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WEIGHING MOLECULES WITH LIGHT

User Manual MassFluidix HC - System add-on for mass photometry

User Manual

Version 6

MassFluidix HC - System add-on for mass photometry

User manual

Warranty

Refeyn Ltd 'MassFluidix HC' system comes with a 12-month parts and labour warranty and has been thoroughly tested against Refeyn's published specification.

The warranty covers the repair or replacement of the instrument or a discretionary refund of the purchase price.

Keep the supplied shipping crate and packing material for the duration of the warranty period, so that in the unlikely event of needing to return the instrument, it can be done so safely.

To qualify under the terms of the warranty, the system must be used in accordance with this guide and any training provided by Refeyn.

Failure to do so may invalidate the warranty and no liability is accepted for loss or damage arising from the incorrect use of the 'MassFluidix HC' system.

Refeyn reserves the right without prior notice to alter the specification of the 'MassFluidix HC' system to improve performance and benefits for the end user.

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1. Safety information

1.1. Supplier information

The 'MassFluidix HC' system Add-on is manufactured by:

Refeyn • Unit 9 • Trade City • Sandy Lane West • Oxford • OX4 6FF • UK

Technical Assistance:

support@refeyn.com
+44 (0) 1865 800175

1.2. Intended Use

The 'MassFluidix HC' system is an addition module to a One^{MP} or Two^{MP} mass photometer and it is not possible to operate as a stand alone device. It is designed for the analysis of low affinity macromolecular interactions and it allows analysis of high-concentrated samples above the measurement range of the mass photometer, thanks to its rapid dilution capability.

The system is for use of trained personnel and in laboratory environment only.

The 'MassFluidix HC' system and its components are intended to be used as General Laboratory Equipment (GLE). They are not intended for use in diagnostic procedures.

Refeyn recommends that the laboratory in which the 'MassFluidix HC' system is used follow the general best practices for mass photometry. This includes among others:

- Using the system in the appropriate lab environment
- Regularly running calibrations
- Following the shelf life for all consumables used with the system
- Running the system only if properly trained on the use of the system

1.3. Mass photometer identification

'MassFluidix HC' system can be supplied as an add-on to One^{MP} or Two^{MP} mass photometers and electrical ratings will depend upon specific mass photometer version. The instrument model is shown on the label on the back of the mass photometer.

- One^{MP} type: new mass photometer model number: One^{MP} MFHC
- Two^{MP} type: new mass photometer model number: Two^{MP} MFHC

1.4. Electrical ratings

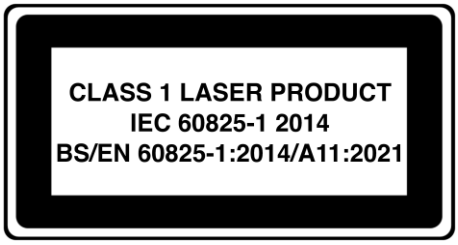
Electrical ratings depend on the type of mass photometer:

MassFluidix HC using One^{MP} (One^{MP} MFHC)	Mass photometer Electronics controllers:	Input 1: 100-240 VAC • 50/60 Hz • 5 A Input 2: 12 VDC • 5 A (Model: HN PowerHNP65-120)	
	'MassFluidix HC' controller box unit:	Input: 24 VDC • 1.75 A (Model: Mascot 3721-24)	
	RX pressure pump:	Input: 24 VDC • 1.75 A (Model: Mascot 3721-24)	

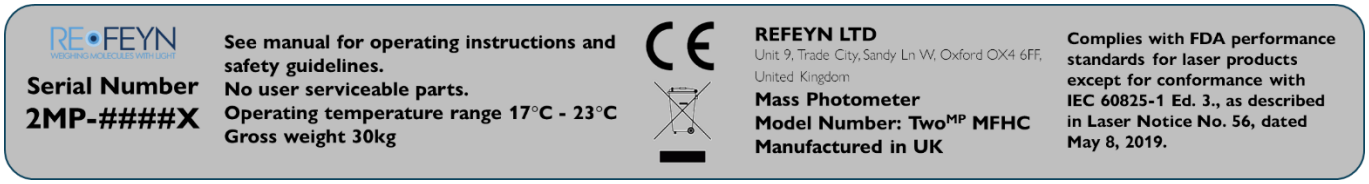
MassFluidix HC using Two^{MP} (Two^{MP} MFHC)	Mass photometer Electronics controllers:	Input Rating: 12V VDC • 13.75 A (Model: HN PowerHNP65-120)	
	'MassFluidix HC' controller box unit:	Input: 24 VDC • 1.75 A (Model: Mascot 3721-24)	
	RX pressure pump:	Input: 24 VDC • 1.75 A (Model: Mascot 3721-24)	

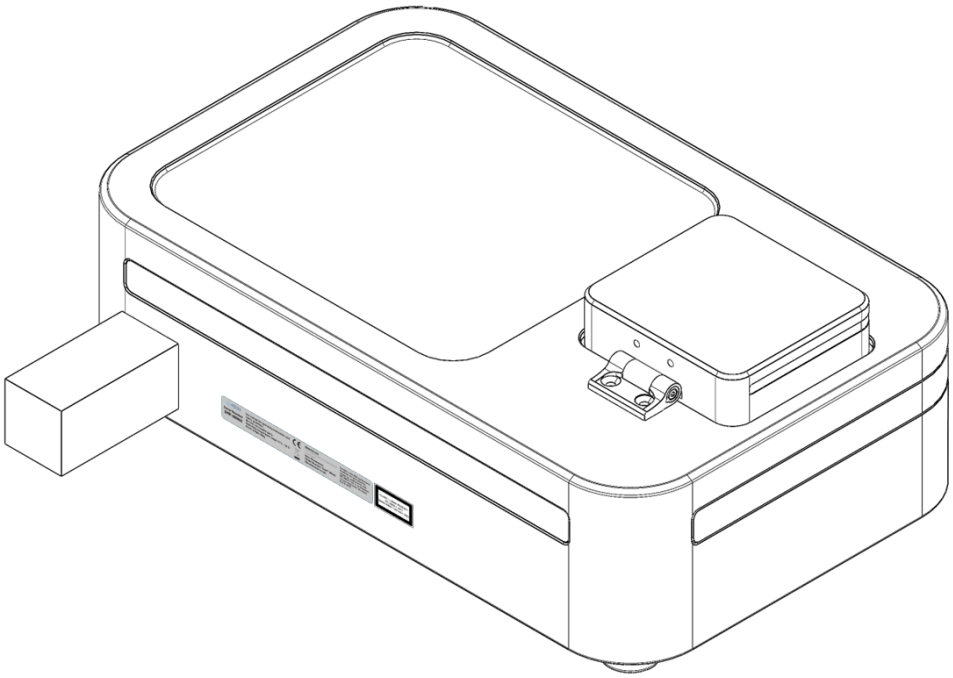
1.5. Laser safety information

Caution – Use of controls or adjustments, or performance of procedures other than those specified herein may result in hazardous radiation exposure.



The Refeyn 'MassFluidix HC' add-on is used in conjunction with One^{MP} MFHC and Two^{MP} MFHC instruments, which are designated Class 1 laser systems during all procedures of operation and maintenance, making them safe to use in an open laboratory environment. The laser safety classification and certification labelling is located on the back of the instrument, as shown on the diagram below.

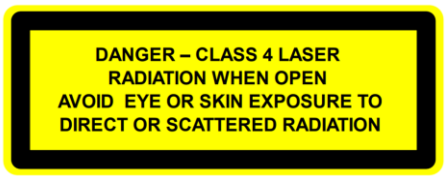


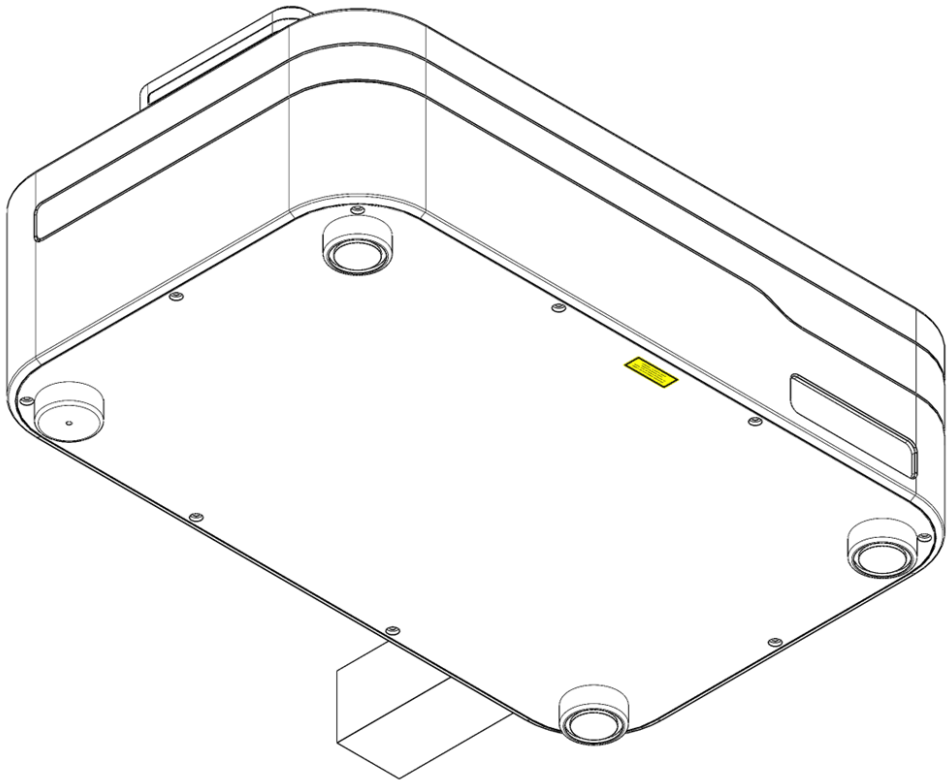


The lid and the sample area of the instruments has been modified to fit with the microfluidic components of the 'MassFluidix HC' add-on, while retaining the Class 1 laser performance. The lid contains 3 gland-based tubing feed throughs on the back. Excessive force on the glands should be avoided. If a gland should ever become loose, please contact Refeyn Technical Support (support@refeyn.com).

Users should not attempt to remove or adjust the instrument lid. If needed, adjustments should be done only by a qualified Refeyn engineer.

Users should not attempt to remove screws securing either the mass photometer enclosure, or the electronics control unit housing. All screws in these housings are security type screws and should not be removed, except by qualified Refeyn Ltd. engineers. The housing panel which gives access to class 4 laser radiation is on the underside of the instrument, and is indicated with the label shown below.





Care should be taken when using the instrument. If there is any indication of a fault resulting in the escape of laser radiation from the unit, switch off the unit (at the power button) immediately and contact Refeyn Technical Support (support@refeyn.com).

Refeyn mass photometers have embedded lasers that are not accessible during operation or maintenance. The non-accessible internal laser parameters are:

MassFluidix HC using One^{MP} (One^{MP} MFHC)	Primary laser:	525 nm (± 7 nm) · 1 W Max · Class 4 · CW
	Autofocus laser:	635 nm (± 5 nm) · 3.5 mW Max · Class 3R · CW

MassFluidix HC using	Primary laser:	488 nm (± 5 nm) • 2 W Max • Class 4 · CW

Two^{MP} (Two^{MP} MFHC)	Autofocus laser:	638 nm (± 5 nm) • 700 mW Max • Class 4 · CW

1.6. Environmental operating conditions

A Refeyn 'MassFluidix HC' instrument is intended for indoor, laboratory use only.

When siting the instrument, it is important that there is sufficient space around the instrument to allow for accessing the tubing, sample carrier and air compressor unit.

Cables other than those specified for use by Refeyn should be kept tidily away from the instrument.

For optimum performance, the 'MassFluidix HC' instrument should be operated in an environment with a stable temperature of 20 °C – 25 °C and with relative humidity <60%.

Standard precautions must be taken to prevent environmental or cross-contamination when collecting/handling samples and swapping sample tubes into the instrument.

1.7. Transporting the 'MassFluidix HC'

The Refeyn 'MassFluidix HC' add-on is intended to be left at the installation location. It can be relocated around the area of installation if there is sufficient space for the tubing to safely reach the connected One^{MP} MFHC or Two^{MP} MFHC and the air compressor cable.

If the 'MassFluidix HC' add-on needs to be moved from the installation location to other areas at the installation site, please take precaution to disconnect all connections tethering the box to the location and consider the reagents exposed to the add-on that may require decontamination or cleaning.

When shipping a MassFluidixs HC system please contact support@refeyn.com for advice.

1.8. Servicing and maintenance

This add-on must only be installed by trained service personnel from Refeyn.

To avoid any personal risk, users are prohibited from accessing the interior compartments related to safety critical aspects. These are enclosures, panels etc. that require a tool such as a screwdriver for access.

If repair is needed, this will be arranged via Refeyn Technical Support and instructions will be given on how to proceed. Until then, the add-on must not be used.

There is no scheduled maintenance required to keep Refeyn Samux^{MP} Mass Photometers in compliance with laser safety regulations.

1.9. Electrical supply to the instrumentation

Refeyn 'MassFluidix HC' add-ons must be operated using the power supplies and cables supplied with the add-on.

Under no circumstances should these cables be replaced with similar cables.

Any deviation from the factory-provided electrical connection and power supply parts may affect the safety of the instrumentation.

If in any doubt about the mains cables feeding electricity to the add-on, please contact Refeyn Technical Support (support@refeyn.com).

Refeyn 'MassFluidix HC' add-ons are intended for research use only for measuring molecular mass distribution of biomolecules in solution and, as such, are to be operated in a laboratory setting in alignment with the environmental requirements specified in this manual.

1.10. Liquids and Personal protective equipment

Due to the nature of the work and the type of liquids used, users should wear appropriate protective goggles, gloves and clothing when performing any steps with the system.

All users must know the hazards presented by the system and the substances they are using and should observe the related Material Safety Data Sheets (MSDSs).

An eyewash facility and a sink should be available nearby. If any substance contacts the skin or eyes, wash the affected area and seek medical attention.

1.11. Removal and disposal of the 'MassFluidix HC' add-ons

At the end of the product life, the add-on can either be disposed of or be recycled.

Refer to local guidelines for disposal and recycling procedures and contact Refeyn if advice is required. Follow guidelines regarding biomolecule contamination and cleaning procedures.

2. Site preparation guide

2.1. Transport requirements

The 'MassFluidix HC' add-on is delivered in a single box with all the necessary components.

If a mass photometer is purchased together with the 'MassFluidix HC' add-on, this will be shipped inside a separate palletised wooden crate (80 cm x 120 cm x 95 cm, W x D x H) weighing approximately 100 kg.

A pallet truck will be required to move the crate. The box and crate should be stored at 10–25°C without major or rapid temperature changes and at <75% humidity prior to unpacking and installation.

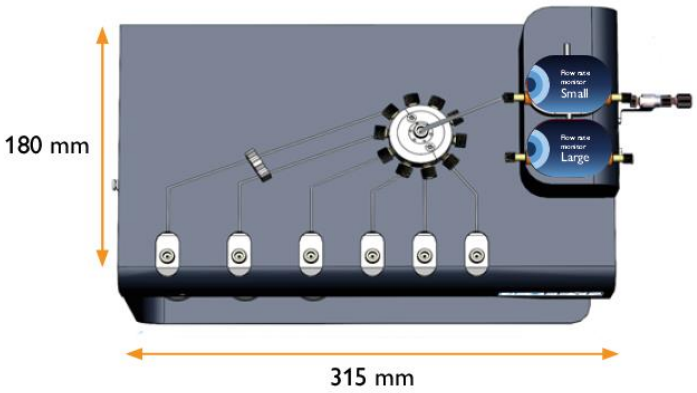
2.2. Shipping box contents

Box 1 - Refeyn 'MassFluidix HC'

- Refeyn 'MassFluidix HC' controller box unit
- Air compressor (RX model)
- Electronic 2-switch shut-off valve
- Outlet flow sensor
- 250 mL buffer reservoir bottle
- (3x) 0.5 mL Eppendorf microcentrifuge tubes
- (3x) 50 mL Falcon centrifuge tubes
- A4 polypropene Tray
- All required tubing, nuts and fittings mentioned in Section 3.1

If a mass photometer is purchased together with the 'MassFluidix HC' add-on, a crate will be shipped containing the mass photometer (and electronic controller unit), a PC and monitor (with mouse and keyboard) as well as an instrument shipping kit (including mains cables, USB cable, immersion oil and instrument power supply).

2.3. Room/site requirements



MassFluidix Controller box unit dimensions

The 'MassFluidix HC' controller box unit add-on has a footprint of 315 mm x 180 mm x 270 mm (W x D x H) and weighs approximately 2 kg.

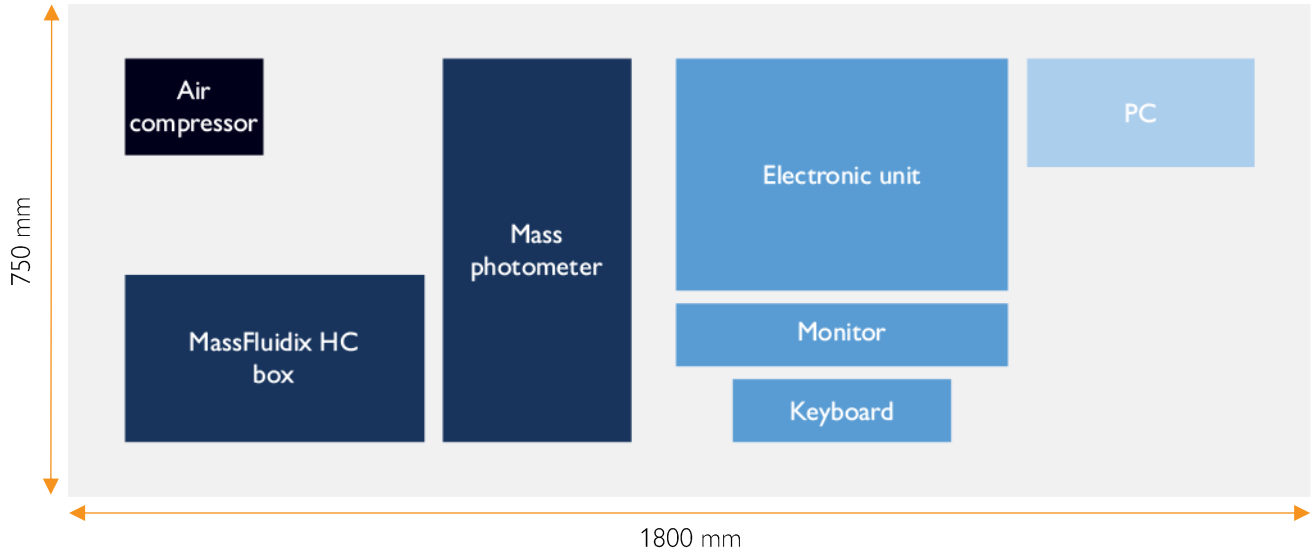
It is important to ensure that there is sufficient clearance at the add-on location site to permit handling the add-on fully. It is recommended to have a space of approximately 1-2 cm between the fluidic system and the mass photometer (as close as possible without touching).

The add-on air compressor unit will sit close and is connected to the controller unit via pneumatic tubing. The compressor should be placed on a nearby shelf or on the benchtop on top of a piece of foam, so the vibrations do not interfere with any of the measurements.

Internet connectivity is essential for the duration of the installation and occasional availability may be necessary for remote access and updates.

The add-on requires 2 additional power outlets in total and 1 additional USB slot.

A clear A4 sized Polypropylene tray is provided with the system. Keep the operational bottles inside the tray to limit spills.



Recommended benchtop positions for the mass photometer, PC and MassFluidix HC components for easy access

3. Getting started

3.1. Tubing connections

The MassFluidix HC system requires four types of tubing:

A. PTFE 1/16" OD (0.2 mm ID) tubing

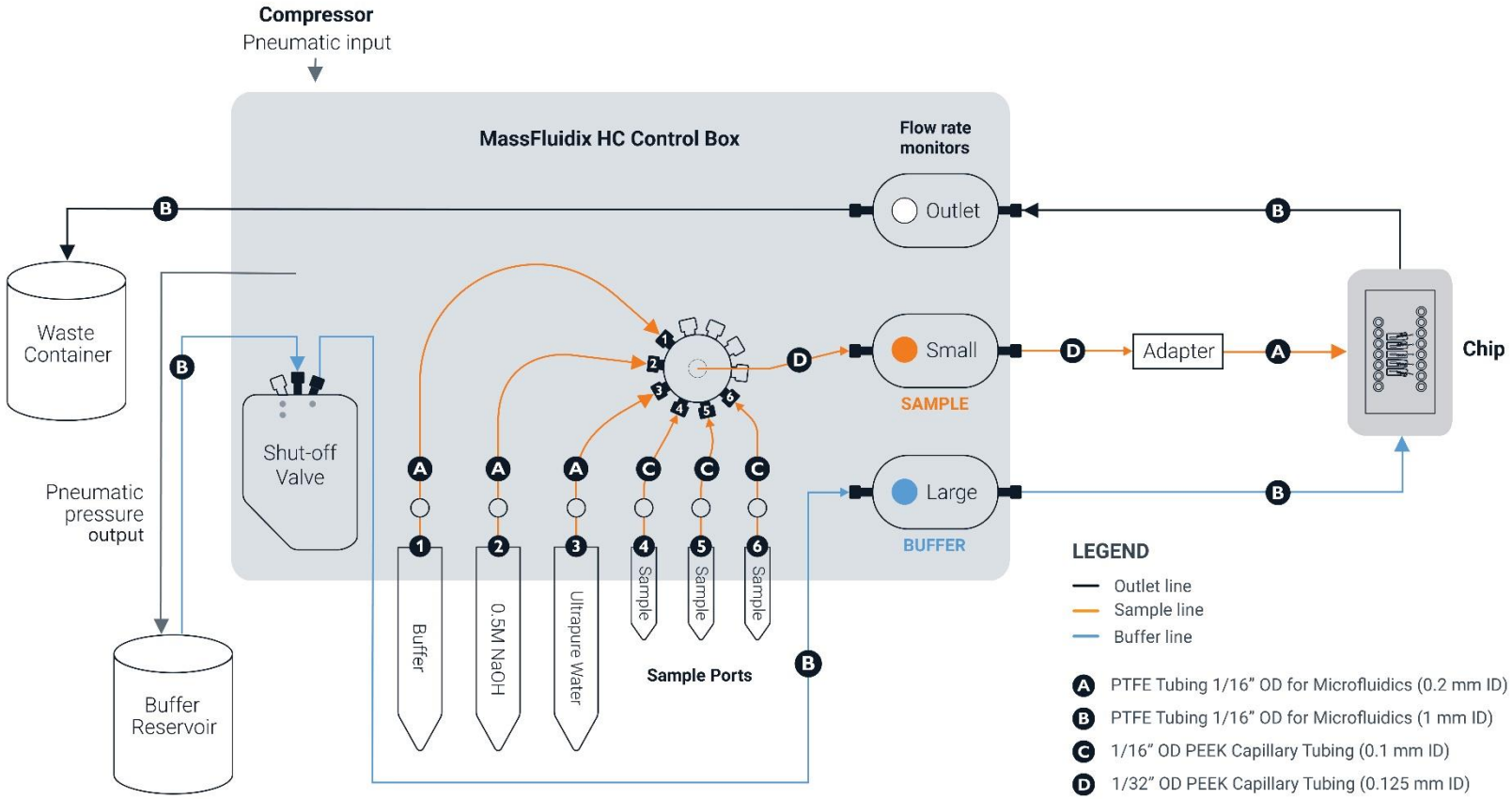
B. PTFE 1/16" OD (1 mm ID) tubing

C. 1/16" OD PEEK Capillary (0.1 mm ID) tubing

D. 1/32" PEEK Capillary (0.125 mm ID) tubing

Only non-flammable PEEK and PTFE tubing are permitted to be used with this system. Fluids should be checked for material compatibility before being used with the system.

The use of the different tubing types is indicated below.



Tubing connections and layout. The type of tubing is indicated on the bottom right.

3.1.1. Connections for tubing types A, B and C

To connect tubing **type A** (PTFE 1/16" OD (0.2 mm ID)), tubing **type B** (PTFE 1/16" OD (1 mm ID)) and tubing **type C** (1/16" OD PEEK Capillary (0.1 mm ID)), do the following:

The tubing connections require a flangeless ferrule on each end that screws in a component, along with a nut.

Flangeless ferrule

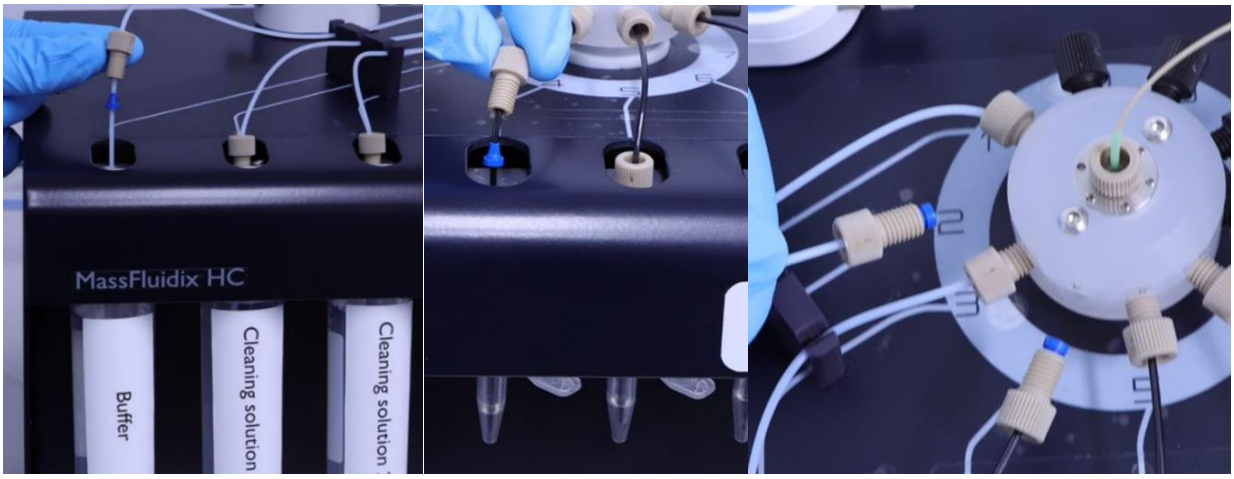
Nut

For tubing types A, B and C, insert the nut with the thread facing outwards on the tubing.

Next, insert the ferrule with the flat end facing outwards. Leave 1-2 mm of tubing to stick out at the end.

Finish the connection by screwing in the nut to the desired space. Make sure the connection is tight.

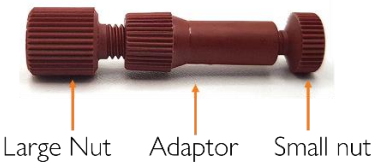
For the tubing going into the 50 mL centrifuge tubes and the 0.5 mL microcentrifuge tubes, leave the appropriate length to reach the bottom of the tubes.



3.1.2. Connection for tubing type D

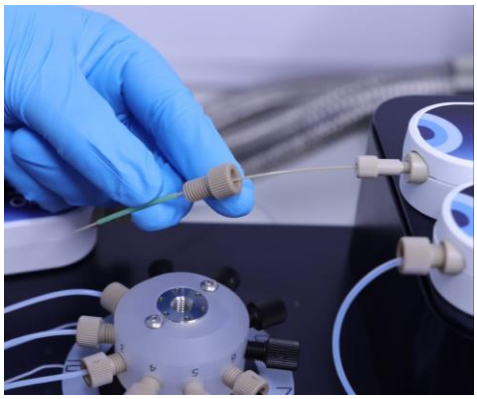
Tubing type D is used for the connection between the multi-switch and the flow sensor, and the flow sensor and adaptor, requiring a few additional components.

The sleeve adapts 1/32" OD tubing to 1/16" OD connectors.
The flat bottom plug is used to align the tubing.
The PEEK adaptor is to connect the different types of tubing.

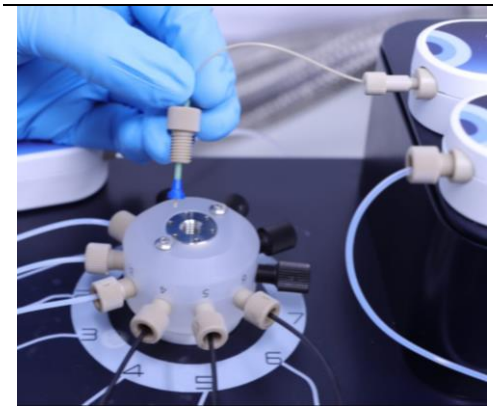


Sleeve *Flat bottom plug* *and* *PEEK Adapter*

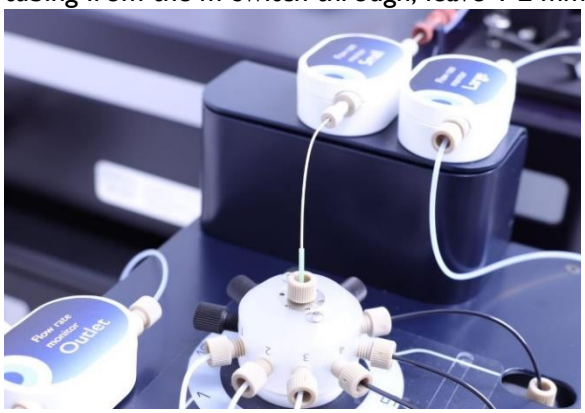
Slide the sleeve over the length of the tubing with a nut.



Add the ferrule over the sleeve, flat end facing outwards. Make sure the sleeve is flat with the ferrule. Leave 1-2 mm of the tubing to stick out at the bottom of the ferrule and sleeve before tightening the nut to the top of the multi-switch. This is the only connection requiring the sleeve.

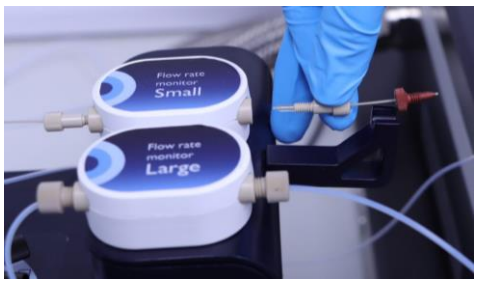


Both sides of the connection to the small flow sensor have their own nut and do not require a ferrule. Thread the tubing from the m-switch through, leave 1-2 mm of it sticking out from the end, and tighten to the flow sensor.

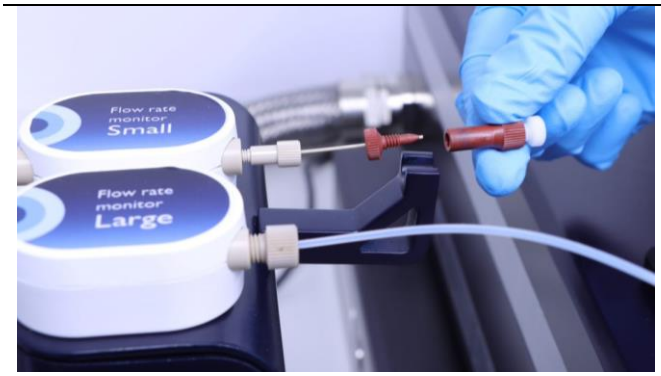


Thread the second Type D tubing section through the nut on the other side of the small flow sensor no ferrule required. Leave 1-2 mm of it sticking out from the end and tighten to the flow sensor.

The tubing coming out of the small flow sensor needs to be connected to the PEEK adaptor. Insert the type D tubing into the small nut of the adaptor assembly, leaving 2-3 mm of it to stick out.



Tighten the flat bottom plug into the wider side of the adaptor and connect the small nut into the adaptor on the narrower end, making sure to tighten them well. This is done to align the type D tubing inside the adaptor using the flat bottom nut.



Insert type A tubing through the large nut of the adaptor assembly with 3-5 mm of it left to stick out. Remove the flat bottom nut and screw the large nut into the adaptor.

The connection with the adaptor is now complete.

Launch the MassFludix Tool from the Tool menu in the Acquire^{MP} menu and initialise the system to confirm system recognition

To complete the set up perform the following **two** cleaning protocols from the MassFluidixs Tool Menu

1. In-between measurement clean
2. Full system clean (through ports 4, 5 and 6)

Guidance on how to do these protocols is in the Cleaning and Maintenance Section in 6.1.4.

3.2. Best Practices for Fluidic Handling with the MP System

Mass Photometer systems are sensitive to fluid deposition inside them. Multiple precautions are in place when a system is converted to MassFluidix HC compatible system, to protect the systems from damage. They are described below.

3.2.1. Software

MassFluidix controls are supported by Refeyn Acquire^{MP} v2024 R2.0 or later, using the MassFluidix Tool.

This includes leak detection during measurements (from monitoring the outlet flow rate), to prevent any leaks leading to system flooding.

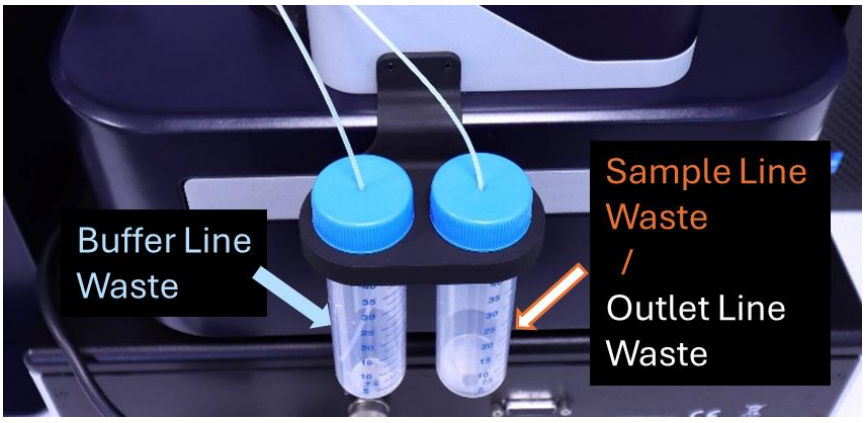
All protocols contain an "abort" option which will stop all fluid flow in case of any potential leakages are noticed as a failsafe.

The cleaning protocols also contain "abort" option if misguided tubing is spotted during a cleaning step. This option is to be used if misplaced tubing is discovered during cleaning. If using Oxygen software, use "reset all pressures" option (refer to section 6.5 for more information).

3.2.2. Importance of Fluid collection

Before starting a measurement, ensure the end of the outline is placed into an appropriate fluid container as indicated below.

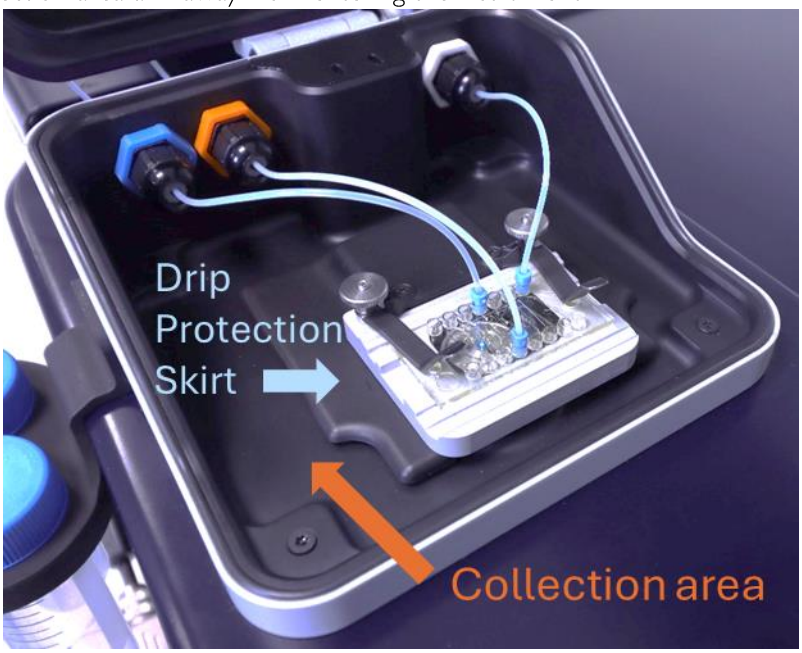
When performing cleaning it is important to pay attention to where the sample and buffer lines are placed. This is indicated in relevant places in this manual, as well as down below.



Never perform cleaning whilst the tubing is still connected to the chip. If the cleaning procedure is started whilst connected to a chip, use the abort option on the protocol. Then, disconnect the tubing and restart the cleaning procedure.

3.2.3. Drip Protection

The OneMP/TwoMP stage of an instrument compatible with a MassFluidix HC add-on has a drip protection skirt that will direct any leaks toward a collection area and away from entering the instrument.



This area should be monitored regularly for any accumulation of liquid. Should liquid be present in this collection area, this can be removed by using an absorbent tissue to wipe away the excess liquid, then cleaned with another tissue soaked with an appropriate cleaning solution (such as Isopropanol) as required.

4. Typical consumables: MassFluidix HC

Further information on what the packages include can be found on Refeyn's website (www.refeyn.com/mass-photometry-consumables). For further clarification, send an inquiry to orders@refeyn.com.

Sample carrier slides and chips – available from Refeyn through orders@refeyn.com

- MassGlass UC Sample Prep Kit – includes Sample Carrier Slides (MassGlass UC), Sample Well Cassettes and Lens Cleaning Tissue. Compatible with protein and AAV samples.
- MassFluidix HC Sample Prep Kit - includes MassFluidix HC chips and connectors

Calibrants

- Proteins
    - MassFerence® P1 – A calibrant protein tailored for One^{MP} and Two^{MP} measurements. Allows calibration suitable for measuring proteins between 90 kDa and 1000 kDa. Available from Refeyn through orders@refeyn.com
    - Chicken albumin, 44 kDa, Sigma A2512
    - Beta-Amylase from sweet potato, 56, 112 and 224 kDa, Sigma A8781
    - Bovine Serum Albumin (BSA), 66 and 132 kDa, ThermoFisher 23209

Others

- PTFE 1/16" OD (0.2 mm ID) tubing, Darwin Microfluidics BL-PTFE-1602-20
- PTFE 1/16" OD (1 mm ID) tubing, Darwin Microfluidics BL-PTFE-1610-20
- 1/16" OD PEEK Capillary (0.1 mm ID) tubing, Darwin Microfluidics ID-1561
- 1/32" PEEK Capillary (0.125 mm ID) tubing, Darwin Microfluidics ID-1576
- SMC pipe cutter 12 mm, RS TK-3
- 50 ml Falcon® Conical Centrifuge tubes (tubes from a different producer might not seal properly)
- 0.5 ml Eppendorf® microcentrifuge tubes (tubes from a different producer might not seal properly)
- Immersion oil, Zeiss Immersol 518 F no. 433802-9010-000
- Lens cleaning tissue. Such as Whatman® lens cleaning tissue, Grade 105, Sigma WHA2105841

5. Experimental guidelines

5.1. MassFluidix Technical Overview and Microfluidic chip handling guidelines

Safety and Handling Considerations

Always use gloves when handling the microfluidic chips.

Be aware the chips are bonded to a glass coverslip and may break if handled incorrectly.

Keep the chips in the delivery packaging to maintain cleanliness and prevent debris from contaminating the coverslip surface. Do not touch the glass bottom where the observation area is with anything other than lens cleaning tissue.

Chips should be cleaned by removing oil after measurements. This is important to prevent old oil from mixing with newly applied oil, which could affect measurements. Refer to Section 6.1.2.

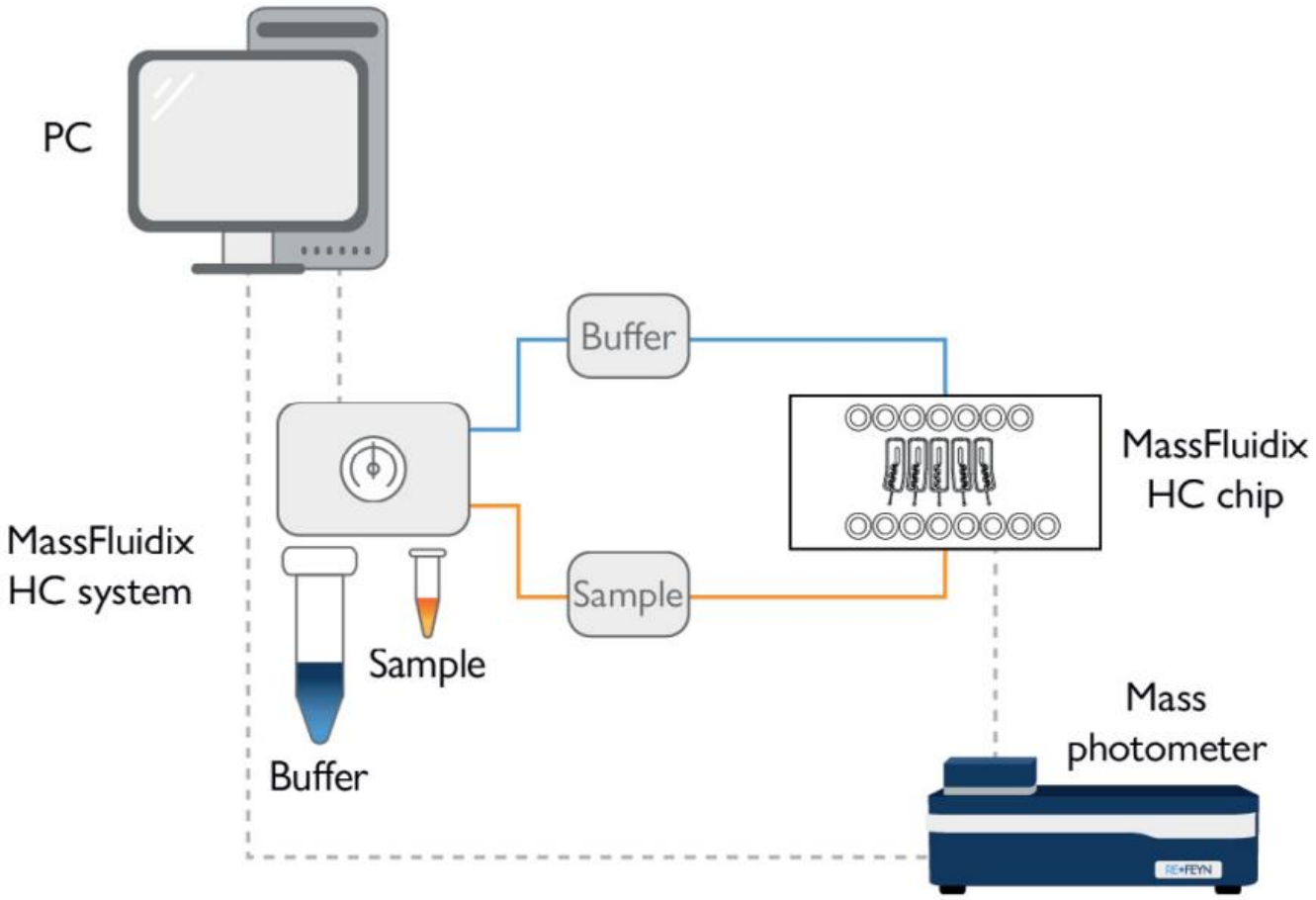
System Design

To expand the concentration range for mass photometry analysis, the MassFluidix HC system uses microfluidics technology to raise the upper sample concentration limit from the nanomolar to the micromolar range. It enables a measurement to be made at a concentration optimal for mass photometry, while capturing the state of a biomolecular system at micromolar concentration.

MassFluidix HC works by rapidly diluting the sample and flowing it across the measurement surface in milliseconds – before the biomolecular system's equilibrium is disrupted by the dilution.

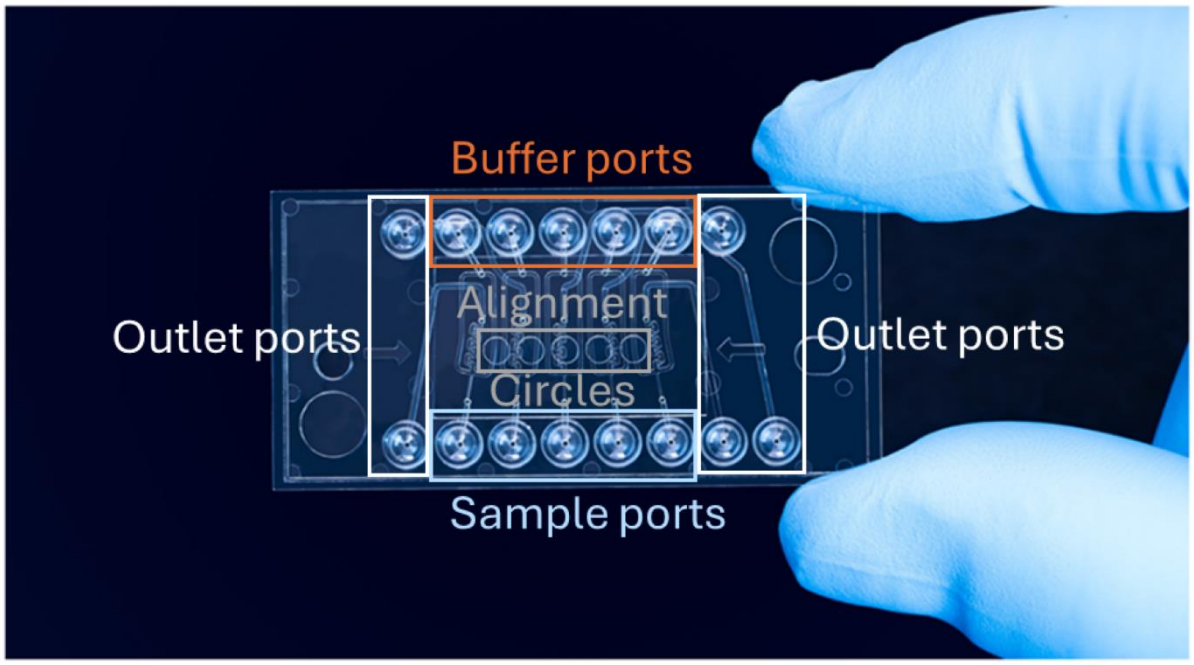
The MassFluidix HC system consists of multiple components: A computer, a central unit, sample and buffer line tubing, and flow rate monitors. These components work together to ensure that the sample and buffer enter the chip at the correct flow rates. The whole system is automatically detected by Refeyn's software and controlled from Acquire^{MP}. This careful control enables rapid sample dilution, which is immediately followed by the mass photometry measurement.

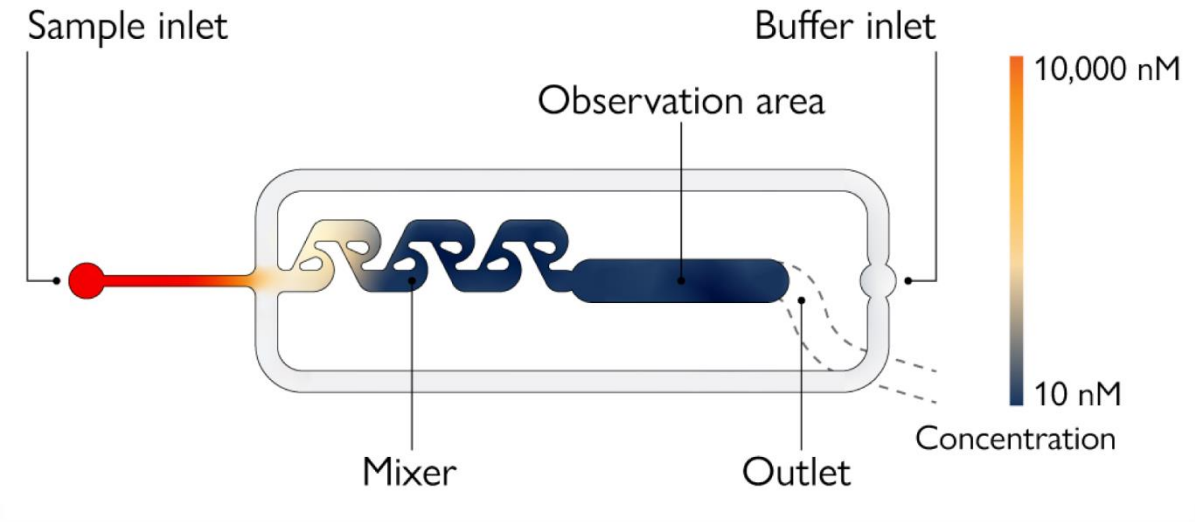
Sample dilution and the mass photometry measurement occur on the MassFluidix HC chip. The dilution factor is set prior to the initiation of the experiment. Dilution takes place when the sample and buffer are combined in a reverse Tesla valve mixer, then the diluted sample flows through the observation area before leaving the chip through an outlet. The mass photometry measurement is done in the observation area. Each chip has a set of five such dilution and measurement channels.



Chip design

The chip consists of ports that connect the fluid lines to the appropriate microfluidic channels. Dilution takes place when the sample and buffer are combined in a reverse Tesla valve mixer, then the diluted sample flows through the observation area before leaving the chip through an outlet. The flow rate of sample and buffer determine the dilution factor, which is performed in less than 37 milliseconds. The mass photometry measurement is done in the observation area. Each chip has a set of five such dilution and measurement channels. Each chip also contains 5 alignment circles used by Acquire^{MP} to automatically align the chip and measure accurately in the observation area of the desired channel.





The dilution range of the chips is x125 – x10 000 times from the starting concentration. Refeyn Acquire^{MP} MassFluidix Tool performs this calculation and control. The dilution factor is calculated in the following way.

$$Dilution\ Factor = \frac{1000(\frac{\mu L}{min})}{Flow\ Rate\ (\frac{\mu L}{min})}$$

If the starting concentration of the sample is known, the diluted concentration in the observation window can be calculated as follows:

$$Diluted\ concentration\ in\ observation\ window = \frac{Stock\ Starting\ Concentration}{Dilution\ Factor}$$

The final diluted concentration in the observation window will be 0.2-5 nM to view a similar number of events to 10 nM on a static coverslip measurement.

The dilution efficiency of the microfluidic chip is confirmed using fluids with viscosities close to water, fluids with increased viscosity may impact the uniformity of the final diluted sample in the buffer.

5.2. Preparation guidelines and Considerations

Before starting an experiment, make sure all of the below is prepared.

 # Preparation

5.2.1 Materials

The following will be needed to run an experiment using the Refeyn 'MassFluidix HC' system. Refer to the section Typical consumables for further information.

(Note the consumables listed below will need to be purchased by the user, i.e not provided with the system)

- MassFluidix Chips and Connectors from Refeyn

- Calibrant, such as MassFerence P1
- 0.5 mL microcentrifuge tubes
- 50 mL tubes
- Standard Laboratory Consumables are also required for this experiment such as:
    - Gloves
    - Pipette tips
    - Lint Free Wipes

5.2.2 Solutions and buffers

The following solutions and buffers are required:

- Ultrapure water
- 0.5 M NaOH
- Sample Buffer

All solutions added onto the system should be sterile or filtered through a 0.2 µm filter to avoid blockages and reduce low mass noise affecting measurement:

- Refeyn recommends the use of PES membrane filters that are within their expiry date.

Measurement Buffers should be validated for use on traditional Mass Photometry for the following reasons:

- To understand how they interact with standard measurements
- To understand the amount of noise they have in them and understanding how this could impact the observation of any species of interest
- For any buffers going into the sample lines that will be diluted, these should be validated at the expected dilution they will be measured at.

To change the buffer within the buffer reservoir, perform a buffer flush, as described in Section 6.1.4

5.2.3 Samples

Sample considerations

The samples used in MassFluidix should consider the following advice:

- Samples must have at least 20 µL volume with a maximum of 400 µL
    - 20 µL sample volume is the minimum required volume at maximum flow rate (7.9 µL / min, 125-fold dilution). This is because there is a risk of the sample tube emptying before sample has reached the observation area leading to air being introduced into the system.
    - Therefore, it is advised to either:
        - Use larger dilution factor with lower flow rates
        - If requiring 125-fold dilution factor, use larger volumes or take only 1 movie before promptly finishing the measurement routine
    - Air in the system can lead to blockages (which can be solved following Section 5.4)
- Samples must have a concentration of at least 100 nM with a maximum of 50 µM
- Be aware that the mass range of MassFluidix HC is 50 kDa - 5 MDa
- Pay attention to the incubation the sample complex has prior to measurement, as this can affect the appearance of interaction species viewed on Mass Photometry
- When measuring macromolecular interactions, it is advised to measure the individual interaction species in the same way as a control (matching concentration, incubation time and buffers used in interaction measurements)

Sample Buffer considerations

If samples contain the following components, we recommend reaching out to a Refeyn Field Application Scientist for more advice about how this may affect measurements or system cleanliness.

The use of nonstandard buffer components may affect the cleanliness of the system and it is advised to perform buffer measurements before the sample is introduced (as guided in Section 4.3.2) to understand any system noise.

Follow advice in Section 6.3.7 if there is increased sample carryover or presence of large number of counts in buffer movie measurements.

Here is some more specific advice on a selection of buffers components:

- Glycerol
  - The final concentration in the measurement should be < 5% as to not affect the refractive index Mass Photometry relies upon.
- Sucrose
  - The final concentration in the measurement should be < 0.1% as to not affect the refractive index Mass Photometry relies upon.
- Detergents
  - All detergents should be validated at intended measurement concentration in traditional Mass Photometry measurements
  - Detergents are likely to introduce a population of counts at a specific mass; more information is available in the form of an application note on detergents available from Refeyn.
- High Salt buffers
  - These can introduce higher amounts of low mass noise.

All Calibrations should be performed in a buffer containing the same concentration of elements as in the sample being measured at the measurement dilution.

5.2.4 Connections

Ensure all tubing connections are correct (Section 3.1)

Ensure the lid to the buffer reservoir is screwed on tight.

Three 50 mL centrifuge tube will be required for position 1, 2 and 3 (corresponding to the multi-switch positions) and three 0.5 mL microcentrifuge tubes will be required for position 4, 5 and 6 (corresponding to the multi-switch positions).

Ensure all centrifuge tubes are screwed onto the fluidic manifold tight, and all microcentrifuge tubes are correctly sealed.

Left: Image showing 50 mL tubes positions (connected by thread on 50 mL tube) Right: Image showing 0.5 mL Microcentrifuge tubes positions (connected using P-cap system)

5.2.5 Tubing

The tubing on the instrument should be changed every 3 months to ensure cleanliness.

Please refer to section 3.1 for guidance about how to replenish the tubing.

5.2.6 Cleaning

To perform cleaning please refer to the Cleaning and Maintenance Section (6.1.4).

Cleaning of the system should be performed:

- After changing the tubing
- After the system has been left idle for >2 weeks
- When performing the measurement protocol

The **measurement protocol** guidelines include cleaning steps as part of the standard measurement protocol.

This is because of the importance of regularly cleaning the Refeyn 'MassFluidix HC' system. Without regular cleaning, measurements will be affected by low mass noise, potentially causing low molecular weight species (50-100 kDa) to be obscured by measurements and increase mass resolution of other species.

5.3. General experimental procedure

> Important: Be aware that mass photometry measurements are temperature sensitive. To ensure most accurate results, Refeyn recommends using buffers and samples at room temperature.

5.3.1. Calibration

It is advised to use static calibrations (which is traditional Mass Photometry measurements on glass slides) for MassFluidix Measurements. Ensure the calibration measurement and any MassFluidix measurements contain no immersion oil bubbles for the best accuracy.

The calibrants used on a One^{MP} or Two^{MP} can also be used with the 'MassFluidix HC' system. The recommended calibrant is MassFerence P1.

- Developed and tested to give in specification (< 5% mass accuracy) results for proteins between the range of 90 – 1000 kDa
- For use outside the stated range, and it is recommended to take a separate calibration measurement of a protein <90 kDa or >1000 kDa to improve the accuracy of results
    - Please contact a Refeyn Field Application Scientist for more advice on calibrations

Some users may be able to enhance the mass accuracy of their measurements through performing calibration measurements in the MassFludix chips, but it is recommended to start with static calibrations first.

- If performing flowing calibration of MassFerence P1, dilute it by a factor of 5 in filtered PBS, e.g. 40 µL MFP1 and 160 µL PBS and measure at the suggested dilution of 1/1000.

5.3.2. Measurement Procedure

MassFluidix Measurements are supported by Refeyn Acquire^{MP} **v2024.R2 or later** software using the MassFluidix Tool

If issues are encountered, refer to Section 5 of this manual or the Refeyn Acquire^{MP} software manual (for software related concerns).

Alternative use of the system with Oxygen Software using Refeyn protocols is detailed in section 6.5 for users that require this.

#	Step description

5.3.2.1 **<u>Start up the system</u>**

Ensure that the system is powered and connected to the PC and that the compressor is powered and set to P1.

Turn on the MassPhotometry System starting Acquire^{MP}.

Open the MassFluidix tool in the Acquire^{MP} Tool menu. This tool guides users through the experiment and cleaning protocols and controls the fluidic and Mass Photometry components.

Alternative usage of the system with Refeyn Oxygen software is detailed in Section 6.5.

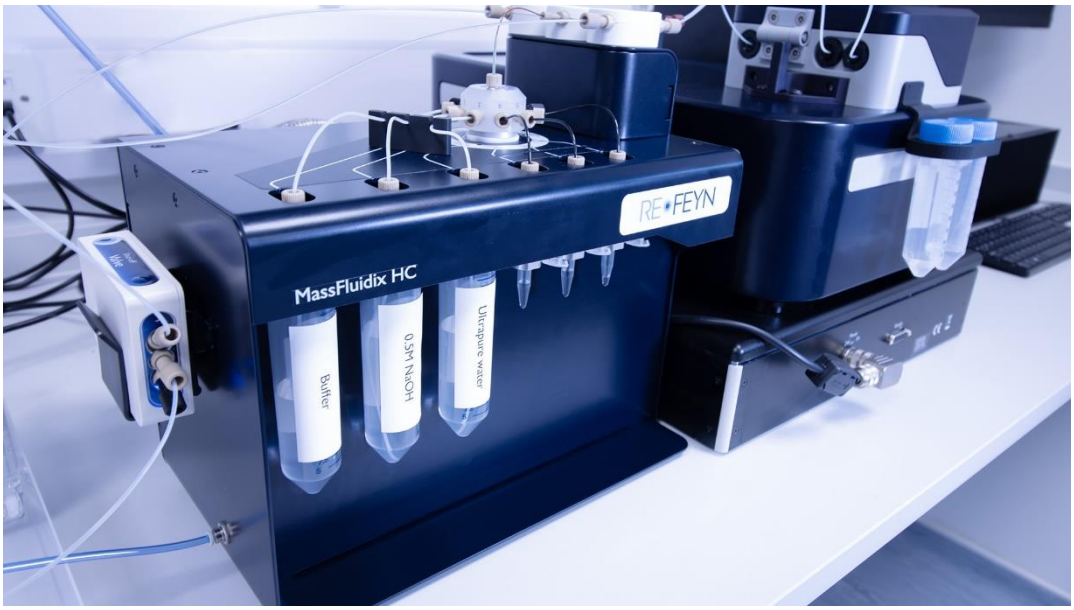
5.3.2.2 Prepare Buffers

Prepare the buffer reservoir bottle with the intended buffer solution

- Ensure fresh solution is used in a clean 250 mL glass bottle
- Use at least 100 mL
- Ensure the lid is securely tightened

Prepare 40 mL of cleaning solutions in 50 mL centrifuge tubes; place the following solutions in the following lines:

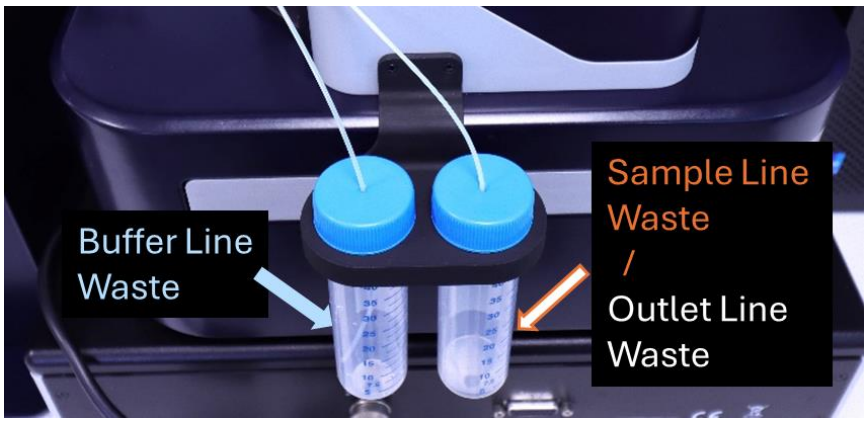
1. The intended buffer solution
2. 0.5 M NaOH (cleaning solution 1)
3. Ultra-pure water (cleaning solution 2)



Leave the same solutions (Buffer, Cleaning Solution 1 and 2) accessible on the benchtop for filling 0.5 mL microcentrifuge tubes with these during the cleaning process.

Prepare 0.5 mL microcentrifuge tubes with the intended buffer for the samples and place them in the sample ports (4-6) that will be used that day.

Ensure the buffer line and sample line (from large and small flow sensors respectively) are placed into the waste containers as shown below



5.3.2.3 Beginning of day clean

This step is to clean the system before first use of the day, as well as prime the sample line with the intended buffer (from Line 1).

Ensure all buffers have been correctly added to the system (as in Step 5.3.2.2)

Ensure the buffer line and sample line (from large and small flow sensors respectively) are placed into the waste containers (see previous step)

Run the protocol on the MassFluidix Tool

If this is not the first clean of the day run the inter-measurement clean (it is the same protocol), More detail can be found in Section 6.1.4.

Please continue to monitor the system throughout the cleaning process

5.3.2.4 Prime Sample lines

In the MassFluidix Tool select the measurement option.

Then prime the sample lines with the sample **buffer:**

- Add sample buffer to the sample port being used for the measurement
- Then prime the sample line according to the instructions given by the MassFluidix Tool

Warning, skipping this step may lead to air being left in the sample line leading to air entering the chip during priming which can affect measurement quality. This step also ensures the protein under test is unaffected by a non-compatible buffer.

5.3.2.5 Connect Sample

Connect the tube containing the sample into the same sample port as primed in step 5.3.2.4.

5.3.2.6 Measurement Set Up

To set up the measurement with the MassFluidix Tool confirm the following:

- Chip Channel selection (1-5)
- Sample Dilution (125-10,000 times)
- Movie Length (Suggested 1 minute)
- Measurement mode
- Image Size
    - (For more details refer to the AcquireMP software manual)

Ensure the channel being used for the measurement has not been used before

Using channels which have previously been primed might output results outside of specification

5.3.2.7 Tubing and connector set up

Direct the tubing to go through colour coded glands in the following suggested way

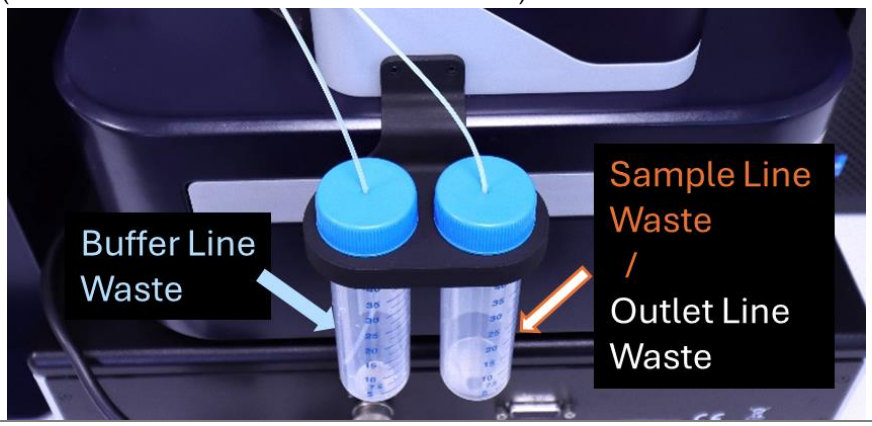
- Unscrew the nut on the glands until loose
- Feed the tubes through the glands in the following manner
    - Blue = Buffer line coming from the Large Flow rate sensor
    - Orange = Sample line coming from the Small Flow rate sensor
    - White = Outlet line going to the Outlet Flow rate sensor
- Tighten the nuts so that the tubes can still move freely but the nut is not loose

After threading the lines through the glands, do the following:

- Wipe them with lint free wipe to ensure they are dry

- Add the mini-luer connectors to the tubing, ensure fresh ones are used each time and the tubing extends 0.5 – 1 mm past the edge of the mini-luer connector as shown in the photos below

Ensure the outlet line is directed into an appropriate waste container; we suggest the Sample line waste also be used for the Outline line (to avoid contamination of the buffer line waste).

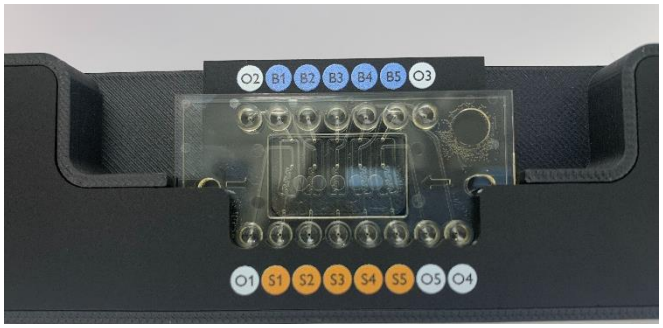


5.3.2.8 Connect the Chip

With the lid of the Mass Photometer open, place the sample preparation plate over the lip of the lower lid.

Place the chip into the sample preparation plate

- When placing the chip in the sample preparation plate ensure the 8 ports fit into the 8 slots.

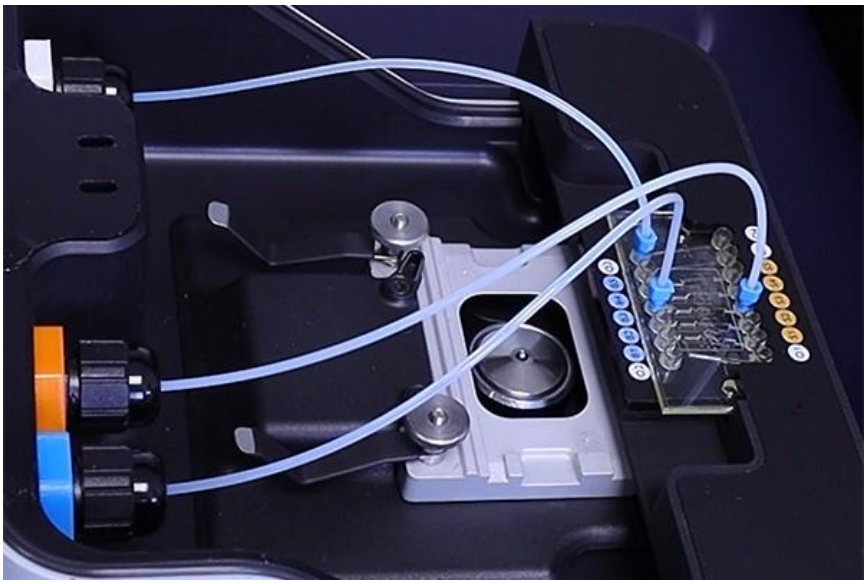


Never connect a chip whilst the chip is on the stage, always use the preparation plate

Connect the chip in the following way on the preparation plate:

- Connect the tubes to the appropriate ports on the MassFluidix HC Chip, which will be guided by the colour coded labels on the sample preparation plate
    - If using Channel **X**, do the following:
        - Outlet Line (tube through white gland) into **OX** (White)
        - Sample Line (tube through orange gland) into **SX** (Orange)
        - Buffer Line (tube through blue gland) into **BX** (Blue)
- Push the connectors firmly into the ports

Once connected this is what the stage area should look like:

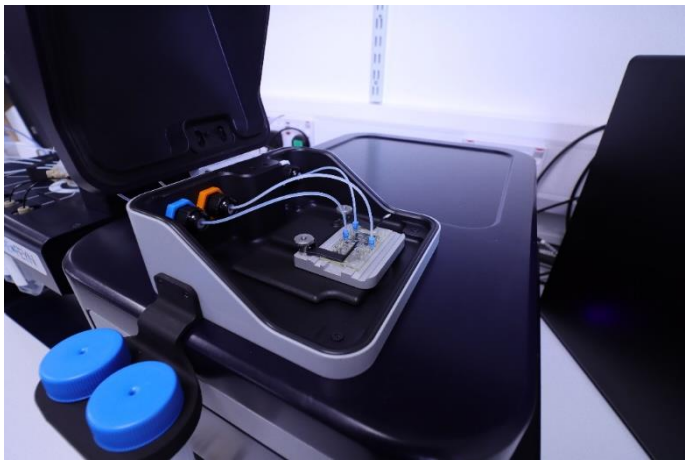


5.3.2.9 Install Chip on the stage

- Add immersion oil onto the objective, using two drops of oil.
- Lift the chip out of the sample preparation plate with care, adjust the tubing through the glands as the chip is moved towards the stage, in order not to strain the tube connections to the chip
- Place the chip onto the stage, locating the chip into the top left corner of the recess on the stage, and fastening the chip down with the clips:
    - Take care to avoid the clips touching any ports or connectors
    - Ensure the clips are sufficiently tightened to keep the chip secure and in the right position

Do not push down on the stage to tighten the clips

- Ensure tubing is adjusted to have an approximately 90° bend in the tubing.
- Tighten the glands to ensure tubing stays in place



5.3.2.10 Chip alignment and channel finding

Align the chip to the measurement channel in the following ways:

- Automatic Alignment feature
- Manual Channel Finding

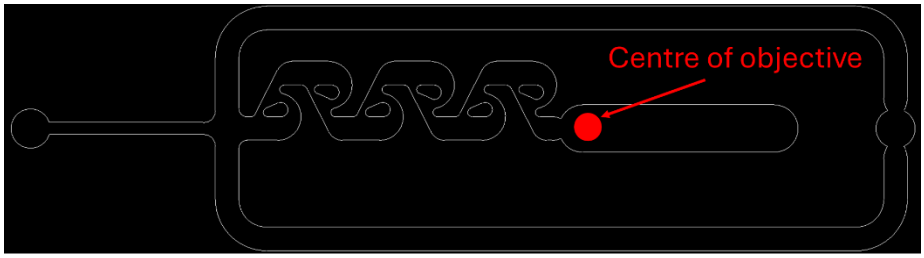
Automatic alignment

- Follow the instruction the software provides (the lid will need to be closed for this step)
- If the chip alignment fails repeatedly, perform Manual Channel Finding (the software will present this as an option)

Manual Channel Finding

- Use the stage movement controls to line up the centre of the objective in the centre of the channel, just after the mixer as indicated below

- Noticing the flicker of light from the auto focus laser as the centre of the objective passes into the observation area of the channel might help.
- Use a magnifying glass to confirm the location of the centre of the objective if possible.



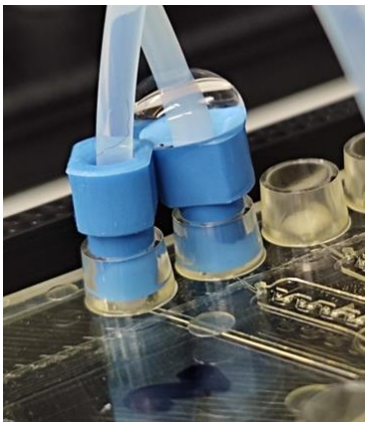
Without correct location the instrument will not be able to find focus in the next steps and the native image will not appear. The channel position can be adjusted once flow has started in the next steps to perform any corrections if the image cannot be viewed or focus cannot be found.

5.3.2.11 Beginning of Flow and buffer movie

Ensure the lid of the Mass Photometer is open to observe the chip for any leaks.

Start the flow and check for any leaks such as those shown below – If leaks are suspected abort the measurement to stop flow.

It is recommended to also view the buffer and pressure graphs (Accessed through the  in the bottom left of the MassFluidix Tool) to confirm the Buffer and Sample line pressures are at 250-400 mBar.



Example of leak

5.3.2.12 Buffer Movie

Close the lid of the instrument.

Find focus and then take buffer movies with these considerations in mind:

- As with traditional Mass Photometry, confirm the native and ratiometric images match what is expected from Mass Photometry. Move around the channel with the stage arrow keys if required.

- Be careful not to move outside the channel remembering the position of the mixer and the narrow width of the channel
- Then moving away from dirt or scratches on the glass it is recommended to move up the channel and away from the mixer

- Pay attention to the native image and sharpness value to confirm the instrument is not losing focus
  - Refer to troubleshooting guide 6.3.2 for more information
- The pressure and flow rate graphs can be observed through toggling the graphs button in the bottom left of the window 
  - This allows monitoring for any suspected leaks or blockages (see troubleshooting section for more details).
  - As well as to confirm stable flow rates.

5.3.2.13 Sample measurement

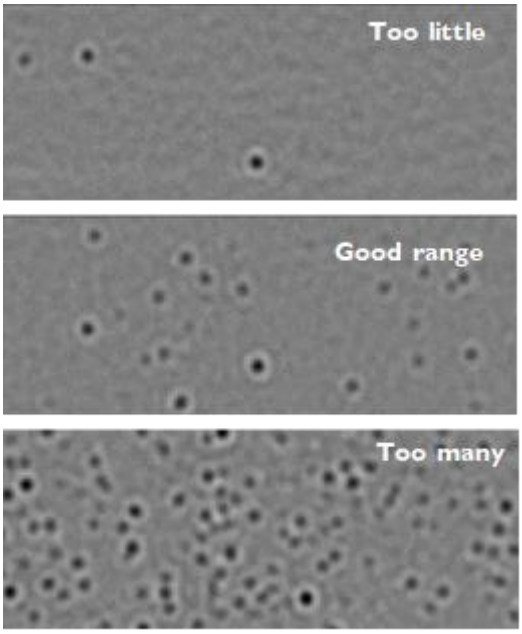
Once the buffer movie has been recorded, move on to sample recording (prompted in the software)

Clicking on the prompt will direct the system to automatically switch over to the sample port previously selected and flow the sample in at 7.9 µL / min (1/125 Dilution).

It will take 1-2 minutes for the sample to reach the observation window, during which time ensure focus has been found and is stable to allow events to be viewed on Ratiometric view. The focus is stable if a clear native image is maintained.

Once the sample is observed, click to confirm the sample has been detected which will prompt the system to go to the previously set target dilution (selected in step 5.3.2.6) and stabilise the flow rate.

- The dilution can now be adjusted to ensure the correct number of counts are present, as indicated below
- Note that the events will rise within the first 30-60 seconds of first seeing events, therefore the dilution factor may require adjusting during this time to provide a stable number of counts.



Ratiometric images of counts indicated the appropriate range

Then take sample movies

- Ensure the image is in focus before taking the movies
- If there are too many counts (see above) for too long this can saturate the surface leading to changes in the results such as more unbinding, lower resolution, or reduction in detection of smaller species

5.3.2.14 End of measurement and Cleaning

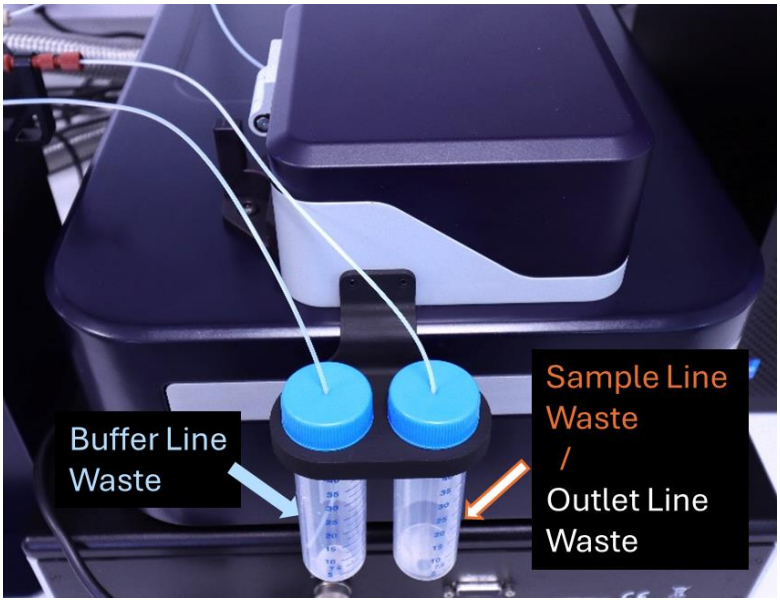
To finish the measurement, press the cleaning option in the MassFluidix Tool

This will cause the system to cease flow automatically, activating the cut-off switch on the buffer line.

Choose which cleaning protocol to perform next:

- Inter-measurement Clean
    - To clean the system between measurements, as well as prime the sample line with the intended buffer (from Line 1) and flush the large buffer line. It cleans the sample line from the M-switch onwards. It can be used if the next measurement will use the same sample from the same sample port, or a different sample from a different sample port.
- Full System Clean

- To clean the entire sample line, from the sample port (4, 5 or 6) onwards. This is important to



avoid contamination of the sample lines. It can be used when changing the samples in the sample ports and at the end of the day or the experimental session.

(These can be performed from the main menu of the MassFluidixs Tool as well)

Run the protocol on the MassFluidix Tool. More detail on the cleaning can be found in Section 6.1.4.3.

Please continue to monitor the system throughout the cleaning process

Disconnect the chip from the stage and do the following:

- Use lens cleaning tissue and Isopropanol to clean the objective
- Clean the glass surface of the chip if the chip is to be used again. This is done as follows:
  - Place the chip down on a lint free wipe, glass side down (remove most of the immersion oil)
  - Place the chip with the surface port side down on a clean area of a lint free wipe
  - With a lens cleaning tissue, wipe the remainder of the immersion oil off the glass surface of the chip
  - Apply Isopropanol to a new lens cleaning tissue and wipe the glass surface with long, firm, slow motion
- Dispose of used chips (all 5 channels been primed or used for measurement) in the appropriate sharps' container
- Discard all the mini-luer connectors
- Direct the tubes into the waste containers

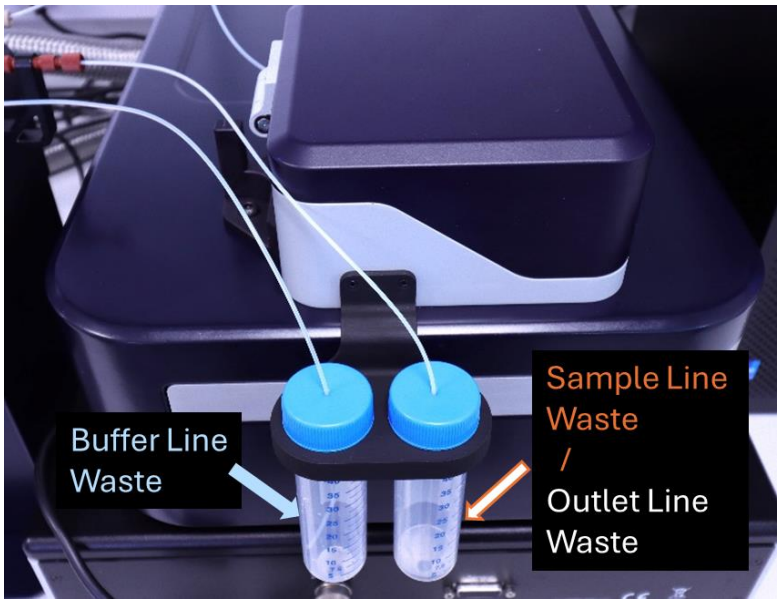
More details on cleaning are included in Section 6.1.4.

5.3.2.15 Inter-measurement Clean

This step is to clean the system in-between measurements, as well as prime the sample line with the intended buffer (from Line 1) and flush the large buffer line.

Ensure all buffers have been correctly added to the system (as in Step 5.3.2.2)

Ensure the buffer line and sample line (from large and small flow sensors respectively) are placed into the waste containers as shown below.



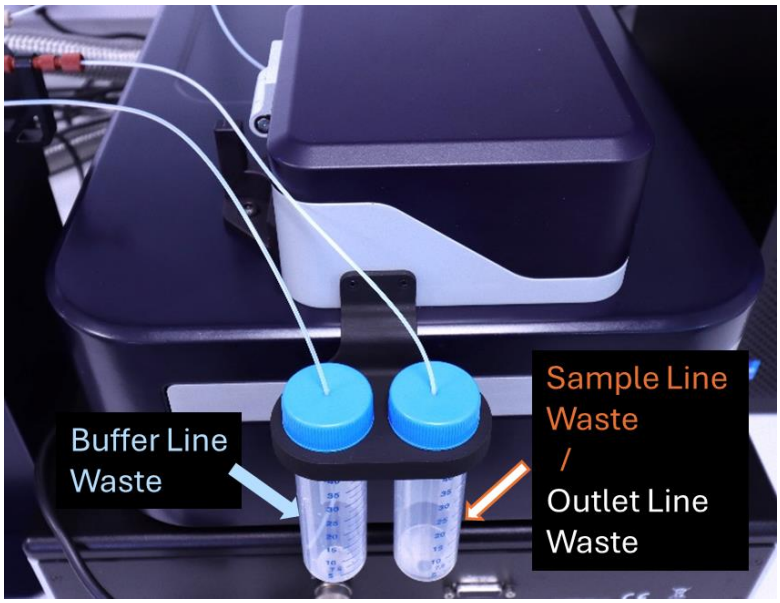
Run the protocol on the MassFluidix Tool. More detail on the cleaning can be found in the cleaning and maintenance section.

Please continue to monitor the system throughout the cleaning process

5.3.2.16 Full System Clean

This will clean the system through the sample line ports, which is important to avoid contamination of the sample lines.

Ensure the buffer line and sample line (from large and small flow sensors respectively) are placed into the waste containers as shown below.



The number of sample ports to clean, which should correspond to the number of sample ports used this day/experiment, can be selected.

The steps (prompted in the software) are (more detail in Section 5):

1) Connect a microcentrifuge with 400 µL of Ultrapure water into appropriate port(s)
    a. Wait 4 minutes per sample line
2) Connect a microcentrifuge with 400 µL of 0.5 M NaOH into appropriate port(s)
    a. Wait 4 minutes per sample line
3) Connect a microcentrifuge with 400 µL of Ultrapure water into appropriate port(s)
    a. Do not reuse the same microcentrifuge tube of ultrapure water
    b. This step also includes a buffer line flush
    c. Wait 4 minutes per sample line

Please continue to monitor the system throughout the cleaning process

5.4. Data analysis

For detailed information on how to analyse the recorded data, refer to the Refeyn Discover^{MP} user manual.

For data, acquired with the MassFluidix HC, it is recommended to record technical repeats on different chip channels to ensure data quality.

It is recommended to always record a measurement of the sample buffer before starting the sample flow to ensure there is a clear separation between the sample and any noise that might be introduced by the buffer.

6. Care, maintenance and troubleshooting

6.1. Cleaning

6.1.1. Objective cleaning

The objective lens must be cleaned after each use of a chip channel, using lens cleaning tissue and isopropanol to avoid immersion oil spilling over the whole objective.

Please refer to the 'Standard operating procedure: cleaning objective' document for further details.

6.1.2. Chip Cleaning

Chips should be cleaned by removing oil after measurements. This is important to prevent old oil from mixing with newly applied oil affecting measurements. This is done as follows:

- Place the chip down on a lint free wipe, glass side down (remove most of the immersion oil)
- Place the chip with the surface port side down on a clean area of a lint free wipe
- With a lens cleaning tissue, wipe the remainder of the immersion oil off the glass surface of the chip
- Apply Isopropanol to a new lens cleaning tissue and wipe the glass surface with long, firm, slow motion.

Dispose of used chips (all 5 channels been primed or used for measurement) in the appropriate sharps container.

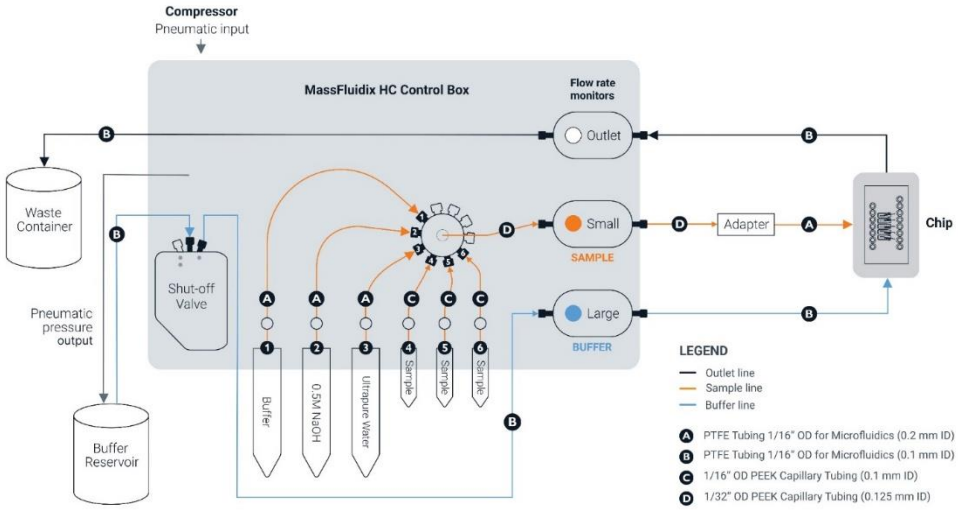
6.1.3. Tubing Changing

The MassFluidix system should have all tubing changed every 3 months to ensure system cleanliness.

Follow the guided steps in Section 3.1, ensuring the system is then cleaned as in 6.1.4 once fresh tubing has been attached.

6.1.4. Instrument cleaning – Internal (Tubing)

This section outlines the ways to clean the tubing of the MassFluidix system.



The effectiveness of the cleaning can be affected by the type of sample ran. If the user notices carryover from a sample that would affect the following measurement results after a clean has been run, it is recommended to repeat the cleaning. If sample carryover is still observed, it is recommended to change the sample line tubing and buffer line tubing (refer to section 6.3.7).

The following procedure should also be done when the tubing has just been freshly attached to the system.

#	Step description

6.1.4.1 Start up the system

Ensure that the system is powered and connected to the PC and that the compressor is powered and set to P1.

Turn on the Mass Photometry System and start Acquire^{MP}.

Open up the MassFluidix tool in the Acquire^{MP} Tool menu. This software provides a guide to cleaning instructions.

If for any reason the usage of the Oxygen software is required, please refer to Section 6.5.

6.1.4.2 Prepare Buffers

Prepare the buffer bottle with the intended Buffer Solution (for the experiment you will perform after cleaning).

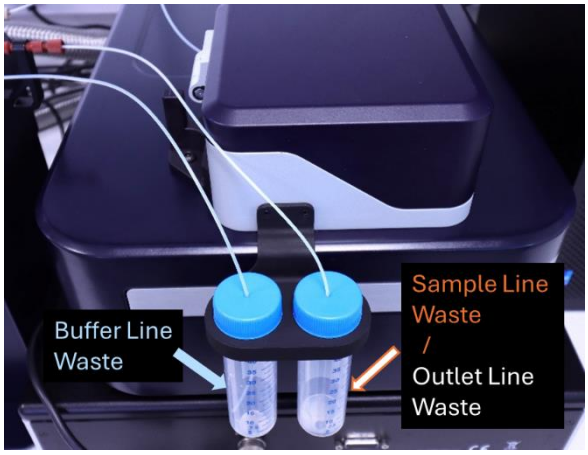
- Ensure fresh solution is used in a clean 250 mL Glass Bottle
- Use at least 100 mL
- Ensure it is correctly tightened

Prepare the 40 mL of cleaning solutions in 50 mL centrifuge tubes, place the following solutions in the following lines:

4. Intended Buffer Solution
5. 0.5 M NaOH (cleaning solution 1)
6. Ultra-Pure Water (cleaning solution 2)

Leave the same solutions (1,2 and 3) accessible on the benchtop for filling 0.5 mL microcentrifuge tubes with these during cleaning process.

Ensure the buffer line and sample line (from large and small flow sensors respectively) are placed into the waste containers as shown below



6.1.4.3 Run Inter-measurement Clean

This step cleans the system from the 3 large reservoirs on the box.

It also primes the sample line with the intended buffer (from Line 1) and flushes the large buffer line.

Ensure all buffers have been correctly added to the system (as in Step 6.1.4.2)

Ensure the buffer line and sample line (from large and small flow sensors respectively) are placed into the waste containers as shown in the previous step.

Run the protocol on the MassFluidix Tool

- This is protocol does the following:
  - 4 Minutes of Ultra-pure water (Through Line 3)
  - 4 Minutes of 0.5 M NaOH (Through Line 2)
  - 4 Minutes of Ultra-pure water (Through Line 3)
  - 2 Minutes of Buffer (Through Line 1 and the buffer line bottle)

Please continue to monitor the system throughout the cleaning process

6.1.4.4 Run Full System Clean

This will clean the system through the sample ports, which often interact with high concentration samples and require frequent cleaning.

Ensure the buffer line and sample line (from large and small flow sensors respectively) are placed into the waste containers as shown in step 6.1.4.2

Select the number of sample ports to clean. Each sample port selected will take 12 minutes.

The steps (prompted in the software), and are as follows:

4) Connect a microcentrifuge with 400 µL of Ultrapure water into appropriate port(s)

a. Wait 4 minutes per sample port being cleaned

5) Connect a microcentrifuge with 400 µL of 0.5 M NaOH into appropriate port(s)
   a. Wait 4 minutes per sample port being cleaned
6) Connect a microcentrifuge with 400 µL of Ultrapure water into appropriate port(s)
   a. Do not ever reuse the same microcentrifuge tube of ultrapure water
   b. This step also includes a buffer line flush
   c. Wait 4 minutes per sample port being cleaned

Please continue to monitor the system throughout the cleaning process

---

6.1.4.5 Buffer Flush

This will pump buffer through the buffer line at 1 mL/min, for 2 minutes.

It is available through the main system menu of the MassFluidix tool.

This is ideal practice for:

- Replenishing a new line to be full of buffer
- Removing stale buffer (buffer that has been left in the buffer line for longer than 1 day)
- Changing the buffer
- Switching to Ultrapure water for long term storage

It is important to ensure the buffer line is placed into a waste container before starting this procedure.

If there is a suspicion of contamination in the buffer line, or want to clean the line, please use the following procedure

1. Remove the current bottle from the system
2. Replace with a bottle with Ultrapure water and perform the flush protocol
3. Replace with a bottle with 0.5 M NaOH and perform the flush protocol
4. Replace with a bottle with fresh Ultrapure water and perform the flush protocol
5. Replace with a bottle with either of the following options:
   a. Ultrapure water for long term storage, then perform buffer flush
   b. Buffer of choice for the experiment, then perform buffer flush

---

6.1.5. Instrument Cleaning – External

Unless otherwise required by local procedures, there is no need to clean the exterior of the 'MassFluidix HC' instrument more than once a week.

Dampen a sheet of absorbent tissue with purified water and wipe all exterior surfaces of the instrument. The absorbent tissue must be moist enough to ensure water is in contact with the exterior surfaces of the instrument. Use further sheets of tissue as required.

The working area around the instrument can be cleaned with standard laboratory materials and methods.

6.2. Long term storage and shipping of System

If the MassFluidix HC system is going to be left idle for more than 2 weeks it is recommended to clean all the tubing and leave ultrapure water in the buffer line and sample line.

If the MassFluidix HC system is going to be left idle for more than 2 months it is recommended to clean all the tubing (as stated above), then switch positions 1 and 2 with 40 mL of ultrapure water and perform the inter-measurement clean again.

When shipping a MassFluidix HC system please contact support@refeyn.com for advice.

6.3. Troubleshooting guide

6.3.1. Air in observation area

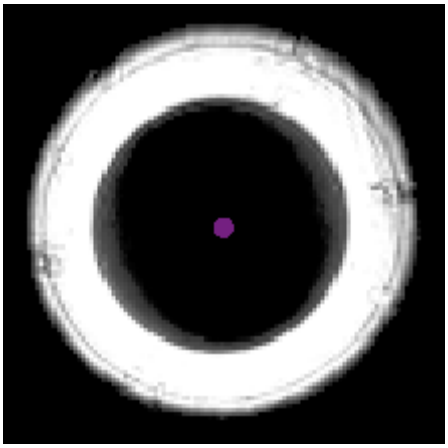
In some instances, a bubble can be introduced that can stay in the observation window before the measurements have started or during measurements.

If this keeps happening, try to assess all connections for any leaks and confirm recommended volumes are being inserted into the system.

First step is to wait 15 seconds to see if the air passes. Sometimes this can result in the surface becoming dirty so it may require moving to a new location in the channel to avoid the dirt. This could increase the low mass noise so consider this during any data analysis.

If there is still air after 15 seconds it could be issues with the buffer line, check the following:

- The buffer reservoir is closed tightly
- The buffer reservoir has sufficient buffer in it
- The buffer line inside the buffer reservoir is sufficiently suspended in the buffer



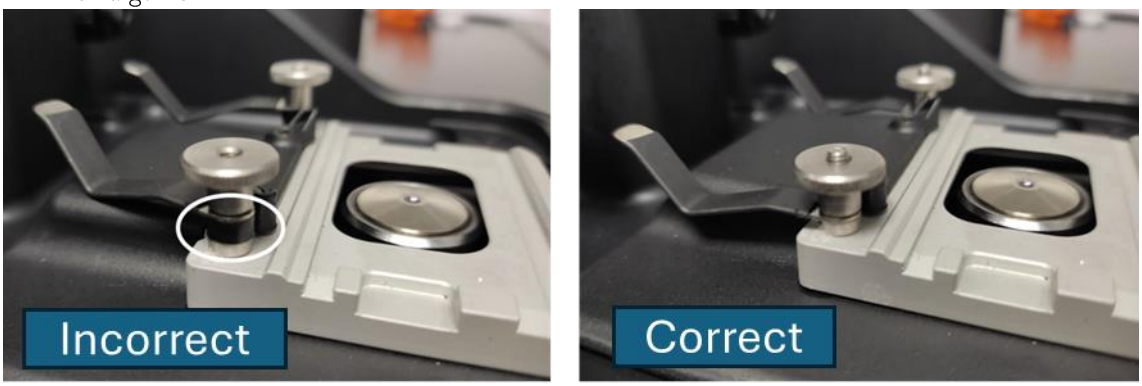
Example of what a bubble caught in the chip will look like in the autofocus ring.

6.3.2. Focus drift during measurements

After focus has been found, the piezo motor may continuously re-adjust to maintain focus. This is done by the fine focus controls which have limits. If the focus cannot be maintained by the fine focus, the sharpness of the measurement will rapidly drop accompanied by losing events on Ratiometric view or a fuzzier native image.

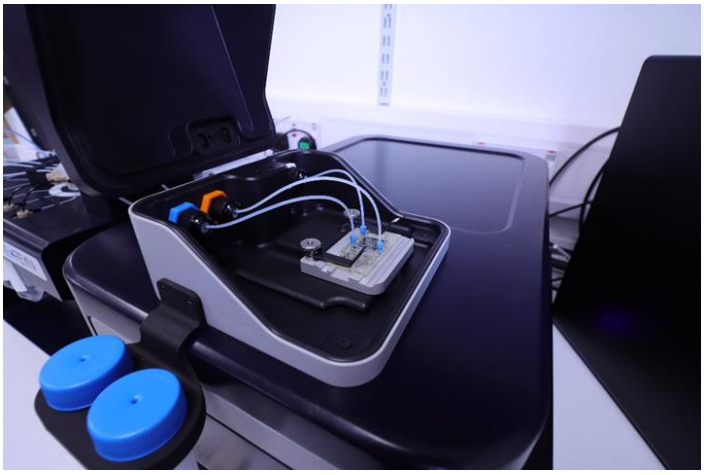
This is often caused by two main things (listed below). Address these issues before attempting to find focus again.

1. The thumb screws not being tight enough – so tighten them further with care not to push down on the stage
    a. If the thumb screws cannot be tightened enough to hold the chip in place, please check the below image for a guide



2. The tubing may be exerting a force on the chip; this could be due to the follow two reasons:
    a. The tubing pointing at >90-degree angle, exerting a downwards force on the chip

i. Follow diagram below making sure the tubes have a similar 90°-degree bend.



b. Or the glands not being appropriately tightened so the lines slowly move, exerting a force on the chip.

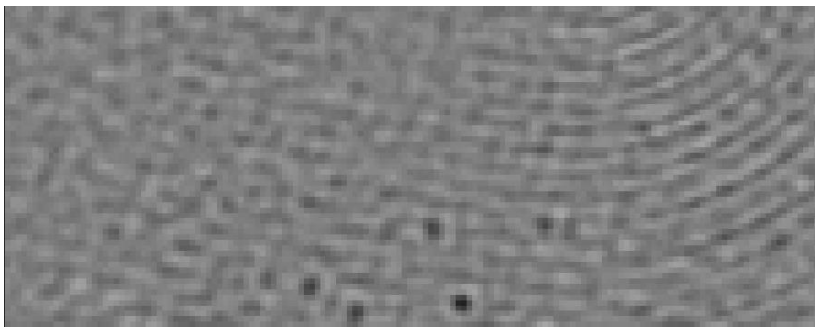
i. Ensure they are appropriately tightened

Focus drift can also be tracked by the fine focus bar (this can be shown by selecting Manual Focus before entering the MassFluidix Tool). If the fine focus does not stabilize in 30 sec – 1 min (indicated by the slider reaching >80 or <20 value) it is an indication that the thumb screws are not tight enough, or the tubing may be exerting a force on the chip.

6.3.3. Bubbles getting into the observation area during measurements

Air can get into the observation area during measurements. There are several points to consider:

1. If the air bubble is small and gets stuck close to the field of view, the user might see noise interference on the ratiometric image in the form of waves appearing over the image.

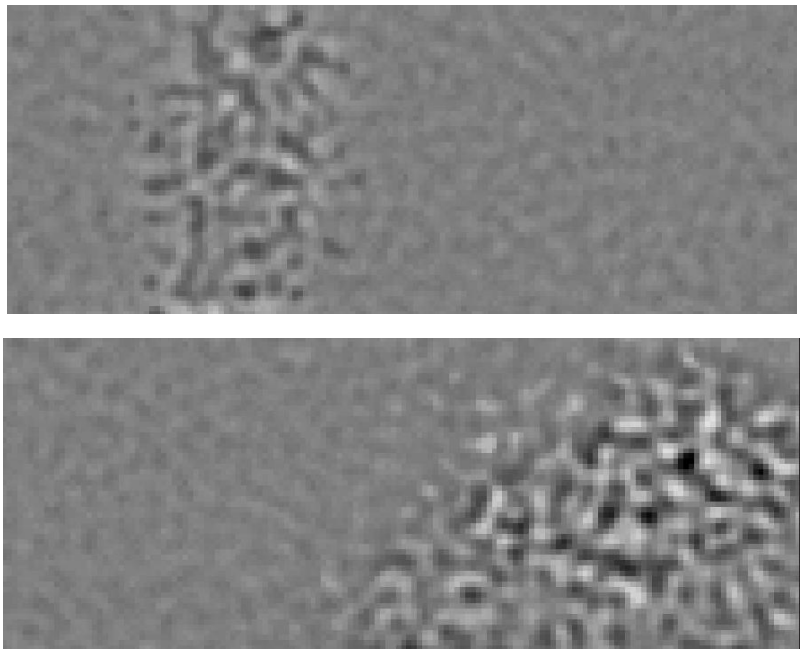


Example of interference noise in the form of waves seen in the FoV due to the proximity of an air bubble.

If this happens before the sample is in the observation area, the user can move away from the bubble using the lateral control buttons in Acquire^{MP} and re-find the focus before the measurement. *Note: stay away from the walls of the observation area.*

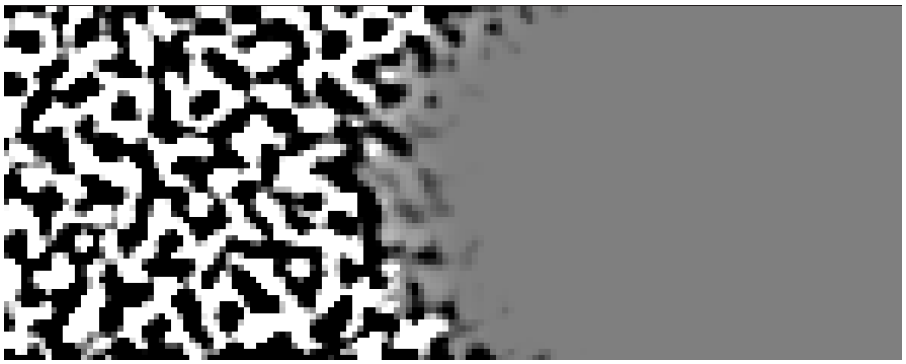
If the sample is already in the observation window, the measurement can be recorded but the user is advised to check the data quality of the movie afterwards for peak broadening, accuracy of the measured masses and peak resolution.

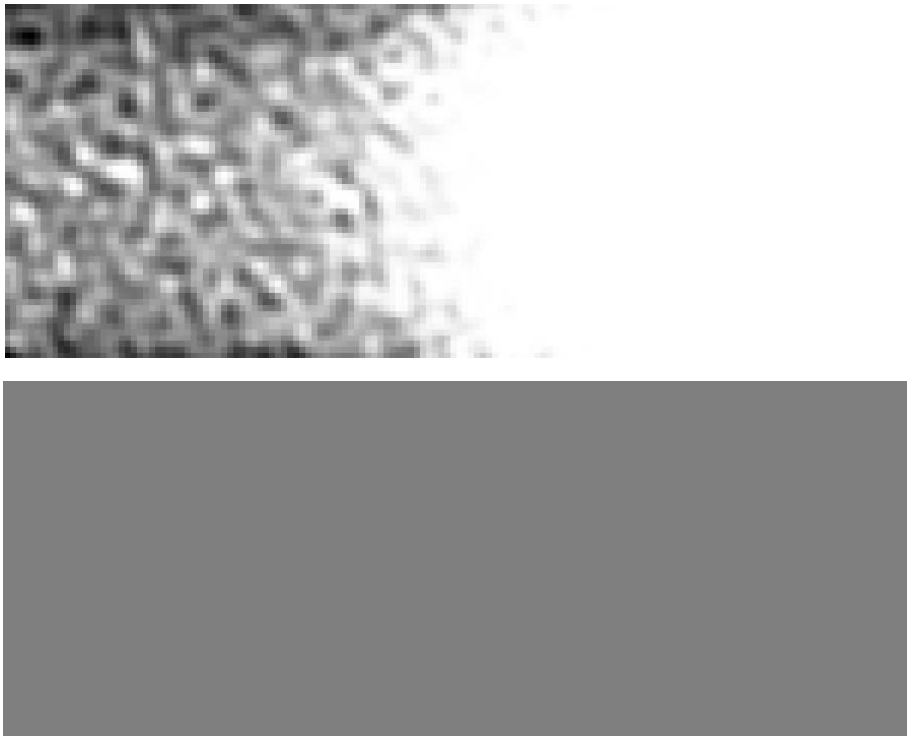
2. Small air bubbles might pass through the FoV. The user will see spots of noise on the ratiometric image for less than a second. The user is again advised to check the data quality.



Examples of small air bubbles passing through the imaged area.

3. Air bubble gets stuck in the observation area. In this case, parts, or all of the image will become greyed out. Again, the user can try to move away from the air bubble and re-find the focus.





Examples of air bubbles affecting the image in ratiometric and native view.

6.3.4. Lack of binding events in the observation window and low sample volume

The time between injecting the sample and observing the events in the observation window will depend on the length of the tubing. varying between 1.5 – 3 minutes. If the user doesn't see events after 4 minutes of waiting, the following is recommended:

- Check the focus has not drifted.
- Check there is no blockage in the tubing lines (indicated by the line pressure – see section 6.4).
- Check the correct sample port is being used.
- Check the starting sample concentration is high enough to be measured with the MassFluidix instrument.
    - This can be done by doing a static measurement of the sample with the same dilution factor measured on the MassFluidix system. If no events are seen on the static measurement, then the sample is too diluted for fluidic measurements.

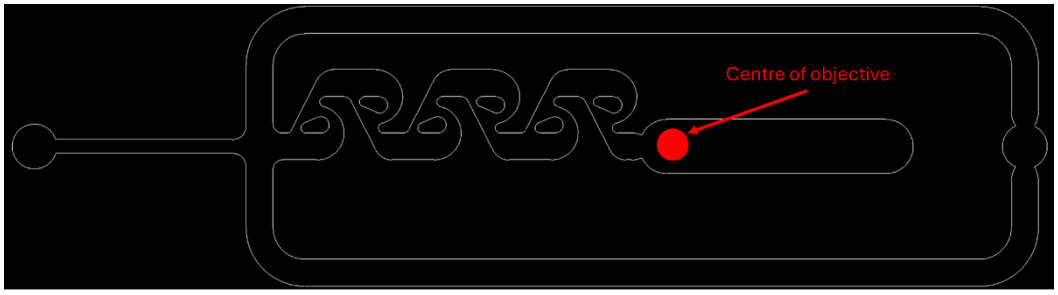
6.3.5. Sample events periodically overwhelming image

Sample events in the image during measurements can appear to suddenly increase or decrease noticeably for a few seconds or an extended period of time. This likely is caused by the air bubbles. Point 6.3.3 should be considered here. The best way to avoid this happening is to prime the system well in the beginning as instructed in section 5.3.2.

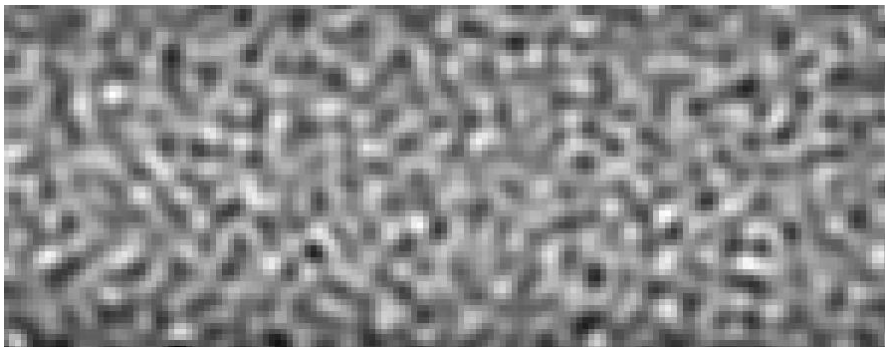
6.3.6. System cannot find focus

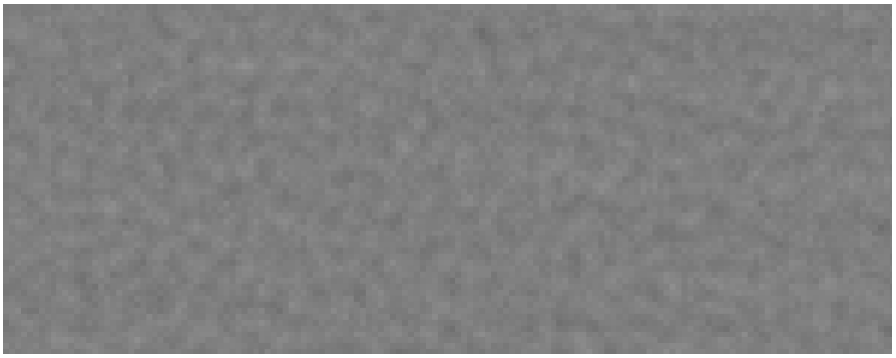
If an error appears after the focusing routine has been run saying 'Acquire^{MP} couldn't find the focus', this may be due to several causes:

- The chip has not been aligned correctly in the channel; ensure the centre of the objective is at the position indicated in the diagram below.



- The chip clamps are not tight enough - the user should open the lid and make sure the chip clamps are well tightened and holding the chip
- The objective may be dirty. Always clean the objective after measurements with lens cleaning tissue and Isopropanol to properly clean the objective.
- The imaged area of the glass is dirty – dirt spots affect the focus of the instrument. User should move to a clean area and redo the focusing routine.





Example of clean regular image in native and ratiometric view (Two^{MP})

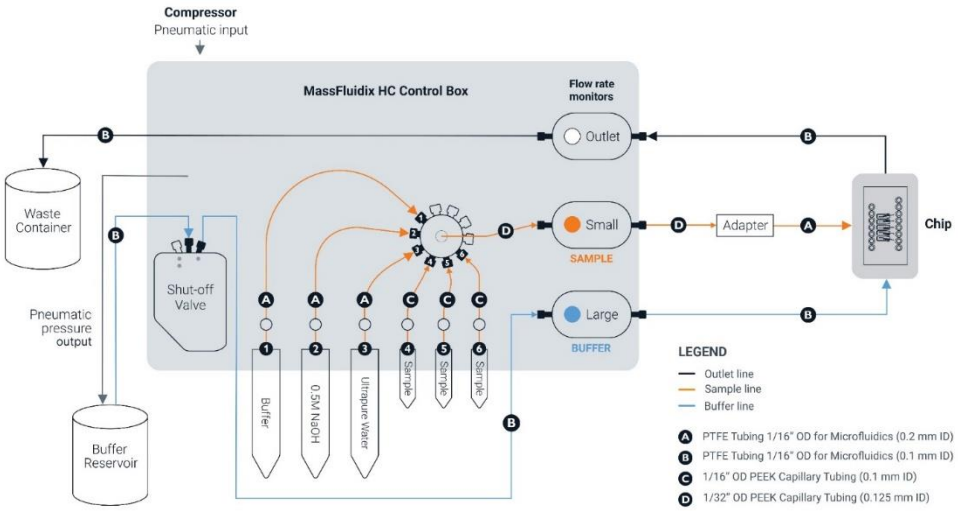
For any issues with the instrument, please contact support@refeyn.com.

6.3.7. Carryover

There should be little to no carryover proteins between the measurements if the cleaning procedure has been run correctly and frequently. Carryover can be checked by recording neat buffer and checking any events that correspond to the masses of previously run samples. Note, low mass noise (~30 kDa) should not be considered as carryover unless it is present in abundant amounts (>500 counts) when previously the system exhibited low amounts of low mass noise.

- If carryover events are observed in the neat buffer measurements, the cleaning protocol should be run again to clear them out of the tubing.
- If carryover is still seen after the second clean, the user can change the sample tubing A and B and run the cleaning protocol again.

- User should check the events are not coming from the buffer itself. This can be done with static measurements of the buffer for confirmation.



6.4. Diagnosing and Solving Blockages

6.4.1. Diagnosing Blockage

Debris or ineffective cleaning could lead to blockages occurring in the tubing. A blockage indication can be one of the following.

Note that pressure values can be viewed in the MassFluidix Tool in the lefthand corner by selecting this option →

1. Sample events not appearing during measurements
    a. Follow the advice in section 6.3.4
2. Sample pressure rising above 500 mbar with 0 µL / min flow rate
3. Buffer pressure rising above 500 mbar
4. No fluids leaving the sample or buffer line during cleaning
5. No flow through the outlet tubing

If there are signs of blockages as indicated above, abort any current protocols then follow the procedure below.

6.4.2. Solving Blockages

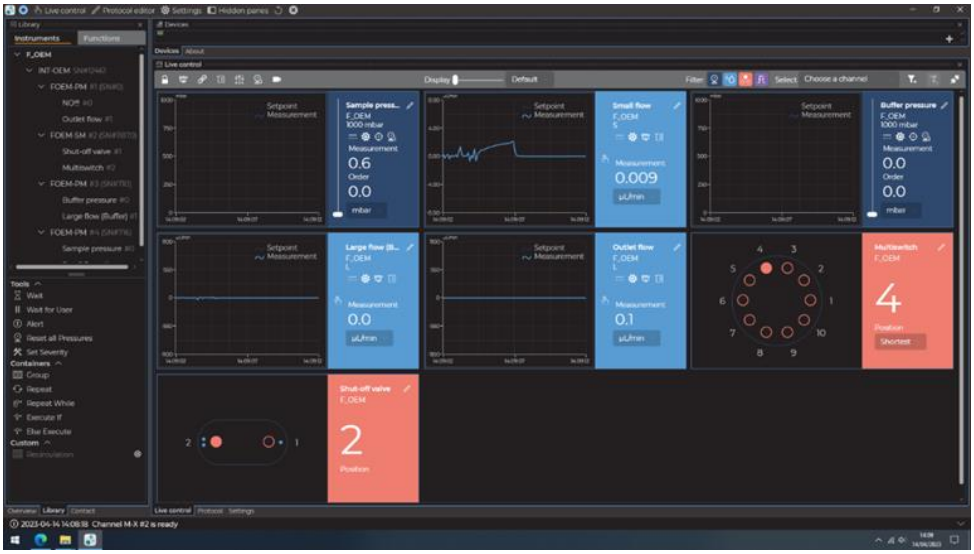
This section requires the use of Oxygen software.

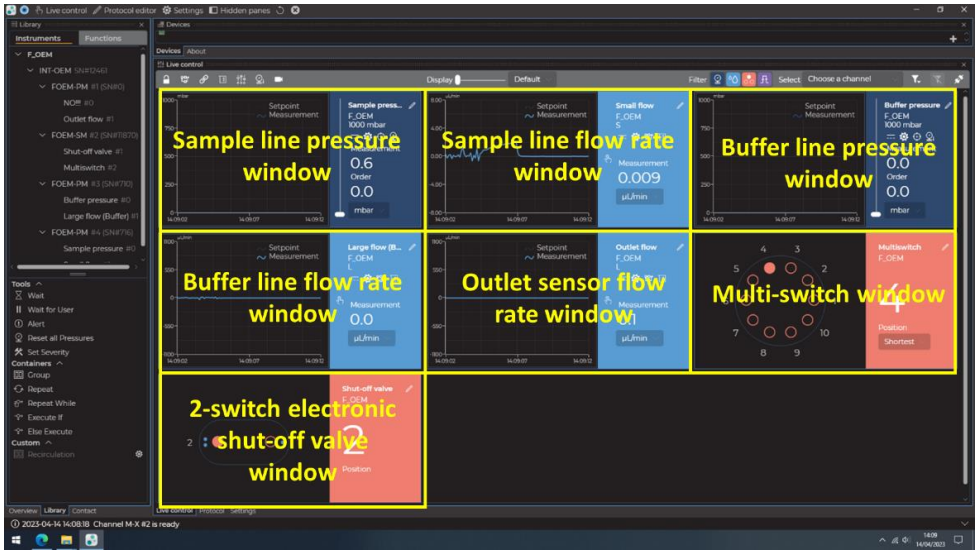
Launch the OxyGEN software and select "Live control" from the pop-up window.

The user interface should show all the elements integrated into the MFx HC system:

- Sample line pressure window
- Buffer line pressure window
- Sample line flow rate window
- Buffer line flow rate window
- Outlet sensor flow rate window
- 2-switch electronic shut-off valve window
- Multi-switch window

(If any elements are missing, please contact Refeyn support: support@refeyn.com).





Oxygen software user interface

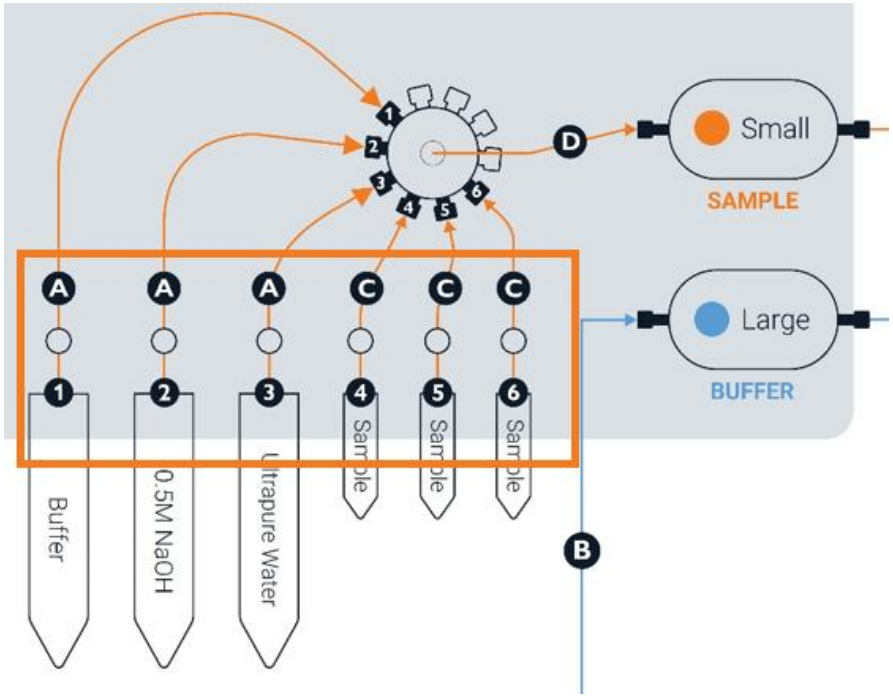
Ensure the system is set up with Ultrapure water in all ports (1 to 6):

- Ports 1-3 containing 40 mL of Ultrapure water in 50 mL Centrifuge Tubes
- Ports 4-6 containing 400 µL of Ultrapure water in 0.5 mL microcentrifuge tubes

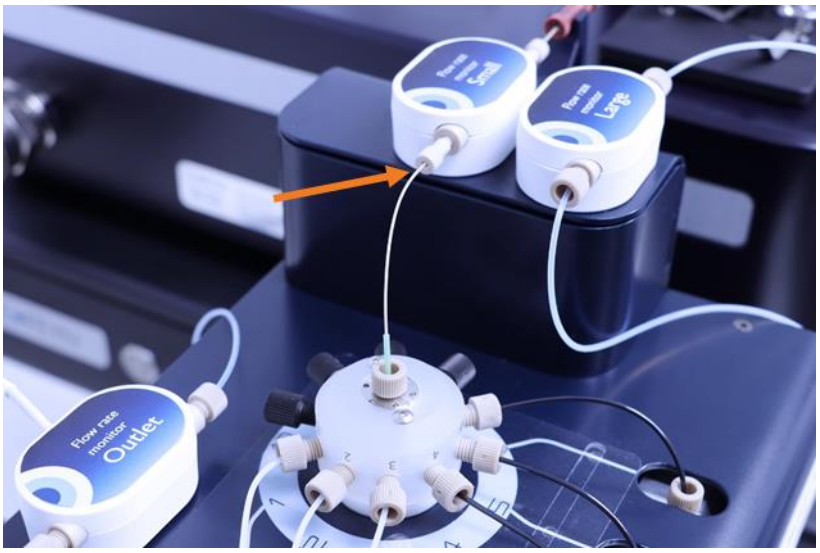
The following process is recommended to determine where the blockage occurs:

#	Step description
6.4.2.1	**Set the multi-switch to position 1 and set the sample flow rate to 7.9 µL/min.** **Wait for approximately 30 seconds to observe the flow rate monitor for detection of flow.** **Move to each position on the multi-switch and observe if flow is detected in each of the positions.**

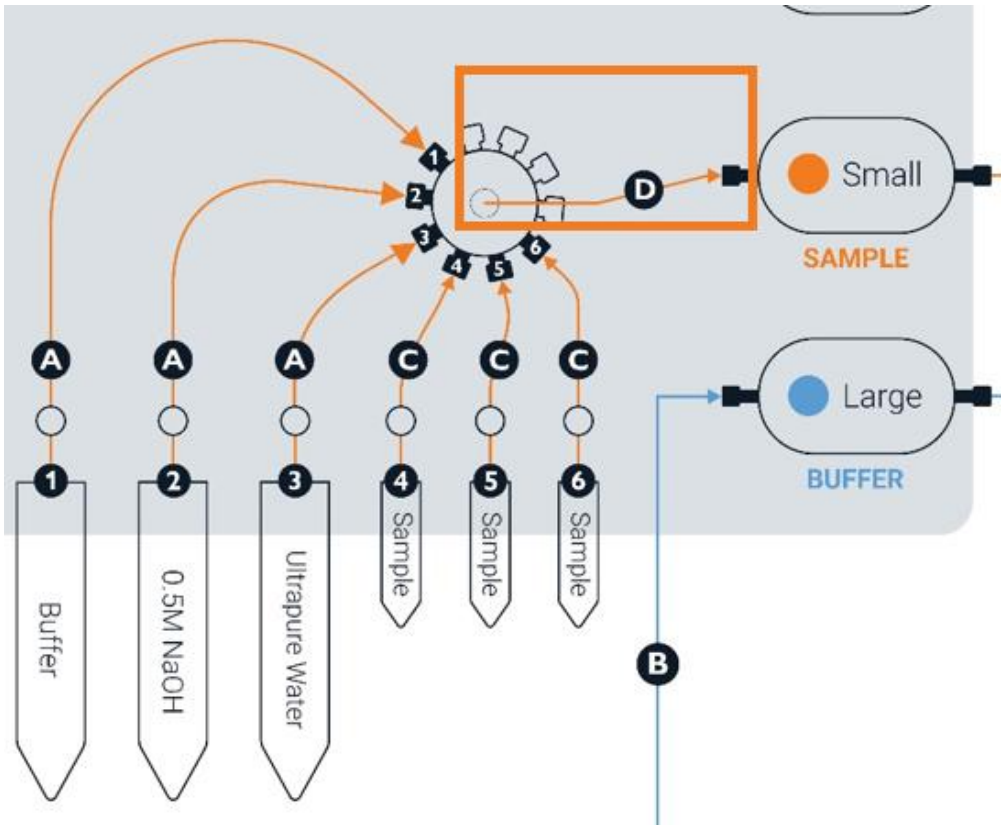
6.4.2.2 If flow is not detected from the one of the switch positions (but is on the other switch positions), stop the sample line pressure (reset pressure), and change the tubing between the port on that position and the multi-switch.



6.4.2.3 If no flow is detected from any of the switch positions, keep the sample line set to 7.9 µL/min flow rate and disconnect the tubing at the small flow sensor and check if fluid is flowing out of the disconnected end.

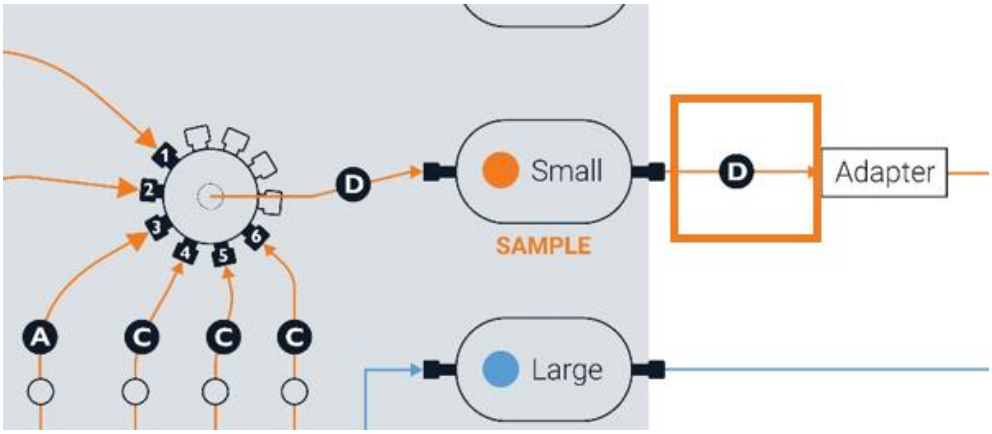


6.4.2.4 If no liquid is observed in step 6.4.2.3, stop the flow and replace tubing between the multi-switch and small flow sensor. Repeat from step 6.4.2.1 to verify the blockage has been removed.

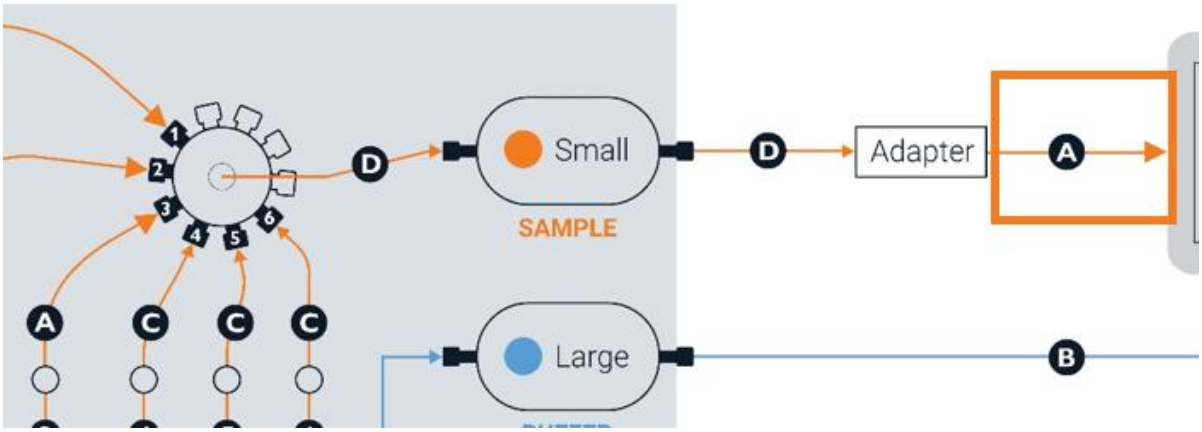


6.4.2.5 If flow is observed in step 6.4.2.3, reconnect the tubing end into Small flow sensor and disconnect the tubing adapter (as pictured). Check for liquid flowing out of the end.

6.4.2.6 If no liquid is observed in step 6.4.2.5, replace tubing between the Small flow sensor and the tubing adapter.



6.4.2.7 If liquid is observed in step 6.4.2.5, replace the remaining tubing after the adapter.



6.4.2.8 If all tubing has been changed, but there is still a blockage, it might be that the end of the tubing was not cut flat but at an angle, preventing a good connection. Use a sharp set of tube cutters to get a neat cut and replace the blocked tubing again.

6.5. Performing a measurement in Oxygen.

Oxygen software is an alternative way to use the system for measurements. This is an unsupported method but can be performed by users more experienced with MassFluidix.

To do this, Acquire^{MP} software will need to be used alongside Oxygen.

Warning: the MassFluidix Tool in Acquire^{MP} cannot be used whilst using Oxygen. This means automatic chip alignment and channel finding cannot be performed.

When using Oxygen, there is more control of the fluidics system shown below. This will allow to control of the following:

Switches:

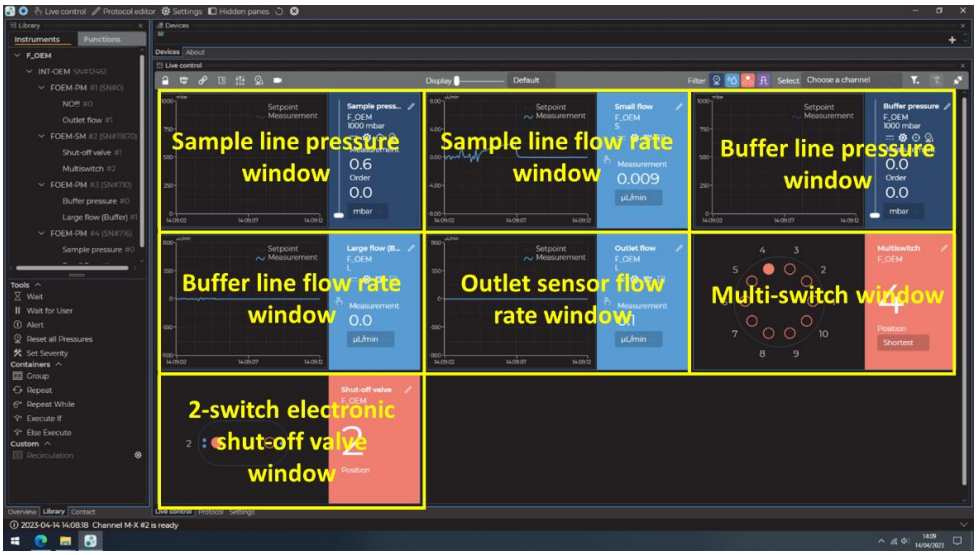
- The 2-switch electronic shut-off valve window
    - This has two positions, open and closed, control the movement in the buffer line
    - Important for stopping back flow from the chip and tubing into the buffer bottle that would cause contamination
- Multi-switch
    - Controls where the sample line draws fluid from
        - From positions 1-6 described in Section 4

Buffer line, Large Flow rate:

- The Buffer line pressure window
    - Controls the pressure of the buffer line
- The Buffer line flow rate window
    - Control the flow rate of the buffer line
    - This is usually set at 1 mL/min for optimal dilution

Sample line, Small Flow rate:

- The Sample line pressure window
    - Controls the pressure of the sample line
        - Some processes are better controlled through pressure than flow rate
- The Sample line flow rate window
    - Control the flow rate of the sample line
    - Important for managing the dilution (Refer to section 4 for more details).



Measurements performed with Oxygen are done with the following Refeyn protocols which can be requested by emailing support@refeyn.com.

- Beginning of Day Clean
- Sample line priming
    - Protocol for each sample line
- Sample line measurement
    - Protocol for each sample line
- End of flow
    - To finish each measurement to avoid contamination of the buffer line
- Inter-measurement clean
- Full system clean
    - Protocol for each sample line

It is recommended to use these protocols when doing measurements to ensure efficient priming of the chip during measurements and effective cleaning. Variation to buffer line and sample line flow rate should be adjusted manually when the protocols allow.

7. General information

7.1. Service and support

Refeyn Ltd offers service and technical support for MassFluidix HC:

Refeyn, Unit 9, Trade City Sandy Lane West Oxford, OX4 6FF UK

Email: support@refeyn.com

7.2. Disposal and recycling

Before disposing of this equipment, check the following:

Check with the appropriate local organization to obtain advice on local rules and regulations about disposal and recycling.

Contact Refeyn before disposal begins (support@refeyn.com).

8. Disclaimer

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