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Introduction

With the µFlow perfusion system we address the need for a perfusion system that can handle micro liter volumes of solutions. Taking technology used in our internal pipette perfusion system (2PK+), we designed the µFlow system. This enables us to bring the reservoirs closer to the front-end manifold to minimize the dead volume and to minimize the reservoir volume.

The µFlow system, available in 4 or 8 channels, is designed to provide a fluid application system that preserves precious drugs and substances and allows them to be applied directly to a single cell or small group of cells under study with imaging or the patch clamp technique. Only micro-dimensional check valves are used in the fluid pathway to prevent back-flow. Approximately 25cm of 100um ID quartz tubing that goes directly from the 0.5ml reservoirs to the 100um output tip that is placed in close proximity to your cell. This way “dead volume” is minimized to about 2uL per channel and there are no electronic valves to increase dead volume or retain sticky substances. For fluidic control we rely on a system of pneumatic control valves that port pressure to the reservoirs to push solution out. The resistance to flow of the small dimension provides the fluidic control.

Typically one reservoir is reserved as a wash reservoir and that will be pressurized with the low pressure output. This pressure becomes active whenever the other valves are off, as long as the pressure switch is on.

We use our VC3 interface to control the valves in the µFlow pressure interface. The VC-3 is a proven valve controller with many convenient features. It is easy to use and very reliable for these types of experiments.
System Components

Pressure Control Interface

The µFlow perfusion system’s pressure control interface is an integral part of the system’s ability to supply pressure to drive the solutions as well as to maintain a balance pressure to stop solution flow.

The **High Pressure**, “Drive Pressure” on the front panel, gives up to 50psi (3.45bar) of pressure. It consists of an analog pressure gauge, a pressure regulator and bleed switch. This pressure is used to push solution out during experiments.

Turning the regulator knob clockwise will allow air pressure to flow into the internal valve manifold. The more pressure used the higher the flow rate. Use the VC3 valve control interface to control the internal valves to start and stop solution flow. The Gauge indicates the amount of pressure present at the valves.

The pressure can be easily released from the system by moving the air switch down. This will bleed the pressure in the system to atmosphere.

The **Low Pressure**, “Balance Pressure” on the front panel (up to 10psi (.690bar)) consists of an analog pressure gauge, a pressure regulator and bleed switch. The balance pressure is used to supply pressure to one reservoir (usually number 1) to provide a wash solution that keeps the other solutions at bay in their respective tubes. It can also be used in other ways in different configuration described later in this manual.

The µFlow rear panel consists of a pressure input that should be connected to a source of clean air. NO FLAMMABLE GASES CAN BE USED, EVER! There is a valve power input, and another plug for a 12V input that comes from the 12V aux output of the VC3 controller that is used to power the valve that controls the low pressure to the wash reservoir. Finally, the multi port tubing connector brings all the pressure to the reservoirs.
Front-End manifold

The Micromanifold® is made up of quartz capillaries bonded together to form as many channels as needed into a single output. The Micromanifold® has been modified to optimized the micro volume perfusion system. The µFlow Micromanifold has 100 micron id channels as well as the tip. The tips are removable to ease cleaning as well as replacing. Due to the small ID’s of the Micromanifold®, it is recommended that they be used with pressure systems.

(image not of the uFlow Micromanifold® which does not have the silicone connectors)

The Micromanifold used in the uFlow (QMM-4/8UF) has extended tubes that go all the way back to the reservoirs. All the tubes come together in the front tip which has an output of 100um, but can be ordered with a 200um tip as well. The front end manifold is mounted in an adjustable holder to allow it to be positioned properly.

Reservoir Manifold

The µFlow reservoir manifold uses 500µL reservoirs that are easily replaced when needed. Solution is placed into the reservoir which is then screwed into one of the ports on the manifold. Female Luer’s are located on the top of the manifold to connect the pressure tubing to each individual reservoir. A built-in compression fitting is used to hold each channel of the Front-end manifold. Each polyimide coated quartz capillary tube is inserted into the compression fitting, through the soft silicone gasket.

The tube needs to be gently pushed through the gasket. Apply a small amount of force to pierce the gasket, and then push the tube all the way down to the bottom of the reservoir, until the tube cannot go any farther. Then tighten up the nut on the compression fitting making it just finger tight.
The uFlow is equipped with micro-dimension check valves that insure that fluids cannot flow back into adjacent reservoirs and contaminate them. These valves are located at the end of the quartz capillary tubes and actually reside in the fluid reservoirs. Micro valves need to be removed and installed whenever the Micromanifold is exchanged or installed.

**Micro Valve at end of quartz capillary tube**

To clean a Micro valve:

Remove the Micro valve from the quartz capillary, and using a syringe with a 1mm ID length of silicone tubing, inject water into the valve. About 1ml is all that is needed to flush out the valve. After flushing you can test the Micro valve by pulling back on the syringe. If the valve is working, the plunger will not remain extended after letting go.

Micro Valves add about 4.5uL to the total volume in each line.
1. **Soft Power Button with LED** - Green LED indicates when system is powered. It is also used to reset the spill sensor alarm.

2. **Channel LED** - Above each Valve button is a green LED that indicates when the power to that particular valve is ON.

3. **Valve Switches** - membrane switch (channels 1-8) for valve activation. Default valve switch setting is ON-OFF.

4. **MOMENTARY** - Sets all Valve switches to “MOMENTARY ON” switches.

5. **SPILL DETECTED indicator** - LED will light when a spill is detected by the spill sensor cable connected to the rear of the controller.

6. **LATCHING** - Sets the controller to operate in Latching mode. When active the valve that is ON will be switched OFF when any other valve is switched ON.

7. **TTL ON** - Sets the controller to accept TTL pulses to control valves via the Digital I/O port on the controller rear panel.
8. **AUX OUT 12V DC**- Port for additional 12V dc valve connection. Positive center pin.

9. **DC Power Input** - 15VDC @ 3.3A.

10. **Power Switch** - Main power switch to turns VC³ controller ON/OFF.

11. **FUSE** - Controller fuse. 3.15A 250 V Fast Blow 5x20mm fuse.

12. **SPILL SENSOR INPUT** – Connector for the spill sensor cable.

13. **GND** - Port (banana jack) to connect controller to ground.

14. **TO VALVES** - DB-9 FEMALE connector to connect controller to valve manifold.

15. **DIGITAL INPUT** - DB-9 MALE connector for TTL input control of valve channel 1-8 via DB-9 to BNC breakout cable.

16. **USB** – port to connect controller to PC via USB cable.

17. **ANALOG IN** - BNC connector to control valves via an analog voltage input in 0.5 V steps with a +/-100mv threshold.

18. **ANALOG OUT** - BNC connector that outputs an analog voltage in 0.5 V/ valve steps.

19. **TTL SYNC OUT** - BNC connector that outputs a TTL high signal whenever a valve is ON.
VC³ Controller Instructions

Power Buttons

The VC³ valve controller has two power switches. The main power is located on the rear of the unit. This rocker switch will turn input power ON or OFF to the system.

The other power switch is a soft power button located on the membrane panel. This switch serves a dual purpose. First it is used to turn power ON or OFF to the membrane panel controls. Secondly it is used as a reset button for the spill sensor. When the spill alarm is activated, press the soft power button to reset it. Activating the soft power button will also calibrate the spill sensor to its new capacitive value. (The sensor should be cleaned and dried before calibration)

Valve Control Modes

The VC³ is a four or eight-channel 12V DC valve controller. The control box has a membrane panel with pushbutton switches to control the valves and other functions. The controller is designed to control in one of three ways, Manual switch, TTL input or Analog input. The controller can also be enabled to control latching valves.

The VC³ is designed with Low voltage circuitry that will automatically drop the valve voltage after opening the valve. This option is for researchers who are concerned about the transmission of heat to solutions from the warming of valves.

Manual ON/OFF Control:

Manually each valve is controlled by an individual membrane panel switch. Each switch is numbered, 1 through 8, corresponding to each valve. Press the membrane switch once to energize “turn ON” the corresponding valve. The valve will remain on until the membrane switch is pressed again. Press the membrane switch again to de-energize, “turn OFF”, the valve. A green LED will indicate when a switch is ON.

The controller is able to activate all eight valves at the same time. This aids in flushing out the reservoirs during a cleaning procedure. It is not recommended to activate more than one valve at a time during an experiment since solution mixing cannot be measured accurately.

Manual MOMENTARY Control:

To enable the membrane switches to act as momentary switches press the “MOMENTARY” button on the controller. A green LED will indicate the momentary setting is enabled. In the momentary mode you must press and hold down the membrane switch to energize, turn ON, a valve. As long as you hold down the switch the valve remains ON. Release the switch and the valve turns OFF.
TTL Mode Control:

The VC\textsuperscript{3} controller has a digital I/O port to enable valve control via TTL signals. This port allows you to control each valve using a single TTL “high” signal. To enable this feature press the TTL ON button on the front panel. A green LED will indicate the TTL mode is enabled. The digital I/O port is a DB-9 Male connector on the rear of the controller.

The DB-9M to BNC breakout cable, included with the system, allows the VC\textsuperscript{3} to be controlled by most acquisitions systems’ digital outputs.

Multiple valve control is possible by using this port. This is useful when priming the system reservoirs and when running a cleaning procedure.

ANALOG Voltage Control:

A BNC connector is used to input an analog voltage to control the valves. An analog voltage from 0.5V to 4.0 V dc in 0.5V steps will control valves 1-8, respectively. There is a +/-100mV threshold.

To operate in this mode, the “MOMENTARY” mode switch must first be enabled. Connect the ANALOG IN port to your acquisition system analog out port via a BNC male cable to control all valves.

Latching Valves Control:

The VC\textsuperscript{3} controller can be set to latching mode. By pressing the “LATCHING” button on the controller the green led will indicate that the controller is in latching valve mode.

When active the valve that is switched ON will be switched OFF when any other valve is switched ON. Only one valve can be ON at a time in this mode. This feature allows for fast manual solution switching by eliminating the need to switch a valve OFF before switching another ON.

Rear Panel Connections

TTL SYNC OUT:

The sync out BNC port can be used as either a TTL marker or to sync (trigger) the controller to another device.

A TTL high (+5V) signal is output whenever a valve is turned ON (energized).
ANALOG Voltage Control:

As stated above (Control Modes section), this feature allows for the control of valves via an analog input. A BNC connector is used to input an analog voltage to control the valves. An analog voltage from 0.5V to 4.0 V dc in 0.5V steps will control valves 1-8, respectively. There is a +/-100mV threshold.

To operate in this mode, the “MOMENTARY” mode switch must first be enabled. Connect the “ANALOG IN” port to your acquisition system analog out port via a BNC male cable to control all valves.

ANALOG OUT:

BNC connector is used to output an analog voltage to representing a valve opening. An analog voltage from 0.5V to 4.0 V in 0.5V steps represents valves 1-8, respectively.

AUX OUT:

The AUX OUT port outputs 12V dc whenever any valve is turned ON. This port can be used to connect an additional 12V dc valve. Center pin on the connector is positive (+).

USB:

The USB connector is used to connect the VC³ controller to a computer. This port is used for programming and maintenance at the factory only.

DIGITAL IN:

The digital input is used to control the valves via a TTL signal. The DB-9 male connector pins 1-8 is used to input TTL signals to valves 1-8, respectively, with pin 9 being common ground.

An optional DB-9F to 8BNC breakout cable can be used to connect the VC³ to an acquisition systems digital output.

**DB-9M to BNC Breakout cable:**

The TTL valve control feature uses a DB-9Female connector as its input. To facilitate connection to the digital outputs of an acquisition system the DB9BNC4 or DB9BNC8 cable can be used.
Cable is color coded according to above image. Red corresponds to valve1, green to valve 2 and so forth. Connect the DB-9Female connector to the rear of the VC³ controller. Connect the BNC ‘s to the digital outputs of your acquisition system.

**TO VALVES:**

This DB-9 Female port is used to connect the controller to the valve manifold (VM-4/8).

A DB-9M/M cable is supplied with the VC³ perfusion system. Pins 1-8 correspond to valves 1-8, respectively. Pin 9 is the common +12 V DC.

**DC POWER:**

The DC power jack is the main power input to connect the universal 15V DC power supply supplied with the VC³ system.

**POWER ON/OFF:**

Power ON/OFF switch will turn the main power to the controller ON or OFF.

**GND:**

The VC³ controller can be connected to ground via a banana jack on the rear panel.

**FUSE:**

The VC³ fuse holder is located on the rear panel and uses 5x20mm fuses. The rated value for the VC³ controller is a 250V 3.15A fuse.

Replace fuse by using a straight edge screw driver to twist off fuse holder cap. Use fuse of stated rated value only.
SPILL SENSOR INPUT:

The VC³ has been designed with a built in spill sensor. This feature will allow for the protection against overflows from chamber or dishes onto expensive optics or electronics.

To use this feature simply connect the spill sensor cable supplied with the VC³ system to this port. Place the sensor wire around the area you wish to protect from liquid spills and turn ON the controller.

The VC³ controller auto calibrates the spill sensor every time it is turned ON. Therefore, it is important that the spill sensor cable be connected before the controller is turned ON.

If a spill occurs the sensor will detect it and two things will happen.
1) There will be a soft power shut down. This means power to the valves will be cut off. The main power will still be ON.
2) An audible and visual alarm will be activated. The audible alarm will be a chirping sound. The visual alarm is a red LED on the controller will blink repeatedly.

To reset the system after a spill

After a spill the alarm will sound and power will be cut to the valves. Follow the procedure below to reset the controller:

1) Press the Soft power switch on the controller panel. This will turn the alarm off.
2) Turn OFF main power on rear of controller.
3) Remove spill sensor cable from controller.
4) It is important to carefully clean and dry the sensor wire after a spill. Depending on what was spilled on the sensor, use distilled water to wash off any solution on the sensor. Carefully dry the sensor with an absorbent towel (paper towel).
5) Once dry replace spill sensor cable on the controller.
6) Turn main power ON from rear of controller.
7) Turn soft power switch ON from controller membrane power.
8) Controller will calibrate itself to the spill sensor cable.
9) System is now ready to be used again.

It is sometimes necessary to turn soft power OFF and ON after following above procedure for the system to calibrate.

1. Turn ON main power on control box by flipping rocker switch on the rear of the control box.
2. Press the soft power button on the control membrane panel. Green LED will light.
3. Your system is ready for manual use. Press membrane panel switches on top of the control box to open/close valves.

To digitally control the valves you will need to use our DB-9F to 4/8BNC breakout cable. Connect the DB-9F to the VC³’s digital I/O port and the BNC connectors your data acquisition system TTL outputs (digital). Follow the steps on your data acquisition system to set up digital pulses.
Assembly Instructions

There are three main components of the uFlow. Each must be placed properly. The VC3 controller should be placed in a location where it can be accessed by hand. The pressure controller will need to be located close to the microscope stage since the air pressure tubes are not very long. Then, the reservoir bracket and the Micromanifold need to be placed on a micromanipulator so that the tip of the Micromanifold can be pointed at the cell(s) under study.

The reservoir bracket and Micromanifold must be mounted so that the Micromanifold can reach the prep, to minimize flow due to gravity mount the Micromanifold level with the reservoirs.

The manifold assembly comes mounted to a 6mm bar that can fit in most manipulators. The entire assembly in an 8 channel system will be about 150g, so be sure that the micromanipulator can handle it. Try to keep the assembly horizontal and move the parts around using the adjustment points shown above to keep the output tip at the same level as the fluids in the reservoirs.
The photo below summarizes the important connections that need to be made on the back of the instruments.

**Flush valve power.** This valve turns on whenever any valve in the system is selected. Power is off when all valves are off.

**Multi tube connector**
This is a locking fitting so the two parts must be pressed together, and then the lock slides to secure the two parts.

Main valve power cable

Input pressure
Experimental setup - Solution Delivery

Overview

The uFlow is a pressure driven solution delivery system that takes advantage of our Micromanifold® technology to create the “sewer pipe effect” whereby there is a large stream of liquid flowing that completely submerges the cell under study. There are no liquid control valves or check valves in the fluid line to keep the volume down to about 2uL per channel. As a result, control comes from switching on and off a series of pneumatic valves that deliver air pressure to the sealed reservoirs on the manifold. The air pressure forces the solution out through the small tube. Since the tubing is small and rather long (25cm) several 2-10psi minimum of pressure is needed to move solution quickly. However, solution can move slowly by other forces such as gravity and diffusion which we will discuss later.

Filling and Installing Reservoirs

Once your uFlow is set up and the manifold is in position, ready to begin puffing, remove the reservoirs by unscrewing them. As you do, check that each capillary tube comes down to the bottom of each reservoir. The wash reservoir may have a bigger volume so that tube will need to extend down farther. Be sure all air switches are turned off when you do this. Fill each reservoir and return it to the manifold and screw it into position. Finger tight is adequate. When all reservoirs are in position, check the tip. It should be below the surface of the prep bath. See that the tip and the fluid in the reservoirs are at about the same height, make any adjustments if necessary.

Priming the System

Next, switch on both air pressure sources. (If no pressure is connected to the system, this will be apparent at this point because as you turn the regulators you will not see any pressure on the gages.) Set the low pressure to about 2-3psi and the high pressure to about 20psi to start. As soon as the low pressure is on, wash solution should start to flow out. You can stop if by turning off the low pressure, or just turning on valve 1.

Once the wash solution is flowing, systematically go through each channel, one by one turning it on with the VC3 controller (It is best to place the controller in Latching mode where the latter valve turns the former off, for this procedure). As you turn on each valve, the 20psi of pressure will be applied and the solution will begin to flow after some air bubbles come out first. If this is your first time setting up the system, you may want to add some food dye in the system to get familiar with the flow. Just for reference, the system will emit about 100uL of plain water at 20psi in about 1 minute. Priming is done when there are no more air bubbles coming out. If an air bubble should get stuck to the top of the applicator tip, you can tap the metal rod with a small screwdriver and it will release.

Output flow can also be observed by lifting the tip out of the bath and observing the formation of droplets at the tip.
The system is delivered in a configuration where reservoir 1 is for flushing the system and helping to prevent cross talk. There are two other ways you system can be configured, they will be discussed later.

Reservoir 1 can also be equipped with a larger reservoir to hold more solution, even though it uses a lower drive pressure than the other reservoirs, it will be the one flowing the most. Set the drive pressure low (1-2psi) to conserve solution.

**Controlling Reservoir 1**

Pressure to reservoir 1 will be cut whenever power is sent to any valve in the system. You can use the button for valve 1 to stop the flow of reservoir 1, or you can just turn off the pressure switch. Depending on your experimental requirements, you can decide whether to keep the flow from reservoir 1 on or off most of the time. Low pressure used to drive the wash from reservoir 1 can also cause cross talk. Keep the tip level with the reservoirs and turn off all pressure when not needed.

**Experimental Protocol**

A typical experimental protocol will involve applying a single dose or multiple doses of solution on to your cell prep. You can do this manually by pressing buttons on the valve controller, or you can use the VC3 software with a USB port to create a sequence, or you can slave the VC3 from another data acquisition system. The amount of pressure you apply determines the flow rate, but you will need to determine what flow rates are best for your prep. Sometimes higher flow rates can cause mechanical artifacts, but you can switch between flows faster at faster flow rates, that is the advantage of more pressure. Higher pressure can lead to more cross talk between reservoirs, so long applications at high pressure are not recommended.

**Grounding**

It is a good idea to ground the VC$^3$8 system to your set-up ground. The grounding connector (banana jack) on the rear of the control box can be used to secure a wire between the VC$^3$8 and your set-up ground.
Daily Usage

Getting the µFlow ready for an experiment

Turn on the VC3 controller. Load solutions into each reservoir. Make sure there is solution in each reservoir and that the perfusion capillary is at the bottom of the reservoir. (There must be solution in each reservoir for proper function.)

Make certain that your source of positive pressure is working and hooked up to the valve manifold.

Once your system is fully assembled and you are ready to start perfusion do a final check to make sure that the valve manifold is close enough to your setup to allow the front-end manifold to reach.

**Important notes:** All tubes must be filled for the unit to operate properly. This means that all reservoirs must be filled and each tube primed and free of air bubbles. Tubes not being utilized can be filled with distilled water.

When filling the reservoirs it is best to use solutions that have been de-gassed. Warm the solutions to a few degrees above room temperature or above the temperature at which they will be applied. Solutions that are not de-gassed run the risk of releasing air bubbles during the experiment as they pass through the small tubes. Solutions should always be filtered before being added to the system.

For priming, we recommend at least 4 psi (.276bar) has been set as the pressure for each reservoir (This is the minimum pressure needed for priming the system, more pressure may be desirable.).

Running Experiments

**Basic Style of Operation**

Depending on which mode of valve control you chose to use, solutions will be delivered to the target whenever a valve is energized. By pressing any of the valve switches on the membrane panel (1-8) solution can be delivered to your target and stopped again manually. TTL and analog voltage protocols can be written in your acquisition system software for automated valve control. Pressure must be applied for application, and shutting the pressure switches will also stop flow.

The uFlow comes set up to use one reservoir (channel 1) as a wash solution, and the other channels for your test substances. Channel 1 will flow whenever the low pressure switch is turned on. Solution will continue to pour out of reservoir 1 until another valve is selected.

Typical experiments involve a series of valve openings and closings that may include a return to the wash solution at any time. However, you can simply put your wash solution in another reservoir and toggle it at the same pressure as the other solutions if you wish.
Controlling the Wash Solution

The wash will stop whenever any valve is turned on. If you want to toggle the wash (reservoir 1) on and off, electrically, simply refer to it as valve 1. Any time valve 1 comes on, the flow out of reservoir 1 will be stopped. You can also stop the flow by switching off the low pressure switch.

Electronic Timing

If you need precise timing, better than you can count yourself, then you should use the analog or digital input on the VC3 controller to trigger the event(s). Simply use the software included with the VC3 controller, or use another data acquisition system that can control analog or digital outputs.
Other System Configurations

There are two other ways that the uFlow can be set up pneumatically.

Configuration A shown below is the default configuration that your uFlow was shipped with. The two other styles, B and C can be utilized. See below for a discussion of the pro’s and con’s of each and how to set them up.

Configuration A is the default set up. You can see that the output of valve 1 is not connected to Reservoir 1. The output of the low pressure valve is ported to reservoir 1. Whenever power is turned on to any valve, power is also turned on to the low pressure valve which stops the low pressure flow to reservoir 1. Pressure to reservoir 1 returns when all the other valves are off, and thus the low pressure valve is off as well. This provides a constant wash-out of the Micromanifold® from Reservoir 1. As soon as another valve is selected. The wash will stop until all valves are off. The wash can be stopped by switching off the Low Pressure Air Switch or selecting valve #1 on the control pad.

Configuration B will port high pressure to all the reservoirs in the manifold. All flow is stopped when all valves are off. All solutions are propelled via the High Pressure. The Low Pressure is not utilized.
**Configuration C** will port Low Pressure to all the reservoirs in the manifold. This allows the user to get more accurate pressure control when pressures under 10psi are used constantly.

Below is a photo of the inside of the rear panel of the uFlow. It is easily accessed by removing the six screws that hold it on. (4 channel system shown)

**Configuration A**

- Output from valve 1 is disconnected and connected to:
- Tube from reservoir 1 is connected to the common port of low pressure valve.
- Low pressure supply feeds to normally open port on low pressure valve.
- High pressure to reservoirs, ported to valve manifold.
- Normally Open ports of each valve bleed to atmosphere.
- High pressure feed input tube.
Eight Channel Configuration A. Shown from the inside. Note the Low Pressure Valves is plugged into the "Balance Pressure 12 Volt Input". Also, Valve Number 1 is unplugged to save air.

For further information, contact support@alascience.com.
µFlow System Maintenance

When using Micromanifolds that have µm ID openings, proper cleaning will increase life span and minimize clogging.

The µFlow does not require any regular maintenance other than routine flushing/changing of the reservoirs, and flushing the Micromanifold® and flushing Micro-Valves to prevent microbial growth and salt or calcium build up. All parts can be externally cleaned with a damp cloth and mild soap or alcohol and water. The system can be cleaned internally with any disinfectant including vinegar. Flush system thoroughly with water after doing so. Strong Clorox solutions are not recommended and no CFCs, acetone or high-grade alcohols are to be used. The reservoirs can be easily replaced when contamination is suspected.

Cleaning Instructions for Micro-Valves are listed in the Micro-Valves Section above.

The Micromanifold must be completely flushed out after each use. This can be accomplished in two ways: first, the reservoirs can be replaced with ones full of distilled water, and a repeating sequence of purging the lines under pressure can be done by hand or through software. Or, you can remove the Micromanifold from the reservoirs and purge it separately in a few minutes. Please see below:

One of the easiest and fastest ways to clean the Micromanifold is to flush it directly with distilled water.

1) Remove the tip, Carefully slide it off as shown on right.
2) Connect a syringe with distilled water to the end of the Micromanifold tubing cluster using a small piece of silicone tubing.
3) Slide the silicone tubing on as you would the tip.
4) Slowly push the water from the syringe into the Micromanifold (see right)
5) Immediately you will see droplets emerging from the back ends of the tubes.
6) After 2-3ml have passed through, your manifold is flushed!
7) Re-assemble the Micromanifold and re-install it in the uFlow as shown on pg. 7

Micromanifolds® can be stored wet or dry. If calcium builds up internally, flush with vinegar for at least 1 hour and then distilled water. Micromanifolds can also be sonicated.
Spill Sensor

The spill sensor is a capacitive element that when wet causes the controller to sound an audible alarm and shuts off power to the valves. If the sensor wire gets wet, it must be completely dry before it can be used in the system again. To dry the sensor wire, use an absorbent towel (paper towel) to carefully remove all liquid from the wire. The system will calibrate itself to the new humidity level of its environment when it is reset. Therefore it is very important that the wire be as dry as possible so it functions properly when a spill occurs.

Technical Specifications

μFlow

<table>
<thead>
<tr>
<th>Specification</th>
<th>Specification Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size LxWxH</td>
<td>15.5cm x 14.2 x 18.3</td>
</tr>
<tr>
<td>Weight</td>
<td>2.2-2.4Kg (4 channel-8 channel)</td>
</tr>
<tr>
<td>Min. Input Pressure</td>
<td>60psi, 4.14bar</td>
</tr>
<tr>
<td>Max. Output Pressure (High Pressure)</td>
<td>50psi, 3.45bar</td>
</tr>
<tr>
<td>Max. Output Pressure (Low Pressure)</td>
<td>10psi, .690bar</td>
</tr>
<tr>
<td>Valve Power requirement</td>
<td>12V</td>
</tr>
<tr>
<td>Average Flow rate through 100um</td>
<td>100uL/sec</td>
</tr>
<tr>
<td>Tube at 25cm length with 20 psi pressure in one minute</td>
<td></td>
</tr>
<tr>
<td>Front end weight 4channel/8 channel</td>
<td>120g/150g</td>
</tr>
<tr>
<td>Length of front end assembly</td>
<td>Total reach: approx. 30cm</td>
</tr>
<tr>
<td>Mounting Rod diameter</td>
<td>¼ inch, approx. 6mm</td>
</tr>
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</table>

Reservoir Information: (Only Sarstedt vials fit on the uFlow)

<table>
<thead>
<tr>
<th>Company</th>
<th>Size</th>
<th>Part #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sarstedt</td>
<td>0.5ml</td>
<td>72.730</td>
</tr>
<tr>
<td>1-800-257-5101</td>
<td>2.0ml</td>
<td>72.694</td>
</tr>
</tbody>
</table>
### VC³ Controller

<table>
<thead>
<tr>
<th>Feature</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power</td>
<td>110/220VAC to 15VDC @ 3.3 A - CE Compliant</td>
</tr>
<tr>
<td>Fuse</td>
<td>5 x 20 mm 3.15A Fuse</td>
</tr>
<tr>
<td>Switching</td>
<td>Manual / TTL logic / Analog voltage</td>
</tr>
<tr>
<td>Event Marker</td>
<td>TTL Pulse / analog voltage</td>
</tr>
<tr>
<td>Spill Sensor</td>
<td>Audible alarm/ power shut off</td>
</tr>
<tr>
<td>Dimensions</td>
<td>7.874&quot; x 5.906&quot; x 2.472&quot;</td>
</tr>
<tr>
<td>Weight</td>
<td>1.8 lbs./0.816 kg</td>
</tr>
<tr>
<td>Connector to valve manifold</td>
<td>9 pin D-sub female</td>
</tr>
</tbody>
</table>
The VC³ perfusion system can be controlled manually, by an analog signal, TTL signal or via the USB port from a PC. To control the VC³ via software you must first install the VC³ program provided with the system on a CD. The following steps should be followed:

1. Place the VC³ CD supplied into the PC CD/DVD player.
2. Go to the ‘My Computer’ and open the CD/DVD device. The VC³ files will be displayed.
3. Run the Setup.exe file to load the VC³ program. Follow the instructions provided.

   a. A window will appear informing you that the VC³ software is preparing to be installed. You can now cancel the installation or proceed with the installation.

      Press Next

   b. Select the shortcuts wanted by checking the box in front of the option.

      Press Next
c. Select the installation folder where the VC³ software will be installed to by using the browse button. The default folder is 
\textit{C:\Program Files\ALA Science\VC3}.

Assign who can use the VC³ program on this PC by selecting \textit{Everyone} or \textit{Just me}.

Press \textit{Next}.

d. The VC³ software is now ready to be installed.

Press \textit{Next}.

e. The VC³ software is being installed to the computer.
f. The VC³ software installation is complete.

Press Close

The VC³ program is now ready to be used.

---

g. Before proceeding any further, the VC³ controller must be connected to the PC via the USB cable supplied. The VC³ system should be ready for use after following the instructions in the System Assembly section of this manual.

Turn on the VC³ controller main power on the rear of the unit.

If this is the first time installing the VC³ software the computer will detect new hardware connected to it.

Go to the USB Driver Installation section and follow the instructions to install the new hardware drivers.

h. After the USB driver is installed the VC³ system is ready to be controlled via the computer software. Go to the Running the VC³ Software section for instructions.
1. When you turn ON the VC³ controller a message will appear at the bottom right corner of your screen. This message indicates that the PC has detected a new piece of hardware.

2. The Windows Hardware wizard will appear. It gives several options to detect the software drivers for the new hardware detected.

Select the last option. **No, not at this time**

Press **Next**

3. The Hardware Wizard will identify the new hardware; VC3-4 automated Valve Controller, and will install the drivers for it.

Select the second option, **Install from a list or specific location (Advanced)**

Press **Next**
4. The Wizard will now search for the drive at a specific location on your PC hard drive.

Select the first option, **Search for the best driver in these locations**
Then check **Include this location in the search**

Press **Browse** and go to the following location on the computer; **C:\Program Files\ALA Science\VC3\Application Drivers**

Press **Next**

5. A Windows warning will appear.

Press **Continue Anyway**

6. The VC\(^3\) driver will now attempt to install.
7. A window asking for the path of the USB driver will appear if the system cannot find the file itself.

Press Browse... and go to location C:\Program Files\ALA Science\VC3\Application Drivers\n
8. Select the following file, CyUSB.sys

Press Open

9. The USB driver for the VC³ will now be installed on the PC.

10. The Hardware Wizard will indicate when the installation is complete.

Press Finish
Firmware Upgrade Installation

First time upgrade

To upgrade the VC³ firmware to a new version for the first time, follow the steps below. If this is not the first time the firmware has been upgraded via this computer, then follow the steps

1. Run the VC³ software. On the computer desktop double click the VC³ icon named VC³ Desktop. This will open the screen shown.

2. Select the Help drop down menu on the top toolbar. Scroll down to Upgrade Firmware select it.

3. A warning window will pop up before the proceeding with the firmware upgrade. Press OK

4. All the buttons on the VC³ Valve Control GUI will be disabled during the firmware upgrade.

5. The Hardware Wizard gives several options to search for the software for the hardware.

   Select the last option No, not at this time
6. The Hardware Wizard identifies the hardware and gives options to install the firmware. Select the second option,

*Install from a list or specific location*

7. Select *Search for the best driver in these locations* option. Check *include this location in the search*.

Browse to `C:\Program Files\ALA Science\VC3\Firmware Upgrade Tools\USB Driver\` for the files required.

8. Since this is the first time this computer will upgrade the VC³ firmware, it must install the driver that will communicate with the VC³ processor. The *USB Bootloader* driver is loaded to the computer. This will allow for future firmware updates.

9. A Windows warning about the software being installed will appear. Press *Continue Anyway*
10. The wizard has completed installing the USB Bootloader.

   Press *Finish*

11. Once the Bootloader driver is installed, the computer will state that the new hardware is ready to use.

12. Now that all the drivers are installed on this computer we can proceed with the firmware upgrade.

   Go to the *Start* and then *Run* on the Windows taskbar.

13. Browse to and open the following file:

   **C:\Program Files\ALA Science\VC3\Firmware Upgrade Tools\setup.exe**

   Press *OK*

14. The USB Bootloader Host Application will be installed.

   Press *Install*
15. Once installed the **USB PSoC Downloader** will detect the **USB Bootloader**.

Press **Download PSoC Configuration to Device** button.

16. Open configuration file located in `c:\Program Files\ALA Science\VC3\Firmware Upgrade Files`

For VC3-4, the file is `vc3-4.dld`
For VC3-8, the file is `vc3-8.dld`

17. Configuration file is downloaded to the VC³ controller.

18. When complete the Downloader will show that no device is detected.

19. The VC³ program will become active once again. All buttons will return to active status.

The VC³ software is now ready to be used.
Firmware Upgrade – not first time

If you previously upgraded the firmware of the VC$^3$ on this computer, then all the drivers and software required to perform the upgrade are already installed. Follow the steps below to upgrade the firmware.

1. Run the VC$^3$ software. On the computer desktop double click the VC$^3$ icon named VC3 Desktop. This will open the screen shown.

2. Select the Help drop down menu on the top toolbar. Scroll down to Upgrade Firmware select it.

3. A warning window will pop up before the proceeding with the firmware upgrade. Press OK

4. All the buttons on the VC$^3$ Valve Control GUI will be disabled during the firmware upgrade.

5. Go to Start and then All Programs on the Windows taskbar. Find the Cypress Microsystems tag and then click on the USB Bootloader Host Application.
6. The USB PSoC Downloader will appear. It will detect the USB Bootloader of the VC³ controller.

Press **Download PSoC Configuration to Device** button.

7. Open configuration file located in `c:\Program Files\ALA Science\VC3\Firmware Upgrade Files\`

   For VC3-4, the file is `vc3-4.dld`
   For VC3-8, the file is `vc3-8.dld`

8. Configuration file is downloaded to the VC³ controller.

9. When complete the Downloader will show that no device is detected.

10. The VC³ program will become active once again. All buttons will return to active status.

   The VC³ is now ready to be used.
Running the Software

To run the VC³ program double click on the VC³ icon on the desktop named VC³ Desktop. This will open the VC3 Valve Command GUI shown below.

The VC³ Valve Command will open with the Download, Play, Return and Loop buttons disabled. Once a sequence is opened or a new sequence is created and downloaded to the VC³ controller the buttons will then become active.

![VC³ Valve Command GUI](image)

Control Buttons - Overview

- **New**
  This button will start a new sequence file. Make sure you the old sequence file before pressing this button.

- **Open**
  This button is used to open a saved sequence file on the computer. The sequence file will have a *.VC3 extension.

- **Save**
  This button will save the current sequence file being used.

- **Download**
  This button will download a sequence loaded in the VC³ Valve Command program to the VC3 controller.

- **Play**
  This button will start running the sequence loaded.

- **Return to Start**
  This button stops and returns the sequence to the first step.

- **Loop**
  This button will enable or disable a loop created in a sequence.
• **Sync**  
  This button will enable/Disable the TTL/Sync Out port on the back of the VC³ controller. When any valve is opened the port will output 5v DC when the sync is enabled. This will be indicated by the button changing color to green.

• **Trigger**  
  This button enables/disables the trigger function. The trigger function is utilized via the analog input port on the back of the VC³ controller. Therefore the analog function will be disabled if an output trigger is used. When the trigger function is enabled the button will be green.

• **Power**  
  This button turns ON or OFF the VC³ controller.

• **TTL In/Out**  
  This button enables/disables the TTL function of the VC³ controller. When enable the green lamp on the controller panel will be ON. The VC³ can then be controlled via the Digital In/Out port on the back of the VC³ controller using the provided DB9 to BNC cable and sending TTL signals from the acquisition system.

• **Latching**  
  This button enables/disables the latching mode of the VC³ controller. The button will go green when enabled. The Latching lamp on the controller will also turn ON. When enabled the valve that is switched ON will be switched OFF when any other valve is switched ON. Only one valve can be ON at a time in this mode. This feature allows for fast manual solution switching by eliminating the need to switch a valve OFF before switching another ON.

• **Toggle/Momentary**  
  This button will switch the valve buttons from being toggle or momentary. When the button is gray, the toggle mode is enabled. In this mode the valve stays ON until the button is pressed a second time. Multiple valves can be ON in this mode.

  When the button is green the momentary mode is enabled. The green lamp on the controller will turn ON. In momentary mode you must left click and hold down button down switch to energize, turn ON, a valve. As long as you hold down the switch the valve remains ON. Release the switch and the valve turns OFF.

• **Valve Buttons**  
  These buttons turn ON/OFF the corresponding valves. The buttons turn green when a valve is ON.
Manual software Control

The VC\textsuperscript{3} can be controlled manually via the software. With the VC\textsuperscript{3} fully assembled, the valves can be controlled by clicking the corresponding valve button to open or close the valve.

There are several modes that can be used to control the valves without using a sequence file.

**Toggle Mode**: The valve buttons function like toggle switches. The Toggle/Momentary button must be gray for toggle mode. Press once to turn ON, press again to turn OFF the valve. Multiple valves can be ON at the same time.

**Momentary**: The valve buttons function like momentary toggle switches. The Toggle/Momentary button must be green for momentary mode. Press and hold to keep the valve ON. Release the button and it will turn OFF. Only one valve at a time can be ON.

**Latching**: The valves act like toggle switches. The Latching button must be displayed in green to enable this mode. Press once to turn valve ON, Press again to turn OFF. The difference is that in this mode pressing a different valve the second time will turn ON that valve and turn OFF the previous valve. Only one valve can be ON at a time.

I/O Functions

**TTL mode**

There are two modes for the TTL In/Out setting.

In the **TTL IN** mode the VC\textsuperscript{3} system is controlled via the TTL I/O port on the back of the VC\textsuperscript{3} controller. The TTL button must be enabled displayed in green. Using the supplied DB-9 to BNC cable TTL signal from an acquisition signal will turn valves ON and OFF.

In the **TTL OUT** mode the TTL I/O port on the back of the controller will output 5v DC when a valve is turned ON to its corresponding pin. The TTL button must be in the up position displayed in gray.
Sync Function

The Sync function outputs 5v DC from the Sync port on the back of the VC³ controller. To enable this function, press the Sync button so it displays in green.

When a sync signal is sent the SYNC indicator box will turn red as long as the signal lasts.

To add a Sync signal during a sequence, check the box under the SYNC label column of the sequence.

Trigger Functions

The VC³ system can be triggered from an external source by enabling this function. The way this function can be used is to use the same port on the VC³ controller that is used for the analog control. This means that the analog input port is disabled and cannot be used while the trigger function is used.

A message is displayed warning of this. Press OK to continue.

When running a sequence it will now be necessary to trigger the VC³ controller when the sequence is started. After pressing the Play button, a message will appear informing that a rigger is required for the sequence to begin.

Send a TTL high signal to the Trigger IN port (Analog IN port) of the VC³ controller to trigger the system to begin the sequence.
Working with a Sequence

Load a Sequence

There are two ways to load a sequence on the VC³ software.

1. The first is to manually insert each step into the sequence. This is done by filling in the cells under **Valve** and **Time** appropriately.

   The valve number to be turned ON is written into the cell below the **Valve** column.

   The time the valve is to remain ON is written into the cell below the **Time** column. The default time units is in millisecond (ms).

   The time units can be changed by placing the cursor on the **Time** label and left clicking.

   The units available are ms, s, MM:SS, HH:MM:SS

2. The second is to load a saved sequence file from the computer by pressing the Open button. Navigate to the folder where the saves sequence files are stored and open one. The default folder for saved sequence sample files is `c:\Program Files\ALA Science\VC3\Examples`.

   The loaded sequence must be downloaded to the VC³ controller by pressing the Download button.

Run a Sequence

Once a sequence is loaded and downloaded, the sequence can be executed. Press the **Play** button to start the sequence.

The **Start** button will change into a **Pause** button. The sequence can be paused at any time while the sequence is running.

Press the **Play** button again to continue running the sequence.
Sequence loops can be inserted into a sequence at any step. This allows for certain steps to be executed a multiple of times before the sequence moves to the next step outside of the loop.

**Insert a Loop**

1. Place the mouse cursor at the step you wish the loop to start. Press and hold the left mouse button and drag to the step you wish the loop to end.

2. The Loop window will open. Here you can specify the number of times the loop will run for.

3. When a loop is inserted in the sequence the steps in the loop will be displayed as blue and dark green.

After inserting the loop, download the sequence to the VC$^3$ controller by pressing the download button.

The loop will now run when the Loop button is enabled.
Remove a Loop

To remove a loop function, place the cursor on the steps in the loop and right click with the mouse.

This will bring up a window asking to remove the loop.

Press **OK** to remove the loop.

Press **Cancel** to leave the loop as is.
**LIMITED WARRANTY for Valves**

ALA Scientific Instruments agrees to warranty Lee Company Valves for 30 days from date of invoice. The Lee Company Certifies the functionality of each valve shipped to ALA Scientific Instruments. The valves are again tested at ALA Scientific when they are incorporated into the different systems that use them. It is recommended that the valves be checked upon receipt to determine any malfunctions. The following are the only agents approved to check valves: distilled water, Nitrogen or purified air. Any other agents used will void the warranty.

Valves that have been used in actual experiments cannot be returned. Individual valves that malfunction within 30 days of invoice date will be replaced on a per case basis. ALA Scientific Instruments limits coverage to include repair or replacement of defective materials at our discretion.

Buyer is responsible for the cost of return shipment. Inspection upon receipt is essential to receiving coverage should the instrument be damaged in shipment. Generally three days after receipt is the limit for such claims with the shipping company.

ALA Scientific Instruments, Inc. is not responsible for damage occurring to, or from the use of this product that is inconsistent with its intended usage or this manual. It is the buyers’ responsibility to make sure that DC valves used with this instrument are run at the proper voltage and to use common sense in the operation of this product. This instrument, or any of its parts, is not approved for clinical use and has not been produced to such standards. Under the law, the BPS system cannot be used on human subjects in any way. It has no clinical applications and is intended as a research instrument only. No guaranty of results is offered or implied by the use of this product. It is intended only for research purposes. Your rights under this warranty may vary from state to state and country to country.

**Warranty for instrumentation**

**ALA Scientific Instruments** agrees to warranty this product for a period of one year from the date of delivery against any and all manufacturer’s defects in material and/or workmanship. Remedy will consist of repair or replacement at ALA’s discretion. All problems should be reported immediately so as not to jeopardize warranty coverage. ALA Scientific Instruments does not assume any liability based on the use of this product, whether correct or incorrect, except as specified under law. Warranty rights may vary from state to state.

ALA Scientific Instruments will not warranty any of the plastic parts including 60cc reservoirs, all tubing and connectors, and magnetic stand parts.

If the product does need repair, it must be returned to the factory freight prepaid (freight collect will be refused) and in clean condition. If returned parts have been in contact with any liquid substance, documentation must accompany those parts, regarding what substances were used.

This product is intended for use in cellular and tissue research only. **THIS EQUIPMENT IS NOT INTENDED OR APPROVED FOR CLINICAL USE IN ANY WAY AT ALL.** Micromanifold™ is a trademark of ALA Scientific Instruments Inc.
## Parts List

### µFlow System checklist

<table>
<thead>
<tr>
<th>Item Description</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALA UFlow __ Pressure Control interface</td>
<td>SN:</td>
</tr>
<tr>
<td>QMM-___UF Micromanifold</td>
<td></td>
</tr>
<tr>
<td>µFlow Solution Manifold</td>
<td></td>
</tr>
<tr>
<td>1/8 ID PVC tubing w/ luer connectors 10 ft.</td>
<td></td>
</tr>
<tr>
<td>DC Power cable w/2.5mm jacks</td>
<td></td>
</tr>
<tr>
<td>2.0ml Res. x 1</td>
<td></td>
</tr>
<tr>
<td>0.5ml Reservoirs × 4 □ 8 □</td>
<td></td>
</tr>
<tr>
<td>Extra Reservoirs 2 x 0.5 ml</td>
<td></td>
</tr>
<tr>
<td>VC³ _____ controller</td>
<td>SN:</td>
</tr>
<tr>
<td>Universal power supply with</td>
<td></td>
</tr>
<tr>
<td>110 VAC power cord</td>
<td></td>
</tr>
<tr>
<td>220 VAC power cord</td>
<td></td>
</tr>
<tr>
<td>DB-9 M-M cable for Valve control</td>
<td></td>
</tr>
<tr>
<td>Spill sensor cable</td>
<td></td>
</tr>
<tr>
<td>DB-9 to ___ BNC breakout cable</td>
<td></td>
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<tr>
<td>USB cable</td>
<td></td>
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<tr>
<td>VC3 Software CD</td>
<td></td>
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<tr>
<td>Instruction manual</td>
<td></td>
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