Mitos System User Guide

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1 Disclaimer

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2 Safety

All Unchained Labs hardware is designed and manufactured to ensure safe operation when used within the prescribed temperature and pressure limits, but the basic responsibility for safety using the equipment rests entirely with the user. This equipment should only be used by competent, suitably trained personnel after they have read and understood the operating instructions and considered any hazards involved.

3 READ BEFORE USE

Always connect the instruments to an earthed AC power outlet. The operating voltage is indicated on the specification sticker. Failure to observe this provision may result in damage to the module, personal injury, or damage to property.

Do not use a replacement mains supply cord that is inadequately rated for this piece of equipment. The ratings for this piece of equipment can be found further in this document.

The system must be sited in appropriate fume extraction cabinet with adequate ventilation if hazardous chemicals are to be used in conjunction with this unit. In the case of accidental spillage, carefully wipe with a dry cloth, considering the nature of the spilled liquid and the necessary safety precautions.

4 Warning and Safety Information

The system should only be used by competent, suitably trained personnel after they have read and understood these safety notes, the Mitos System manual and considered any hazards involved.

If training is required to run the system, Unchained Labs should be contacted to arrange for training of the system or of its components.

The hardware supplied with the system should not be opened (chassis/casework) and/or otherwise dismantled. Any repair work or investigation into hardware or hardware failures should be conducted by Unchained Labs only.

When moving or relocating the system, the hardware should be separated into its modular form and each module moved/relocated individually.

The user must have a complete understanding of the experiment planned to be run and carry out a risk assessment before starting. Risks to be evaluated include (but are not limited to): use of Pressurized gas, hazardous and corrosive chemicals, and chemical compatibility.

Place the instrument on a solid, horizontal, fire-proof surface. Ensure the area around the unit is clear. The system should be used in an appropriate environment such as a fume hood if venting gases.

Appropriate Personal Protective Equipment (PPE) must be worn at all times when operating the system, considering temperature, pressure, and chemical hazards. Safety googles (minimum of Class 1 EN 166 F 3 or equivalent) should be worn when handling hazardous fluids.

Comply with all safety and accident prevention regulations applicable to laboratory work. Operate in full compliance with local safety codes and rules.

Do not connect a pressure supply over 11 bar gauge to the Mitos System. This may result in the failure of the Mitos System and injury to the user.

If the glass pressure chamber is scratched or cracked, do not continue to use the P-Pump. This may result in failure of the P-Pump and injury to the user. Please contact Dolomite for replacement of the glass vessel.

Inert gases must be used when flammable fluids are used with the system.

Flow Sensors are highly sensitive measurement devices for ultra-low flow rates. To assure precise and drift-free flow measurements mechanical stress needs to be avoided. For this reason, only tighten fittings by hand to assure precise flow measurement.

5 Cleaning and Maintenance

The Mitos System utilizes microfluidic technology and microfluidic devices in its system configuration. As a result, care must be taken to ensure that the system is handled in a safe and clean environment to reduce instances of contamination or external debris (fibres/dust) entering the system.

Users are recommended to filter all fluids used where possible with a 0.2 µm filter and to ensure that the work area is cleaned regularly.

Cleaning should only be performed by personnel trained in such work, and who are aware of the possible dangers involved. The Dolomite P-Pump (and all associated hardware) has not been designed for sterilization by an autoclave.

Repair should only be attempted by qualified service personnel or under guidance by Unchained Labs. Opening any module may invalidate the warranty.

Before cleaning or maintaining the equipment ensure all pressure is safely vented from the P-Pump chambers, the pressure supply to the system has been switched off and the input tube removed.

Parts should not be repaired by the user.

5.1.1 Returning Equipment

Equipment which has been contaminated with, or exposed to, bodily fluids, toxic chemicals, or any other substance hazardous to health must be decontaminated before it is returned to Unchained Labs or its distributor.

Please contact support@unchainedlabs.com for further help.

5.1.2 Waste Electrical and Electronic Equipment (WEEE) statement

Unchained Labs is compliant with the EU directive on waste electrical and electronic equipment (WEEE) please refer to https://www.unchainedlabs.com/ for directions and information on end-of-life policy.

6 Specifications

Operating Pressure	0 - 10 bar
Supply Pressure (PFS)	> 1 Bar < 11 Bar
Flow Rate Range	70 nL/min to 5 mL/min (Flow Rate Sensor dependent)
Sample volume	Accepts a wide range of vials and vessels from 100 µl to 30mL (higher on request)
Component Dimensions	1300 mm (L) x 600 mm (W) x 600 mm (H)
Intended use space requirements	Recommended 200 cm (access is required to the rear and front of the system)
System Weight	5 Kg (Dolomite Imaging Pack excluded) 15 Kg (Dolomite Imaging Pack included)
Voltage Input	100 V – 240 V AC, 50 – 60 Hz
Working Temperature Range (external)	5 - 50 °C
Maximum Relative Humidity	80 %
Communication	DB9 to USB via Dolomite Flow Control Center Software
Wetted Material	Glass, FEP (tubing), FFKM, FKM, PEEK and PTFE
Tubing Dimensions	1/16" OD x 0.25 mm ID

7 Quick Start Guide

This user guide has been prepared to provide Users with guidance on system assembly, instruction on use of the system via software (Flow Control Center Software) and system maintenance/troubleshooting. The Mitos System enables the production of microparticles and droplets using a single junction microfluidic chip (lab scale). Particle/droplet size can be fine-tuned by varying process parameters such as total flow rates, flow rate ratios, microfluidic chip and reagent parameters e.g. concentrations.

A quick start guide is provided to help with setting up the Mitos System in a timely manner, but detailed information on each component can be found later in the guide.

7.1 Hardware Composition

The Mitos System is comprised of the following pieces of hardware:

- 3 x P-Pumps
- 3 x Sensor Interfaces
- System Controller
- Imaging pack (containing High-Speed Microscope and Camera; X-Y Stage) (Optional)

This hardware is connected via a calibrated tubing network and microfluidic chips purchased as part of an application specific pack to enable the formation of PLGA particles, Hydrogel particles, Aqueous Droplets and Double Emulsions. Hardware is controlled via the Dolomite Flow Control Center Software (FCC) that is installed on the System PC. The software allows for easy control and optimization of flow rates, flow rate ratios and pressure to generate droplets/particles for the ideal size and composition.

7.2 Application Packs

The following applications packs are compatible with the Dolomite Mitos System and are available for purchase (Table 1). Each pack includes flow rate sensors, microfluidic chips, calibrated tubing, and reagents required to achieve droplets/particles within the specified size range.

Part Number	Description
3201094	PLGA Particles 10-60 µm Application Pack
3201095	PLGA Particles 40-140 µm Application Pack
3201096	PLGA Multiple Emulsions 40-140 µm Application Pack
3201097	Hydrogel Particles 10-60 µm Application Pack
3201098	Hydrogels Particles 40-300 µm Application Pack
3201099	Polyacrylamide and Polystyrene Particles 40-200 µm Application Pack
3201100	Aqueous Droplets 10-60 µm Application Pack
3201101	Aqueous Droplets 60-300 µm Application Pack
3201102	Aqueous 2 Reagent Droplets 50-400 µm Application Pack
3201103	µEncapsulator 20-60 µm Droplets Application Pack

Table 1 List of Application Pack Compatible with Mitos System

7.3 Hardware Schematic

When assembling the system, its useful to understand the overall configuration to ensure you are using the space you have available effectively. The diagram below illustrates the assembly of the Mitos System when used in conjunction with the Aqueous Droplets 40-300 µm Application Pack – system schematics for other application packs can be found in their specific datasheet.

- P-Pump 1: Continuous phase
- P-Pump 2: Dispersed Phase

- P-Pump 3: Dispersed/Flushing Phase
- Sensor Interface: Each P-Pump requires a Sensor Interface to facilitate communication between the Pump and Flow Rate Sensor.
- Flow Rate Sensors: Able to monitor the flow of fluids within the range specified on the sensor.
- Microscope: Visualization of the junction in real time

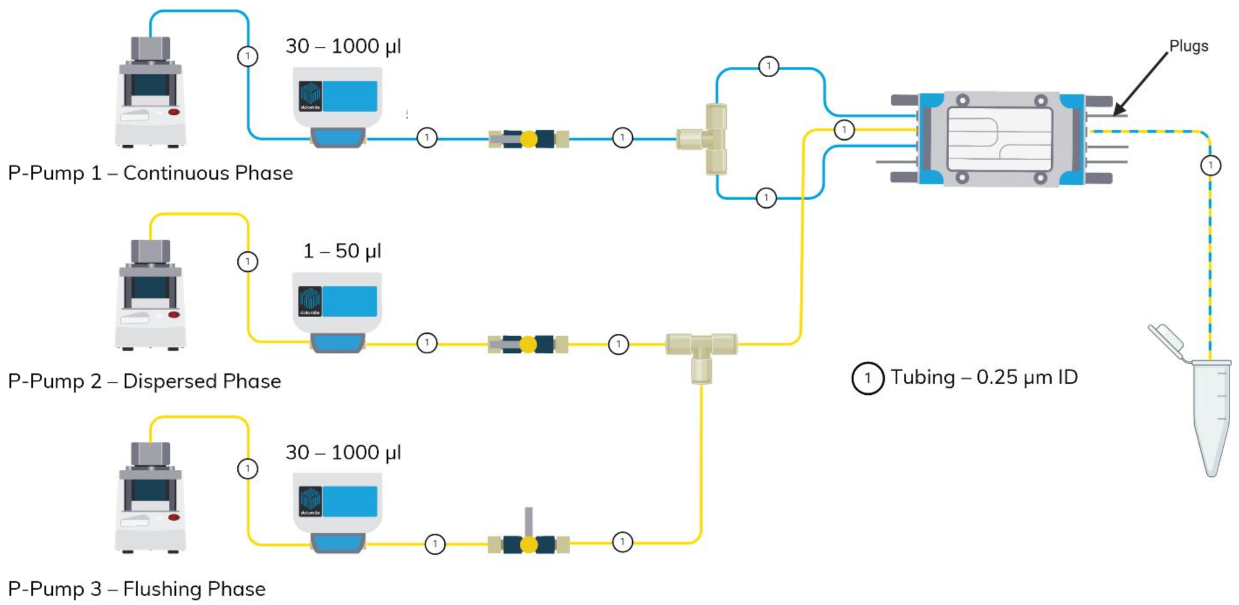


Figure 1 Schematic of Mitos System in Conjunction with the Aqueous Droplets 40-300 µm Application Pack (3201101)

7.4 Hardware Connections

Hardware should be assembled as shown in Figure 2. Depending on the space available, the System PC can be placed on the left or right of the system.

To achieve the correct set-up for the system, the following steps should be followed:

Step 1: Connect a Flow Rate Sensor (taken from Application Pack) to each P-Pump using a Sensor Interface. The P-Pump controlling the Continuous Phase will require the highest Flow Rate Sensor if multiple types are supplied.

Step 2: Place the X-Y stage on the base of the stand Microscope Stand. Place the Light Controller on top of the X-Y stage.

Step 3: Locate and connect the power cables, communication cables and pneumatic tubing required for the system.

Figure 2 Assembly of the Mitos System without Fluidic Connections.

7.5 Connecting the Microfluidic Chip

Microfluidic Chips have different geometries but are connected to the Mitos System in a similar manner. Detailed information about how to connect different Chips can be found in Application Pack datasheets.

Step 1: Locate the Tubing Kit, and H interface or Top Interface. The Tubing Kit is supplied with the Linear Connectors pre-assembled.

Step 2: Connect the tubing labelled 1A to P-Pump 1 (Continuous Phase Pump) by threading the tubing through the P-Pump lid and screwing in the dip tube fitting (Figure 3). Connect the fitting on the opposite end of 1A to the RIGHT side of the Flow Rate Sensor.

Figure 3 Diptube Fitting with Tubing.

Step 3: Repeat the process and connect tubing 2A to P-Pump 2 and 3A to P-Pump 3.

Step 4: Connect the tubing labelled 1B to the LEFT input on Flow Sensor 1.

Step 5: Connect the tubing labelled 2B to the LEFT input on Flow Sensor 2.

Step 6: Connect the tubing labelled 3B to the LEFT input on Flow Sensor 3.

Step 7: Place the Chip in the Interface and evenly screw the Linear Connector(s) in place.

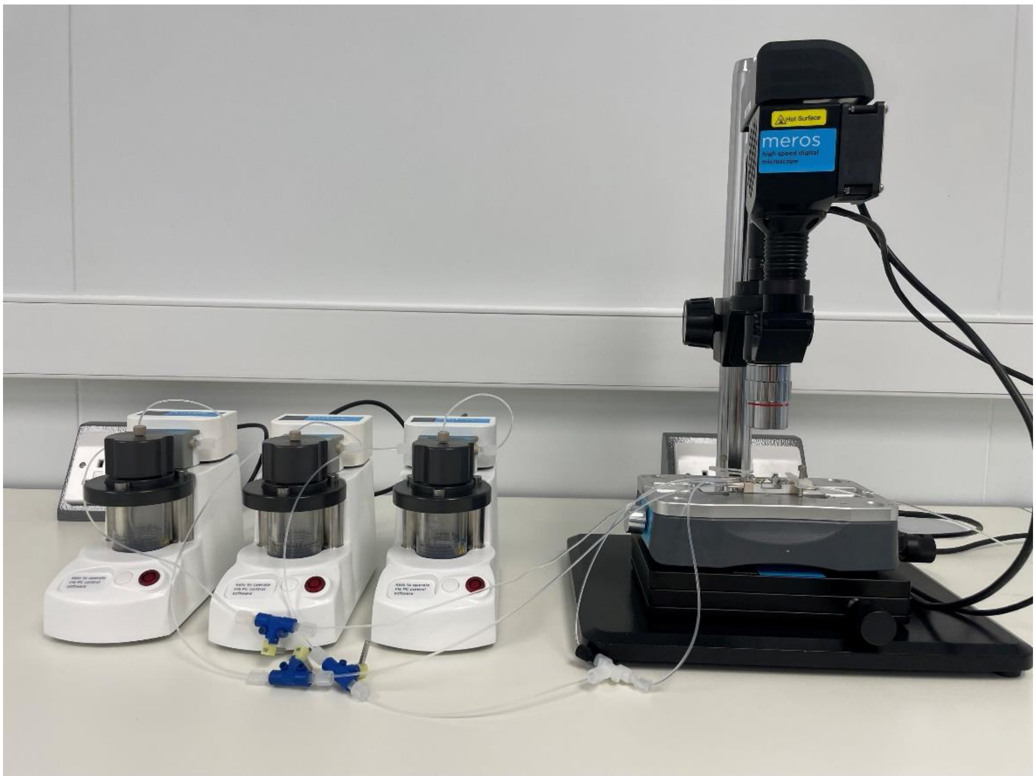


Figure 4 Mitos System Complete with Fluidic Connections.

8 P-Pump

The P-Pump delivers fluid by a pressure driven pumping mechanism. The User sets the chamber pressure via the software, which determines the flow rate of liquid from the chamber. The pressure pump can be controlled in pressure mode or, when coupled with a flow rate sensor, monitor, and control the flow of fluids. The P-Pump chamber is designed to hold up to 30 mL of fluid.

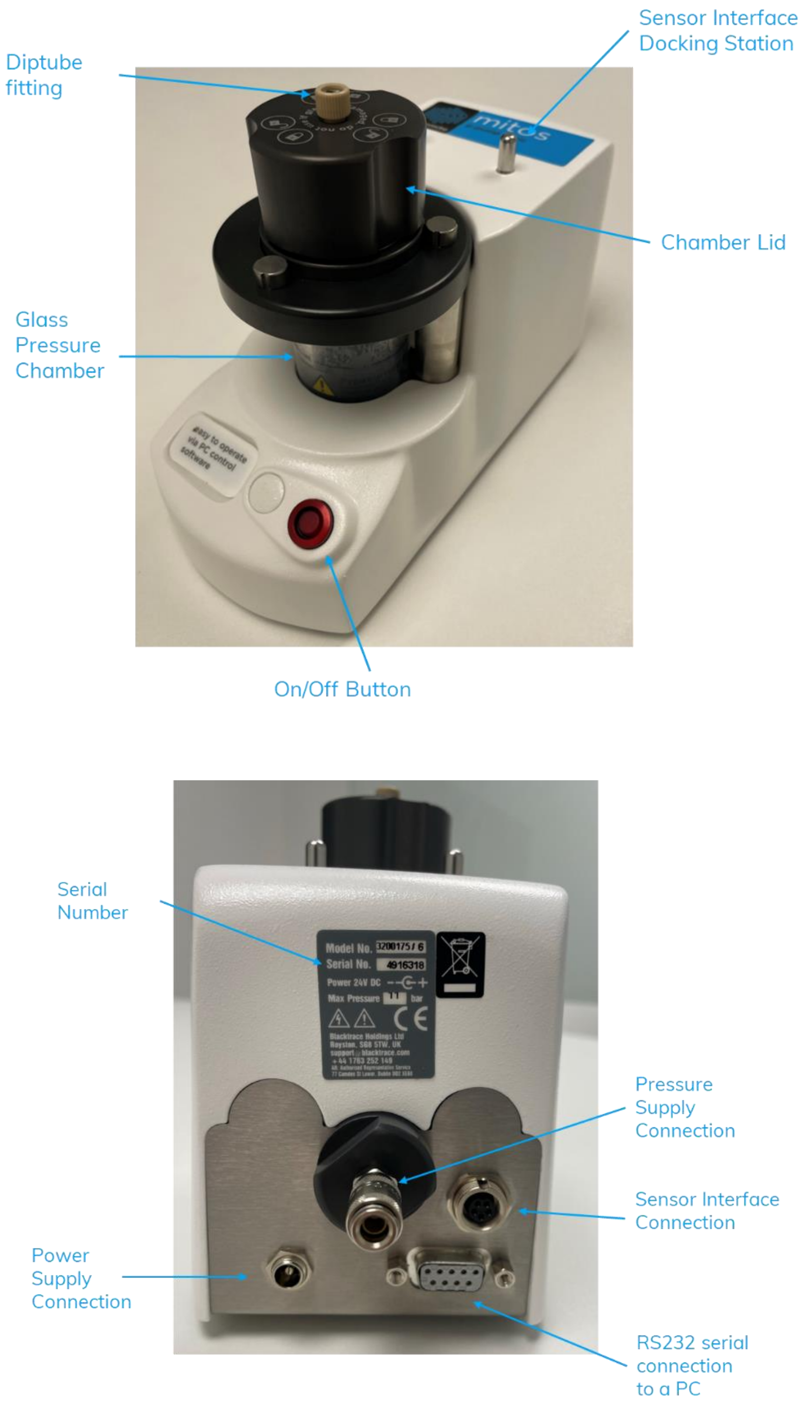


Figure 5 P-Pump

The following accessories are supplied with the Mitos P-Pump and are required for its successful installation:

- Pneumatic supply cable
- Tubing FEP 1/16” X 0.25 mm ID
- Power Supply Unit
- Mains IEC Lead

Important: Fluids cannot be poured directly into the chamber of the P-Pump. Instead, fluids should be loaded into the chamber via vials. Vials much have a diameter smaller than 30 mm and a height of less than 88 mm.

8.1 Pneumatic connection

The Mitos P-Pump is designed to work with a supply pressure up to 11 bar. This can be from a standalone compressor, in-house system supply or from a gas cylinder fitted with a suitable pressure regulator. Depending on fittings from the supply, this must terminate in a 6mm push fit pneumatic fitting.

Note: Explosive or ignitable gases/vapors should not be used – Users are responsible for the risk assessment of combinations of specific driving fluids with pressurized gases.

A female pneumatic push fitting that accepts a Male SS fitting can be located on the back of each P-Pump. The pneumatic cable supplied with each P-Pump is pre-fitted with the male connection. T-pieces are used to make connections between the 3 units and supply pressure. Once connected the pneumatic tubing network should resemble Figure 6.

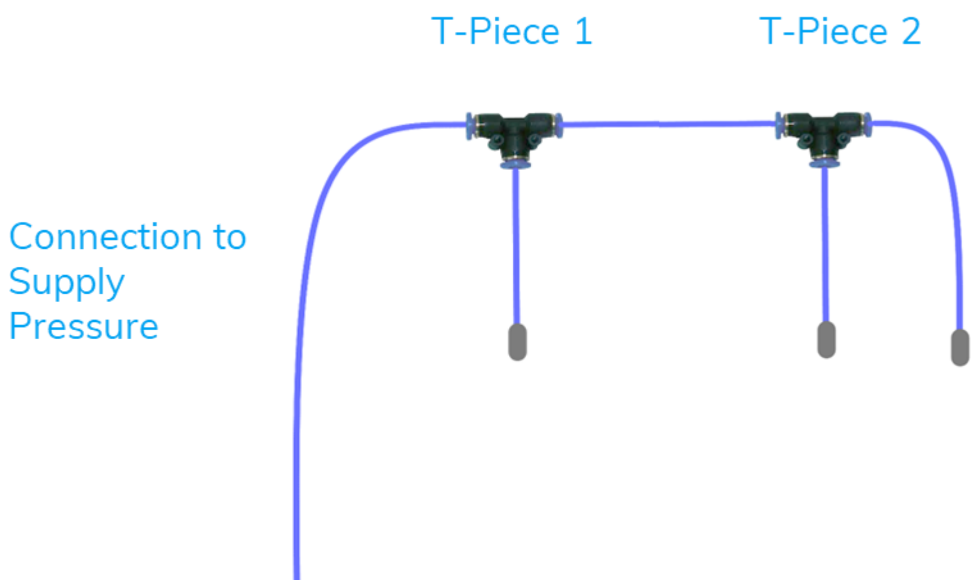


Figure 6 Pneumatic Tubing Connections

To form the connections the below steps should be followed:

Step 1: Locate 3 reals of blue pneumatic tubing and 2 T-pieces.

Step 2: Cut a 25 cm length of pneumatic tubing (including the Male SS fitting) from each set of pneumatic tubing.

Step 3: Connect a cut piece of tubing to each Mitos P-Pump by inserting the metal fitting into the push fitting on the back of the Pump.

Figure 7 P-Pump pneumatic tubing connection

Step 4: Connect a T-piece to the free end of the pneumatic tubing attached to P-Pumps 1 and 2

Step 4: From the left-over tubing, cut a 15cm piece and connect the two T-pieces.

Step 5: Attach Pump 3 to the remaining input on T-piece 2.

Step 6: Using the remaining tubing, insert one end into the remaining port on T-piece 1 and connect the free end to the compressed air supply.

To disconnect the pneumatic tubing, ensure the supply pressure is turned off and pull down on the collar of the metal push fitting to release the tubing.

8.2 Power and Communication Connections

The P-Pump has one LED power switch located on the front of the Pump. Connect the provided mains cable to a power outlet and the 1-pin socket at the rear of the module. Push the power button on the front of the Pump to power on the unit. Each P-Pump will require its own power cable.

> **Note:** It is recommended to assemble all hardware, including sensor interfaces and flow rate sensors before powering on the P-Pumps to ensure all components are directly connected.

Figure 8 P-Pump Power Connection

The P-Pump is designed to operate solely via PC control using the Dolomite Flow Control Center Software (FCC). The unit connects to the PC via an RS232 to USB cable. Using the white communication cable provided, connect RS232 to the DB9 port on the back of the Pump and USB to the PC.

> **Note:** Most PCs only feature 2 USB ports, it is advised to utilize the USB hub supplied with the system to ensure there are enough USB connections for the whole system.

Figure 9 P-Pump Communication Cable Connection

8.3 Loading Fluid into Chamber

All sample fluids must be placed within a suitable vial within the chamber of the P-Pump. The lid of the Pump is illustrated with a locked and unlocked symbol. When the locked symbol is aligned with the metal pin on the front of the chamber, the lid is locked.

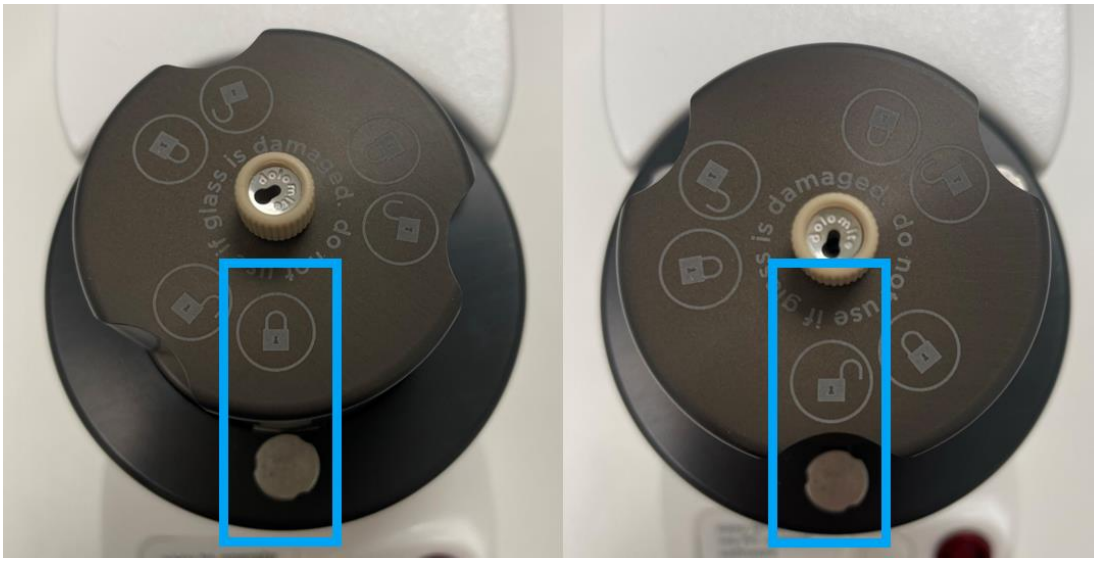


Figure 10 P-Pump Lid in locked and unlocked position

To unlock the lid, there is a 2-stage opening process:

Step 1: Push down and rotate the lid anticlockwise.

Step 2: Pull upwards, slightly turn anticlockwise and pull upwards again to remove the lid.

Vials can then be placed inside of the chamber. For small volumes, the system is supplied with a pack of vessel holders which allow, for example, Eppendorf's to be placed inside the chamber and remain upright. **Vials much have a diameter smaller than 30 mm and a height of less than 88 mm.**

To lock the lid:

Step 1: Align the unlocked symbol with the metal pin on the chamber.

Step 2: Push down and turn clockwise.

Step 3: Push down further and rotate clockwise until the lid clicks into position. The locked symbol should now be aligned with the metal pin (Figure 10).

NOTE: When tubing has been assembled, guide the tubing into the vessel as the chamber lid is put into position.

9 Sensor Interface

Flow control mode allows a flow rate to be selected instead of a pressure and the P-Pump pressure is automatically adjusted to achieve the target flow rate. The benefit of flow control mode is that it accounts for changes in the flow resistance of the system to maintain constant flow rate.

To operate in flow control mode, a Flow Rate Sensor must be connected to the P-Pump using a Sensor Interface (Figure 11).

Figure 11 Sensor Interface

Step 1: Use the circular multi-pin cable attached to the Sensor Interface to make connection to the P-Pump.

Figure 12 Sensor Interface Connection to P-Pump

Step 2: House the Sensor Interface on top of the P-Pump using the 2 docking pins on the top of the Pump (Figure 5).

Sensor Interfaces are not P-Pump specific; any Interface can be connected to any P-Pump.

10 Flow Rate Sensors

Flow Rate Sensors are provided as part of the Application Packs. Dolomite offers a range of Flow Rate Sensors (Table 2) and must be used in combination with a Sensor Interface. The flow range in which a sensor can accurately measure flow rate can be located on the sensor label (Figure 13). The arrow on the label illustrates the direction fluid must pass through the sensor to get an accurate reading.

Part Number	Flow Rate Sensor Range
3200096	0.2 – 5 mL
3200097	30 – 1000 µL
3200098	1 – 50 µL
3200099	0.4 – 7 µL
3200100	70 – 1500 nL

Table 2 Flow Rate Sensor that may be provide within the Application Packs

Figure 13 Example of a Flow Rate Sensor

To connect a Flow Rate Sensor to a Sensor Interface:

Step 1: Pull down the metal clip on the underside of the Sensor Interface.

Step 2: Slot the Flow Rate Sensor into the Sensor Interface and release the clip. The clip should latch with a click sound.

Figure 14 A Flow Rate Sensor connect to a Sensor Interface

Generally, the P-Pump controlling the flow of the continuous phase used within experiments requires a high flow rate than the dispersed phase. The Flow Rate Sensor provided in the Applications Pack which measures the highest flow rates should be paired with P-Pump 1.

10.1 Fluid Calibrations

The flow rate generated at a chosen pressure is greatly affected by not only the resistance within the system, but also the viscosity of the fluid.

10.1.1 Pre-set Fluids

Flow Rate Sensors supplied with the Mitos System are calibrated to provide an accurate flow rate reading when used with 5 common fluids:

Abbreviation	Full Fluid Name
H20	Water
FC40	FC-40 Fluorinated Liquid
Oil	Mineral Oil
Novec	Novec 7500
Hexa	Hexadecane

Table 3 Fluid calibrations available in the Flow Control Center Software

When first connected, water is the fluid calibration automatically selected by the sensor. When in idle mode, fluid calibration can be change using the Flow Control Center Software – more information can be found in Section 14.3.1.

10.1.2 Alternative Fluids

The Flow Rate Sensors are calibrated for water and 4 common fluids. To obtain accurate flow rates for alternative fluids, it is necessary to create a calibration curve comparing the displayed flowrate against actual flowrate. For each Flow Sensor type, it is necessary to pump the selected fluid through the flow sensor at a minimum of 5 different flow rates in the range and record the displayed value. For example, with the 1 – 50 µL/min sensor, displayed readings could be recorded at flow rates of 5, 15, 25, 35 and 45 µL/min. This data can be plotted as a curve of actual flow rate against displayed flow rate. Alternatively, the correction factor can be calculated by dividing actual flow rate by displayed flow rate. The correction factor can then be plotted against displayed flow rate and used to adjust the flow rate values for this selected fluid. An example of a calibration curve can be found below. For more information contact Support@Unchainedlabs.com.

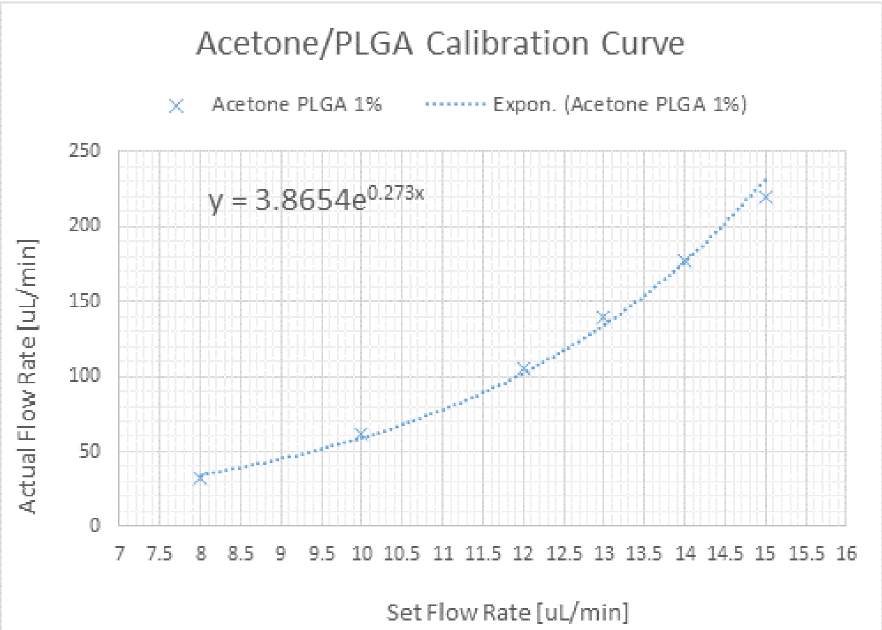


Figure 15 Example of a Calibration Curve

11 Tubing Connection

The Mitos System is delivered with a set of calibrated tubing (encompassing FEP tubing (1.6 mm OD, 0.25 mm ID)), required to transport fluids through the system, to the microfluidic chip for mixing, and sample collection. The assembly of this tubing is critical to ensure fluids are directed to the correct channels within the chip. The tubing pack supplied with the system will be application specific, with components such as 2-way in-line valves and T Connectors (Figure 16) pre-fitting in the correct locations within the network to correlate with specific chip configurations.

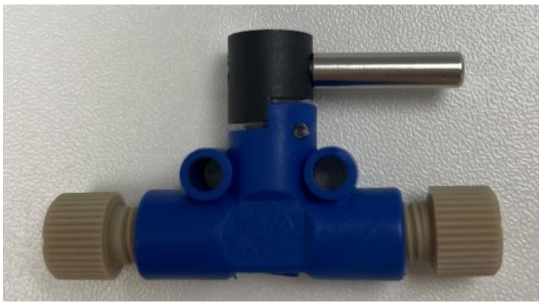


Figure 16 2-Way in-line valves and T-connectors are pre-assembled in Calibrated Tubing Network

A Linear Connector (Figure 17) will arrive pre-assembled at the end of the Tubing. The Linear Connector works alongside a Chip Interface to seal the chip/tubing assembly. Further details about this connection can be found in Section 12.

Figure 17 Linear Connector

11.1 Connecting P-Pump to Flow Rate Sensor

Even though the tubing packs supplied with the system may differ, the installation of the tubing follows the same steps.

Step 1: Locate the Tubing Pack supplied with the system. Within this tubing pack there is calibrated tubing with the following labels:

- 1A: To connect P-Pump 1 to Flow Rate Sensor 1
- 2A: To connect P-Pump 2 to Flow Rate Sensor 2
- 3A: To connect P-Pump 3 to Flow Rate Sensor 3

Step 2: Remove yellow plugs from the inputs on either side of all Flow Rate Sensors.

Step 3: Small range Flow Rate Sensor (70 - 1500 nL, 0.4 - 7 µL, 1 - 50 µL) are supplied with Convertors (3200285). Attach a Convertor either side of the Flow Rate Sensors by gently screwing the coned 6-40 fitting into the Flow Rate Sensor (Figure 18).

Figure 18 a) Flow Rate Sensor Convertor b) 2 Convertors connected to a Flow Rate Sensor

Important: Careful not to overtighten when screwing fittings into Flow Rate Sensors. All fittings should be finger tight.

Step 4: Unscrew the dip tube fitting from P-Pump 1 and locate tubing labelled 1A. Insert the free end of the tubing (at an angle) through the larger hole in the dip tube fitting. Thread ~15 cm of tubing through the fitting (Figure 3).

Step 5: Thread the tubing through the hole in the P-Pump lid and tighten the dip tube fitting in place. Check the tubing is below the surface of liquid within the Pump.

Step 6: Lock the tubing into place by pushing the tubing into the small hole of the dip tube fitting (the cleat). When the Pump is pressurized, the tubing will not be ejected (Figure 19).

Tubing not secure

Tubing secure

Figure 19 Tubing Threaded Through Dip Tube Fitting

Step 7: Tighten the fitting on the opposite end of the tubing to the RIGHT side of Flow Rate Sensor 1 (or attached to Convertor if using)

Figure 20 Fluidic Connection between P-Pump and Flow Rate Sensor when using Large Sensor (left) and Small Sensor (right)

Step 7: Repeat the process with tubing labelled 2A and 3A for P-Pumps 2 & 3 respectively.

11.2 Connecting Flow Rate Sensor to Linear Connector.

Step 1: Locate the Linear Connector with the following tubing pre-fitted:

- 1B: To connect Flow Rate Sensor 1 to Linear Connector
- 2B: To connect Flow Rate Sensor 2 to Linear Connector
- 3B: To connect Flow Rate Sensor 3 to Linear Connector

Note – as the tubing has the Linear Connector attached already, locate this temporarily onto the bench surface during the connection process. Wipe down the area with a clean wipe to limit exposure of components to dust/debris which may enter the system and result in blockages.

Step 2: Tighten the ¼”-28 fitting attached to 1B into the LEFT side of Flow Rate Sensor 1 or left Flowrate convertor (Figure 21).

Figure 21 Fluidic Connection between Flow Rate Sensor and Linear Connector when using Large Sensor (left) and Small Sensor (right)

Step 3: Repeat this process for the 2B tubing for the connection to Flow Rate Sensor 2.

Step 4: Repeat this process for the 3B tubing for the connection to Flow Rate Sensor 3.

The tubing network for the system is now complete, with the remaining connections to be made to the Microfluidic Chip via the Linear Connector.

12 Linear connectors, Interfaces and Chip configurations

The Mitos System connects the hardware modules together via Calibrated Tubing. More specifically, the Calibrated Tubing provides the means to direct fluid into and out of the microfluidic chips for reagent mixing and sample collection.

Each microfluidic chip connects via the same set of hardware, though different chips require reconfiguration to ensure the intended channels/geometry is used. Please note that the chips provided with the system will be dependent on the Application Pack selected.

The calibrated tubing supplied with the system will be pre-assembled within the Linear Connector to match the configuration of the Microfluidic chips supplied with the chosen Application Pack. The design of some microfluidic chips (typically those that generate small droplets/particles) require fluids be inserted and removed via the top of the chip. To achieve this, a Top Interface is required. Edge connection chips insert fluids laterally into the microfluidic chip and requires a H-Interface (Figure 22).

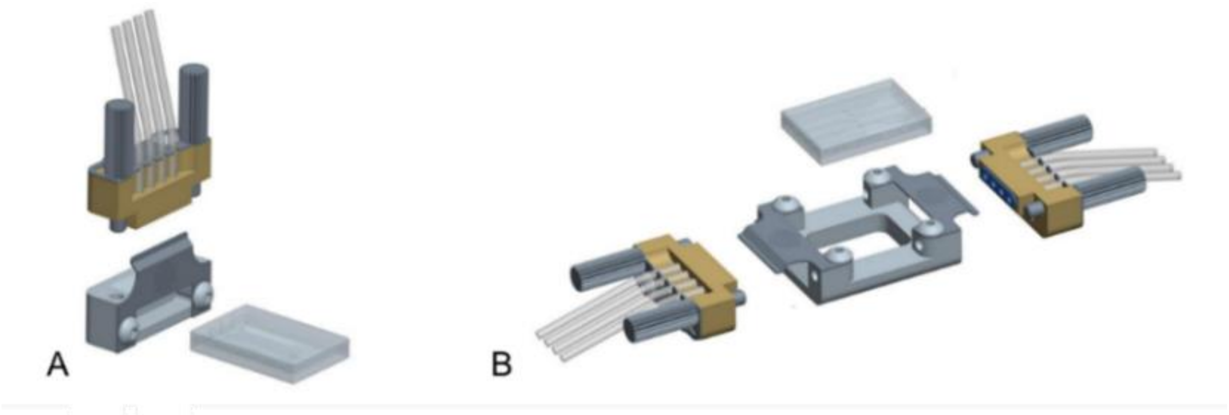


Figure 22 Chip Top Interface and one Linear Connector 4-Way in A; Chip Interface H and two Linear Connector 4-Way in B.

12.1 Linear Connector

For both Surface Connection and Edge Connection Chips, the interface between the tubing and chip itself remains the same, utilizing either a single Linear Connector, or a set of Linear Connectors. While the Linear Connector(s) arrive pre-assembled, it is necessary to understand the assembly/ disassembly process to facilitate changing to a different Chip type or when replacing tubing. Users should also check the correct gasket for their chosen application has been included in the assembly. Applications using fluorinated oil as a fluid should use a brown FKM gasket, whereas any other application requires a black FFKM gasket. Both gasket types will be provided with the Linear Connector.

The Linear Connector is comprised of the following components:

i. **Thumbscrews** – used to tighten the connector to the interface and seal against the chip.

ii. **Gasket** – sealing interface between the chip and linear connector, made of black FFKM or brown FKM.

iii. **Tubing Cleat** – retains tubing to prevent disconnections.

Figure 23 Components of Linear Connector

Step 1: Slide the end of the tubing into the correct Linear Connector Port (Figure 23). Insert the tubing so that this is "above" the Tubing Cleat to prevent damage to the external surface of the tubing. Tubing should extend past the end of the Linear Connector approximately 0.5 cm. Refer to the schematic provided with the Application Pack Datasheet to confirm tubing configuration.

Step 2: Push the Tubing into the Tubing Cleat to prevent this from moving whilst the remaining tubes are assembled.

Step 3: Repeat this process for all remaining Fluidic Tubes, ensure that the Tubing is inserted into the Linear Connector in the correct position for the experimental fluid being used.

Step 4: If Linear Connector ports remain empty after assembling all tubing, fill these ports with Plugs.

Step 4: With the tubing inserted, the Linear Connector Gasket can be installed. Take the black/brown Gasket and slide this over each of the tubes until the seal is loosely in place.

Step 5: Temporarily remove the tubing from the Tubing Cleat – doing so will allow the tubing to be pushed into place. Using a flat clean surface, press the Linear Connector down to leave a flat seal with tubing flush to the end of the seal (as shown in Figure 24).

Figure 24 Fully Assembled Linear Connector with tubing flush with gasket

12.2 H Interface

Each edge connection chip requires a Linear Connector on both sides of the chip to form a seal over the channels. The Linear Connectors tighten to a H Interface which houses the glass Microfluidic chip using the Thumbscrews to evenly apply pressure across the face of the chip to deliver fluids to the microfluidic channels.

When handling microfluidic chips, Users should wear gloves and work in a clean environment to minimize the chance of introducing dust/debris into the system.

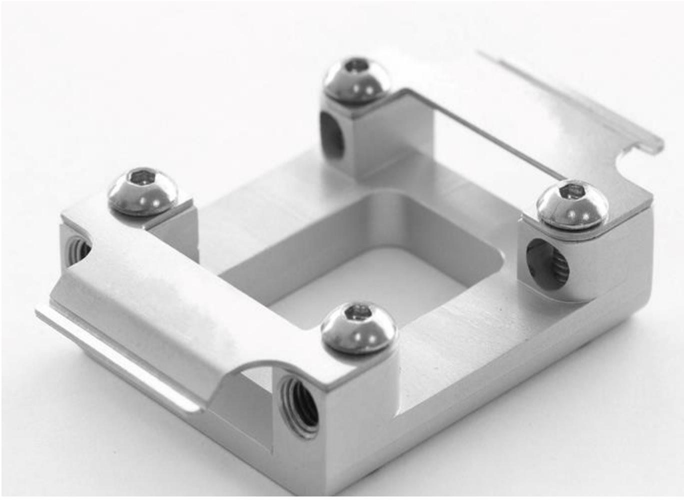


Figure 25 H Interface

Step 1: Locate the two Linear Connectors and H interface. Note: If the tubing as has already been assembled with the hardware modules, the Linear Connectors will already be located at the terminations of the tubing.

Step 2: Remove the Chip from the Gel-Pak box (retain the boxes to store the chips after use).

Step 2: Insert the Chip into the H Interface (Figure 26).

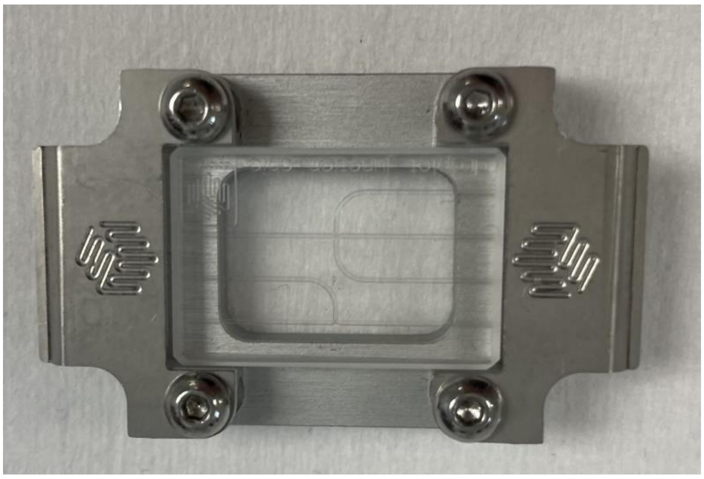


Figure 26 H Interface with Microfluidic Chip Inserted

Step 3: Locate the Inlet Linear Connector and Outlet Linear Connector (dependent on Chip used).

Step 4: Tighten each side of the Linear Connector evenly until hand tight. No tools should be used to tighten the Thumbscrews. Ensure that the Chip remains in place while tightening to prevent this from falling out before fully sealed (Figure 27).

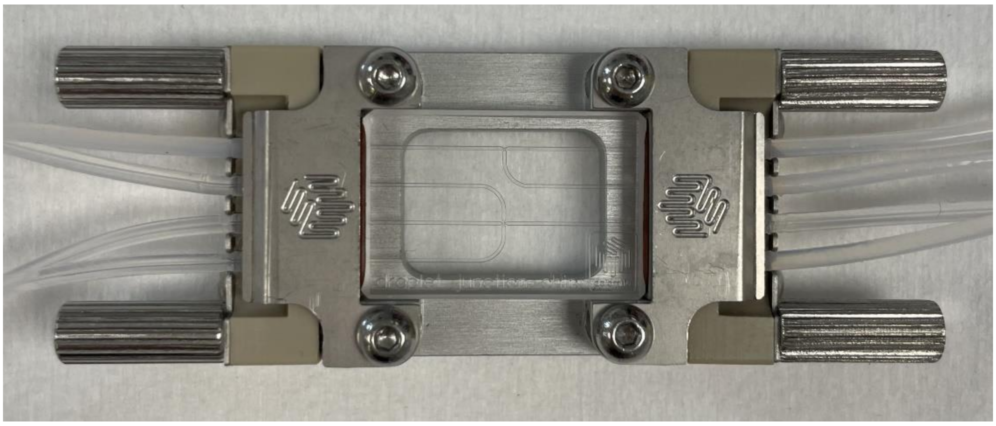


Figure 27 Chip Assembly

Step 5: To check the sealing is oven across the chip, turn the interface over and visually check for any large gaps between the Linear Connectors and the H Interface. If present, unscrew the Linear Connectors slightly and reassemble.

Step 6: Place the assembly on the Microscope Light Stage, using the clips to keep the Chip in place (Figure 28). The fluidic network is complete, place the end of the output tubing into a collection vessel.

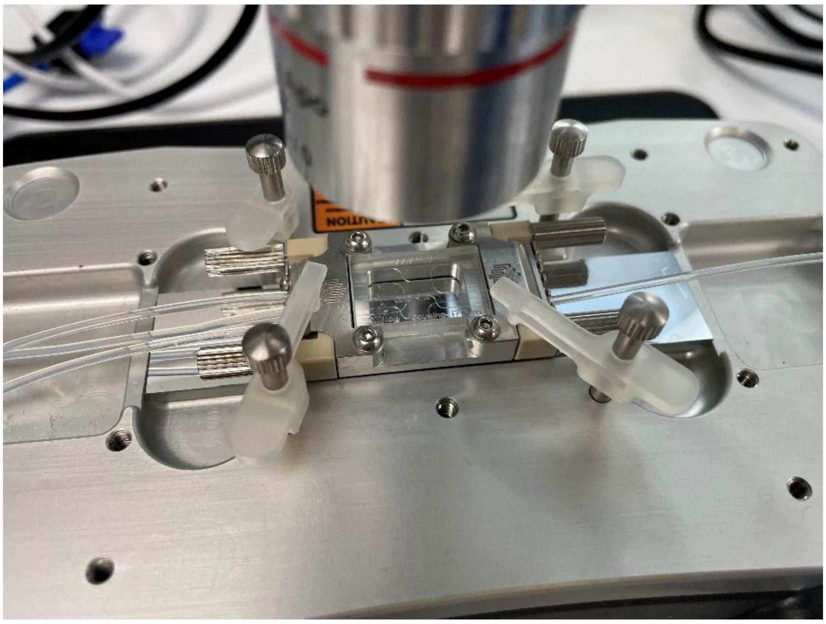


Figure 28 Chip assembly on Microscope Light Stage

12.3 Top Interface

The Top Interface works in conjunction with a single Linear Connector, providing fluidic connections to the top surface of glass microfluidic chips.

Step 1: Locate the Top Interface and Linear Connector (ensure the tubing has been assembled to correctly match the chip inputs/outputs. Figure 29)

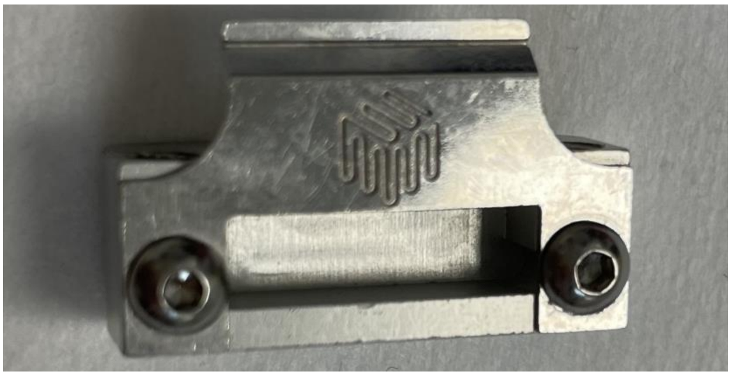


Figure 29 Top Interface

Step 2: Remove the Chip from the Gel-Pak box (retain the boxes to store the chips after use).

Step 3: Push the Chip into the slot in Top Interface ensuring the holes are facing upwards (Figure 30).

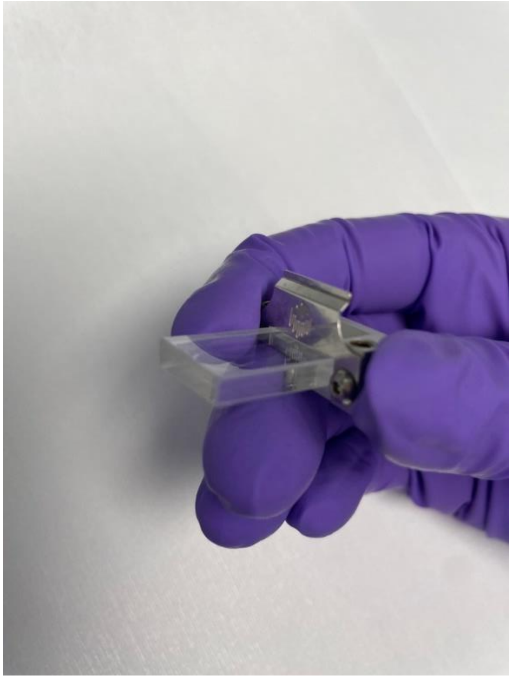


Figure 30 Chip Inserted into Top Interface

Step 4: Attach the Linear Connector and tighten the screws in tandem to ensure even compression of the gasket across the Chip inlets. Tighten until hand tight – tools should not be used (Figure 31).

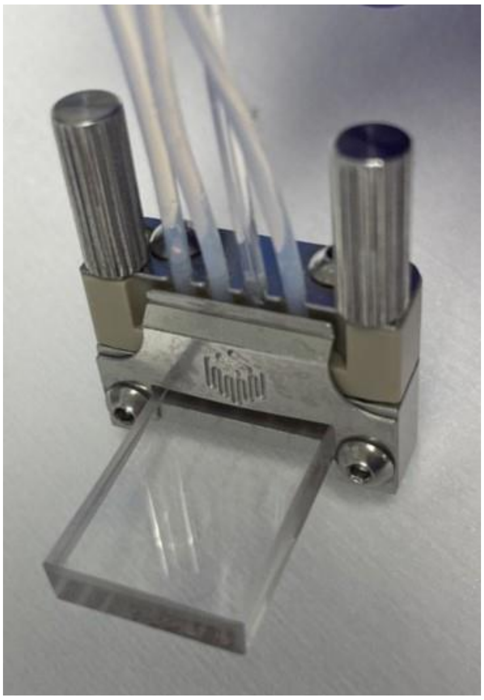


Figure 31 Chip assembled with Top Interface and Linear Connector

Step 5: Place the assembly on the Microscope Light Stage, using the clips to keep the Chip in place. The fluidic network is complete, place the end of the output tubing into a collection vessel.

13 High-Speed Microscope and Camera

The High-Speed Microscope and Camera works in combination with the X-Y stage to give real time visualization of the chip junction and channels.

Step 1: Locate the Microscope (3200531) and X-Y Stage (3200600).

Step 2: Place the X-Y stage on top of the base of the Microscope Stand and Light controller above the X-Y stage (Figure 32).

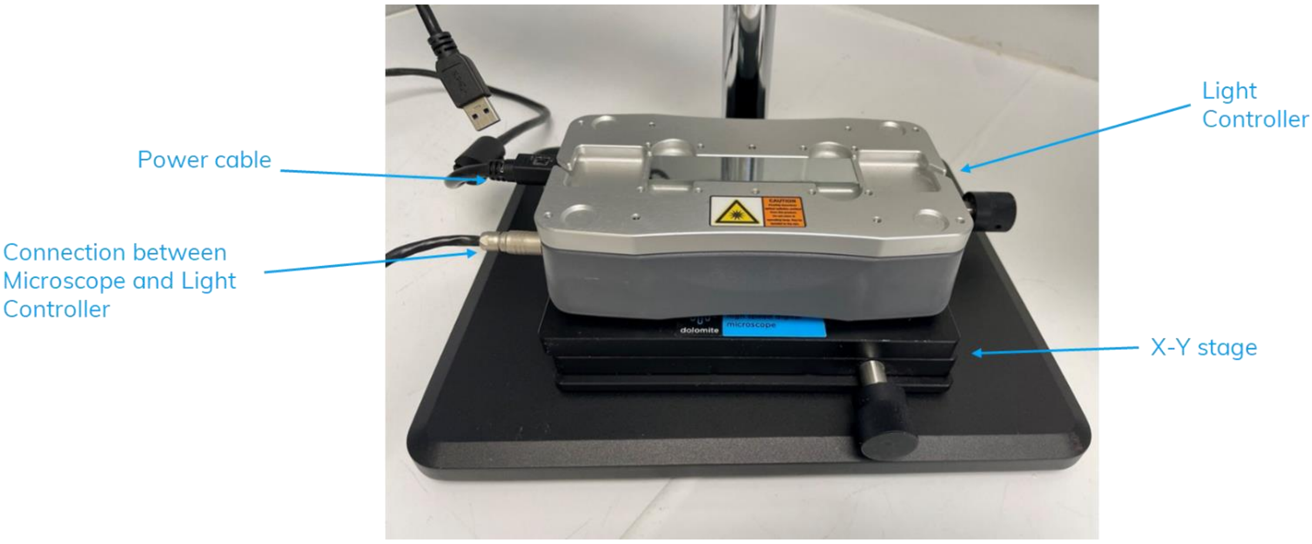


Figure 32 X-Y stage and Light Controller on Top of Microscope Base Plate

Step 4: Connect the Microscope to the Light Controller using the Fan Cable connected to the Microscope.

Step 5: Connect the Microscope to the PC using the Black USB cable attached.

Step 6: Plug the power cord into a mains socket, and attach the power cable to the microscope light controller. Turn on the power switch.

Step 7: Place the mirror slide in the recess on top of the Light Controller. Screw the Clips either side of the mirror, these will be used to hold the Microfluidic Chip in place during experiments.

14 Dolomite Flow Control Center (FCC)

Dolomite Flow Control Center (FCC) is the software platform used to control the Mitos System. The Mitos System can only be controlled via the software and cannot be operated directly via hardware.

The software is pre-loaded on the PC supplied with the system, however if Users require a copy of the software on additional PCs, a download link can be requested from Support@Unchainedlabs.com. Be aware that the system is only compatible with Windows and should be used with a Windows 10 operating system.

14.1 FCC Overview

When starting FCC, the User will be greeted with the Home Screen (Figure 33). Users can link directly to the Dolomite Website from this page.

To the top left, the "About" section details the specific version number of the Flow Control Center as well as the option to disable the Home Screen at start-up, Lastly, the License Manager is an area which allows different software licenses to be loaded. Licenses are provided alongside the µ-Encapsulator application pack, giving users access to automated protocols.

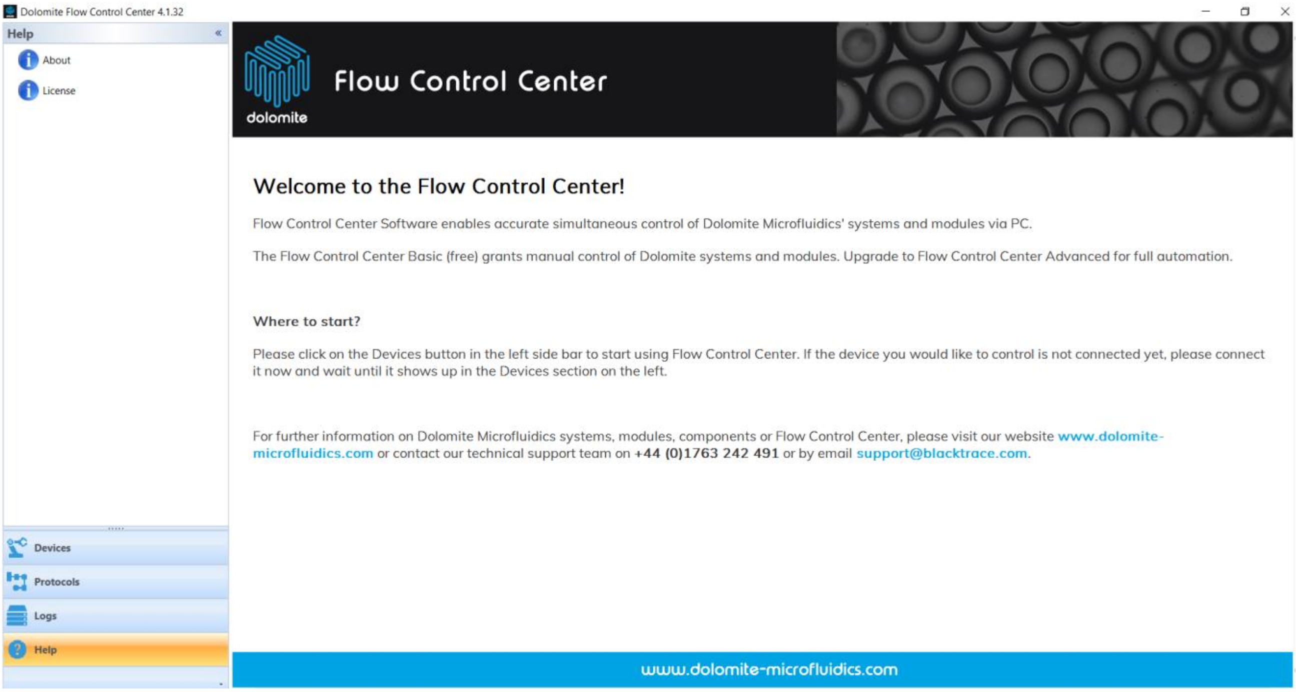


Figure 33 Flow Control Center

To access the functionalities of the software, the User can choose from a variety of tabs located on the bottom left of the software. The different tabs serve different functionalities:

I. **Devices** - Allows for control of all hardware devices connected to the PC.

II. **Protocols** – Allows users of the µ-Encapsulator application pack to run experiments using defined protocols.

III. **Logs** – Experimental data collected whilst running the system which is saved automatically.

IV. **Help** – return to Homepage.

14.2 Connecting Devices

The Mitos System will need to be powered on and connected to the PC (or provided USB Hub) for the Flow Control Center to recognize that the hardware is available to use. Hardware should be powered on prior to opening the software on the System PC

Step 1: Ensure the P-Pumps and Microscope are connected to the PC via the supplied communication cables. Power on all hardware.

Step 2: Open Flow Control Center on the system PC.

Step 3: Navigate to the Device tab on the bottom left of the Home page (Figure 34).

Figure 34 Devices Tab in FCC

Step 4: Devices that are connected and powered on will populate within the software. As the P-Pumps are controlled individually they will appear within their own individual user interface alongside the Microscope. Flow Rate Sensors will not appear in their own windows (Figure 35).

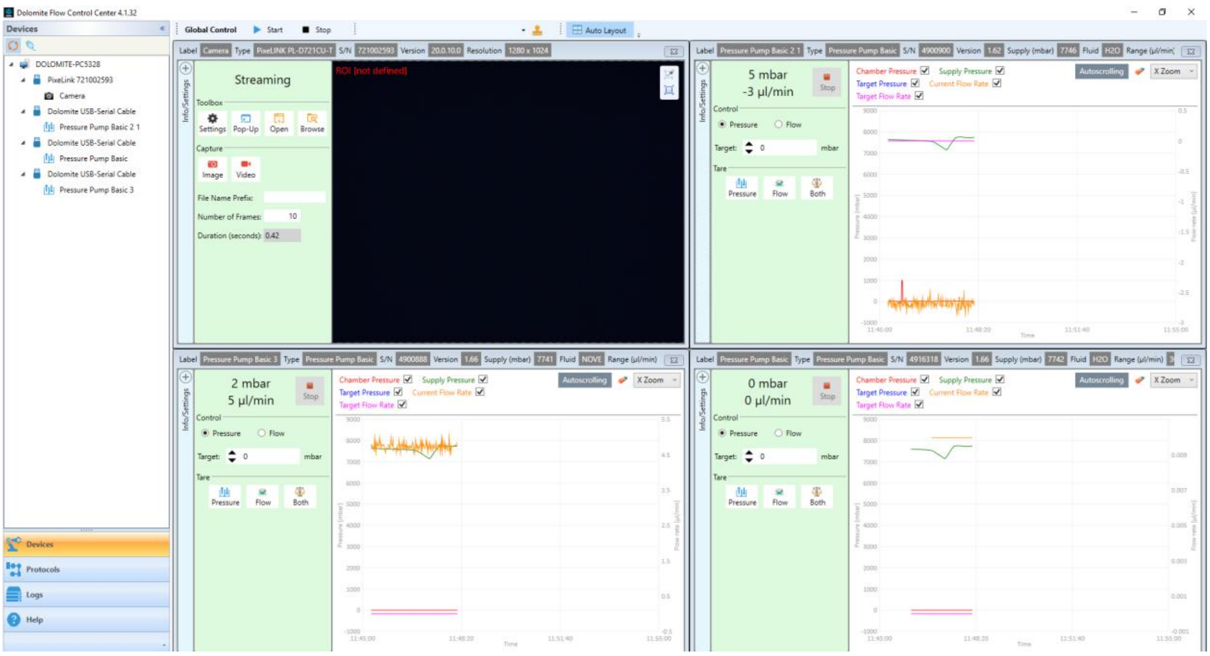


Figure 35 Flow Control Center with Mitos System Hardware Connected

14.3 P-Pump Device View in FCC

When connected, each P-Pump will populate in FCC as individual control windows, as seen in Figure 36.

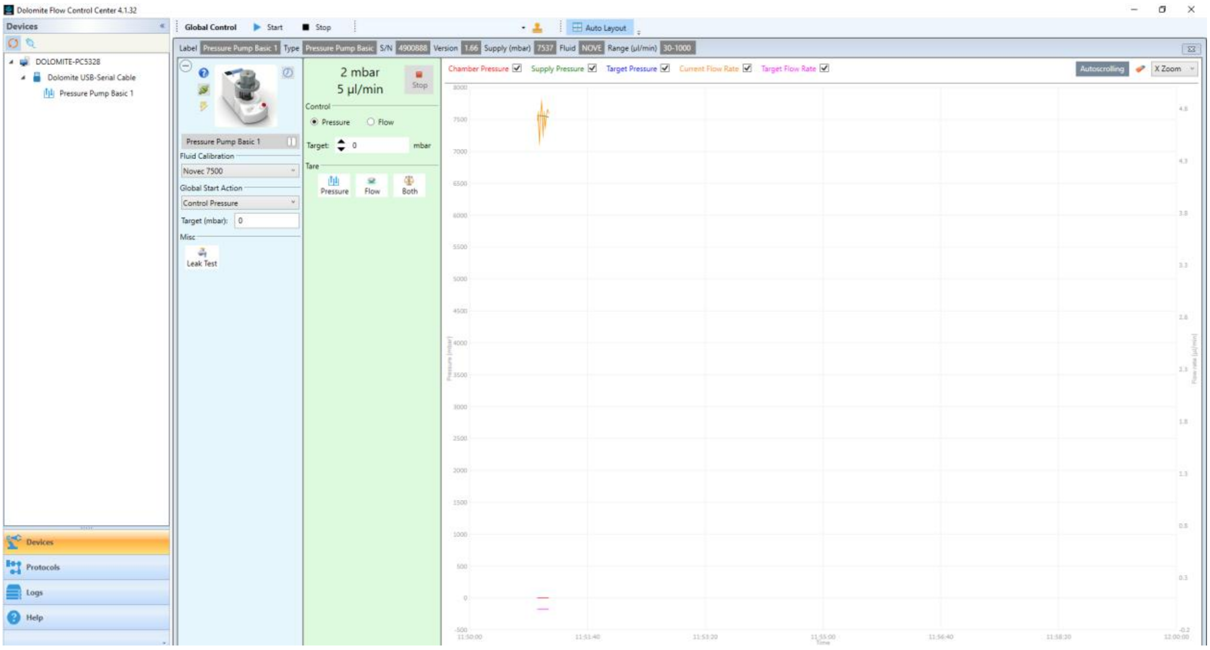


Figure 36 P-Pump Device View

The top of each control window contains the following data about each Pump.

Label – Automatically generated when first assembling the system but can be changed if required (between 3-50 characters; no special characters).

Type – Pressure Pump Basic.

Serial Number – Can be used to identify which control window belongs to which Pump – serial numbers are located on the back of each P-Pump.

Version – Hardware version

Supply pressure – Current supply pressure of system in mbar. Supply pressure of the system should not exceed 11 bar.

Fluid – Set fluid calibration of Flow Rate Sensor.

Range – Accurate detection range of Flow Rate Sensor.

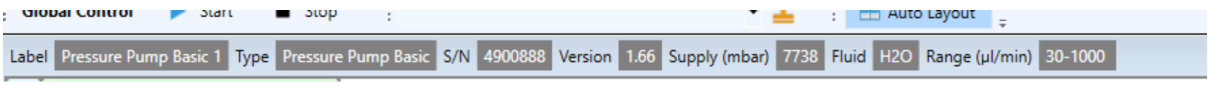


Figure 37 P-Pump Device Information

14.3.1 Information and Setting

Expanding the information and settings panel allows the user to alter some of the data listed above (Figure 38).

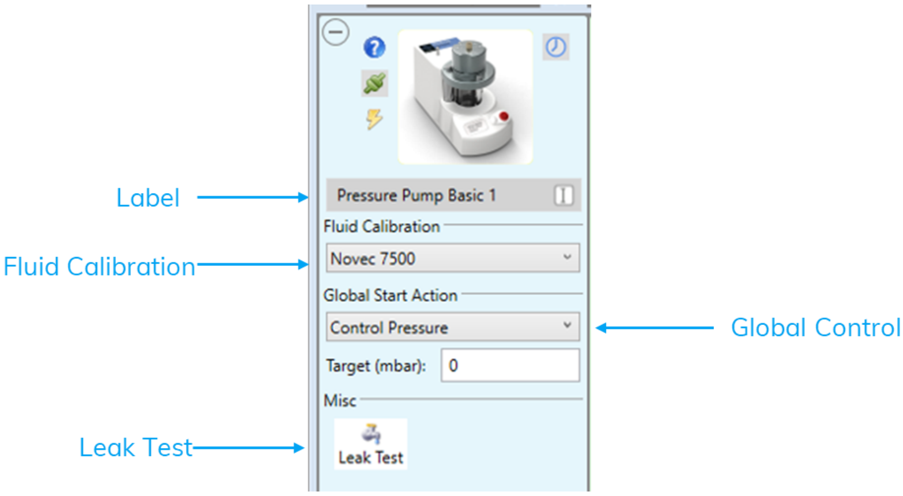


Figure 38 Information and Settings options

14.3.1.1 Changing P-Pump Label

To change the label of a P-Pump:

Step 1: Select the Cursor icon as seen in Figure 38, the box should turn yellow.

Step 2: Type in new label, Users may find it useful to use the name of the fluid loading in the P-Pump.

Step 3: Press 'enter' on the keyboard to confirm the change.

Step 4: The label will be update in both the information and settings panel as well as at the top of the control window.

14.3.1.2 Fluid Calibration

Flow Rate Sensors will automatically be calibrated for Water when first installed in the system. The User has the option to select 4 other common fluids: Novec 7500, Mineral Oil, Hexadecane or FC-40.

To select a different calibration fluid

Step 1: Locate the 'Fluid Calibration' section of the information and settings panel.

Step 2: Click on the arrow and select Fluid from dropdown menu.

Step 3: The Flow Rate Sensor will be updated, and 'Fluid' will change at the top of the control window.

14.3.1.3 Performing a leak test

Leak tests can be performed to ensure the Pressure Pumps are sealing correctly. It is recommended a leak test be performed after initial set up of the system, and every 6 months as general maintenance.

Step 1: Plug the chamber lid by inserting a FEP plug into the dip tube fitting.

Step 2: Turn on supply pressure.

Step 3: Click the 'Leak Test' button. The Pump will be pressured to a low pressure (~700 mbar) for 1-2 mins followed by a high pressure (dependent on supply pressure) for 1-2 mins.

Step 4: Results of the leak test will appear as the image below. When performing leak tests on multiple Pumps, complete one at a time.

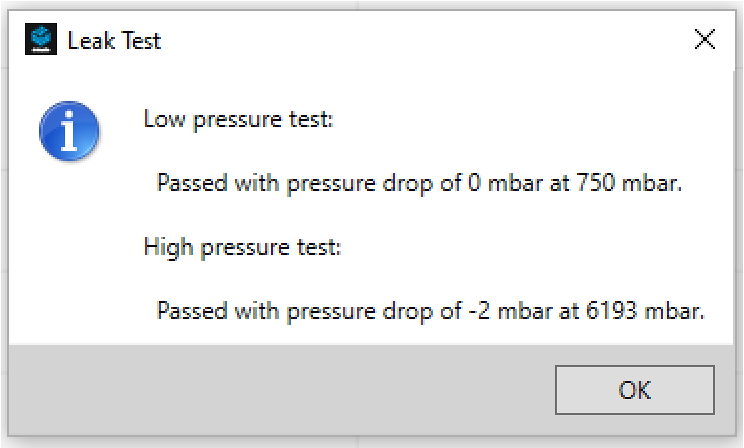


Figure 39 Leak Test Results

14.3.2 Control Pannel

Within Flow Control Software, there are a range of options to control the Pressure Pump. Each feature provides a specific function.

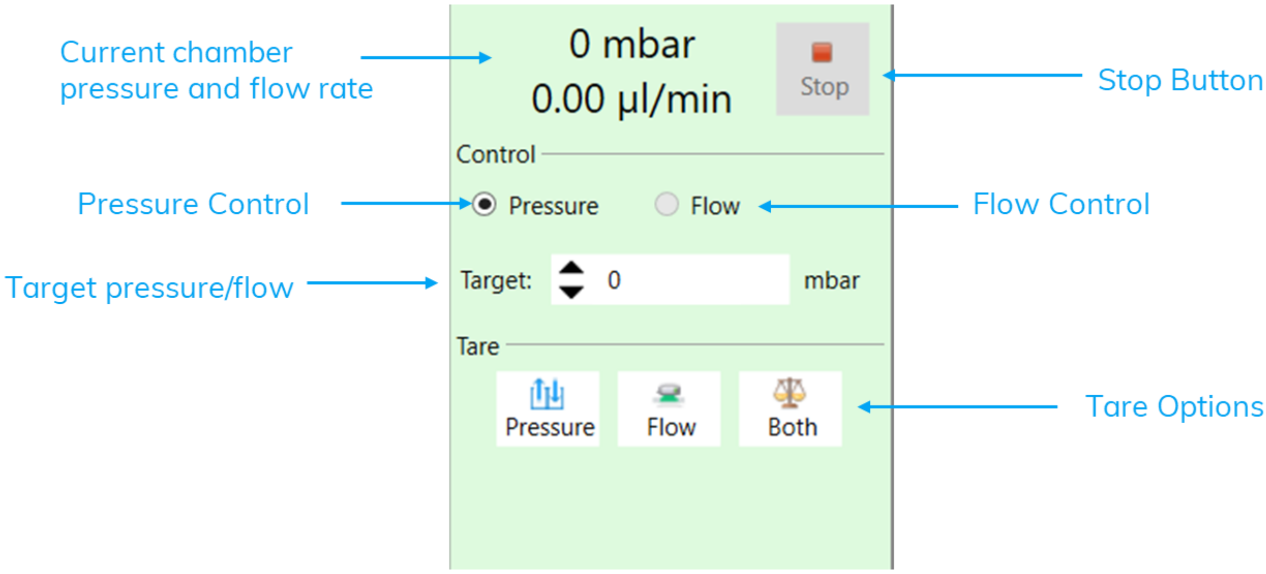


Figure 40 P-Pump Control Panel in Flow Control Center

Below is a description of the terms, and a detail of the action that will occur on selection of each option:

- The numbers at the top of each panel show the current chamber pressure and flow rate.
- **Stop** - Click this button to stop this pump. Only this pump will stop, any other pumps connected will continue to run.
- **Pressure** - Click in the box to select pressure control mode.
- **Flow** - When a Flow Rate Sensor is connected to the Pump, click in the box to select flow control mode. If no Flow Rate Sensor is connected, the flow box is greyed out.
- **Target** -Type in a value and press enter to set the target pressure in the chamber.
- **Up/down** arrow - Right click here to select a pressure change increment of 1, 10, 100 or 1000mbar. A left click up/down will change the pressure accordingly.
- **Tare Options**
    - Pressure – Tare for pressure only. There must be **no Supply Pressure** to perform this function.
    - Flow – Tare for flow only. There must be **no Flow** through the P-pump to perform this function.
    - Both – Tare for pressure and flow at the same time.

Note: If the Flow Rate Sensor/Sensor interface is not connected correctly to the P-Pump the options to tare for Flow and Both will be greyed out.

14.3.3 Data Logging

When a Pump is used via the Flow Control Center Software, data is logged in real time on-screen. In particular, the graph will populate with the Chamber Pressure, Supply Pressure, Target Pressure, Current Flow Rate and Target Flow Rate. This data will continue streaming and show the current values when the top right icon “Autoscrolling” is displayed. If the graph is inspected by the User, the graph will stop “Autoscrolling” and instead show an arrow icon. Clicking this will restore the “Autoscrolling” mode. Clicking the eraser icon in the top left will clear the graph display (note that this will note delete any logged data).

Figure 41 Example Graph Produced in FCC

14.4 Pressure Control Mode

In pressure control mode, a constant pressure is set and maintained.

Note: The supply pressure must be greater than 500mbar above the required P-Pump pressure.

To set the chamber pressure:

Step 1: Ensure Pressure Mode is selected in the Control Panel

Step 2: Entre value into the Target Box and press enter.

Step 3: Change the target pressure by using the arrows to adjust up or down as required or type a new value into the target box.

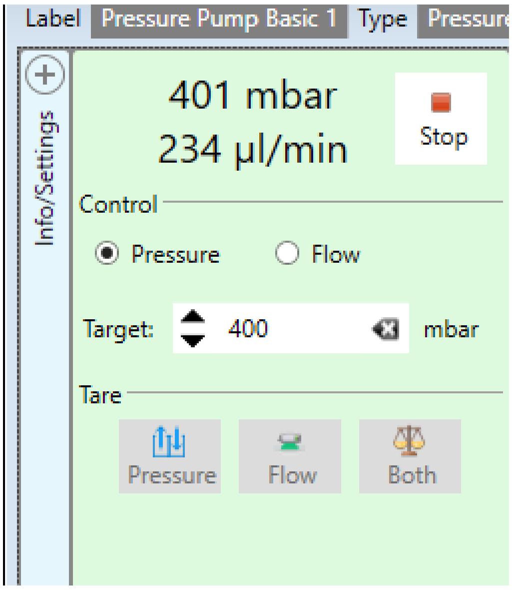


Figure 42 Pressure Control Mode

Even though the P-Pump is controlled in pressure mode, flow rate will also be reported (but not maintained).

14.5 Flow Control Mode

In flow control mode, the chamber pressure is adjusted to maintain a constant flow. Running in flow control mode is useful to regulate droplet production if the lab temperature changes. In this way, the system compensates for small changes in the viscosity of the liquids used.

Note: The supply pressure must be greater than 500mbar above the required P-Pump pressure to maintain the programmed flow rate.

To set flow rate:

Step 1: Ensure Flow Mode is selected in the Control Panel

Step 2: Entre value into the Target Box and press enter.

Step 3: Change flow rate by typing a new value into the Target Box.

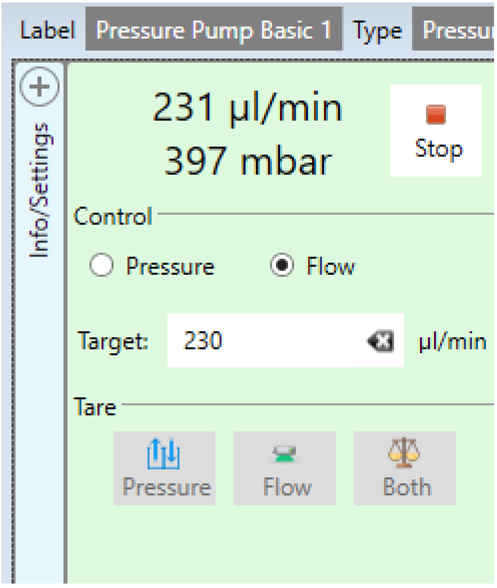


Figure 43 Flow Control Mode

It is recommended to run the system in pressure mode until stable and then switch to flow mode. Control the P-Pumps in pressure mode, adjusting pressure until the desired flow rate is reached. Change to flow control mode by select Flow in the control panel.

14.6 High-Speed Microscope and Camera in FCC

The High-Speed Microscope and Camera allows the mixing of reagents at the junction to be visualized in real time. When connect to the Flow Control Software, the Microscope will appear in a separate window and a number of controls become available to the User.

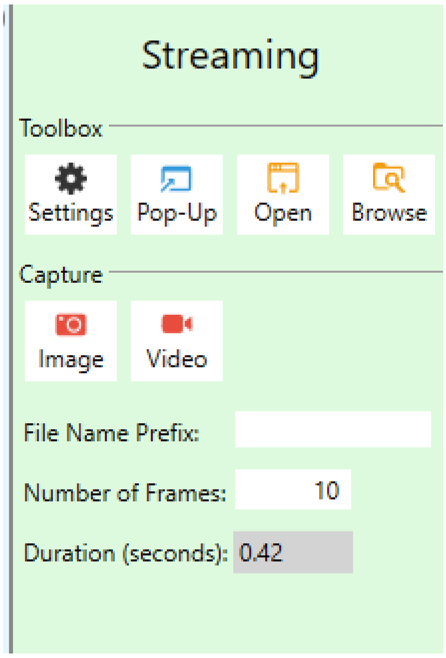


Figure 44 Microscope Control in FCC

- **Pop-Up** - Opens a screen where just the live image can be seen without clutter.
- **Settings** - Click on the Settings button to open a new window, see Figure 45
    - Exposure Time - The sharpest images will be taken with the smallest exposure time and this is especially true when looking at droplets being produced in the order of kHz. Alternatively, select the tick box to automatically determine the exposure time.
    - Gain – Useful to increase when light levels are low.
    - Frame Rate - This is the number of frames captured per second (FPS) during video capture. An increase in the FPS will slow down the apparent movement of the droplets.
    - Gamma - This setting increases contrast e.g. improves the definition.
    - Image Flip – Flip the orientation of the image
    - Region of Interest (ROI) - Use this feature to select the region that you wish to view/capture. Note that the larger ROI, the larger the file size and the lower the frames/second rate.
    - Undo Region of Interest - Returns the ROI to full screen.
- **Open** - Open the default folder where images are stored.
- **Browse** - Change the default folder where images are stored.
- **Image** - Click to capture a single image.
- **Video** - Click to capture video.

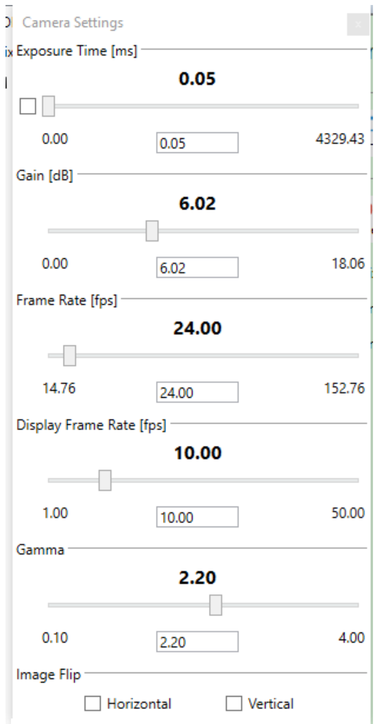


Figure 45 Microscope Settings

14.7 Log Files

Whilst running, all data is saved and stored in a log file on the local area of the PC. These are saved as CSV files and can be accessed via the logs tab in the bottom left of the Flow Control Center Software. The log files can be accessed for later use to compare the physical output and the values entered for that specific experiment for any data analysis or reporting required.

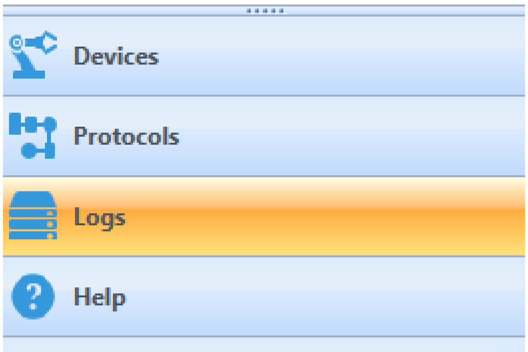
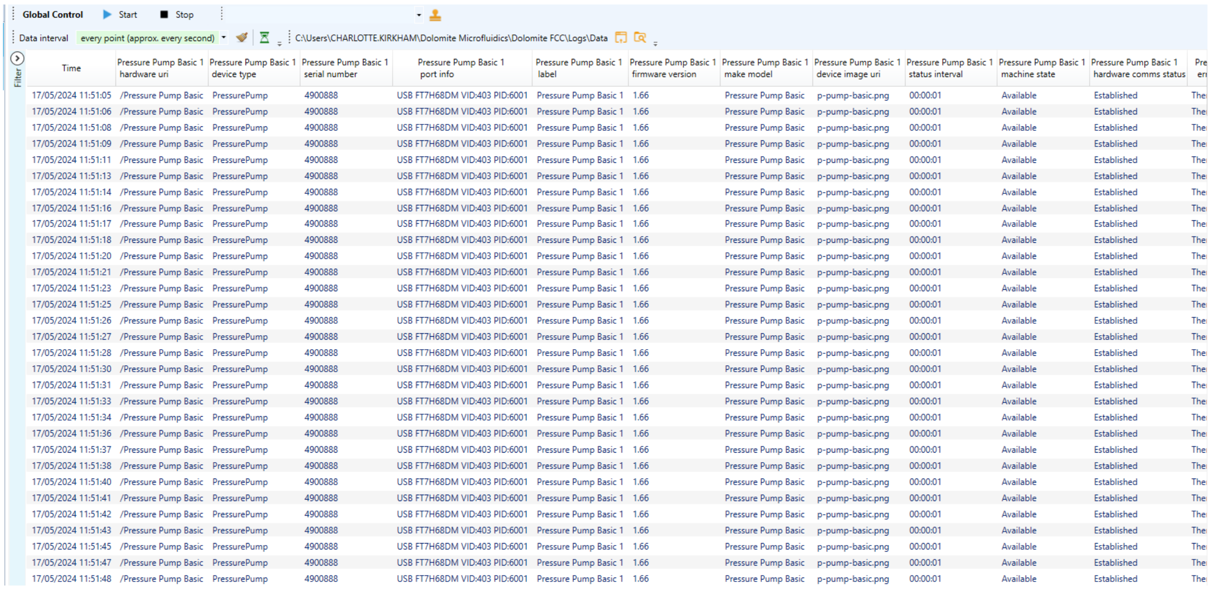


Figure 46 Logs tab in Flow Control Center

FFC will save the logs to a standard location on the PC: C:\Users\USER NAME\Dolomite Microfluidics\Dolomite FCC\Logs\

This location can be accessed via the “Open Data Log” button (folder icon with an UP arrow) and the location can be changed to a different folder location by selecting a location on the PC using the “Browse for Data Log Folder” button (folder icon with a magnifying glass).



Time	Pressure Pump Basic 1 hardware uri	Pressure Pump Basic 1 device type	Pressure Pump Basic 1 serial number	Pressure Pump Basic 1 port info	Pressure Pump Basic 1 label	Pressure Pump Basic 1 firmware version	Pressure Pump Basic 1 make model	Pressure Pump Basic 1 device image uri	Pressure Pump Basic 1 status interval	Pressure Pump Basic 1 machine state	Pressure Pump Basic 1 hardware comms status	Pre er
17/05/2024 11:51:05	/Pressure Pump Basic	PressurePump	4900888	USB FT7H68DM VID:403 PID:6001	Pressure Pump Basic 1	1.66	Pressure Pump Basic	p-pump-basic.png	00:00:01	Available	Established	The
17/05/2024 11:51:06	/Pressure Pump Basic	PressurePump	4900888	USB FT7H68DM VID:403 PID:6001	Pressure Pump Basic 1	1.66	Pressure Pump Basic	p-pump-basic.png	00:00:01	Available	Established	The
17/05/2024 11:51:08	/Pressure Pump Basic	PressurePump	4900888	USB FT7H68DM VID:403 PID:6001	Pressure Pump Basic 1	1.66	Pressure Pump Basic	p-pump-basic.png	00:00:01	Available	Established	The
17/05/2024 11:51:09	/Pressure Pump Basic	PressurePump	4900888	USB FT7H68DM VID:403 PID:6001	Pressure Pump Basic 1	1.66	Pressure Pump Basic	p-pump-basic.png	00:00:01	Available	Established	The
17/05/2024 11:51:11	/Pressure Pump Basic	PressurePump	4900888	USB FT7H68DM VID:403 PID:6001	Pressure Pump Basic 1	1.66	Pressure Pump Basic	p-pump-basic.png	00:00:01	Available	Established	The
17/05/2024 11:51:13	/Pressure Pump Basic	PressurePump	4900888	USB FT7H68DM VID:403 PID:6001	Pressure Pump Basic 1	1.66	Pressure Pump Basic	p-pump-basic.png	00:00:01	Available	Established	The
17/05/2024 11:51:14	/Pressure Pump Basic	PressurePump	4900888	USB FT7H68DM VID:403 PID:6001	Pressure Pump Basic 1	1.66	Pressure Pump Basic	p-pump-basic.png	00:00:01	Available	Established	The
17/05/2024 11:51:16	/Pressure Pump Basic	PressurePump	4900888	USB FT7H68DM VID:403 PID:6001	Pressure Pump Basic 1	1.66	Pressure Pump Basic	p-pump-basic.png	00:00:01	Available	Established	The
17/05/2024 11:51:17	/Pressure Pump Basic	PressurePump	4900888	USB FT7H68DM VID:403 PID:6001	Pressure Pump Basic 1	1.66	Pressure Pump Basic	p-pump-basic.png	00:00:01	Available	Established	The
17/05/2024 11:51:18	/Pressure Pump Basic	PressurePump	4900888	USB FT7H68DM VID:403 PID:6001	Pressure Pump Basic 1	1.66	Pressure Pump Basic	p-pump-basic.png	00:00:01	Available	Established	The
17/05/2024 11:51:20	/Pressure Pump Basic	PressurePump	4900888	USB FT7H68DM VID:403 PID:6001	Pressure Pump Basic 1	1.66	Pressure Pump Basic	p-pump-basic.png	00:00:01	Available	Established	The
17/05/2024 11:51:21	/Pressure Pump Basic	PressurePump	4900888	USB FT7H68DM VID:403 PID:6001	Pressure Pump Basic 1	1.66	Pressure Pump Basic	p-pump-basic.png	00:00:01	Available	Established	The
17/05/2024 11:51:23	/Pressure Pump Basic	PressurePump	4900888	USB FT7H68DM VID:403 PID:6001	Pressure Pump Basic 1	1.66	Pressure Pump Basic	p-pump-basic.png	00:00:01	Available	Established	The
17/05/2024 11:51:25	/Pressure Pump Basic	PressurePump	4900888	USB FT7H68DM VID:403 PID:6001	Pressure Pump Basic 1	1.66	Pressure Pump Basic	p-pump-basic.png	00:00:01	Available	Established	The
17/05/2024 11:51:26	/Pressure Pump Basic	PressurePump	4900888	USB FT7H68DM VID:403 PID:6001	Pressure Pump Basic 1	1.66	Pressure Pump Basic	p-pump-basic.png	00:00:01	Available	Established	The
17/05/2024 11:51:27	/Pressure Pump Basic	PressurePump	4900888	USB FT7H68DM VID:403 PID:6001	Pressure Pump Basic 1	1.66	Pressure Pump Basic	p-pump-basic.png	00:00:01	Available	Established	The
17/05/2024 11:51:28	/Pressure Pump Basic	PressurePump	4900888	USB FT7H68DM VID:403 PID:6001	Pressure Pump Basic 1	1.66	Pressure Pump Basic	p-pump-basic.png	00:00:01	Available	Established	The
17/05/2024 11:51:30	/Pressure Pump Basic	PressurePump	4900888	USB FT7H68DM VID:403 PID:6001	Pressure Pump Basic 1	1.66	Pressure Pump Basic	p-pump-basic.png	00:00:01	Available	Established	The
17/05/2024 11:51:31	/Pressure Pump Basic	PressurePump	4900888	USB FT7H68DM VID:403 PID:6001	Pressure Pump Basic 1	1.66	Pressure Pump Basic	p-pump-basic.png	00:00:01	Available	Established	The
17/05/2024 11:51:33	/Pressure Pump Basic	PressurePump	4900888	USB FT7H68DM VID:403 PID:6001	Pressure Pump Basic 1	1.66	Pressure Pump Basic	p-pump-basic.png	00:00:01	Available	Established	The
17/05/2024 11:51:34	/Pressure Pump Basic	PressurePump	4900888	USB FT7H68DM VID:403 PID:6001	Pressure Pump Basic 1	1.66	Pressure Pump Basic	p-pump-basic.png	00:00:01	Available	Established	The
17/05/2024 11:51:36	/Pressure Pump Basic	PressurePump	4900888	USB FT7H68DM VID:403 PID:6001	Pressure Pump Basic 1	1.66	Pressure Pump Basic	p-pump-basic.png	00:00:01	Available	Established	The
17/05/2024 11:51:37	/Pressure Pump Basic	PressurePump	4900888	USB FT7H68DM VID:403 PID:6001	Pressure Pump Basic 1	1.66	Pressure Pump Basic	p-pump-basic.png	00:00:01	Available	Established	The
17/05/2024 11:51:38	/Pressure Pump Basic	PressurePump	4900888	USB FT7H68DM VID:403 PID:6001	Pressure Pump Basic 1	1.66	Pressure Pump Basic	p-pump-basic.png	00:00:01	Available	Established	The
17/05/2024 11:51:40	/Pressure Pump Basic	PressurePump	4900888	USB FT7H68DM VID:403 PID:6001	Pressure Pump Basic 1	1.66	Pressure Pump Basic	p-pump-basic.png	00:00:01	Available	Established	The
17/05/2024 11:51:41	/Pressure Pump Basic	PressurePump	4900888	USB FT7H68DM VID:403 PID:6001	Pressure Pump Basic 1	1.66	Pressure Pump Basic	p-pump-basic.png	00:00:01	Available	Established	The
17/05/2024 11:51:42	/Pressure Pump Basic	PressurePump	4900888	USB FT7H68DM VID:403 PID:6001	Pressure Pump Basic 1	1.66	Pressure Pump Basic	p-pump-basic.png	00:00:01	Available	Established	The
17/05/2024 11:51:43	/Pressure Pump Basic	PressurePump	4900888	USB FT7H68DM VID:403 PID:6001	Pressure Pump Basic 1	1.66	Pressure Pump Basic	p-pump-basic.png	00:00:01	Available	Established	The
17/05/2024 11:51:45	/Pressure Pump Basic	PressurePump	4900888	USB FT7H68DM VID:403 PID:6001	Pressure Pump Basic 1	1.66	Pressure Pump Basic	p-pump-basic.png	00:00:01	Available	Established	The
17/05/2024 11:51:47	/Pressure Pump Basic	PressurePump	4900888	USB FT7H68DM VID:403 PID:6001	Pressure Pump Basic 1	1.66	Pressure Pump Basic	p-pump-basic.png	00:00:01	Available	Established	The
17/05/2024 11:51:48	/Pressure Pump Basic	PressurePump	4900888	USB FT7H68DM VID:403 PID:6001	Pressure Pump Basic 1	1.66	Pressure Pump Basic	p-pump-basic.png	00:00:01	Available	Established	The

Figure 47 Flow Control Center Data Log Files

15 Mitos System Set Up, Priming and Experiments

Before completing an experiment, the User should perform the following actions and input parameters. While desired pressures and flow rates will differ based on application and User, the system should always be primed with experimental reagents before use. The primary function of this is to remove air from the system. All fluids should be filtered with a 0.2 um filter before use to minimize the risk of debris being introduced into the system.

Step 1: Turn on all Hardware, ensuring modules are connected to the PC using the cables provided with the system.

Step 2: Load Flow Control Center Software on System PC.

Step 3: Change Label of each P-Pump (if necessary).

Step 4: Confirm correct calibration fluid selected for each Pump.

Step 5: Without a supply pressure, Tare all P-Pumps for pressure and flow.

Step 6: Provide supply pressure (max 11 bar).

Step 7: Load reagents in P-Pump chambers using glass vials. Ensure tubing is below liquid level.

Step 8: Prime Continuous Phase tubing to remove air from the system. Set a target pressure of 2000 mbar using Flow Control Center Software, open 2-way in-line valve and wait for fluid to exit via the Linear Connector. Close in-line valve and stop P-Pump.

Step 9: Repeat the priming process of the remaining P-Pumps.

Step 10: Connect microfluidic chip to the system using the correct interface, as detailed in Section 12.

Step 11: Place Chip assembly on Microscope Light Stage to visualize junction.

Step 12: Place free end of Output tubing in a collection vessel.

The system is now set up to complete an experiment, Users can select and adjust pressure and flow rate as outlined in Section 14 to create droplets/particles.

Step 1: Close all in-line valves

Step 2: Set a target pressure on the Continuous P-Pump, open the 2-way in-line valve.

Step 3: Wait until the Chip is fully wetted, and fluid is passing through the output tubing.

Step 4: Adjust target pressure until desired flow rate is reached, change to flow control mode.

Step 5: Set a target pressure on the Dispersed Phase P-Pump(s). Open the 2-way in-line valve(s).

Step 6: Adjust target pressure until desired flow rate is reached, change to flow control mode.

Step 7: Collect emulsion in collection vessel.

When enough emulsion has been collected:

Step 1: Revert to pressure mode,

Step 2: Close all 2-way in-line valves.

Step 3: Press ‘STOP’ in each P-Pump control window.

Step 4: If using one P-Pump as a Flushing Pump. Set a target pressure of 2000 mbar, open the 2-way in-line valve, and allow the solvent to backflow through all the channels of the chip. After 1-2 mins, close the 2-way in-line valve and press ‘STOP’ in the P-Pump control window.

16 System Cleaning and Maintenance

To ensure that the Mitos System continues to operate at its best performance, the system should be cleaned after each use. The tubing and microfluidic chips are cleaned at the same time, first by flushing with a detergent, followed by Water, followed by IPA/Ethanol, and then finally dried using Air. All cleaning fluids should be filtered with a 0.2 µm filter before use to minimize the risk of debris being introduced into the system.

Step 1: Remove all fluids from the P-Pump chamber. Unscrew the input Linear Connector from the Chip assembly.

Step 2: Remove the fluid that remains in the tubing by closing the P-Pump lid, pressuring each Pump to 2000 mbar, and sequentially opening each in-line valve until all fluid has been removed. When all the fluid has been removed, close all in-line valves.

Step 3: Refill each P-Pump with Detergent (must use a vial). Prime all tubing with detergent, as described in Step 8 of Section 15. 1% SDS is suggested as the detergent.

Step 4: Re-connect the Linear Connector to the Chip and Interface. Place output tubing in Waste vessel.

Step 5: Set target pressure of all P-Pump to 2000 mbar. Open all in-line valves and allow the detergent to flow through the Chip for 2-3 minutes.

Step 6: Repeat this process using filter Water as the cleaning fluid.

Step 7: Repeat process using filtered IPA/ethanol as the cleaning fluid.

Step 8: Finally, dry the Chip and tubing by removing all fluids from the P-Pumps, purging any fluid remaining in the tubing and attach the Linear Connector to the Chip. Pressurize the P-Pumps to 2000 mbar – ensuring no liquids in the chambers. Stop the P-Pumps when the chip is dry, confirm by visually checking the channels of the Chip using the Microscope.

Step 9: Return the Chip to its original Gel-Pak for storage.

Step 10: Linear Connectors should be stored on lint-free tissue and/or in bags to prevent dust entering the system.

17 Microfluidic Chip Cleaning

The Mitos System utilizes a series of microfluidic chips of varying geometries, designs, and junction depths. Given the use of these Chips, there may be occasions when the Chips experiences blockage or debris which has the potential to interrupt/stop the flow of fluid through the chips.

Supplied within each Mitos System is a Chip Flushing Kit. The flushing kit contains a pre-assembled H-Interface or pre-assembled Top Interface and set of Linear Connectors as well as lengths of tubing (supplied with fittings and ferrules already connected).

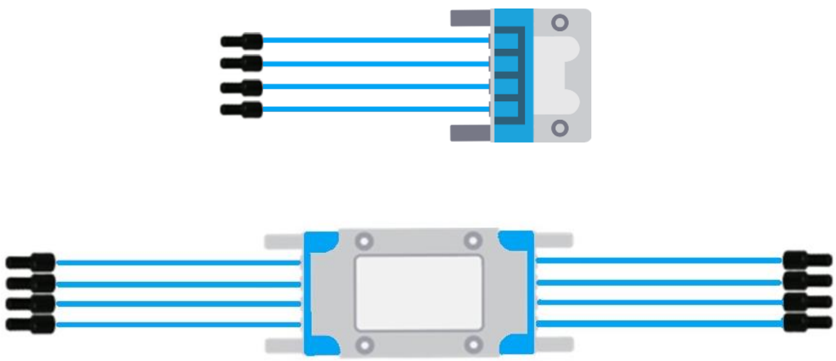


Figure 48 Chip Flushing with Top Interface and H Interface

It is important to factor in the coating of the chip when considering suitable cleaning fluids. Microfluidics chips can have a fluorophilic, hydrophilic or hydrophobic coating, this will be clearly labelled on the chip packaging. Glass is naturally hydrophilic, whereas a coating is applied to create a hydrophobic or fluorophilic channel. These coatings can be removed if strong acids/bases are used for cleaning.

Please refer to the table below before cleaning chips as some liquid may not be compatible.

Fluid	Hydrophilic	Hydrophobic	Fluorophilic
Water	✓	✓	✓
Acetone	✓	✓	✓
Ethyl Acetate	✓	✓	✓
Toluene	✓	✓	✓
Acetonitrile	✓	✓	✓
Isopropyl Alcohol	✓	✓	✓
Dichloromethane	✓	✓	✓
Hexadecane	✓	✓	✓
0.1 M HCL	✓	✗	✗
1 M HCL	✓	✗	✗
0.1 M NaOH	✓	✗	✗
1 M NaOH	✓	✗	✗

Most commonly, debris will default to the smallest feature of the Chip which (typically the junction). Accessing the junction (and more specifically the blockage in question) may require the fluid to be injected into the Chip in a specific direction. Figure 49 an example of a Chip which has a blockage (fiber) at the junction.

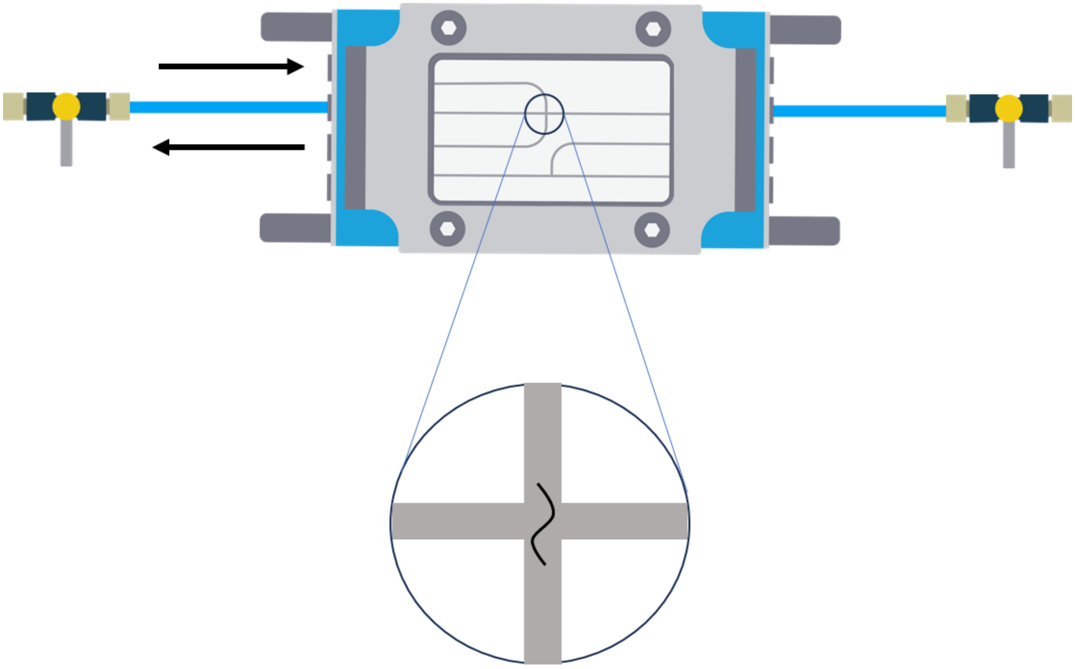


Figure 49 Droplet Chip wit Fiber Blockage at Junction

Some microfluidic chips contain on-chip filters within their inlets, to help prevent debris from fluids from entering the chip. When attempting to unblock a chip with these filters, aim to force the debris out via the output channel. Debris that is caught within the on-chip filters may be reintroduced into the chip junction during subsequent uses.

Step 1: Visualize the Chip via the Microscope to better understand the location of the debris and therefore more efficiently target the blockage.

Step 2: Utilizing the supplied In-Line Valves, connect the valves to the inlets/outlets of the Chip which do not require flushing. The valves provide an effective way to limit flow into/out of the Chip and force fluid to target a specific channel.

Step 3: Taking the supplied Female to Female Luer Fitting, attach the fitting to the tubing in the direction of flow required.

Step 4: Fill a 1/3 mL Luer Lock Syringe with filtered DI Water/Ethanol and connect this to the Female to Female Luer Fitting as shown in Figure 50

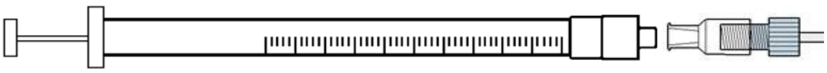


Figure 50 Luer Lock Syringe Connection to a Female to Female Luer Fitting

Step 5: Inject the solution into the Chip to clear the debris, confirming via the microscope.

Step 6: Empty the syringe and fill with air to clear out the remaining fluid from the Chip – repeat 2/3 times to leave the Chip dry.

Step 7: Store the Chip into the supplied Gel-Pak box for later use.

In some cases, polymers can build up on the channels of the chip, disrupting the flow of fluids. Users can identify this by the attraction of the dispersed phase to the walls of the output channel.

Step 1: Load the solvent used in the experiment into all P-Pump chambers, prime the tubing network and attach the Chip via the correct interface.

Step 2: Flow the solvent through the chip at ~1 mL/min for 5 minutes.

Step 3: Remove the solvent from the P-Pumps and push air through the system until the Chip is dry.

Step 4: If there remain issues, the Chip can be flushed with a weak acid e.g. 0.1 M NaOH followed by a flush with DI water. However, this should only be done with hydrophilic chips as the acid will remove the coating from hydrophobic and fluorophilic chips.

18 Technical Support

For Technical Support, please email support@unchainedlabs.com; for all other queries or questions relating to the system please email info@unchainedlabs.com.

19 Troubleshooting

	Issue	Cause	Solution
1	**P-Pump/Microscope is not powering on**	The P-Pumps and Microscope have a mains power switch and an LED Power switch.	Ensure both power switches are in the on position
2	**Hardware is not detected by Flow Control Center Software**	FCC requires each piece of hardware used in the system to be connected to the PC.	Confirm that the P-Pumps are connected to the system PC via the white serial cables provided and that the Microscope is connected to the PC via the USB cable.
3	**Cannot tare or set a flowrate using FCC**	P-Pumps are not detecting the Flow Rate Sensors	Check the connection between the Sensor Interface and P-Pump, remove and reconnect if necessary. Confirm the Flow Rate Sensor is connected correctly to the Sensor Interface.
4	**Fluid leaks from the fittings/connections**	The system can be Pressurized up to 10 bar and requires fittings to be sufficiently tightened	Confirm that each of the fittings is tightened into the respective location. If necessary, replace the fittings/tubing if the leak persists.
5	**P-Pump failed leak test**	The chamber is not airtight.	Confirm the chamber has been sealed by inserting an FEP plug into the diptube fitting. Ensure the diptube fitting is fully screwed into the P-Pump lid.
6	**P-Pump has reach target pressure but there is no flow through system**	No fluid is entering the tubing, or the flow pathway is blocked	Ensure that tubing is below the fluid level within P-Pump Chamber. Confirm 2-way inline valve has been opened. If overtightened, the gasket within the Linear Connector may compress the end of the tubing and prevent fluid from entering the chip. Disassemble and then reassemble the chip within the H interface.
7	**When using FCC, there is an over pressure error**	Overpressures occur if the flow path is blocked and or the required flow rate is too high relative to the fluids or microfluidic chip used or combination thereof.	Confirm that the microfluidic chip being used are unblocked (replace or clean if necessary). Lower the flow rates in the system. If the error persists even at low flow rates, consider replacing the system tubing and or testing the system tubing sequentially to locate the source of the over pressure.
8	**When in flow control mode, the system remains unstable**	P-Pumps are unable to reach/maintain target flow rates	Target flowrate requires a pressure higher than the supply pressure. In general, the system should be stable in pressure control mode before switching to flow control mode. Confirm correct calibration fluid has been selected in FCC.

9	**Unable to input desired target pressure**	Target pressure too high for current supply pressure	Target pressure must be 500 mbar lower than current supply pressure. Input a lower target pressure, or increase supply pressure (max 11 bar)
10	**Air bubbles entering the Chip**	Chip assembly not sufficiently sealed and/or fittings require tightening.	Confirm each of the fittings are tightened into the respective location. Ensure tubing is flush with linear connector gaskets and sealing is even around H Interface.

If the above pre-requisites have been confirmed correct, contact support@unchainedlabs.com