.

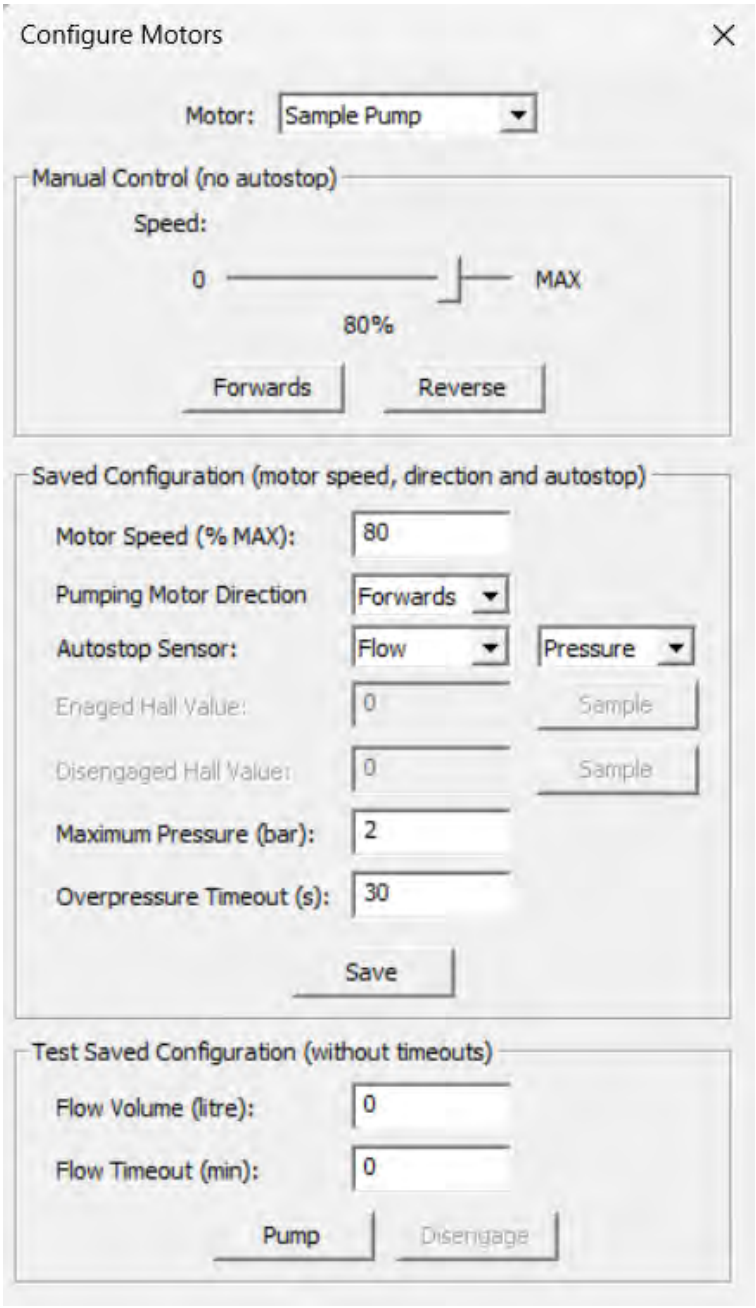


2. Select Sample Pump in the Motor drop-down menu. This allows direct control of the sample pump.



3. Prepare a syringe with clean water that will be used to prime the sample pump
4. Disconnect the exhaust line from the sample pump and connect the syringe directly to the exhaust of the pump.

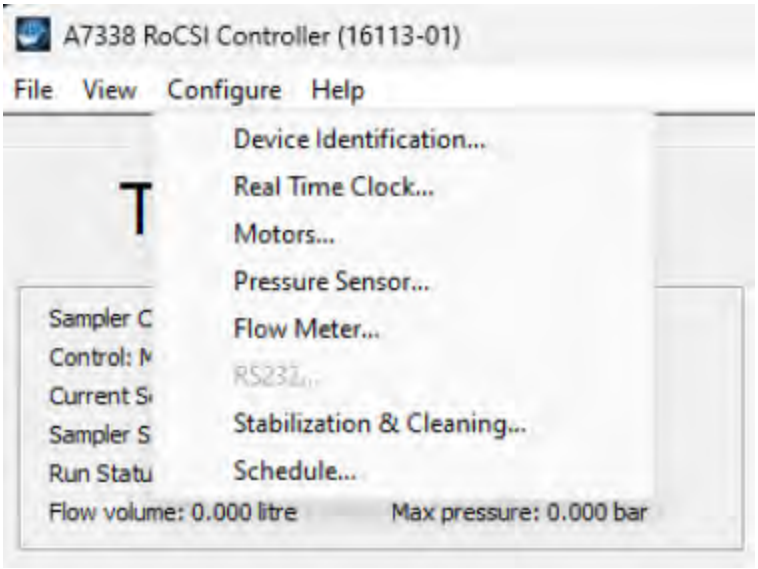
5. In the Manual Control section, set the Speed slider to 80% and click Reverse.



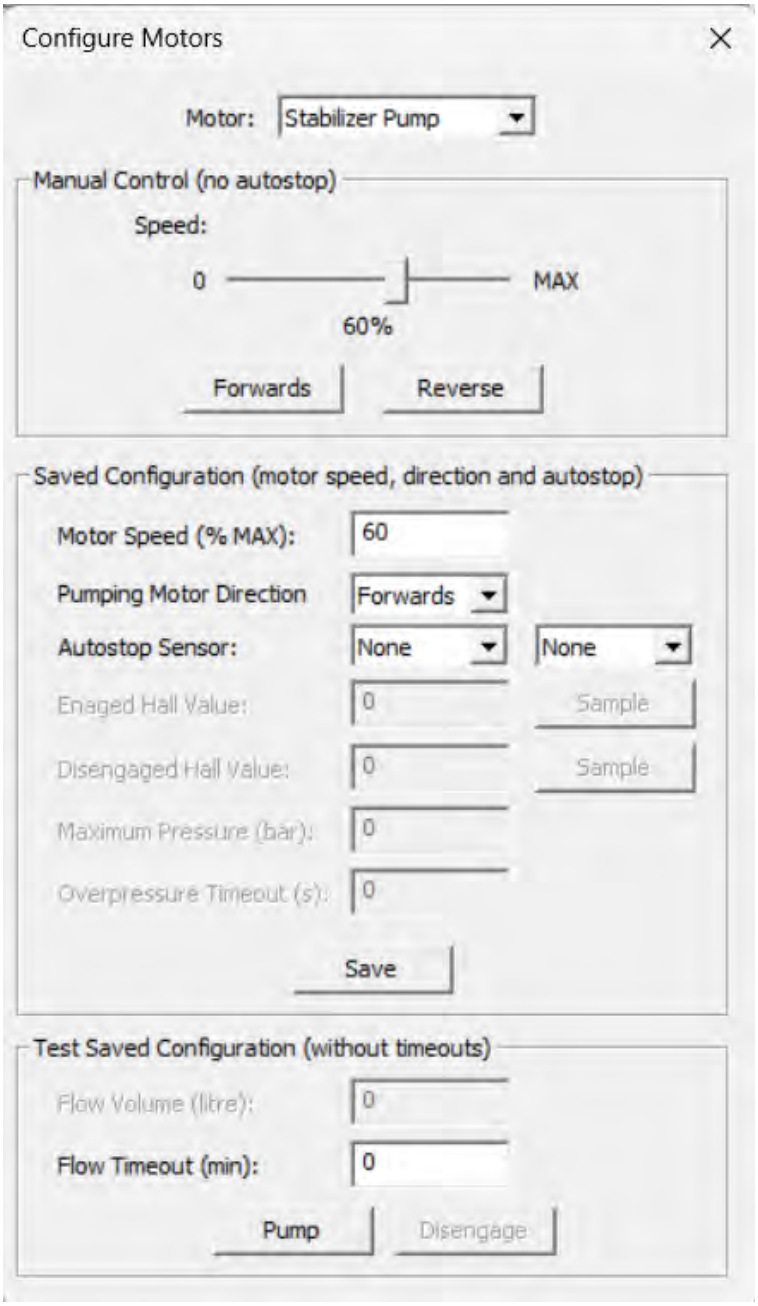
6. Apply some light pressure to the syringe and inject water into the pump until it is moving water successfully. Be aware that, with no cartridge installed and engaged, water will flow from the injector onto the bench.
7. Click Stop, once the pump is primed.
8. Remove the syringe from the pump exhaust and reconnect the exhaust tube.

Priming the stabilizer pump:

1. Navigate to the Configure/Motors... menu.



2. Select Stabilizer Pump in the Motor drop-down menu. This allows direct control of the stabilizer pump.



3. Prepare a syringe with clean water that will be used to prime the sample pump
4. Disconnect the intake line from the stabilizer pump and connect the syringe directly to the intake of the pump.

5. In the Manual Control section, set the Speed slider to 60% and click Forwards.
6. Apply some light pressure to the syringe and inject water into the pump until it is moving water successfully. Be aware that, with no cartridge installed and engaged, water will flow from the injector onto the bench.
7. Click Stop, once the pump is primed.
8. Remove the syringe and reconnect the stabilizer bag line to the pump.

Priming Sample, Stabilization and Cleaner Lines

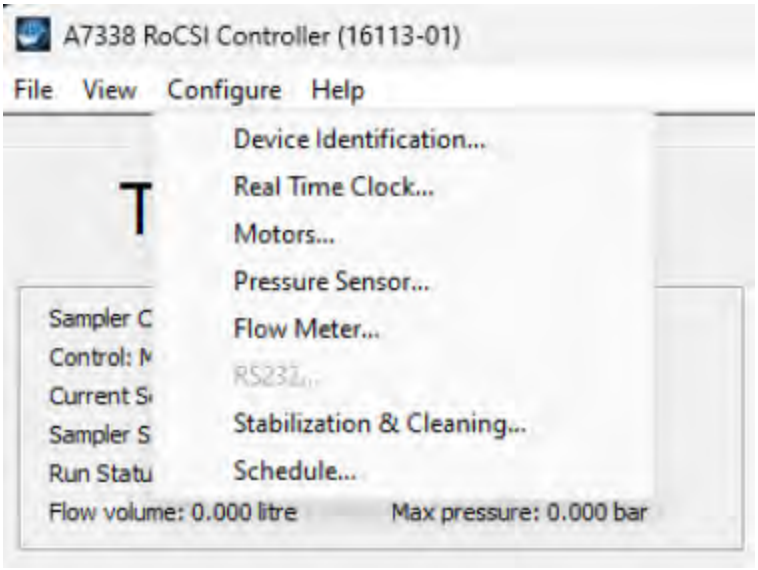
Prior to deployment or running samples, the sample inlet, cleaner inlet and stabilization lines should be primed to remove air bubbles. These processes will use commands located in the **Configure/Motors...** menu. Please note that priming can be a wet process and should be performed in an appropriate area.

Priming the Stabilizer line:

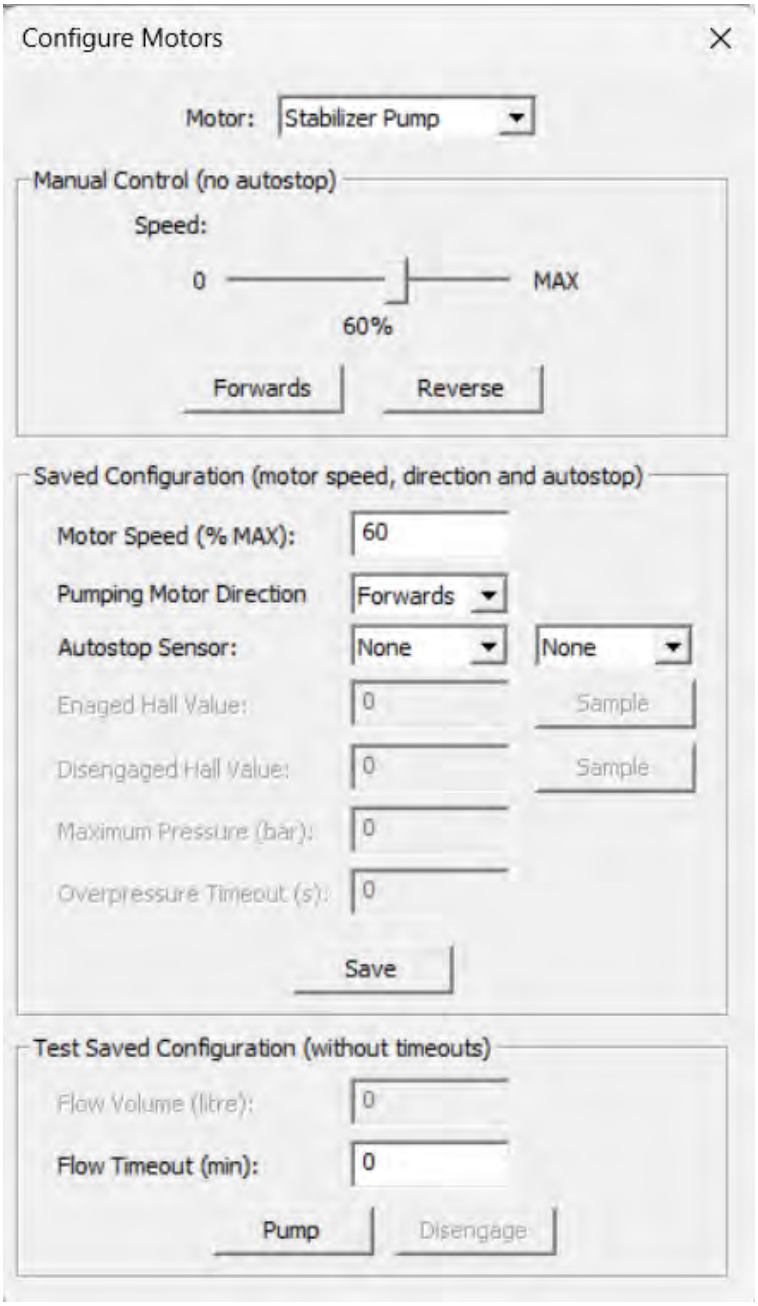
1. First, connect a full bag of stabilizer to the stabilizer bag line. Ensure that clips are

disengaged and the connection is secure.

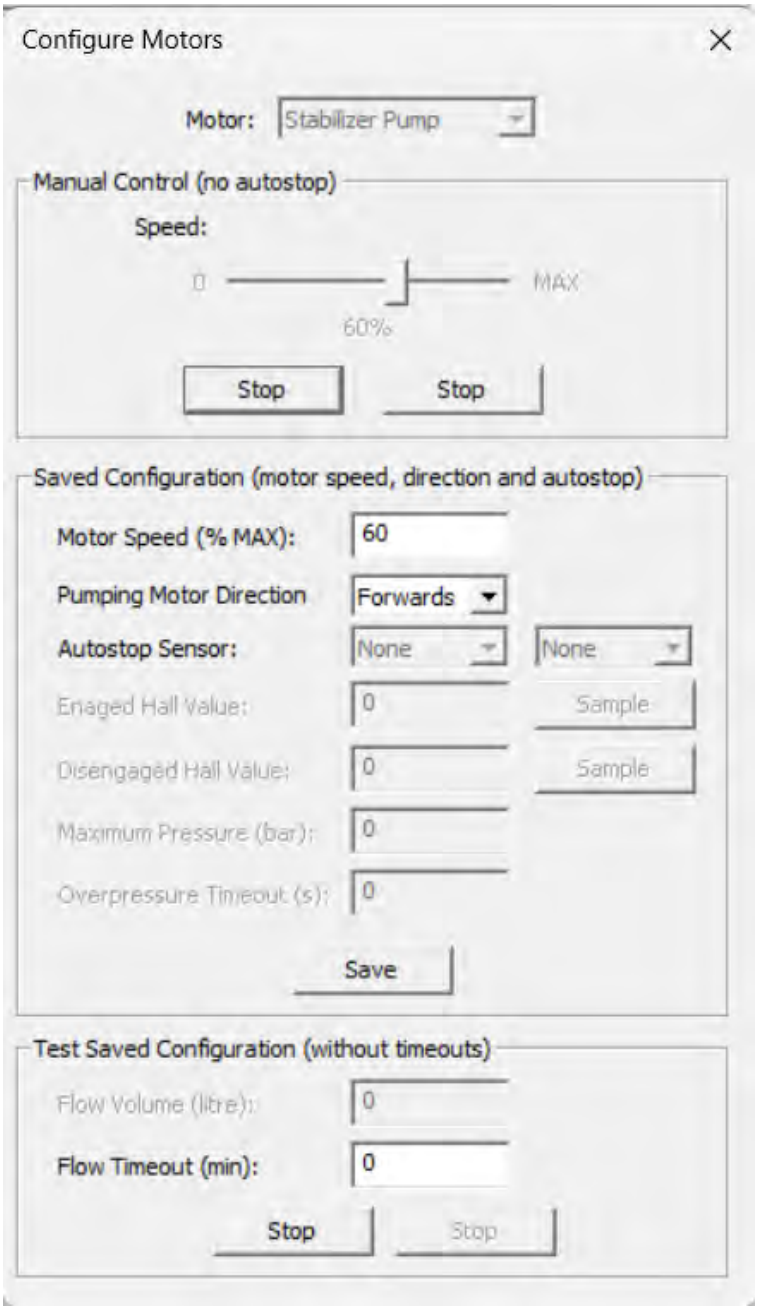
2. Navigate to the **Configure/Motors...** menu.



2. Select Stabilizer Pump in the motor selection menu.
3. Set the speed to 60% using the Manual Control speed slider.



4. Click the Forwards button to start pumping stabilizer into the system.

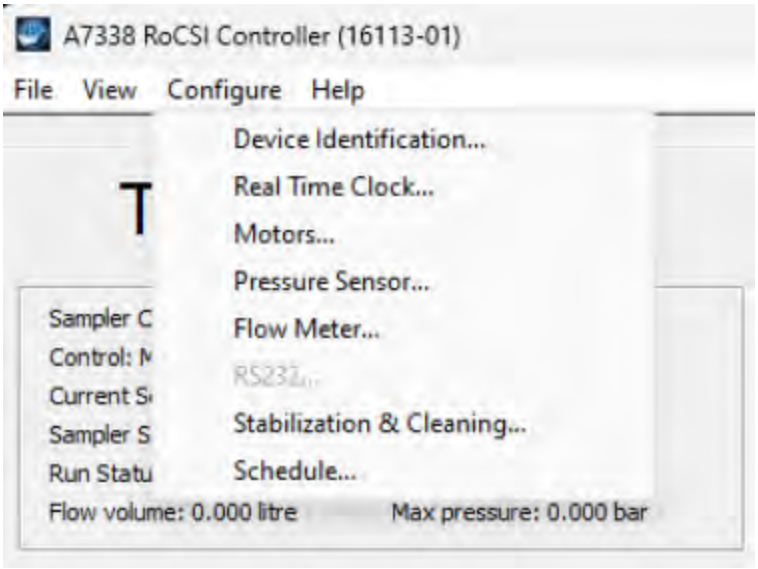


5. Click the Stop button when stabilizer begins to drip from the stabilizer injector. The line is now primed and ready for deployment.

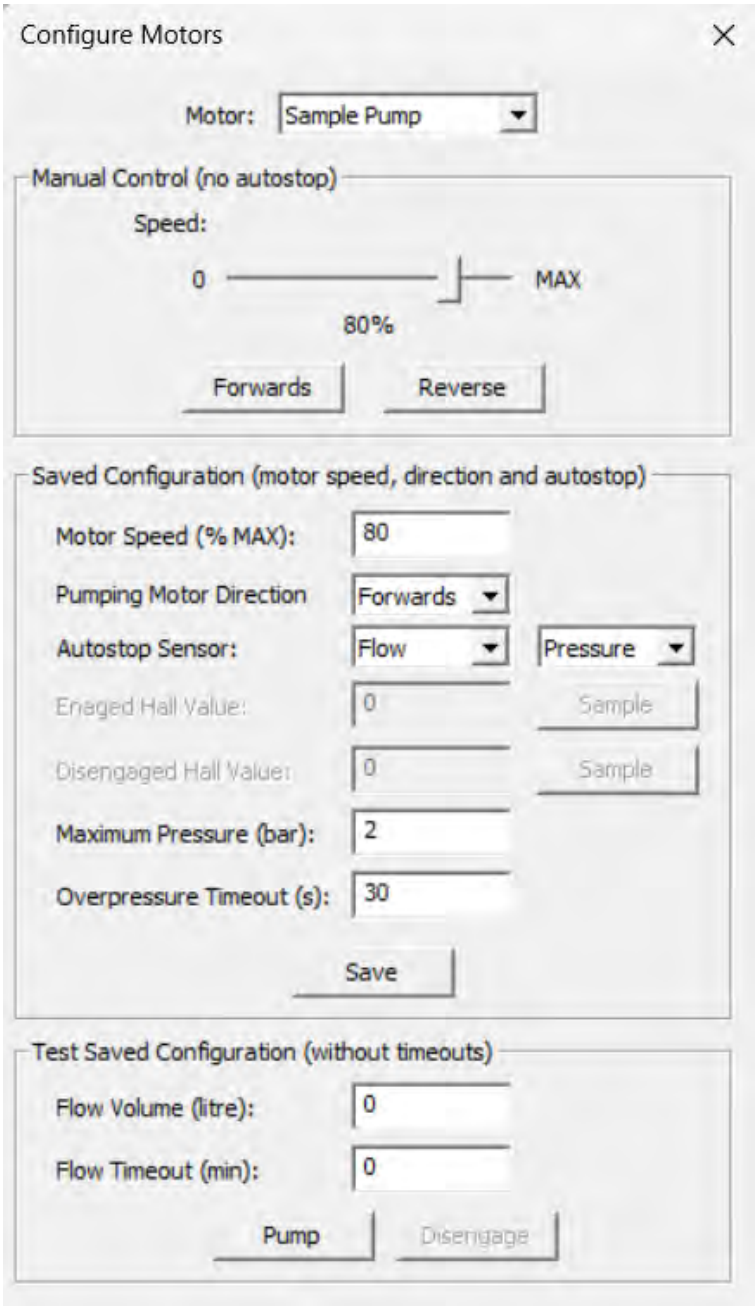
Priming the Cleaner line:

1. First, connect a full bag of cleaner to the cleaner bag line. Ensure that clips are disengaged and the connection is secure.

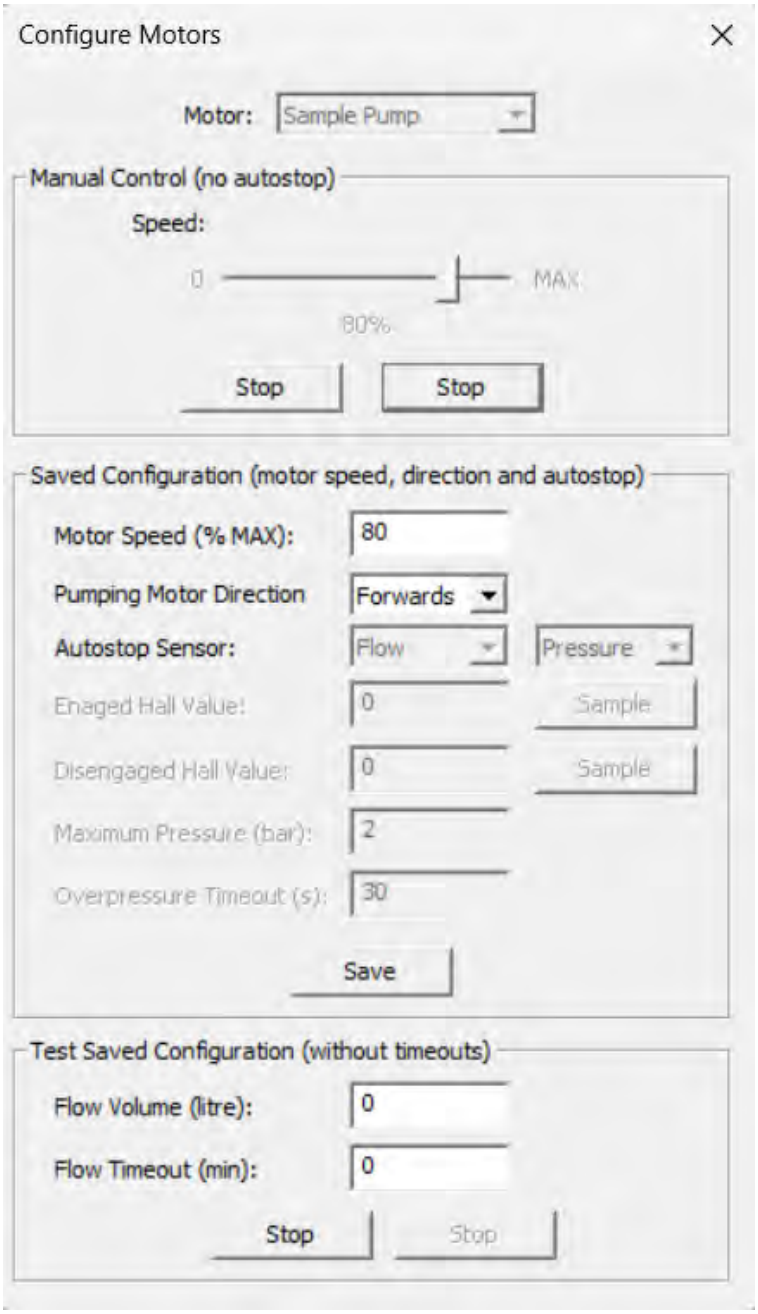
2. Navigate to the Configure/Motors... menu.



2. Select Sample Pump in the motor selection menu.
3. Set the speed to 80% using the Manual Control speed slider.



4. Click the Reverse button to start pumping cleaner into the system.

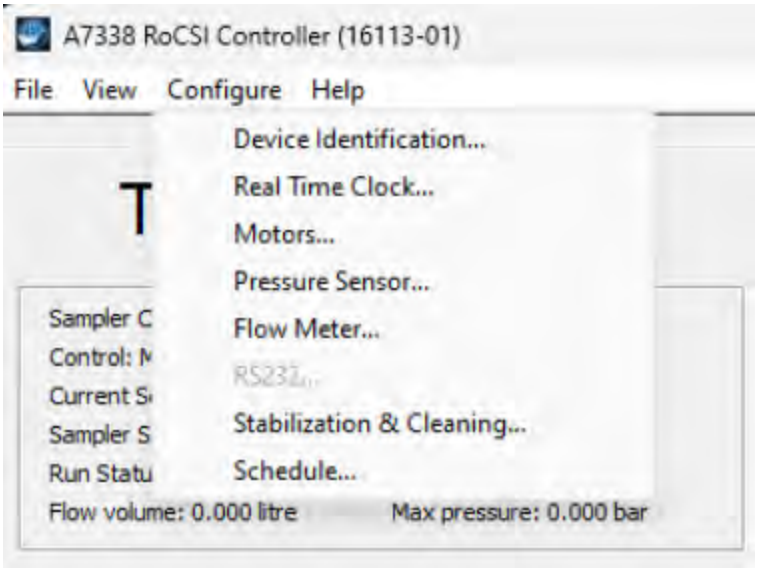


5. Click the Stop button when cleaner fills the cleaner line and enters the main plumbing. The line is now primed and ready for deployment.

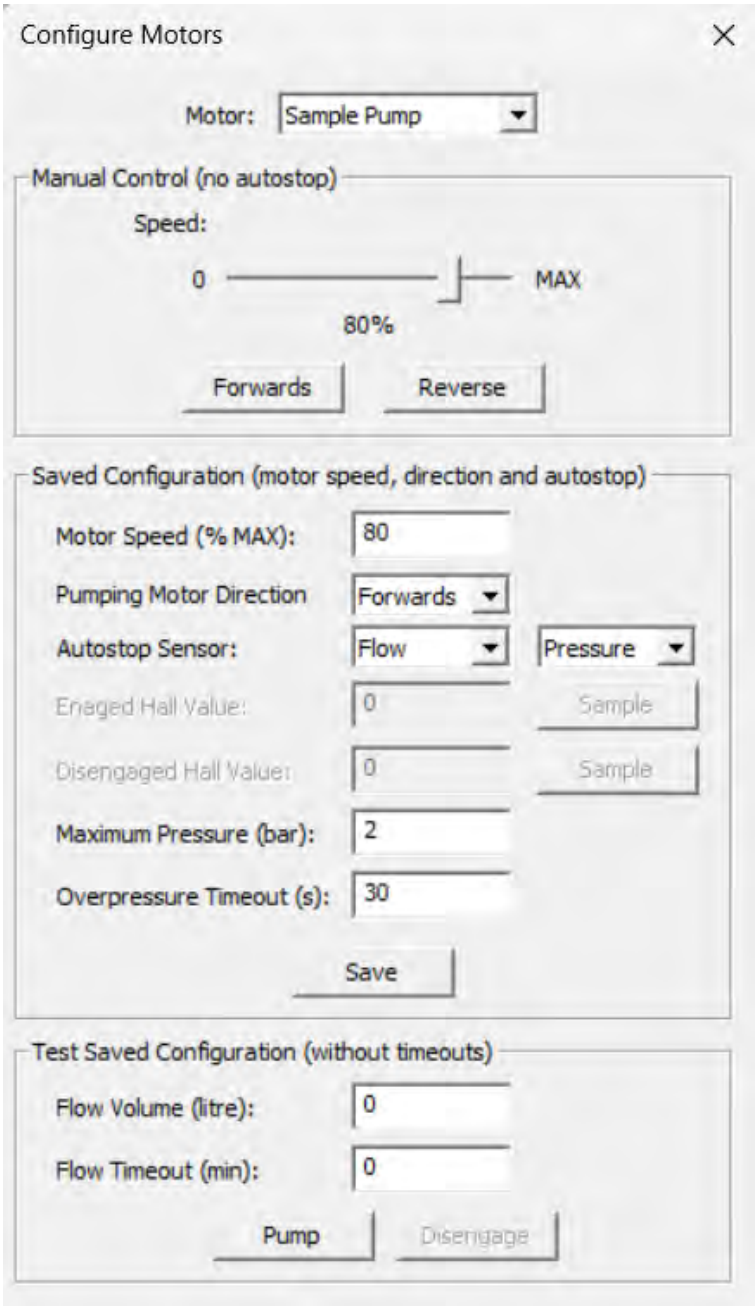
Priming the Sample line:

1. After priming the cleaner bag, it is recommended to flush the system and prime the sample inlet.

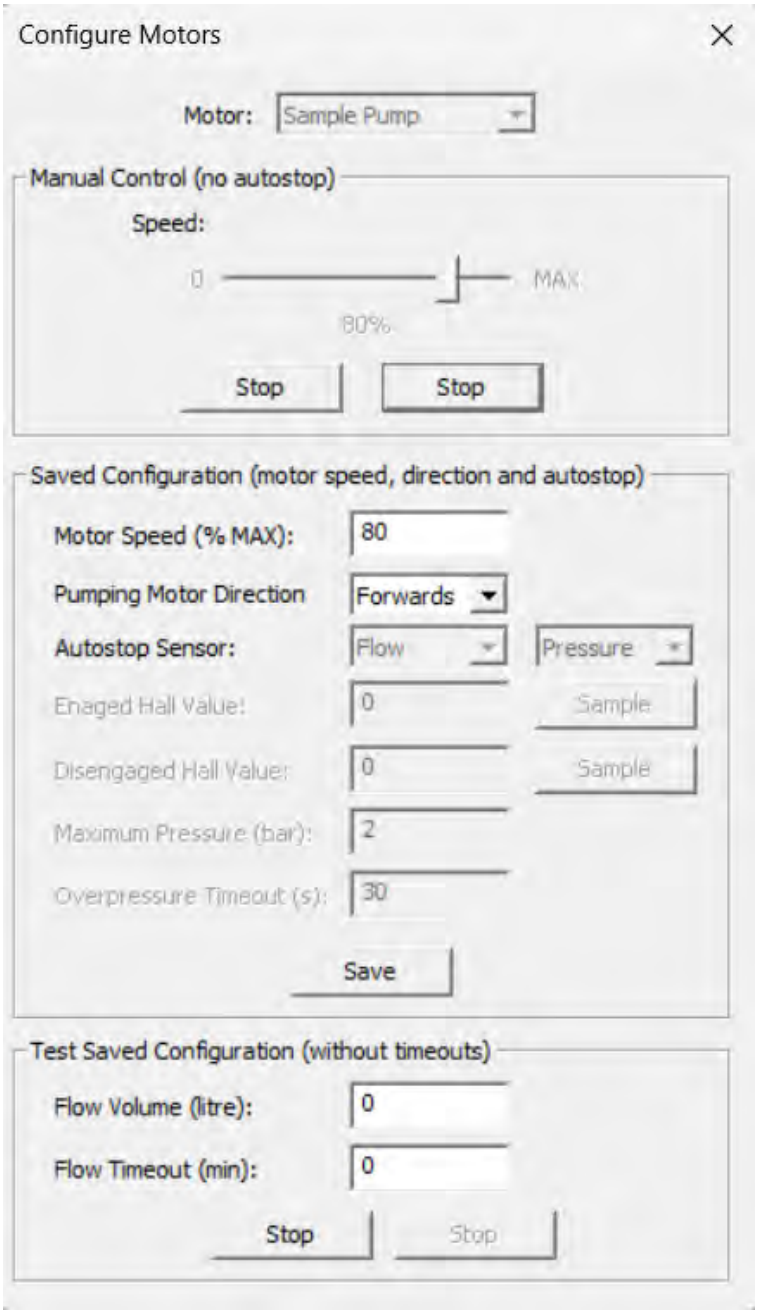
2. Prepare a container of clean water and submerge the sample inlet in the priming water. Another container may be used to collect the exhaust.
3. Navigate to the **Configure/Motors...** menu.



4. Select Sample Pump in the motor selection menu.
5. Set the speed to 80% using the Manual Control speed slider.



6. Click the Forwards button to start pumping sample into the system.



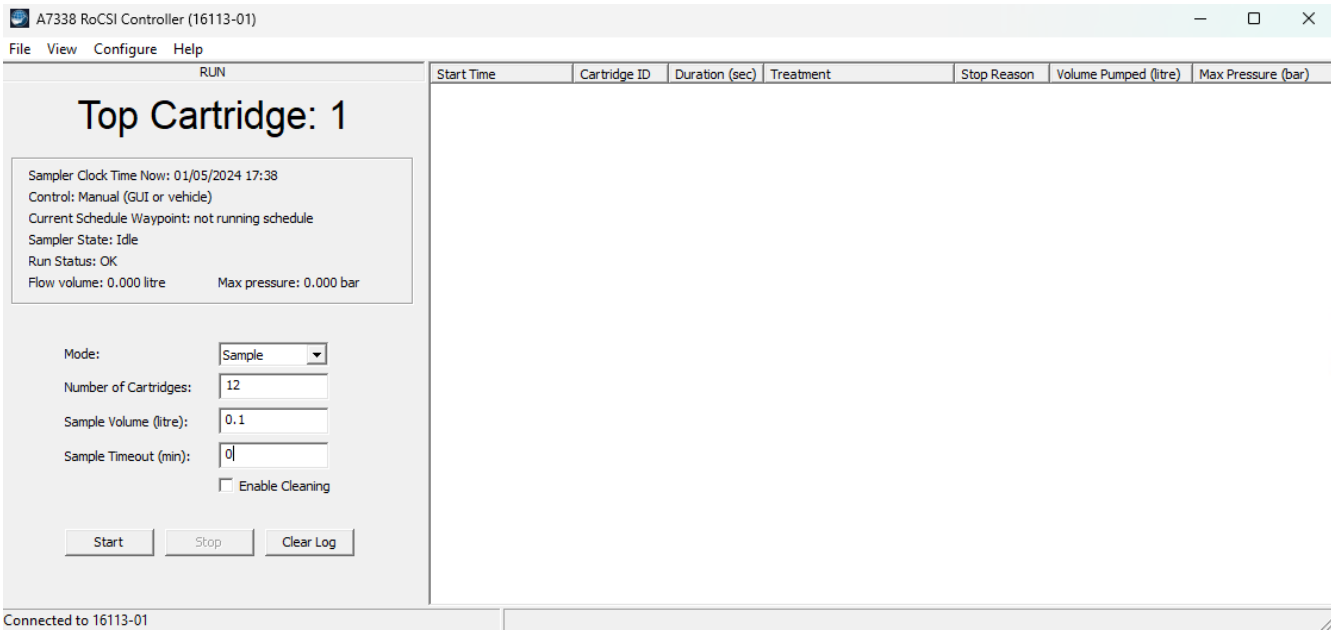
7. Click the Stop button when sample fills the inlet line and begins to drip from the sample injector. The line is now primed and ready for deployment.

Priming Cartridges

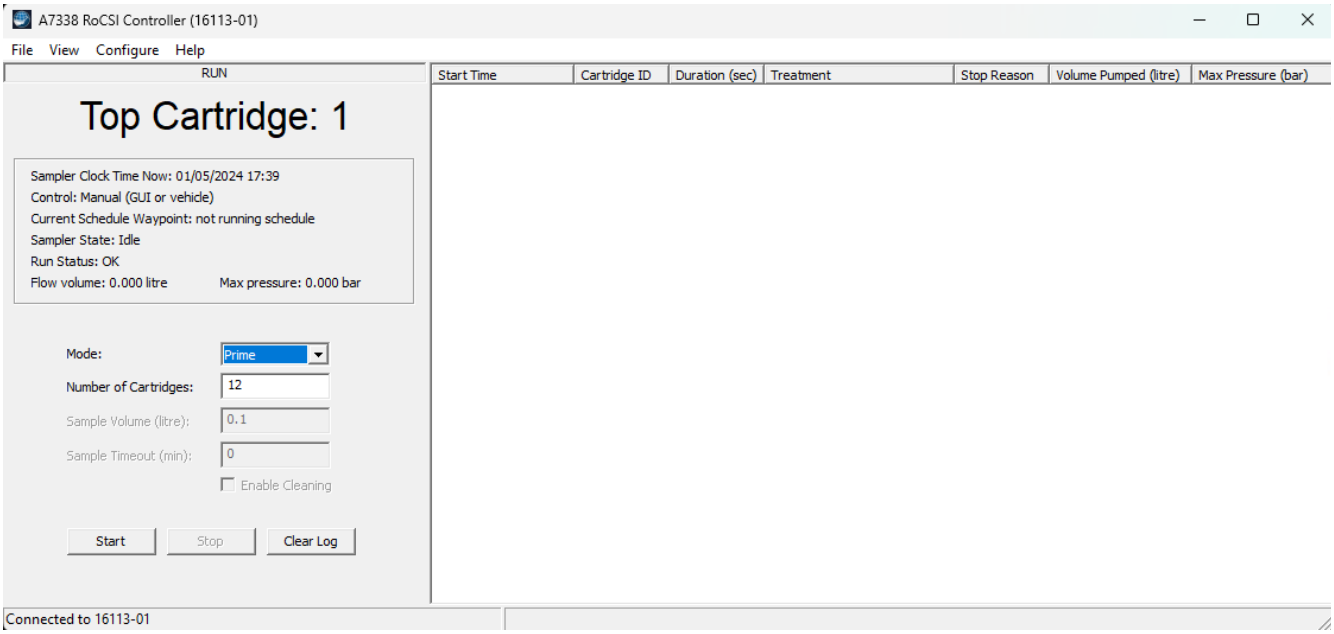
1. **For deployments underwater, the cartridges must be pre-filled with Milli-Q water before deployment.** This can be done in the lab with a syringe, but it is also possible to use the

sampler itself by connecting the sample inlet tube temporarily to a container filled with Milli-Q water and running the sampler with the **Mode** set to **Prime**.

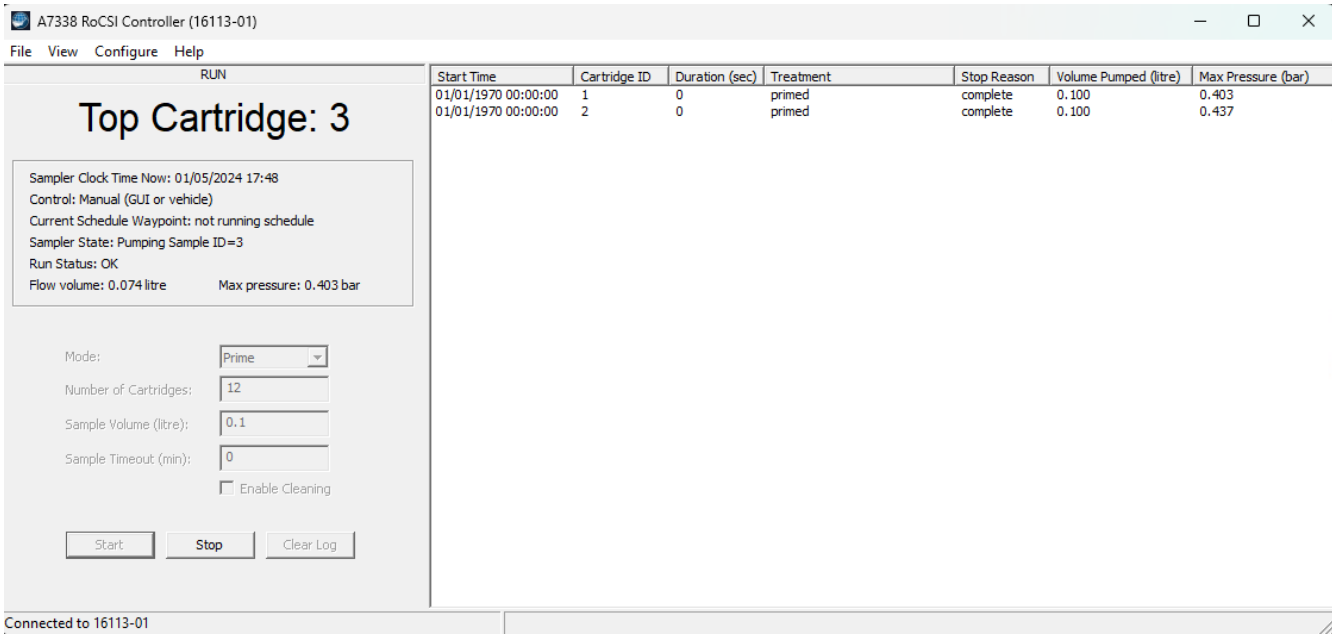
2. Priming uses parameters set under the **Sample** mode. Set the mode to **Sample** and set the priming volume (sample volume) to **0.1** Liter (this is the minimum value allowed by the software).



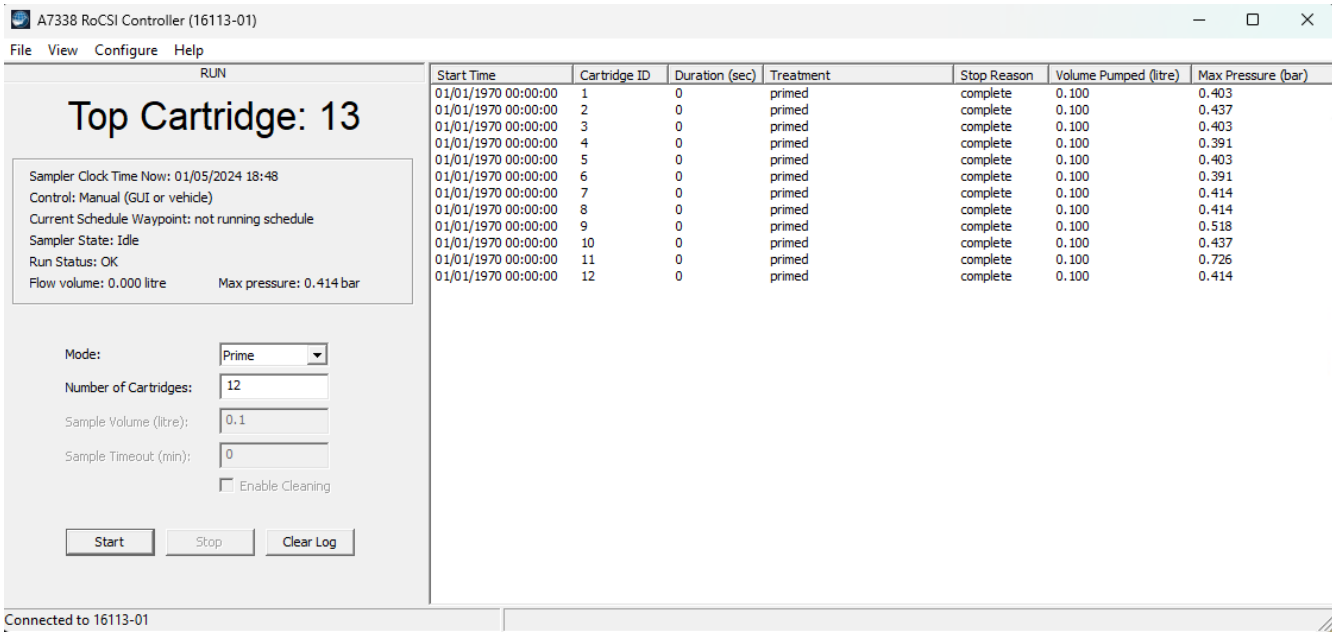
3. Change the mode back to **Prime** and select the number of cartridges that will be primed. In this example, all cartridges of the 12-sample chain will be primed. This means that at least 1.2 L of priming water should be available to RoCSI at the inlet.



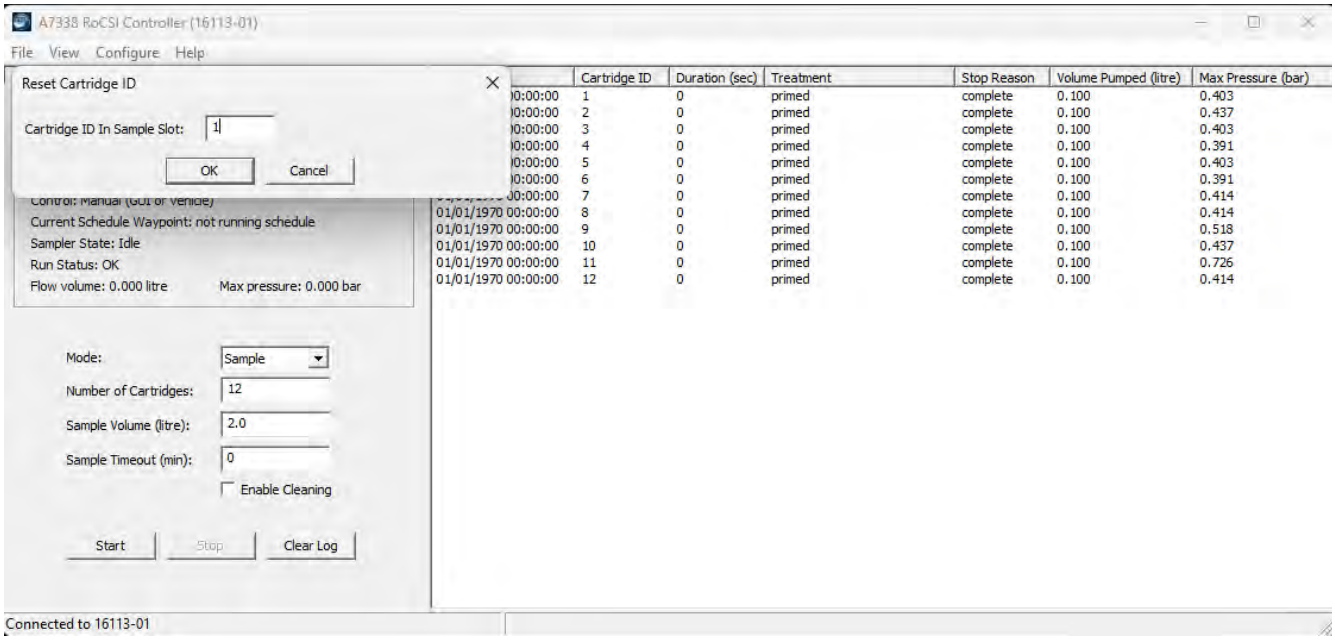
4. Click **Start** to begin the priming sequence. If errors occur during priming, the sequence will halt in order to troubleshoot the issue.



5. Start time is ignored in the sample log, resulting in the generic time tag of 01/01/1970 00:00:00 for each priming event. However, the sample log will record the volume pumped, maximum pressure and status for each cartridge.

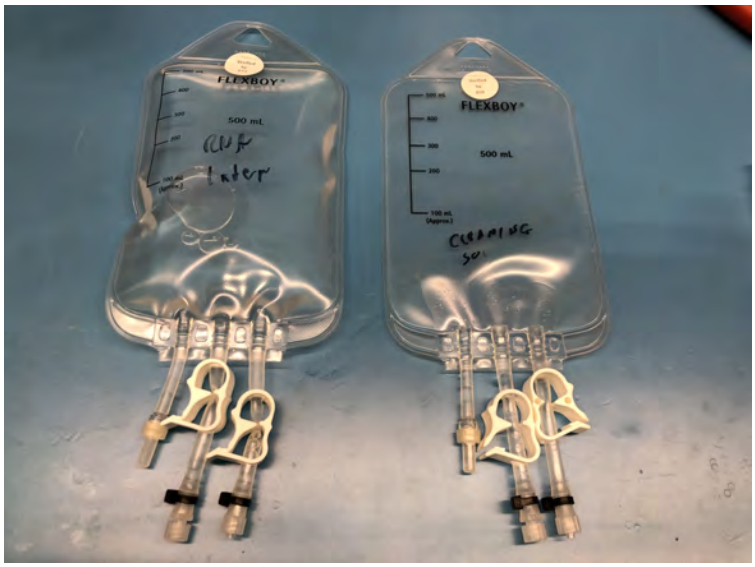


6. Once priming is complete, change the cartridge number back to 1; the RoCSI is now prepared for sampling.



Filling Reagent Bags

RoCSI requires reagents stored in plastic IV bags. Depending on the number of samples, required stabilizer and expected number of cleaning cycles, these bags may contain varying volumes of stabilizer or cleaning agent. RoCSI ships with 1 L bags as default in order to cover most deployment needs. Also supplied is a syringe with Luer Lock fittings used to fill the bags with fluid and also to remove air after filling.



Fill each bag according to expected reagent use, and connect to the marked inlet tubes at each end of the instrument.

- The stabilizer bag connects to the inlet of the stabilizer pump on the left hand side of the RoCSI.
- The cleaner bag connects to the inlet of the sample injector, attached to a one-way valve on the right hand side of the RoCSI, near the pressure sensor.

Be careful to work in a clean area with gloves to reduce contamination risk during filling of reagent bags. For most applications, RNAlater will be an appropriate stabilizer and 2.5% bleach is an acceptable cleaning agent.

Sampling

RoCSI sampling times are variable – based on a nominal flow rate of 60 to 70 mL/minute. Depending on the filter type (Sterivex™ 0.22 or 0.45 µm) as little as few hundred mL or as much as a couple of liters (if using the larger pore size in oligotrophic waters) can be achieved before clogging.

A serial interface allows you to start and stop sampling and perform on demand sample preservation.

Sampling software setup consists of the following:

Setting the Flow Volume and Maximum Pressure

Setting the Real Time Clock

Configuring Stabilization & Cleaning

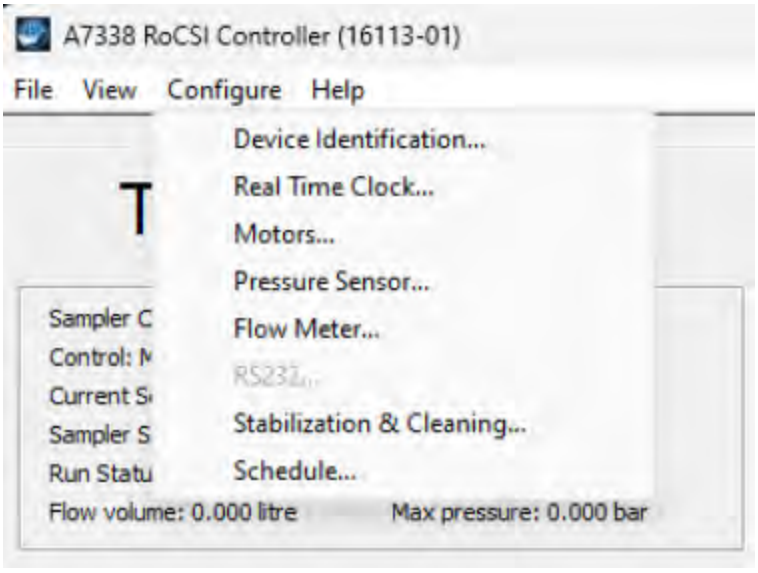
then;

Manually Triggered Sampling or,

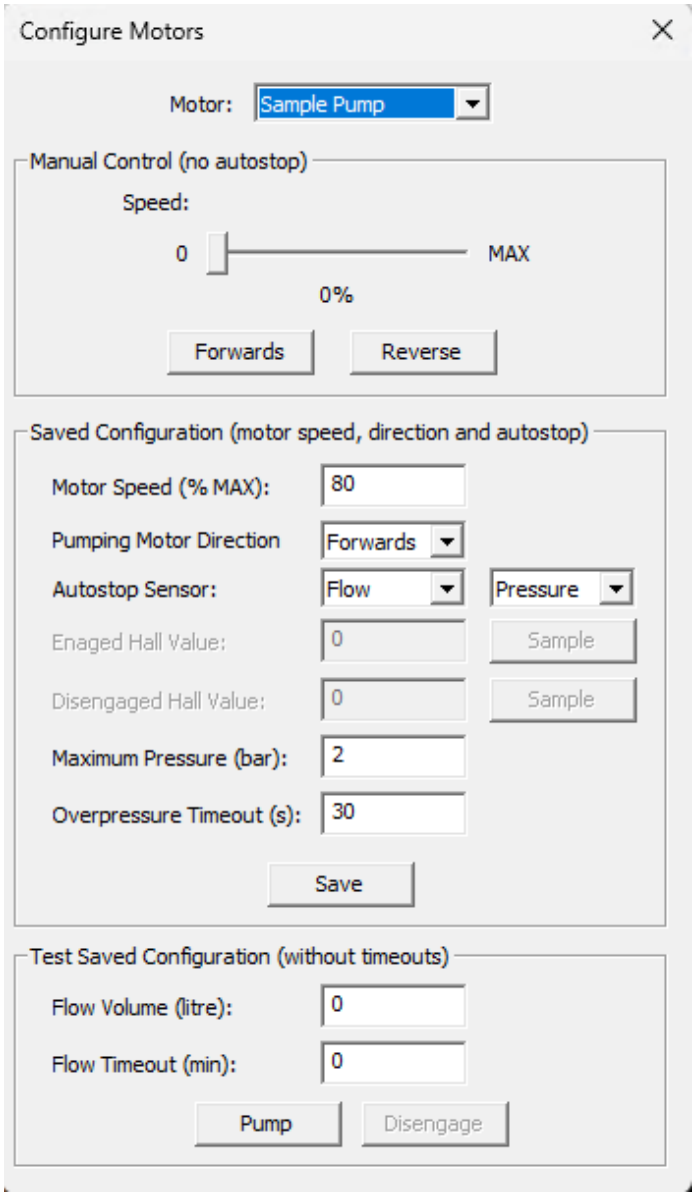
Configuring the Sample Schedule

Setting the Flow Volume & Maximum Pressure

These rarely changing settings are set in the motor configuration which you can access from the **Configure/Motors** menu in the application's menu bar.



1. Change **Motor** to **Sample Pump** as indicated.



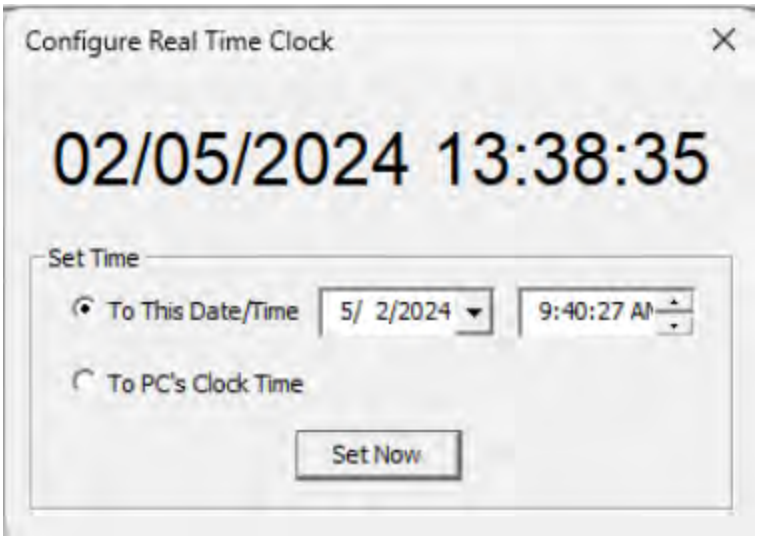
2. Change the **Autostop Sensor** to **Flow**. To disable the flow meter, set this value to **None** and use **Flow Timeout;** be aware that the flow rate will be reduced as the Sterivex™ filter unit clogs.
3. Set the **Maximum Pressure** and **Overpressure Timeout** (i.e. how long to let the sample be at the over-pressure condition before stopping the pump) and click **Save**. Leave other settings as configured.
4. The **Flow Timeout** ensures that the RoCSI doesn't get stuck on a cartridge that is allowing only minimal flow but not exceeding the pressure limit. The flow rate through a new filter will be around 1 ml/s (if you leave the pump motor speed configured as 80%) but this flow rate will drop as a filter clogs.

Setting the Real Time Clock

The sampler has a Real Time Clock to provide date/time for scheduling and for time-stamping the

samples. The clock time is set through the **Configure/Real Time Clock** menu.

The sampler has no concept of time zone but it is recommended that the sampler time is set to UTC. The real time clock has its own battery to maintain time even when the 12 Vdc supply and the USB power are turned off.

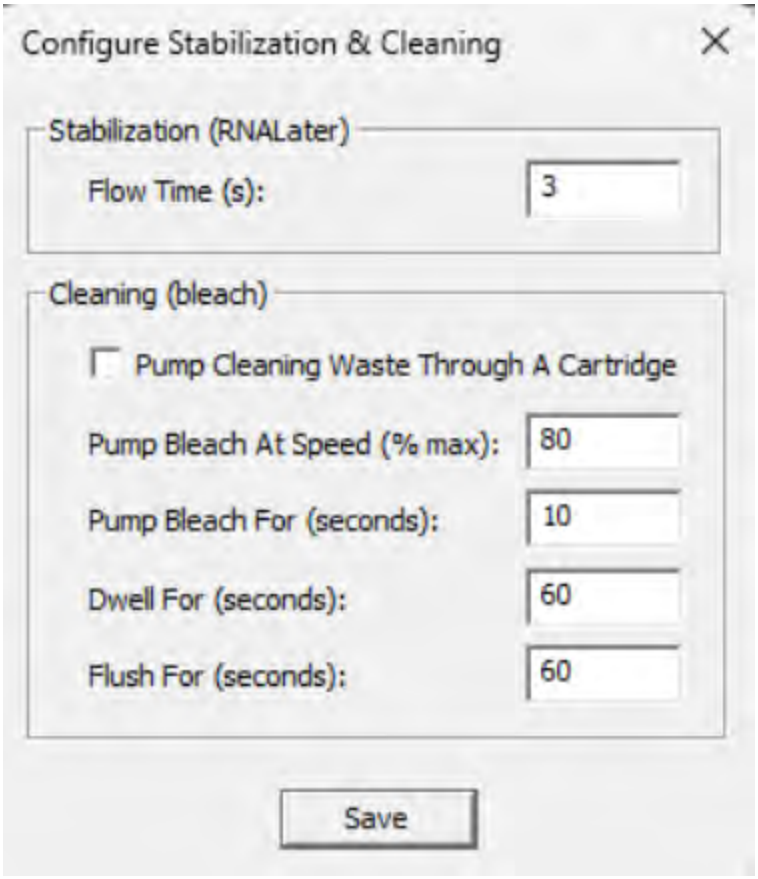


Configuring Stabilization & Cleaning

RoCSI can be configured to pump a cleaning fluid such as 2.5% bleach through the sample intake periodically. The waste cleaning fluid can either be discarded to the exhaust, or pumped through a cartridge into the waste outlet, wasting a cartridge. This option is set in the **Configure Stabilization & Cleaning** menu. If waste is pumped through the cartridge this will be indicated in the sample log.

The pumping flow time for stabilization is also set in this window. Typically a user will only need a few seconds of pumping to fully stabilize samples in the cartridge after sampling. A setting of "0" will disable stabilization for all samples.

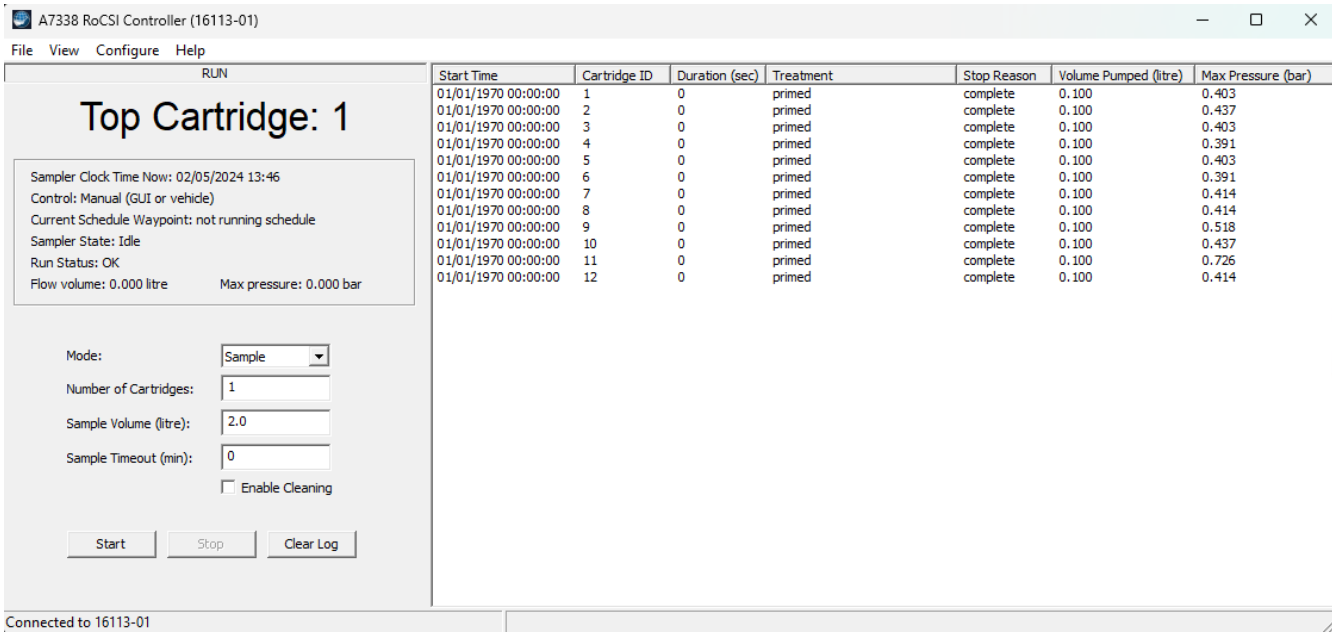
Typically, a cleaning cycle will pump at 80% pump speed (the same as sampling) for 10 or more seconds (depending on the intake tube length - allow enough time to purge the entire intake), dwell for 1 minute and flush with sample water for an additional minute. These settings are global and will be used any time a cleaning cycle is initiated.



Manuallly Triggered Sampling

If the graphical user interface (GUI) is available to the user for sampling, then sampling may be performed "on-demand" using **Sample** mode. Set the parameters for sampling (number of cartridges, sample volume and sample timeout) and click **Start** to begin one or more samples. Enable cleaning (if checked) will initiate the cleaning protocol defined in the **Configure\Stabilization & Cleaning** menu before acquiring the sample.

The current sample will run with controls previously defined in the **Configure\Motors** menu, and below the mode setting. Click **Stop** at any time to terminate the sample; be aware that this will halt an entire sample sequence and no stabilization will be performed on the current sample.



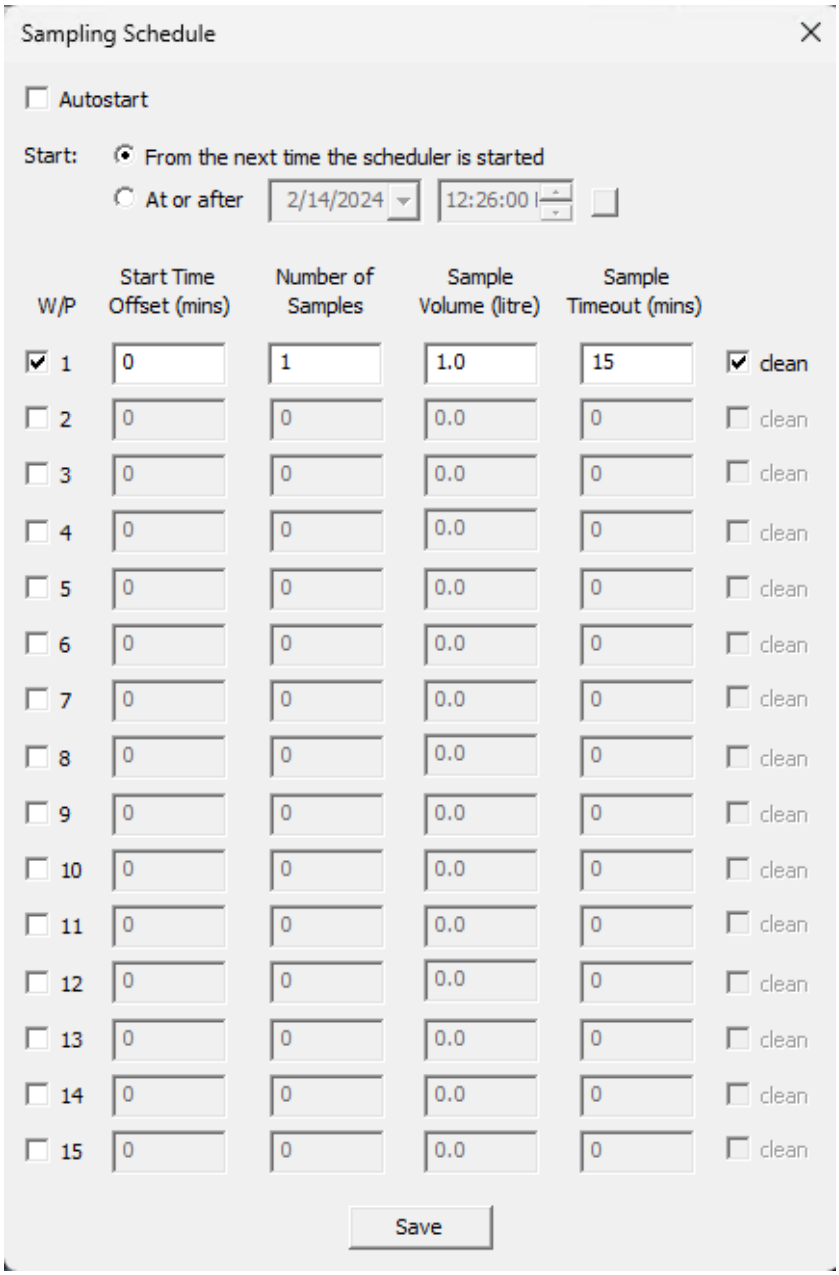
Configuring the Sample Schedule

In its current stage of development, the RoCSI eDNA sampler has scalable sample capacity based on a modular, expandable sample chain. Each “link” in the chain comprises a sample cartridge and clip, with each cartridge representing a single discrete sample. If using the RS232 communications channel to trigger samples, then capacity is only limited by available cartridges in the chain; that is, samples are initiated “on demand” from a secondary controller and metadata are stored for each sample locally on the RoCSI device. However, if operating autonomously, according to a pre-defined schedule, then the RoCSI is limited to 15 discrete sampling events, or “waypoints.” A waypoint may be configured to collect multiple sequential samples, again limited by available cartridges, but only 15 discrete, calendar-based events are able to be programmed in the native scheduler

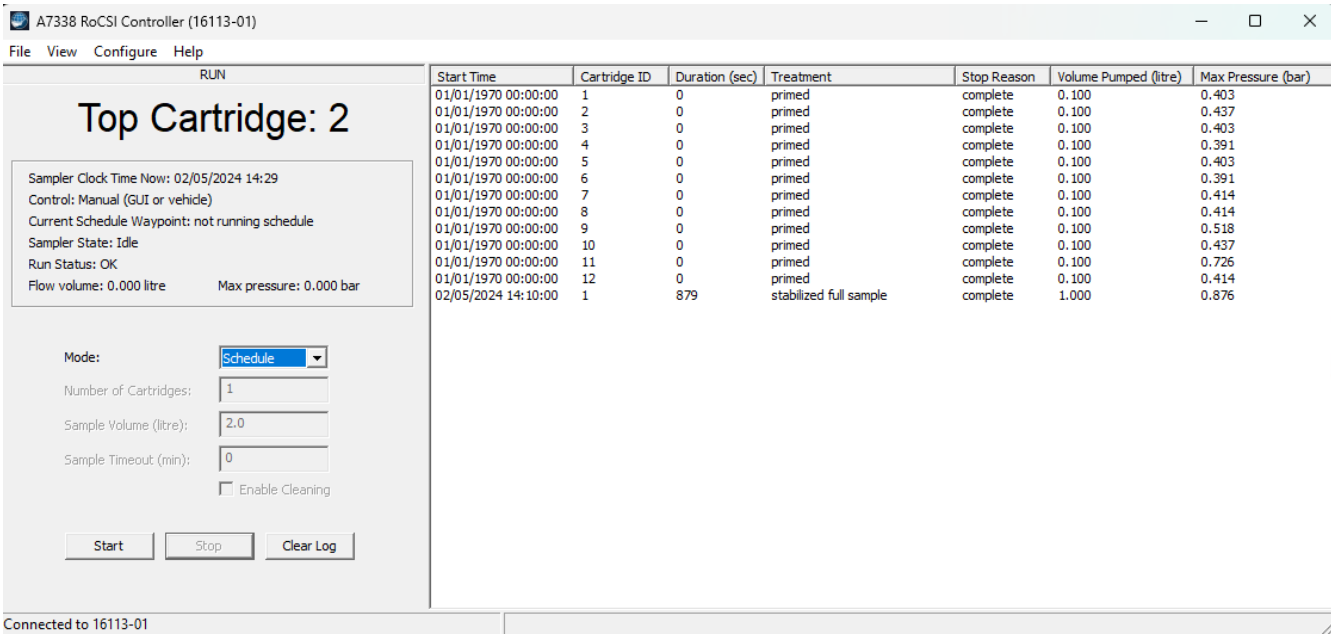
1. To schedule RoCSI to collect samples, go to the **Configure** menu and select **Schedule...**
2. The Sampling Schedule allows for 15 waypoints (or bursts) of samples. All sample times are based on the scheduled start, either set manually via the **"At or After"** selection calendar, or set to start automatically from the **sample** mode menu in the **RUN view**.
3. To schedule a waypoint, click the number on the left to activate it and then set the Start Time Offset, number of sequential samples to run at this waypoint, the sample volume for each sample, and the Sample Timeout for each sample. Also, click the **clean** checkbox to program a cleaning cycle before this waypoint (if desired).
    a. Please note that each waypoint should be scheduled with enough time to finish the sample or sequence of samples. When the next waypoint time is reached, any currently running samples will be terminated and stabilized immediately in order to run the samples defined by the next waypoint.
4. Checking the **Autostart** checkbox will command the sampler to begin the saved schedule as

soon as power is applied, whether connected to a PC or not.

5. Click **Save** to save the current schedule.



6. To initiate the sample schedule, close the Schedule window and return to the RUN view.
7. Change the mode to **Schedule** and click **Start** to initiate scheduled sampling. When the sample or burst of samples is complete, the schedule may be stopped by clicking the**Stop** button. The sample log will contain information on the sample including the waypoint time, duration of sample collection, treatment status, reason for stopping a sample, the volume pumped and the maximum pressure recorded during sampling.



Advanced Software Settings

RoCSI is shipped with Advanced Settings correctly configured at the factory and do not usually require changes.

This section is provided to help make running repairs or alterations during a deployment if necessary.

Calibrating the Motor's Hall Effect Sensors

RoCSI has five motors:

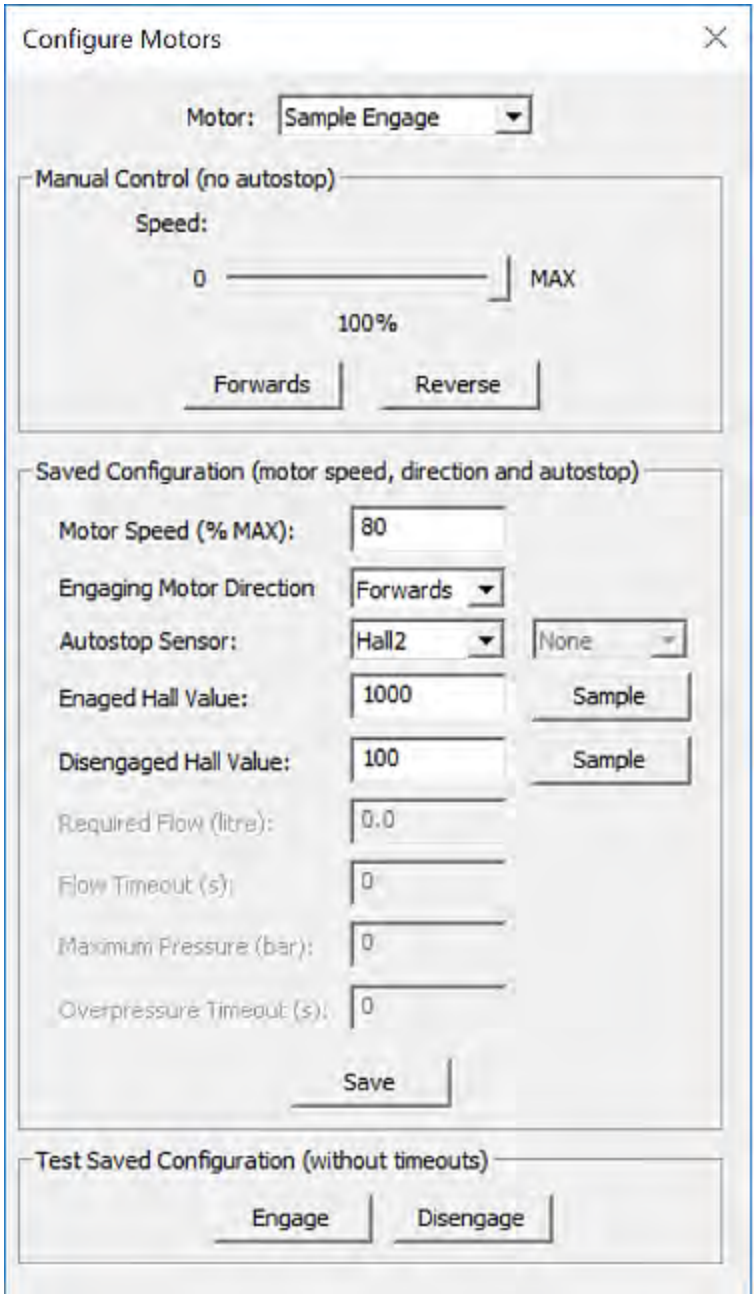
- One to rotate the cartridge magazine,
- One to operate the sample engage mechanism,
- One to operate the stabilizer cartridge engage mechanism, and
- Two to operate the sample and stabilizer pumps.

When a new sampler is being commissioned, each of these motors needs to be configured in the firmware.

The **Configure/Motors** menu allows control of the five motors independently to set and test the configuration for each motor.

Please note that these settings should only be adjusted if the RoCSI is not performing optimally, or during troubleshooting. Factory settings are generally appropriate and long lasting.

Start by selecting the motor to control in the **Motor** drop-down list at the top.



The **Manual Control** simply starts and stops the requested motor. The motor will not stop automatically and there are no interlocks, it is the user's responsibility to ensure that the requested motor can be operated safely (for example making sure the injector nozzles are disengaged from the cartridge before attempting to rotate the cartridge magazine).

Sample / Stabilizer Engage Motors

The motors that control the sample and stabilizer engage motors that move the nozzles backwards and forwards use Hall Effect sensors to determine when they have reached their engaged or disengaged position. To calibrate these two sensors, select the **motor**, set the **speed** to 100% and select the correct **Autostop Sensor** (Hall1 for sampler, Hall2 for stabilizer). Drive the motor using the manual controls to its disengaged position and stop the motor, then click **Sample**

to take a measurement. Then drive the motor using the manual controls to its engaged position and click **Sample** to take a measurement. Finally click **Save**. To test the configuration you can use the **Engage/Disengage** buttons at the bottom of the dialogue box which will stop the motors automatically using your new configuration.

Cartridge Load Motor

The motor that controls the rotation of the magazine motor is set in the same way (using Hall3), but to determine the engaged position, drive the motor until the drive pin that rotates the magazine is in its lowest position and take a sample at that point, this is the **engaged** position. The **disengaged** position is just very slightly (a few degrees) beyond the engaged position and it's easier just to type it in manually than to try to stop the motor there: just subtract about 100 from the engaged value (and don't press Sample). Test by pressing **Disengage** then **Engage** and check the magazine rotates by exactly one cartridge. You may need to tweak the Hall Sensor values to get it running reliably. Click **Save** when you are done.

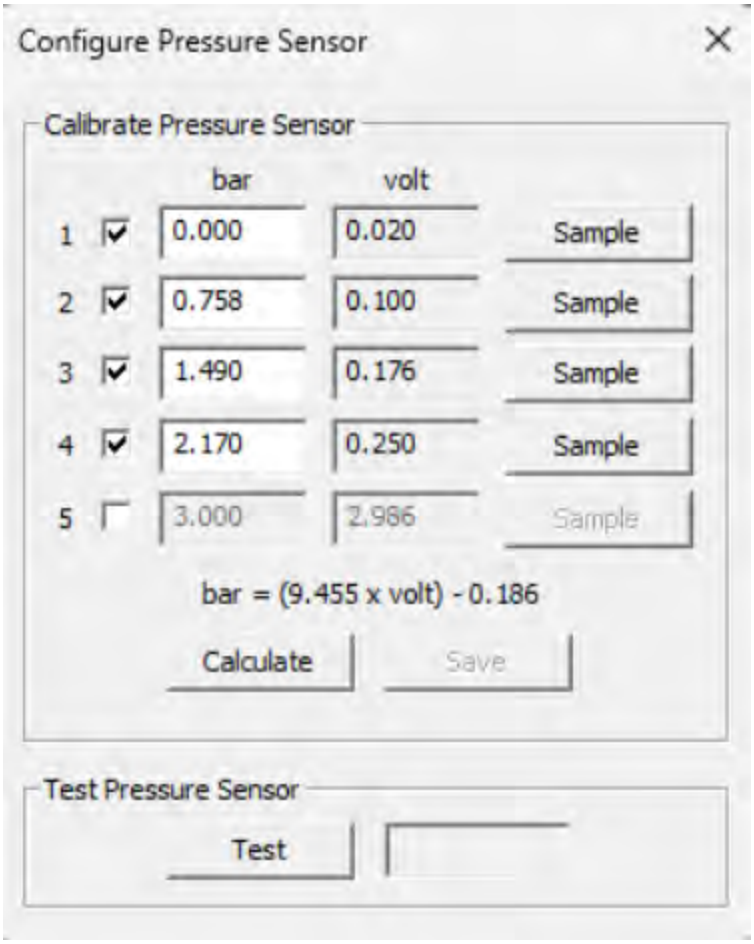
Pump Motors

The sample pump motor configuration (flow volume and max pressure) have already been covered in the section Setting the Flow Volume and Maximum Pressure. To achieve a flow rate of about 1ml/s the pump speed should be set to 80%. The sample pump uses Pressure Sensor 2 (and Pressure Sensor 1 is currently unused). Click **Save** when done.

The stabilizer pump motor does not have a flow meter or pressure sensor but a flow timeout should be set to program how much stabilizer will be pumped through each cartridge to give the required volume; assuming about 1ml/s – it will likely only be a few seconds. Click **Save** when done.

Calibrating the Pressure Sensor

RoCSI has support for two pressure sensors. Pressure Sensor 1 is currently not fitted. **Pressure Sensor 2** is used to monitor the fluid pressure in the sample line. When a new sampler is being commissioned (or a sensor is changed) this pressure sensor must be calibrated through the **Configure/Pressure Sensor** menu.



The pressure sensor is rated for 2 bar. The sensor calibration is linear but the user may capture up to five samples across this range and the software will choose the best fit.

The pressure sensor must be electrically connected to the sampler to calibrate it but you will need to temporarily disconnect the fluid hoses from it (make a note of which is the positive side of the sensor, i.e. the side connected to the filter input).

You will need to connect a water-filled syringe and a reference pressure sensor to the positive side of the pressure sensor only. A handheld pressure calibration device is used at the factory.

If a retort stand is available this is a more convenient way to maintain steady pressures with the syringe than by hand. Do not apply pressure with a syringe without the reference pressure sensor attached because one can easily exceed 2 bar even by hand **(the pressure sensor will be damaged beyond 4 bar**).

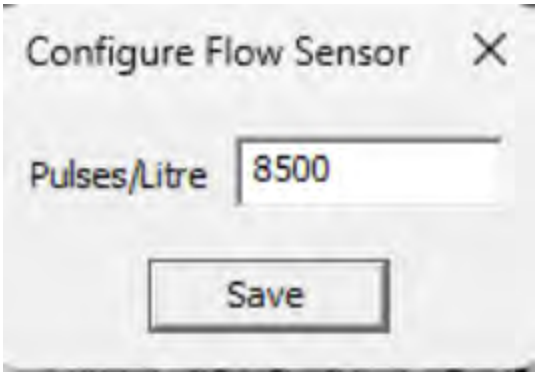
To capture the calibration points follow these steps:

1. Apply the pressure at roughly equal intervals from 0 to 2 bar.
2. Press **Sample** to capture each point in turn from the sampler's pressure sensor and type in the corresponding reference pressure manually.
3. Click **Calculate** then **Save** when you are done.
4. Test the calibration by applying a pressure, clicking on **Test** and comparing it with the

reference sensor.

Calibrating the Flow Meter

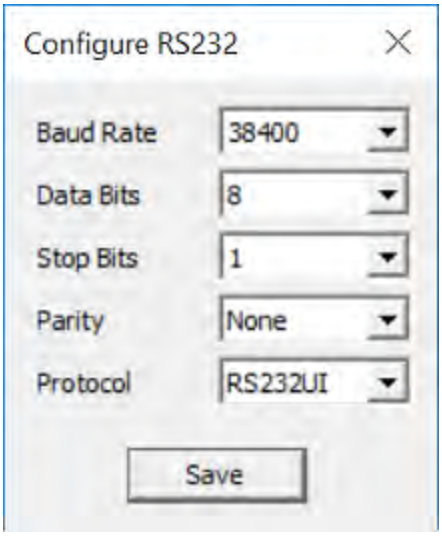
The micro-count DC gear flow meter (in the sample line only) outputs pulses that are counted by the sampler's electronics to determine the amount of sample that has passed through it. Nominally it flows ~0.111mL/pulse but this will need to calibrated on a new sampler. Go to **Configure/Flow Meter**. Set a value of 10000 initially and click Save. In the Motors Configuration make sure the **Sample Pump** is configured to use the **Flow** sensor and set the **Required Flow** to one litre. From the **Run** view, run a single sample and catch the exhaust in a measuring cylinder. When the sample is complete check the actual volume and adjust the calibration value as necessary.



Configuring the RS232 Interface

RoCSI has an RS232 interface which is configured through the **Configure/RS232** menu. At present there are three RS232 interface protocol options:

- "None"
- "RS232UI"
- "IATLANTIC"



The **RS232UI** protocol option supports the RS232 graphical user interface application (**benchmaps_rs232ui.exe**). If you choose to use that application instead of the USB one (e.g. on

an ROV) you will need to provide your own deck lead that connects to the RS232 RX & TX pins instead of the USB pins in the sampler's connector (see Deck Lead in RoCSI specifications).

You will also need an RS232 port (or RS232-USB adapter) on your computer and to know which COM port it is using so you can connect to the right port when you run **benchmaps_rs232ui.exe**. The required serial port settings are shown above (the software will set these by default).You can only change the RS232 configuration using the USB application.

The **IATLANTIC** option is provided for communications integration with vehicles or secondary controllers. It allows the sampler to be started and stopped by sending the text commands START<CR><NL> or STOP<CR><NL> (where <CR> and <NL> are the ASCII carriage-return and new-line characters) to the RS232 interface. There is no response. These very basic commands have the same effect as pressing Start and Stop in the Run View in the graphical interface. You can configure any serial settings you like but 9600 baud, 8 data bits, 1 stop bit and no parity are the defaults. See the appendix RoCSI-Autosub5 RS232 Comm Protocol for details.

Deployment Exit Conditions

Deployment exit conditions (pressure, time and flow) are critical to understand for maintaining sample viability.

Setting the over-pressure threshold to 1 bar is recommended, as higher thresholds can damage biology in the the sample.

Sample Viability

Please note that deployment duration is typically limited by the efficiency of preservative over time. Typical deployments may not exceed one month, if using RNAlater, as longer deployments may not return viable samples due to longevity/effectiveness of the preservative. Other preservatives may offer longer term sample viability, but should be tested and confirmed by the user.

The RoCSI system is highly configurable to accommodate diverse sampling regimes and strategies. Achievable sample volume will be dictated by the porosity of the filter, the environmental conditions of the deployment and the maximum allowable filter pressure. RoCSI is capable of sampling and filter loading up to 2.5 bar, though .7 to 1 bar is recommended for most applications in order to protect the filter material or biology.

RoCSI may terminate a sample based on the filter pressure, a sample timeout, or a newly triggered event. In these cases, RoCSI may report the sample as "incomplete" although it will still preserve the sample regardless of the end condition. These "incomplete" samples should be considered successful in most cases, assuming appropriate preservation.

In addition, samples that report "over-pressure" before achieving the full sample volume should also be considered successful samples. The filter has collected enough material that the pressure limit has been exceeded, but the sample is still preserved and should provide useful data.

Sample Time Outs

A sample timeout allows for an "emergency stop" of the sample if it has not terminated under normal conditions and is running longer than expected. Even if a sample times out, stabilization will still be attempted on that sample.

Pre-Deployment Checklist

Prior to the sampler being deployed or operated a few pre-flight checks should be made.

- Ensure all plumbing connections are secure.
- Check that any electrical connections are secure and replace the protection cap on the Subconn MCBH8M sampler to PC bulkhead connector.
- Ensure that the cartridge chain and filter cartridges are seated correctly.
- Run through 2 complete cycles of the cartridge chain before taking any samples.
- Ensure that the stabilizer and cleaner lines have been primed.
- Make sure the sample inlet is submerged in ‘sample’, and that the stabilizer and cleaner containers are filled.
- For deployments at depth, check Sterivex™ filters are all primed with Milli-Q before taking samples.

Start up Procedure

RoCSI start up procedures consist of the following:

- Assemble cartridges with new Sterivex™ filter units.
- Check that each cartridge is assembled correctly using gauge.
- Load cartridge chain with all cartridges.
- Connect RoCSI to the 12 Vdc power supply and laptop using USB deck lead.
- Turn on power supply and open the **benchmaps_usbui** software.
- Load the cartridge chain and run load/unload mode.
- Configure the sample schedule.
- Change mode to **SAMPLE** after checking mission parameters (volume and max pressure).

Troubleshooting Guide

RoCSI troubleshooting tips can assist with unexpected sampler performance. For technical support, contact McLane for assistance.

Cartridges not loading

Occasionally the cartridges may appear to stall and not load. RoCSI has been designed to try to load and will eventually do so provided the cartridges have been assembled into the cartridge chain correctly.

If the sampler fails to load it is most likely due to one or more cartridges not being assembled as per instructions. Review Assembling New Filter Cartridges.

Pumps

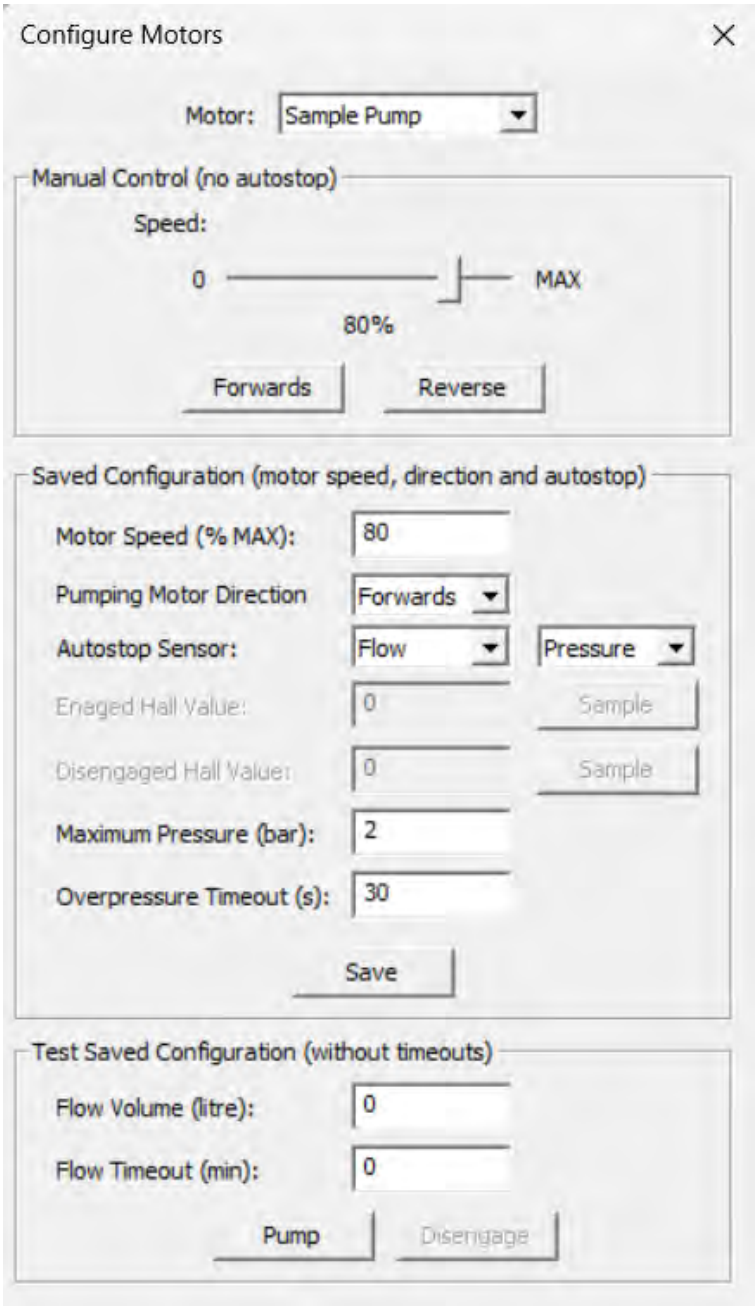
Periodically, the tubing for the peristaltic pumps should be replaced. See Changing Peristaltic Tubing for instructions on replacement.

Additionally, when the pumps are left dry for long periods of time, the peristaltic tubing becomes sticky and will prevent the pump from moving water. In this case, each pump, sample and stabilizer, should be manually primed. See Priming the Pumps for instructions.

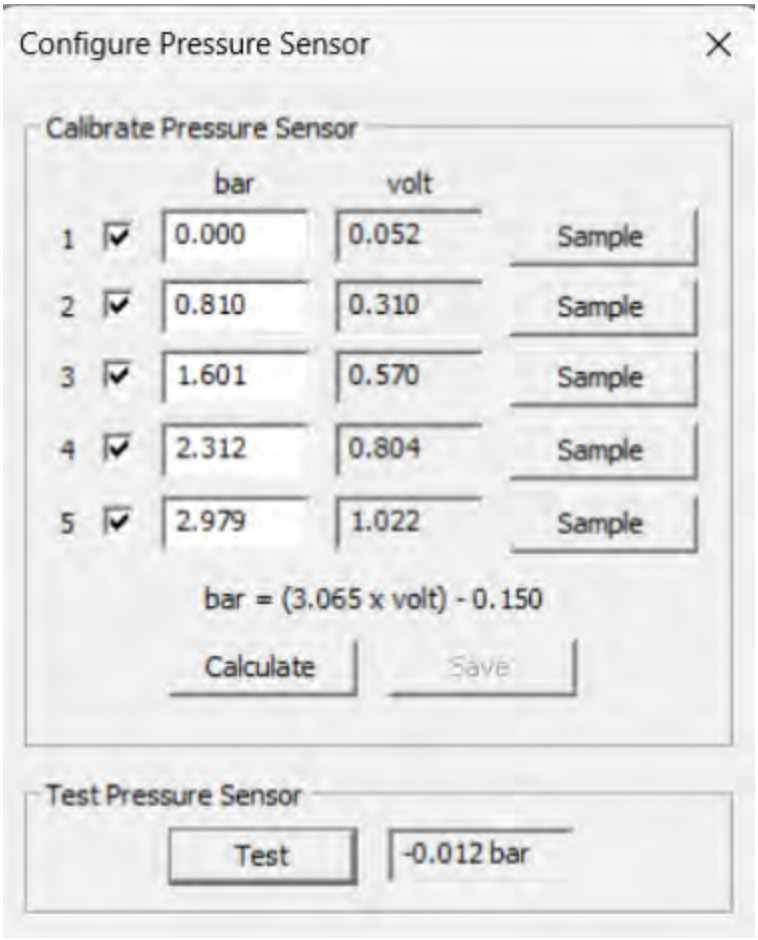
Pressure Sensor

If there are problems with the pressure sensor, first check the diagnostics, then check the plumbing of the lines and ensure that the sample line is going into the pressure sensor (see the schematic of RoCSI fluidic system).

Check that there are no disruptions to the flow (i.e. no pressure on the tubing or blockages). If the problem is not solved, check the configuration under **Configure/Motors** then **Sample Pump**. **Autostop Sensor** should be set to **Flow** and **Pressure**, with the **Maximum Pressure** set to 1 bar (typical for most sampling regimes), and an **Overpressure Timeout** set to 30 seconds.



The sensor may be tested by navigating to the **Configure/Pressure Sensor...** menu. Click the **Test** button to see the current pressure value. At ambient, it should read close to 0 bar.



Flow Meter

The flow meter does not function well if there are bubbles in the system as it is a gear flow meter. Therefore, for accurate volume readings, it is important to avoid air in the system. Check that the sample inlet is drawing in sample when running a sample. If bubbles do get in the system, these might be removed by gently flicking the tubing until they disappear but it may take a bit before the flow meter works again.

Sample Injectors

If the needle free valves on each end of the cartridge are not sufficiently engaged (or over engaged) then they will not allow flow. If the injectors need adjustment, see the section on calibrating motors hall effect sensors.

RoCSI Maintenance & Storage

Proper maintenance after every deployment is critical for ensuring smooth operation and long instrument life. This section provides guidance on the maintenance recommendations immediately following the recovery, when the instrument is back from sea, and when preparing for long-term storage or transport.

Steps may include:

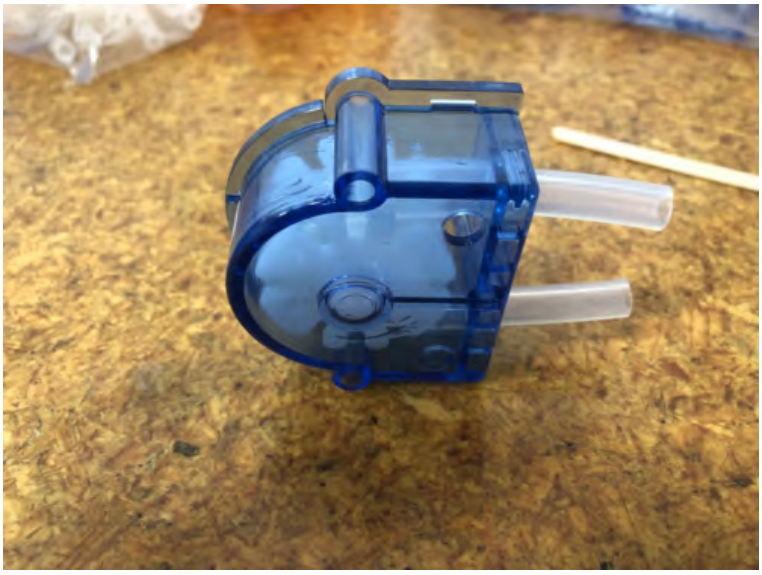
- Thoroughly rinse the instrument.
- Flush and purge all fluid lines of water.
- Reuse the shipping crate for storage and transport.

RoCSI Maintenance & Storage topics
Storage and Transport

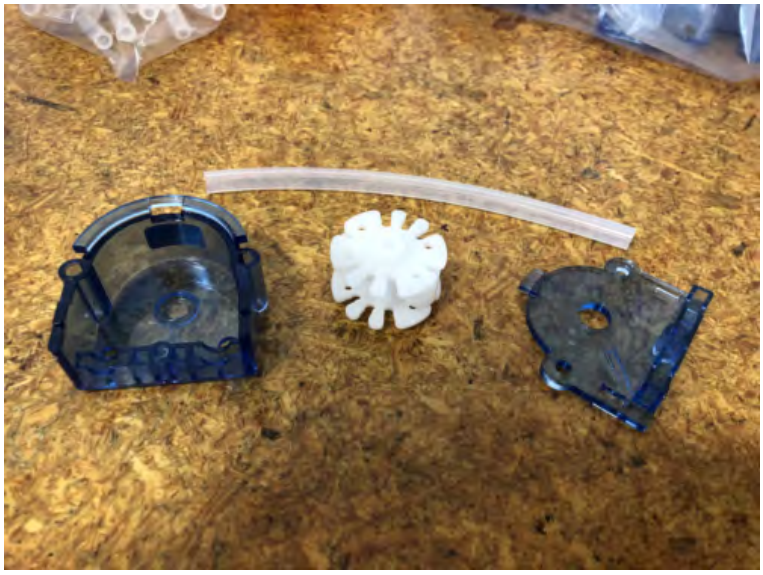
Changing Peristaltic Tubing

Spare peristaltic tubing is included in the toolkit. Follow these steps to remove/disassemble the pumps for tubing replacement.

1. First, disconnect the tubing from the intake and exhaust of the pump that requires service.
2. Remove the two screws that secure the pump head to the pump mount. Set the screws aside for reuse.
3. Gently pull the pump head straight off of the mount and drive shaft.



4. Separate the two halves of the pump housing. They are clipped together.



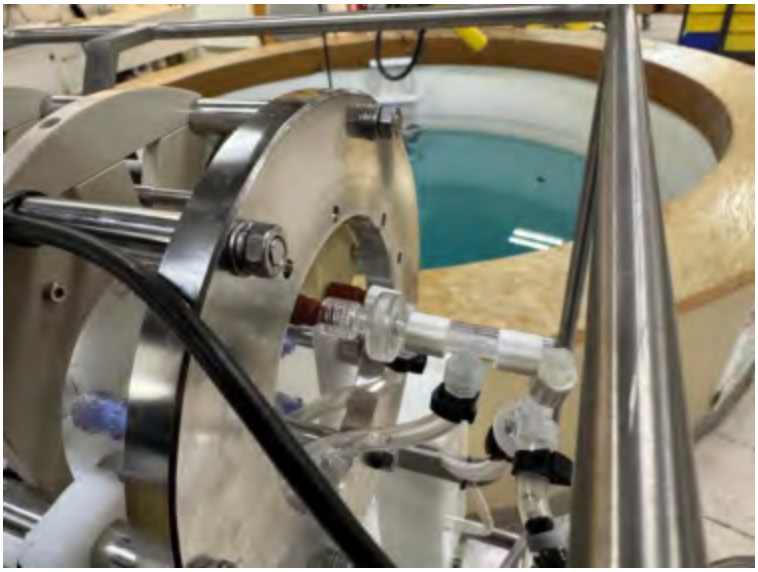
5. Remove the used tubing from the pump and disconnect any fittings that will be reused.
6. Fold the new peristaltic tubing piece and wrap it around the rotor.
7. Place the rotor and tubing back into the front half of the pump housing making sure the rotor clicks into place.
8. Please note that the 'D' cutout for the drive shaft is different sizes on each side of the rotor. The larger cutout must be facing the rear of the pump head assembly.

9. Replace the rear pump housing to secure the rotor and tubing in place.
10. Replace the tubing fittings and tube clamps if not already installed. Note the orientation and type of fitting according to the image below.
11. The pump head must now be reattached to the pump mount while engaging the D-shaft that drives the rotor. It may be necessary to rotate either the pump or the drive shaft to engage correctly.
12. Secure the pump head to the pump mount using the two M4 screws and lock-washers.

Changing Out Check Valves

Replacing check valves is recommended prior to each deployment. Spare check valves are included with the toolkit. Additional check valves are commercially available, or available from McLane.

The main intake check valve will periodically need replacement. It is attached directly to the inlet side of the sample injector.



A second check valve is attached to the cleaner inlet, and will only need replacement occasionally.

Storage and Transport

The shipping case is a reusable international container. Always use the original shipping case for transporting the RoCSI or when storing long term.

It is advisable to clear out any water from the RoCSI plumbing before long term storage of the instrument. This may be achieved by using clean, lab-grade compressed air or nitrogen to clear out all sample lines.

Post-Deployment Cartridge Processing

The level of cleanliness of the RoCSI, its plumbing and sample cartridges can affect downstream processing steps. A clean and flushed system will generally provide better collection and preservation, avoiding contamination or sample corruption. It is the responsibility of the user to maintain a clean system and to properly clean components between deployments. The following is an example of one method that is appropriate for cleaning the sample cartridge housings and fittings.

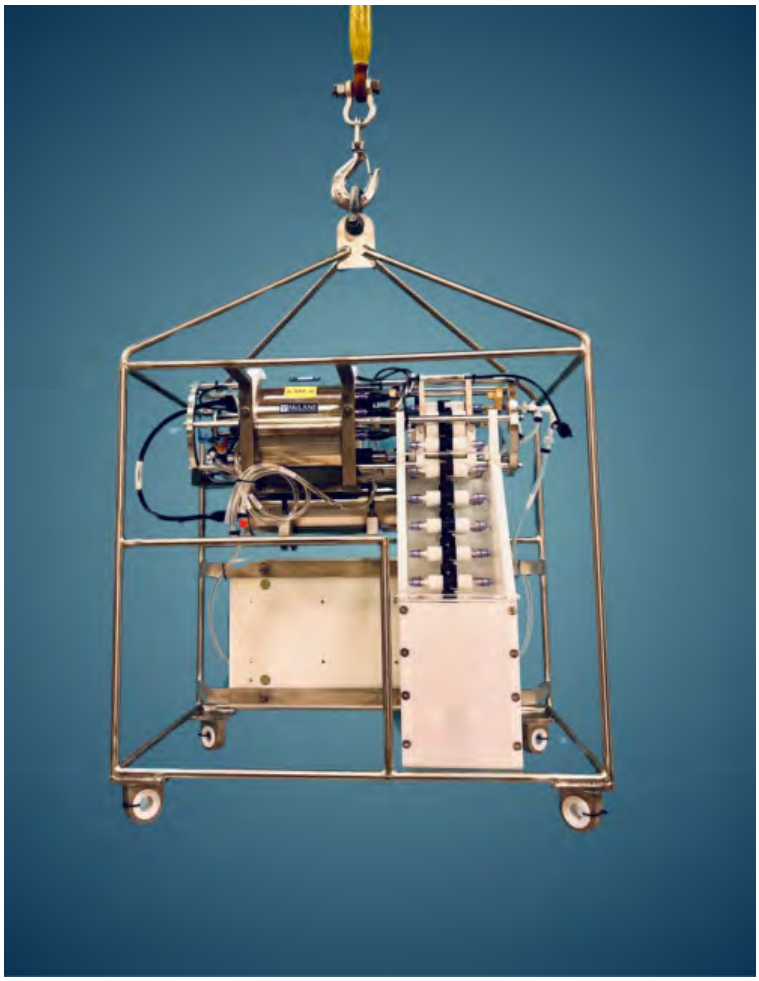
1. First, wash all components in a mild detergent solution to remove residue or bio-fouling.
2. Place all washed components in a tray of 2.5% bleach solution and soak for 30 minutes.
3. Fully rinse all parts using clean water (such as Milli-Q) using at least 3 water exchanges.
4. Leave parts in clean water to soak for at least 12 hours.
5. After soaking in water, allow parts to dry in a clean location (such as a laminar flow hood) on paper towels.
6. Parts must be fully dried before reuse.

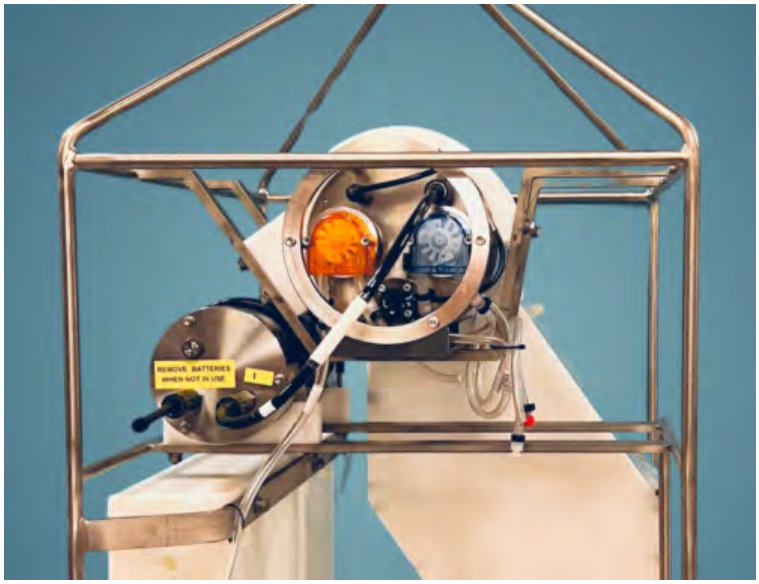
Appendix

This Appendix provides additional details including information about the RocSI moored configuration and use with the external battery.

Moored Configuration

RoCSI is available as a fully self-contained, moored system including reagent bag storage, external battery housing and cartridge containment (hopper), all mounted on a 316 stainless steel or titanium frame.





The cable that connects the RoCSI to the battery housing allows for communications pass-thru when using batteries. Please note that, if not using battery power, the communication cable should be directly connected to the RoCSI. The pass-thru cable does not allow external power when connected to the battery housing communication bulkhead.

External Battery

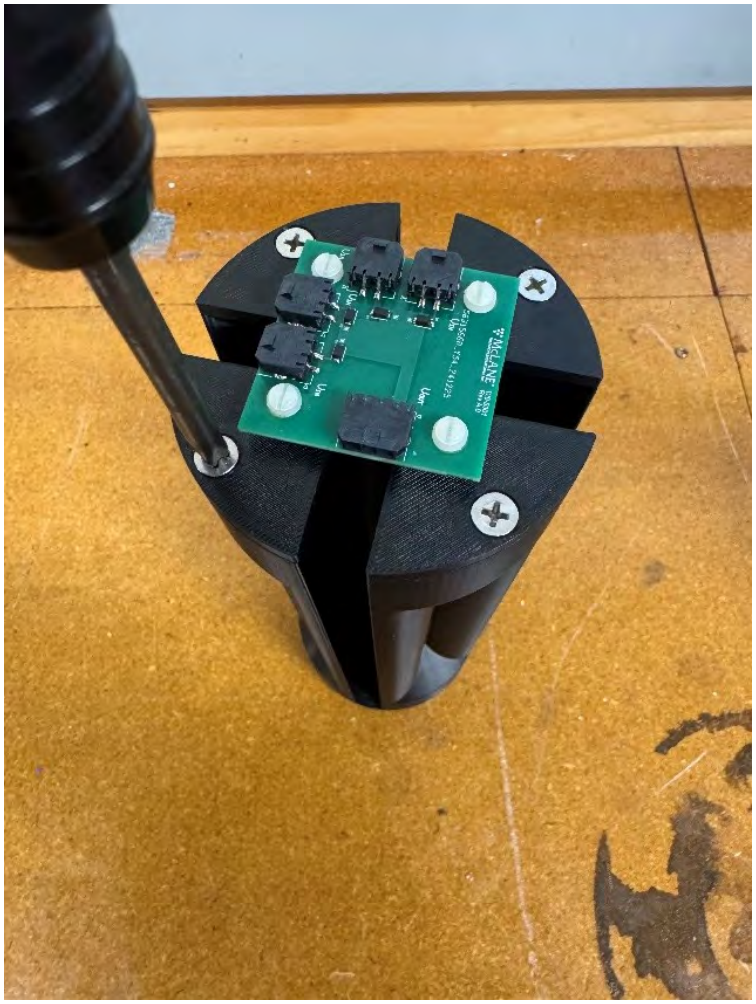
The external battery option includes a pressure housing and battery holder designed to accept 4 custom lithium primary battery packs. The bulkhead connections on the battery case allow for communications pass-thru while the RoCSI is powered by the batteries. If using external power (such as a power supply), plug directly into the RoCSI device; the communications bulkhead on the battery housing will not pass external power by design.

Never power the RoCSI using less than 4 battery packs. Never mix new and used battery packs. Always remove batteries from the pressure housing when not in use. Batteries should be stored in a cool, dry storage area, protected from excess moisture or humidity. Never store batteries in direct sunlight or in high heat. Dropped or damaged batteries should never be used with the RoCSI.

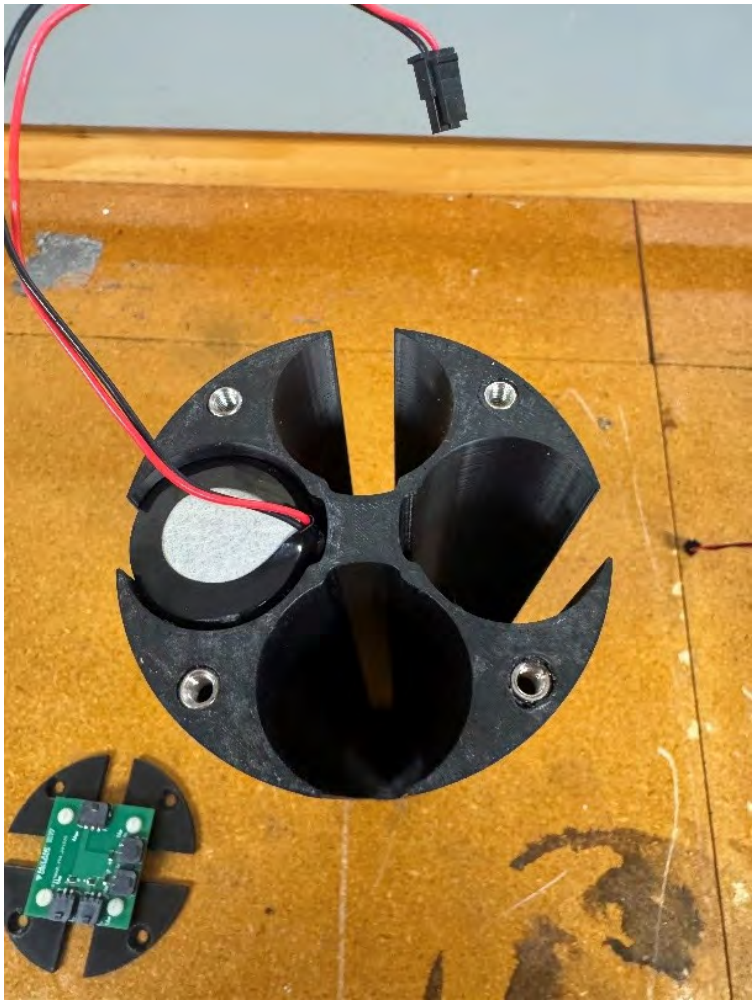
Installing Battery Packs:

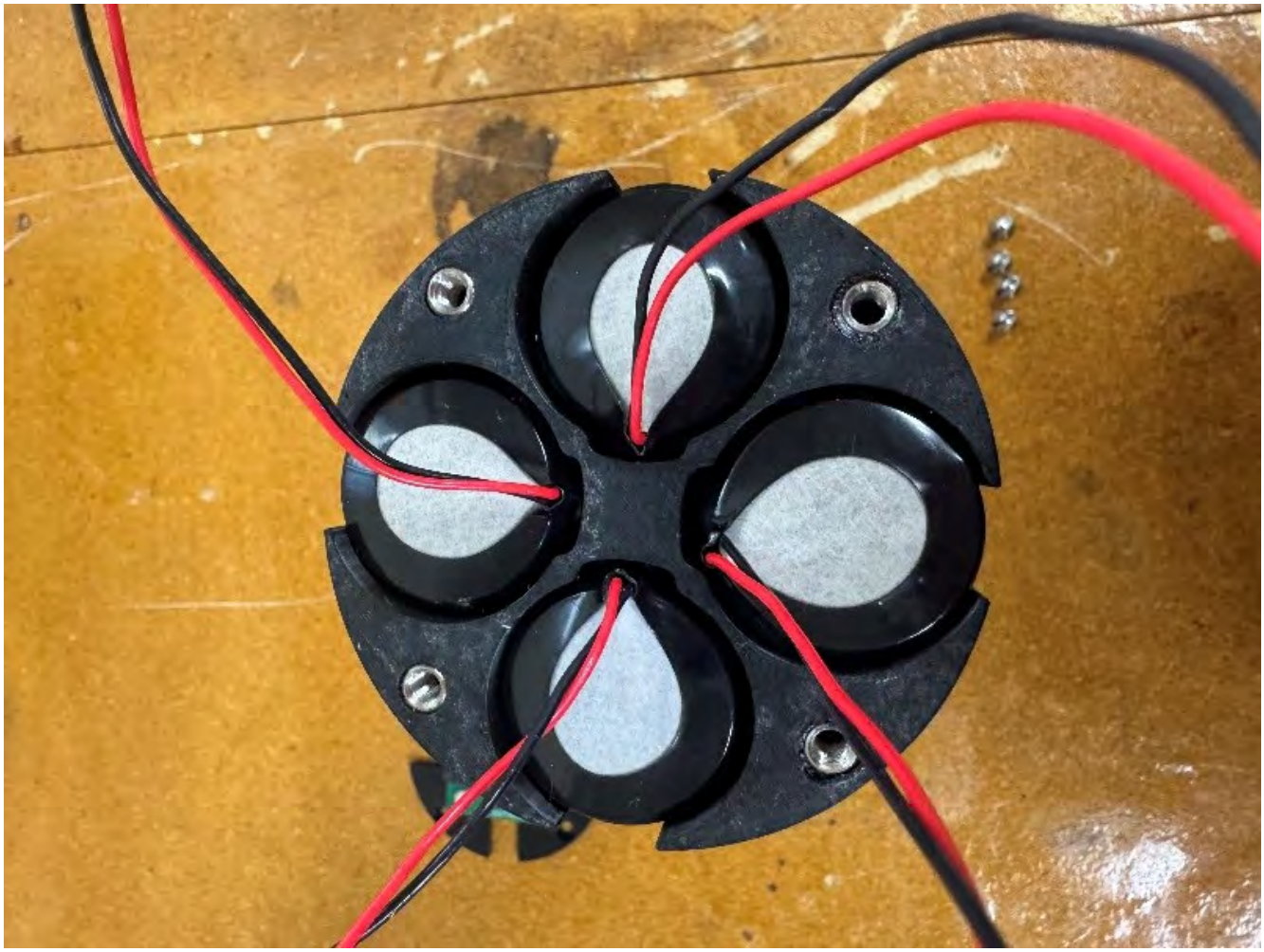
1. Remove the 3 screws on the battery housing end cap.

2. Remove the end cap from the housing.
3. Remove the battery pack holder from the battery housing. Unscrew the four Phillips head screws that secure the top plate to the battery pack holder.

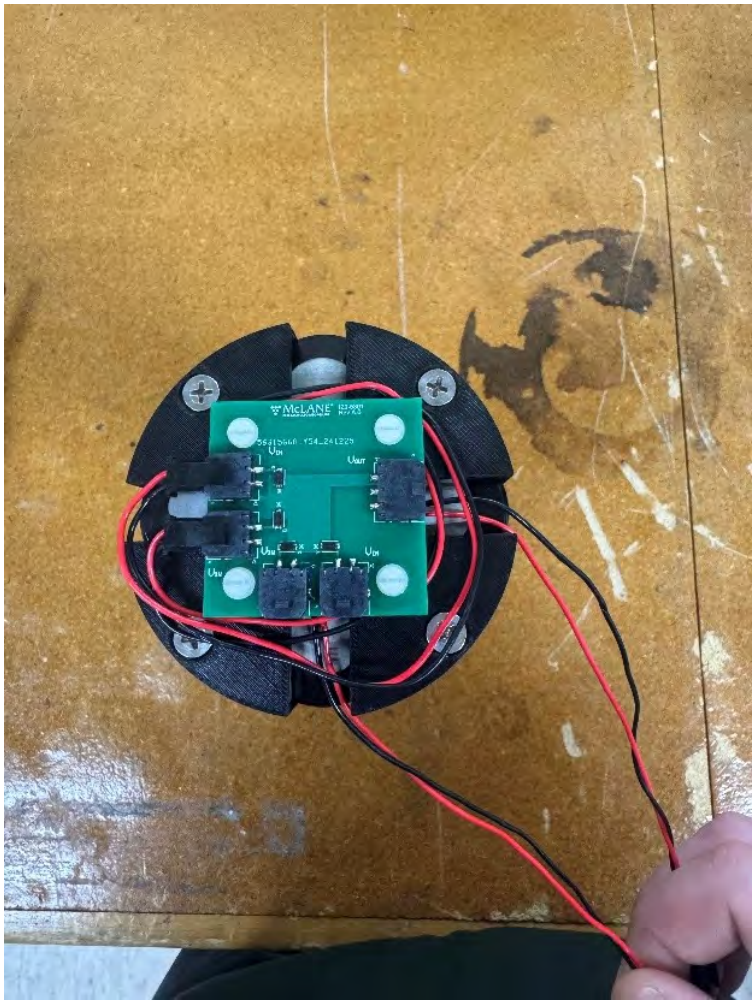


4. With the top plate removed, add the M4501 batteries to the battery pack. Ensure the wires of the battery are facing the center of the battery pack holder. This is to prevent the filled battery pack holder from getting stuck in the battery housing. Place the top plate back on the battery holder and screw the plate back onto the holder.

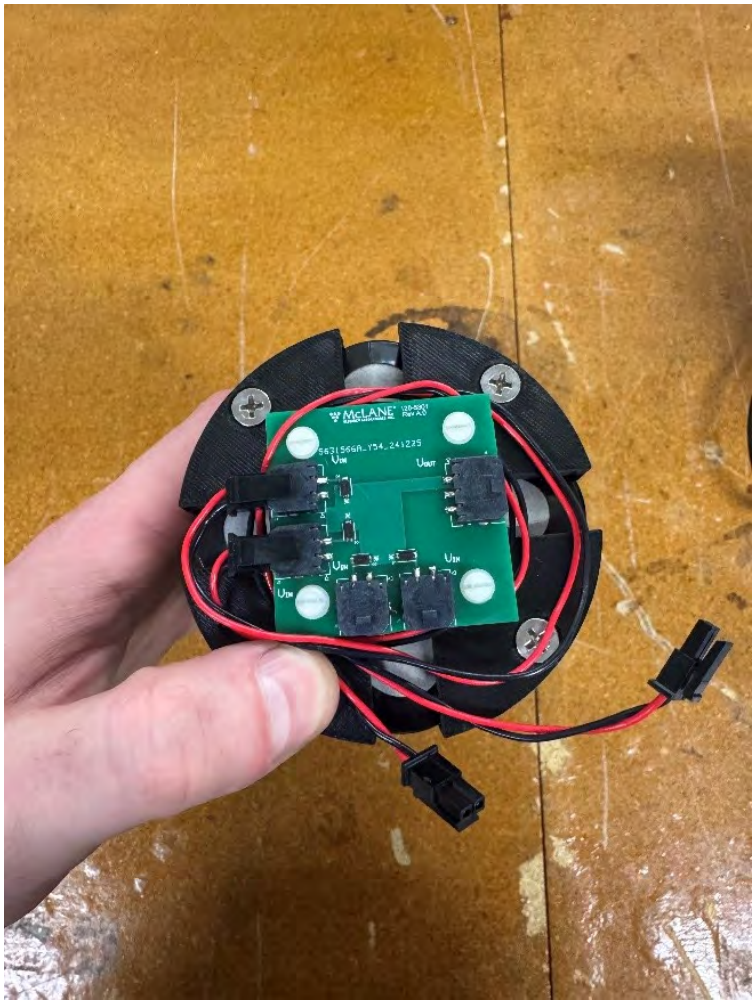




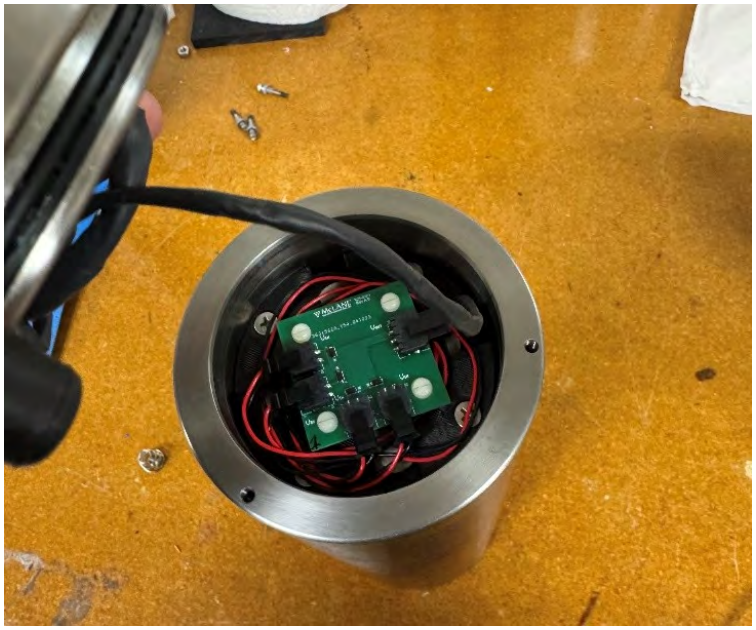
5. Take the 4 headers from the batteries and wrap them under the control board wrapping them clockwise. Two of the headers should line up with the J3 & J4 connectors of the control board.

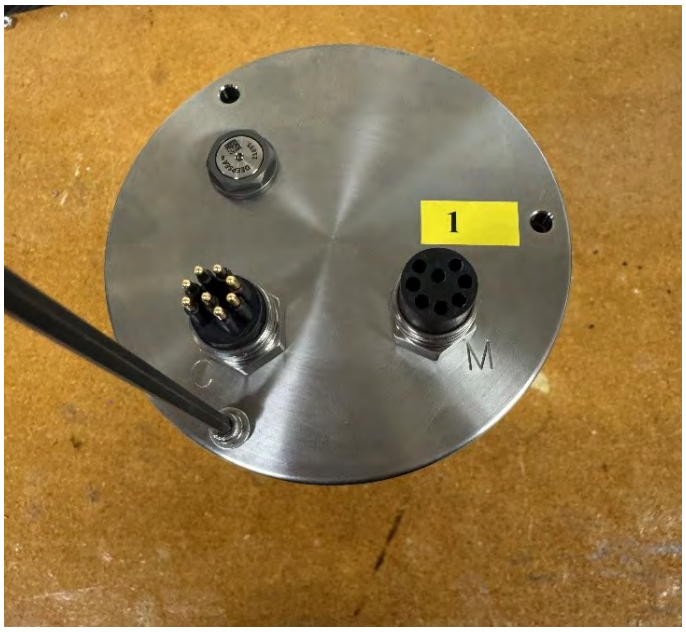


6. Wrap the remaining two headers counter clockwise and the two headers should line up with the J1 & J2 connectors on the control board.



7. Insert the fully loaded battery pack into the housing. Ensure that the corner between J2 & J3 lines up with the sticker on the housing and end cap. This is to ensure the rubber bumpers on the end cap do not land directly on the connectors. Plug in the 3-pin header on the bulkhead to J5 on the control board. Align the end cap sticker ("1") with the housing sticker.





Note: It is important to ensure that the controller housing is sealed correctly before performing operations with the RoCSI. O-rings and sealing surfaces should be regularly cleaned with isopropyl alcohol. Inspect O-rings for signs of wear and the presence of any foreign material (which can cause leaks). Look for small cracks and feel for grit, sand, or hair. Lubricate with a thin coating of provided Parker O-Lube as necessary. Spare O-rings are provided in the toolkit and additional O-rings may be purchased from McLane.

Keep hard objects such as tools or shackles away from the anodized controller housing. Scratches from these objects will localize galvanic action and can cause deep crevices or pitting.

Use caution to avoid scratching the O-rings, end cap, or the sealing surface of the vessel where the O-rings sit. Scratches could cause leaks.

Once the battery holder has been installed and connected to the end cap:

8. Carefully inspect the O-rings on the end cap for any foreign material or residue. Clean with a lint-free wipe and lubricate as needed.
9. Carefully inspect the O-ring sealing surface on the controller housing tube. Wipe away old or excess lubricant and verify the gland is clean and clear of debris or scratches. Lubricate this surface with a thin film of Parker O-Lube.
10. Slide the end cap into the controller housing, ensuring that the O-rings evenly enter the housing. Also, be careful to line up the index mark ("1") and confirm that the rubber bumpers will press against the battery holder and not the diode PCB or battery connectors.
11. Gently push on the end cap so the O-rings are completely engaged in the housing and no gap remains between the housing tube and the end cap.
12. Rotate the end cap to align the end cap bolt holes with the controller housing tube.
13. Install the end cap hardware. Ensure that the screw holes have plastic insulators installed before adding hardware. These screws should also be lubricated with non-metal anti-seize compound.
14. Secure the end cap screws and tighten enough that the split lock washers compress under the bolt heads. Be careful not to over-tighten.

Battery Pack Removal:

Take care in maintaining, operating, and opening the pressure housing. A pressure relief valve (PRV) on the controller housing end cap releases automatically at a pressure differential greater than 10 psi. The PRV has a center hole. A pressure release valve screw is included in the toolkit.

WARNING - Turning the release valve adjusts release pressure.

Always use proper care and attention when handling high-pressure vessels after deployment!

Treat the controller housing as pressurized until manually vented by the pressure relief valve.

1. Slowly pull on the pressure relief valve using the supplied pressure release valve tool to release any vacuum or built-up pressure in the housing.

2. Loosen each end cap bolt a few turns at a time in a star pattern.
3. When pressure is equalized, remove and place the end cap hardware somewhere safe. Typically plastic inserts have a snug fit and will remain in the end cap.
4. Grasp the end cap lip with fingertips and pull the end cap out of the housing. The end cap to housing seal is tight and sometimes difficult to open. Do not use a tool to pull open the housing. The end cap O-rings can be damaged if objects are used to separate the end cap from the housing. Be aware that the battery pack is connected to the end cap. Do not pull on the wires or connector when removing the end cap.
5. Disconnect the 3-pin connector from the battery control board and set the end cap aside.
6. Gently pull out the battery holder from the pressure housing.
7. Disconnect the 4 battery packs from the battery control board.
8. Remove the 4 screws from the top battery holder cap and remove the cap.
9. Remove the battery packs.

10. Recycle or dispose of used batteries according to local laws and regulations.

RoCSI-Autosub5 RS232 Comm Protocol

RoCSI-Autosub5 RS232 Interface

Purpose

This document defines a basic RS232 interface between the RoCSI eDNA sampler and the Autosub5 vehicle.

Contents

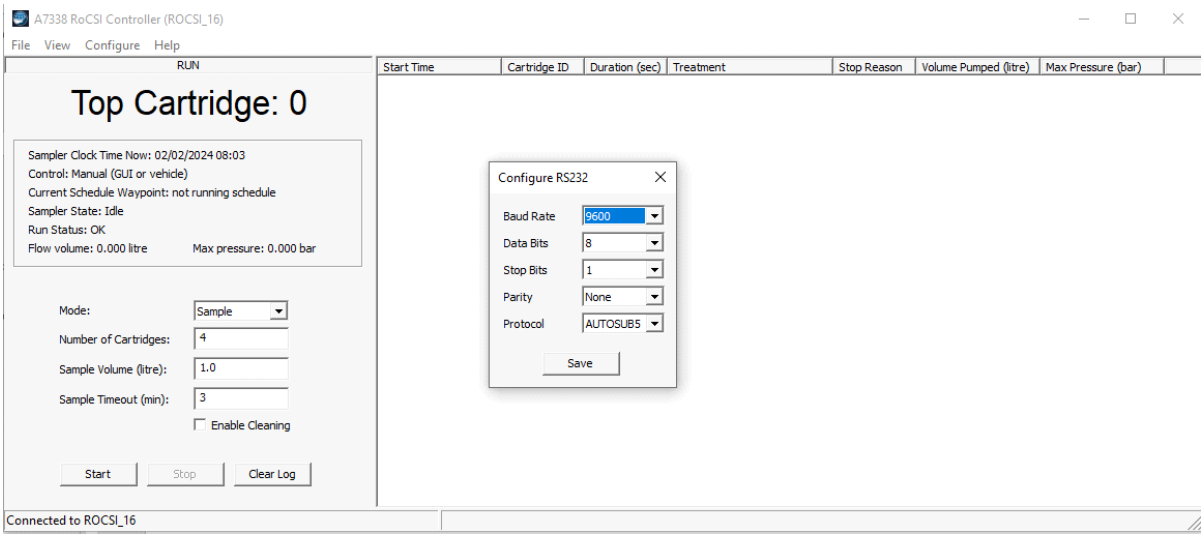
1 Common Message Protocol

In what follows, the VEHICLE is assumed to be Autosub5 and the SAMPLER is RoCSI.

1.1 RoCSI setup

1.1 RS232 Configuration

Using the USB version of Benchmaps, select **Configure** > **RS232.** Set the RS232 settings to



The RS232 connection protocol and settings are:

- 9600 bps
- 8 Data bits
- 1 Stop bit
- No Parity
- AUTOSUB5

Note: TX (from the SAMPLER) voltage levels will be around ±5V

Select **Save** to store these settings.

IMPORTANT – a power reset is necessary before attempting to communicate. Be sure to remove the external power source and the USB connection for a full power cycle. Wait at least 1 minute before reconnecting power.

1.2 Protocol

All communication between the VEHICLE and the SAMPLER consists of a command sent by the VEHICLE to the SAMPLER followed by a response sent by the SAMPLER to the VEHICLE.

The SAMPLER never transmits to the VEHICLE except in response to a command. On power-up, the SAMPLER will be ready to communicate with the VEHICLE within 500ms. After that, the SAMPLER will respond to all commands within 500ms and if it fails to do so, the VEHICLE should either retry the command, or power-cycle the SAMPLER.

All command packets from the VEHICLE to the SAMPLER and response packets from the SAMPLER to the VEHICLE consist of a binary packet of size 32 bytes. Unused bytes after the packet CRC (see below) will be set to zero in command and response packets.

The SAMPLER will allow the VEHICLE 100ms to transmit a complete command packet from receipt of its first byte to receipt of its command terminator. If this limit is exceeded, the SAMPLER will discard the packet without responding. This is to reduce the likelihood of responding to noise on the RS232 RX line. The VEHICLE is expected to do the same with response packets.

The SAMPLER will only respond to valid command packets.

1.3 Data Types

All command and response packets contain packed binary data. All multi-byte numeric parameters in the packets are little-endian. The following data types may be used in this document:

uint8	Unsigned 8-bit integer
int8	Two's complement signed 8-bit integer
uint16	Unsigned 16-bit integer
int16	Two's complement signed 16-bit integer
uint32	Unsigned 32-bit integer
int32	Two's complement signed 32-bit integer
float	IEEE 754 32-bit floating point number
double	IEEE 754 64-bit floating point number

1.4 Version

All commands described in this document are currently at version 1.

1.5 Sequence Number

All command and response packets contain a sequence number parameter. This is an arbitrary integer in the range 0-255 that must match between a command packet and its corresponding response packet to ensure that the response packet received is for the command packet just sent (e.g. following a timeout). The sequence number is generated by the VEHICLE and will typically start at zero, increment with each command sent and wrap back to zero after reaching 255.

1.6 CRC (checksum)

All command and response packets also contain a CRC (checksum) parameter. This is an unsigned 16-bit value calculated using the CRC16 CCITT algorithm (with initial value 0x0000) from all bytes in the command or response packet preceding (and excluding) the CRC parameter itself. Example code for this algorithm is given below but libraries for SD card drivers or XMODEM protocol drivers would also include a reliable implementation. Beware that not all implementations available on the Internet are reliable.

1.6.1 Function

```
uint16_t crc16_ccitt(const uint8_t *data, uint32_t data_length)
{
```

```
uint32_t i;
uint16_t crc;
crc = 0;
for (i = 0U; i < data_length; i++)
{
crc = (crc << 8) ^ crc16_ccitt_table[(crc >> 8) ^ data[i]];
}
return (crc);
}
```

1.6.2 Lookup-table

```
static const uint16_t crc16_ccitt_table[] =
{
0x0000, 0x1021, 0x2042, 0x3063, 0x4084, 0x50a5, 0x60c6, 0x70e7,
0x8108, 0x9129, 0xa14a, 0xb16b, 0xc18c, 0xd1ad, 0xe1ce, 0xf1ef,
0x1231, 0x0210, 0x3273, 0x2252, 0x52b5, 0x4294, 0x72f7, 0x62d6,
0x9339, 0x8318, 0xb37b, 0xa35a, 0xd3bd, 0xc39c, 0xf3ff, 0xe3de,
0x2462, 0x3443, 0x0420, 0x1401, 0x64e6, 0x74c7, 0x44a4, 0x5485,
0xa56a, 0xb54b, 0x8528, 0x9509, 0xe5ee, 0xf5cf, 0xc5ac, 0xd58d,
0x3653, 0x2672, 0x1611, 0x0630, 0x76d7, 0x66f6, 0x5695, 0x46b4,
0xb75b, 0xa77a, 0x9719, 0x8738, 0xf7df, 0xe7fe, 0xd79d, 0xc7bc,
0x48c4, 0x58e5, 0x6886, 0x78a7, 0x0840, 0x1861, 0x2802, 0x3823,
0xc9cc, 0xd9ed, 0xe98e, 0xf9af, 0x8948, 0x9969, 0xa90a, 0xb92b,
0x5af5, 0x4ad4, 0x7ab7, 0x6a96, 0x1a71, 0x0a50, 0x3a33, 0x2a12,
0xdbfd, 0xcbdc, 0xfbbf, 0xeb9e, 0x9b79, 0x8b58, 0xbb3b, 0xab1a,
0x6ca6, 0x7c87, 0x4ce4, 0x5cc5, 0x2c22, 0x3c03, 0x0c60, 0x1c41,
0xedae, 0xfd8f, 0xcdec, 0xddcd, 0xad2a, 0xbd0b, 0x8d68, 0x9d49,
0x7e97, 0x6eb6, 0x5ed5, 0x4ef4, 0x3e13, 0x2e32, 0x1e51, 0x0e70,
0xff9f, 0xefbe, 0xdfdd, 0xcffc, 0xbf1b, 0xaf3a, 0x9f59, 0x8f78,
0x9188, 0x81a9, 0xb1ca, 0xa1eb, 0xd10c, 0xc12d, 0xf14e, 0xe16f,
0x1080, 0x00a1, 0x30c2, 0x20e3, 0x5004, 0x4025, 0x7046, 0x6067,
0x83b9, 0x9398, 0xa3fb, 0xb3da, 0xc33d, 0xd31c, 0xe37f, 0xf35e,
0x02b1, 0x1290, 0x22f3, 0x32d2, 0x4235, 0x5214, 0x6277, 0x7256,
0xb5ea, 0xa5cb, 0x95a8, 0x8589, 0xf56e, 0xe54f, 0xd52c, 0xc50d
0x34e2, 0x24c3, 0x14a0, 0x0481, 0x7466, 0x6447, 0x5424, 0x4405,
0xa7db, 0xb7fa, 0x8799, 0x97b8, 0xe75f, 0xf77e, 0xc71d, 0xd73c,
0x26d3, 0x36f2, 0x0691, 0x16b0, 0x6657, 0x7676, 0x4615, 0x5634,
0xd94c, 0xc96d, 0xf90e, 0xe92f, 0x99c8, 0x89e9, 0xb98a, 0xa9ab,
0x5844, 0x4865, 0x7806, 0x6827, 0x18c0, 0x08e1, 0x3882, 0x28a3,
0xcb7d, 0xdb5c, 0xeb3f, 0xfb1e, 0x8bf9, 0x9bd8, 0xabbb, 0xbb9a,
0x4a75, 0x5a54, 0x6a37, 0x7a16, 0x0af1, 0x1ad0, 0x2ab3, 0x3a92,
0xfd2e, 0xed0f, 0xdd6c, 0xcd4d, 0xbdaa, 0xad8b, 0x9de8, 0x8dc9,
0x7c26, 0x6c07, 0x5c64, 0x4c45, 0x3ca2, 0x2c83, 0x1ce0, 0x0cc1,
```

```
0xef1f, 0xff3e, 0xcf5d, 0xdf7c, 0xaf9b, 0xbfba, 0x8fd9, 0x9ff8,
0x6e17, 0x7e36, 0x4e55, 0x5e74, 0x2e93, 0x3eb2, 0x0ed1, 0x1ef0
};
```

See example message and CRC result in Section 3.

2 SAMPLER Commands

2.1 START (1)

Requests the SAMPLER to start taking a number of samples each from a specified flow volume. If any given sample doesn't process the required volume within the timeout, it should be terminated early and preserved and any remaining samples should be processed as normal. The SAMPLER will return a response if and only if the command packet is valid. If the SAMPLER is already running or there is insufficient supply voltage to run the sampler this command will return an error. The START operation itself is asynchronous and the VEHICLE should use the STATUS command to monitor progress if this command returns successfully.

Command packet format:		
Parameter	Description	Type
<CMD>	Command ID	uint8 (1)
<SEQ>	Sequence number (see above)	uint8 (0-255)
<CLEAN>	Cleaning flag: if true (1) clean before sampling	uint8 (0-1)
<COUNT>	Number of samples to collect (i.e. cartridges to process)	uint8 (0-255)
<VOL>	Volume to process per sample (ml)	uint16 (0-65535)
<TIMEOUT>	Timeout (min) per sample if flow volume isn't reached	uint16 (0-65535)
<TSTAMP>	Timestamp (VEHICLE time as seconds since Unix epoch)	uint32
<CRC>	CRC checksum (see above)	uint16
padding	zero-valued bytes to end of packet	

Response packet format:		
Parameter	Description	Type
<CMD>	Command ID (same as in command packet)	uint8 (as command)
<SEQ>	Sequence number (same as in command packet)	uint8 (as command)
<STATUS>	Command exit status (0 = command succeeded, 1 = command failed, other values reserved)	uint8 (0-255)
<CRC>	CRC checksum (see above)	uint16
padding	zero-valued bytes to end of packet	

2.2 STOP (2)

Requests the SAMPLER to stop sampling and preserve the current sample (if any). The SAMPLER will return a response if and only if the command packet is valid – the STOP operation itself is asynchronous and the VEHICLE should use the STATUS command to monitor progress if this command returns successfully. The sampler may take up to 180 [TBC] seconds to complete the asynchronous STOP operation.

Command packet format:		
Parameter	Description	Type
<CMD>	Command ID	uint8 (2)
<SEQ>	Sequence number (see above)	uint8 (0-255)
<CRC>	CRC checksum (see above)	uint16
padding	zero-valued bytes to end of packet	

Response packet format:

Parameter	Description	Type
<CMD>	Command ID (same as in command packet)	uint8 (as command)
<SEQ>	Sequence number (same as in command packet)	uint8 (as command)
<STATUS>	Command exit status (0 = command succeeded, 1 = command failed, other values reserved)	uint8 (0-255)
<CRC>	CRC checksum (see above)	uint16
padding	zero-valued bytes to end of packet	

2.3 STATUS (3)

Requests the current operational status of the sampler. The SAMPLER will return a response if and only if the command packet is valid.

Command packet format:		
Parameter	Description	Type
<CMD>	Command ID	uint8 (3)
<SEQ>	Sequence number (see above)	uint8 (0-255)
<CRC>	CRC checksum (see above)	uint16
padding	zero-valued bytes to end of packet	

Response packet format:		
Parameter	Description	Type
<CMD>	Command ID (same as in command packet)	uint8 (as command)
<SEQ>	Sequence number (see above)	uint8 (as command)
<STATE>	Run state of sampler (see below)	uint8 (0-255)
<CARTRIDGE>	Cartridge ID currently in sample slot uint16	(0-65535)
<VOLTS>	Supply voltage (volts)	float
<TEMP>	Housing temperature (degrees celsius)	float
<RH>	Housing relative humidity (percent)	Float

<CRC>	CRC checksum (see above)	uint16
padding	zero-valued bytes to end of packet	

Sample run states currently defined are shown below (others may be added in future). The sampler will enter Idle state on power-up (i.e. when 12 Vdc supply enabled) and on completion of START or STOP commands only.

0	Unknown
1	USB power only (i.e. supply less than 6V, enough to give status info but not run)
2	Idle
3	Loading a cartridge
4	Engaging a cartridge for sampling
5	Disengaging a sampled cartridge
6	Engaging a cartridge for preservation
7	Disengaging a preserved cartridge
8	Pumping sample through a cartridge
9	Pumping preservative through a cartridge
10	Cleaning the sample lines
11	Waiting for next sample time

3 Example message and CRC results

3.1 Status Message

Parameters:

<ID>	3
<SEQ>	0

uint8 byte array (hex)

0x03 0x00

Resulting CRC = 0x53 0x55

Message to send (Hex)

0300 5355 0000 0000 0000 0000 0000 0000 0000 0000 0000 0000 0000 0000 0000 0000

3.2 Start Message

Parameters:

<ID>	1
<SEQ>	0
<CLEAN>	1
<COUNT>	12
<VOL>	1000
<TIMEOUT>	30
<TSTAMP>	1706782210 (01-FEB-2024 10:00:00 GMT)

uint8 byte array (hex)

0x01 0x00 0x01 0x0C 0xE8 0x03 0x1E 0x00 0x02 0x6E 0xBB

Resulting CRC = 0x90 0x66

Message to send (Hex)

0100 010C E803 1E00 026E BB65 9066 0000 0000 0000 0000 0000 0000 0000 0000 0000

3.3 Stop Message

Parameters:

<ID>	2
<SEQ>	0

uint8 byte array (hex)

0x02 0x00

Resulting CRC = 0x62 0x66

Message to send (Hex)

0200 6266 0000 0000 0000 0000 0000 0000 0000 0000 0000 0000 0000 0000 0000 0000