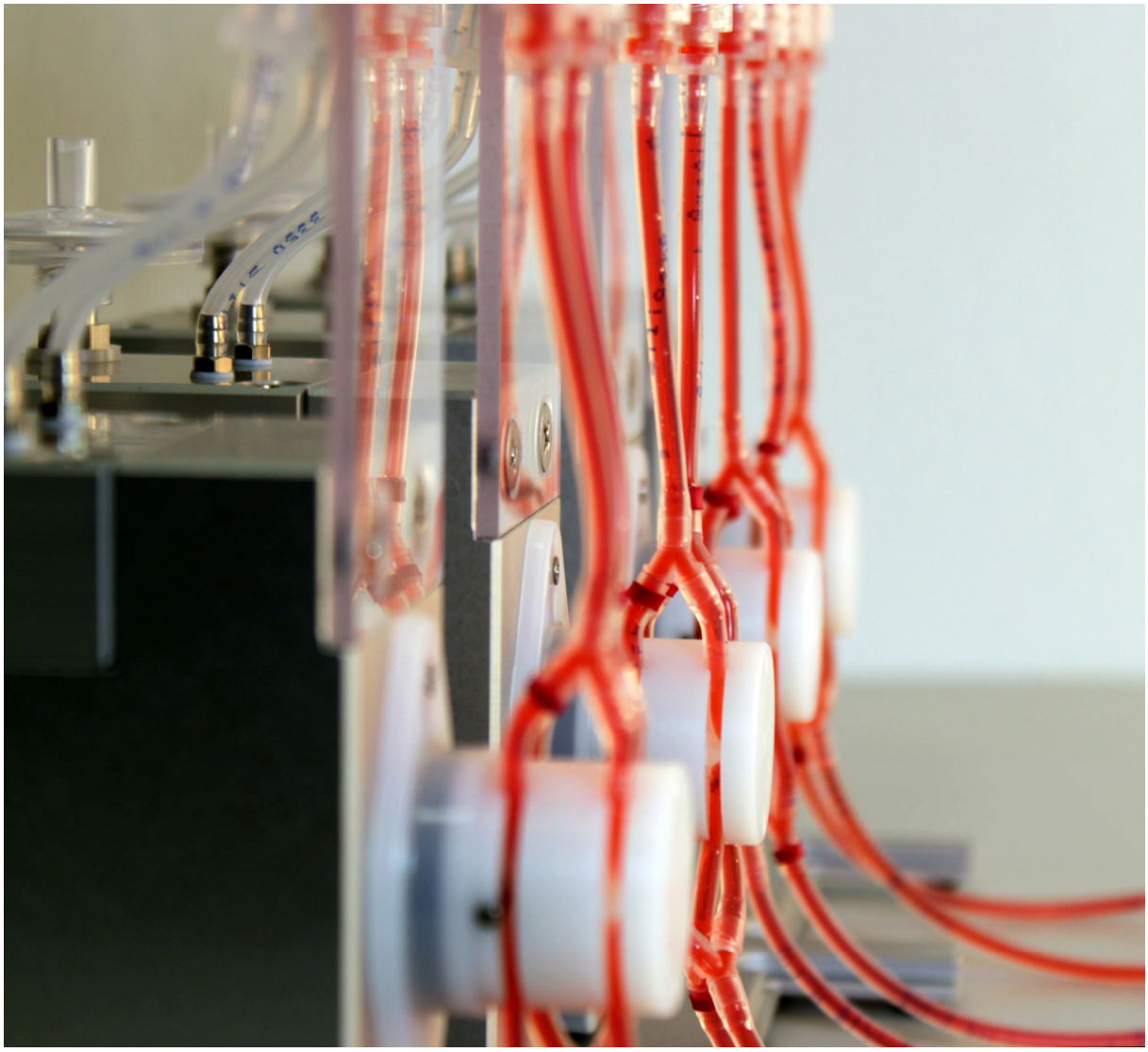
ibidi Pump System Instructions

Version 1.5.1



Contact

ibidi GmbH
www.ibidi.com

Am Klopferspitz 19
D-82152 Planegg
info@ibidi.de
phone: +49 89 5204617 0
fax: +49 89 5204617 59

Dear User,

The idea of the ibidi perfusion system was formulated by scientists working with µ-Slides for perfusion studies. Below are their main requirements for a pump.

1. Simultaneous culture and observation of cells under perfusion conditions.
2. The range of applied shear stress should simulate the physiological spectrum of veins and arteries.
3. The system should be easy to setup under sterile conditions.
4. Mechanical stress to suspended cells should be minimized to avoid destruction and non-specific activation of the cells (white blood cells are affected by mechanical squeezing).
5. The amount of medium needed to perform perfusion assays should be slight, therefore saving valuable reagents. Small volumes also allow for measuring soluble factors produced by cells.
6. Unidirectional flow for long term studies (multiple weeks).
7. Oscillating flow should be possible to mimic turbulent flow situations.

The initial idea was to place a peristaltic pump directly in an incubator. However, this produced too much heat in the incubator and overheated the cells. Furthermore, it was learned that when the peristaltic mechanism squeezes the tubes, it also applied this pressure to the suspended cells. With these findings, it was decided to look for another method and the idea to use air pressure was formulated.

The initial idea for an air pressure pump included the use of two reservoirs, one serving as source of medium and one for waste. Unfortunately, this was incompatible with the idea of using minimal amounts of medium. To apply 10 dyn/cm² for 24 hours in the µ-Slide I 0.4 Luer it would require roughly 11.5 liters! The solution was alternating pumping from one reservoir to the other. But the question remained; "Could the system achieve unidirectional flow?" ibidi engineers worked on this design and came up with an answer: pinch valves.

The main idea was to split the system into two pieces; a portable Fluidic Unit and the pump itself as an air pressure generator. The design of the Fluidic Unit as a separate piece has many advantages. Because it is a closed system and connected to the pump by air tubing and low voltage electrical cable, it can easily be disconnected without losing sterility. This allows the user to work with the unit under sterile conditions as well as keep it in a standard incubator while running the experiment. After preparation, the Fluidic Unit can easily be reconnected to the pump, which is stored outside the incubator. During experiments the Fluidic Unit can be placed close to the microscope, making live cell imaging possible.

ibidi would like to thank Roland Nitschke and Fruzsina Kotsis (both from the University of Freiburg, Germany) for one year of extensive prototype testing. Their troubleshooting and suggestions were invaluable. Also, a special thanks to Natalia Cockcroft (British American Tobacco, R&D Centre, Southampton, UK) who pointed out that the advantage of our system is that it is possible to measure multiple experimental endpoints such as:

- Real-time monitoring of morphological changes in living cells
- Profiling of secreted proteins in conditioned media
- Cell adhesion
- Gene expression profiling after cell detachment.

Thank you for purchasing the ibidi Pump system. Best success with it and please give us feedback!

Table of contents

Safety Considerations

To ensure operation safety, the ibidi Pump System must be operated correctly and maintained according to a regular schedule. Carefully read to fully understand all safety precautions in this manual before operating the instrument. Please take a moment to understand what the signal words **WARNING!**, **CAUTION** and **NOTE** mean in this manual.

Safety symbols

WARNING!

A **WARNING!** indicates a potentially hazardous situation which, if not avoided, could result in serious injury or even death.

CAUTION

A **CAUTION** indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury. It may also be used to alert against damaging the equipment or the instrument.

Do not proceed beyond a **WARNING!** or **CAUTION** notice until you understand the hazardous conditions and have taken the appropriate steps.

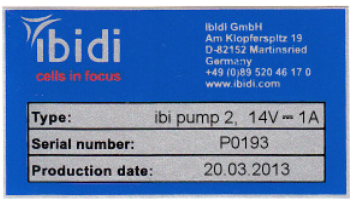
NOTE

A **NOTE** provides additional information to help the operator achieve optimal instrument and assay performance.

Read manual label. This label indicates that you have to read the manual before using the ibidi pump. This label is positioned at the backside of the device.

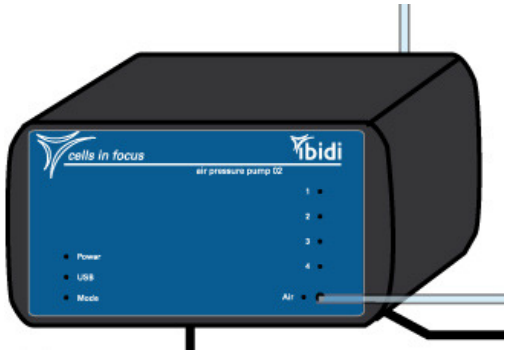
Warnings

Warning messages in the text are shaded orange.

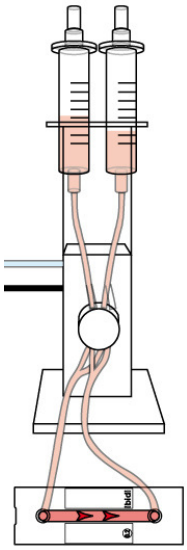


Identification label. This label is positioned at the backside / rear panel of the device.

Nomenclature



IBIDI PUMP



FLUIDIC UNIT

WARNING! Do only operate the ibidi pump with the delivered external power supply (Sinpro M/N:SPU26-106, 14V). Do only use the delivered cables and plugs. If not doing so you risk electric shock and fire.

WARNING! Do not operate the ibidi pump with substances and under conditions which do cause a risk of explosion, implosion or release of gases. Only operate the ibidi pump with aqueous solutions.

CAUTION Ensure that the external power supply is well accessible. The ibidi pump has to be installed in a way that it does not hinder the access to the external power supply.

CAUTION The weight of the ibidi pump is approx. 3 kg. If you move the instrument it poses a risk of personal injury or damage to the instrument.

CAUTION The manual opening of the ibidi pump is not allowed. Manual opening pose a risk of personal injury or damage to the instrument. Contact ibidi service personnel if you need to open the instrument.

CAUTION The ibidi pump can build up overpressure up to 100 mbar. Do not unplug fluidic connections during operation of the pump. Pressurized liquid can shoot out of the tubes and wet surrounding equipment. Leaking water can lead to a short circuit in the external power supply or nearby electrical equipment.

CAUTION Only ibidi staff is allowed for servicing and opening of the ibidi pump. Unplug the external power supply before servicing the instrument, unless otherwise noted. Connect the equipment only to the delivered external power supply (Sinpro M/N:SPU26-106, 14V). Do not use extension cords. Have an electrician immediately replace any damaged cords, plugs, or cables. Not doing so poses a risk of personal injury or damage to the instrument.

CAUTION Do not use the ibidi pump in the cold room.

CAUTION Unplug the external power supply of the ibidi pump, when the ibidi pump is not in use.

CAUTION Do not use ethanol or other types of organic solvents to clean the ibidi pump as they may remove the instrument paint.

CAUTION Use the ibidi pump only for cell experiments with aqueous solutions.

CAUTION Do not use the ibidi pump with hazardous substances or substances/materials which pose a risk of infections.

Regulatory Statement

In preparation

Specifications

Electrical Input:

External power supply: 100 V / 47 Hz - 240 V / 63 Hz (Sinpro M/N:SPU26-106)

ibidi Pump

Electrical input
Supply voltage: 14 V DC
Standby current: 60 mA
Max. current @max. air flow: 600 mA
Max. current with 4 fluidic units: 1000 mA

Environmental
Operating temperature 15 – 40°C (indoor use only)
Storage temperature -5 – 50°C
Humidity 80 % RH up to 31°C, 30 % RH up to 40°C
Operating altitude max. 2000 m

Size
Width 170 mm
Depth 230 mm
Height 90 mm
Weight 2.4 kg

Pressure
Pressure range 0 … 100 mbar
Recommended range 5 … 95 mbar

Fluidic Unit

Electrical input supplied by pump
Switching current 110 mA (1 fluidic unit), 200 mA (2-4 fluidic units)
Hold current (state 2) 120 mA
Typical current @ 20 mbar 130 mA (state 1) / 250 mA (state 2)

Environmental
Operating temperature 15 – 45°C (indoor use only)
Storage temperature -5 – 50°C
Humidity up to 100% (non-condensing)
Operating altitude max. 2000 m

Size
Width 85 mm
Depth 135 mm
Height 250 mm
Weight 1.1 kg

Connections

All ingoing and outgoing connections can be found at the rear and the front panel of the ibidi pump.

Name	Function
Air front port	Connection for the pressurized air for pumping liquids
USB	Socket for connecting the USB cable to the PC/Notebook. To setup a computer communication to the ibidi pump, the USB cable must be connected.
Power connector	The connector to the external power supply (Sinpro M/N:SPU26-106, 14V)
Air rear port	Connection for the pressurized air to incubator (positive pressue)
Electric cable plugs	Electric connection of the to the Fluidic Units

Preface

This manual is your guide for using the ibidi Pump for flow chamber experiments with the ibidi channel slides. It instructs first-time users on how to use the instrument, and serves as a reference for experienced users.

Before using the ibidi pump, please read this instruction carefully, and make sure that the contents are fully understood. This manual should be easily accessible to the operator at all times during instrument operation. When not using the instrument, keep this manual in a safe place. If this manual becomes lost, order a replacement from ibidi GmbH.

Notices

1. ibidi shall not be held liable, either directly or indirectly, for any consequential damage incurred as a result of product use.
2. Prohibitions on the use of ibidi software
    - Copying software for other than backup
    - Transfer or licensing of the right to use software to a third party
    - Disclosure of confidential information regarding software
    - Modification of software
    - Use of software on multiple workstations, network terminals, or by other methods
3. The contents of this manual are subject to change without notice for product improvement.
4. This manual is considered complete and accurate at publication.
5. This manual does not guarantee the validity of any patent rights or other rights.
6. If an ibidi software program has failed causing an error or improper operation, this may be caused by a conflict from another program operating on the notebook (PC). In this case, take corrective action by uninstalling the conflicting product(s).
7. ibidi is a registered trademark of ibidi GmbH in Germany and other countries.

Limited Warranty

Products sold by ibidi, unless otherwise specified, are warranted for a period of one year from the date of shipment to be free of defects in materials and workmanship. If any defects in the product are found during this warranty period, ibidi will repair or replace the defective part(s) or product free of charge.

THIS WARRANTY DOES NOT APPLY TO DEFECTS RESULTING FROM THE FOLLOWING:

1. IMPROPER OR INADEQUATE INSTALLATION.
2. IMPROPER OR INADEQUATE OPERATION, MAINTENANCE, ADJUSTMENT OR CALIBRATION.
3. UNAUTHORIZED MODIFICATION OR MISUSE.
4. USE OF UNAUTHORIZED TUBES OR FLUIDC CONNECTORS
5. USE OF CONSUMABLES, DISPOSABLES AND PARTS NOT SUPPLIED BY AN AUTHORIZED IBIDI DISTRIBUTOR.
6. CORROSION DUE TO THE USE OF IMPROPER SOLVENTS, SAMPLES, OR DUE TO SURROUNDING GASES.
7. ACCIDENTS BEYOND IBIDI'S CONTROL, INCLUDING NATURAL DISASTERS.

This warranty does not cover consumables like channels slides, tubes, fluidic connectors and the like.

The warranty for all parts supplied and repairs provided under this warranty expires on the warranty expiration date of the original product. For inquiries concerning repair service, contact ibidi after confirming the model name and serial number of your ibidi Pump.

Installation Requirements

To ensure operation safety, observe the following conditions:

1. Do only operate the ibidi Pump with the delivered external power supply (Sinpro M/N:SPU26-106, 14V).
2. Ensure that the external power supply is well accessible. The ibidi pump has to be installed in a way that it does not hinder the access to the external power supply.
3. Only operate the ibidi Pump with the delivered PC (Notebook).
4. Only operate the ibidi Pump with original ibidi channel slides.
5. Operate the ibidi Pump in a temperature range of 15 – 40°C (Fluidic Unit 15 – 45°C).
6. Operate the ibidi Pump in a humidity range of 0 – 80 % RH, up to 31°C or 30% RH up to 40°C. The Fluidic Unit can be operated in 100% humidity (non-condensing).
7. Operate the ibidi Pump at altitudes of less than 2000 m.
8. Do not operate the ibidi Pump under conditions which pose a risk of explosion, implosion or the risk of the release of gases.
9. Avoid strong magnetic fields and sources of high frequency. The ibidi Pump may not function properly when near a strong magnetic field or high frequency source.
10. Avoid vibrations from vacuum pumps, centrifuges, electric motors, processing equipment and machine tools.
11. Avoid dust and corrosive gas. Do not install the ibidi Pump where it may by exposed to dust, especially in locations exposed to outside air or ventilation outlets.
12. For cleaning the ibidi Pump, only use water.
13. Do not install the ibidi Pump in a location where it may be exposed to direct sunlight.
14. Install the ibidi Pump in a horizontal and stable position. (This includes a table, bench or desk upon which the instrument is installed).
15. Install the ibidi Pump in a location that allows easy access for maintenance.

NOTE: The above conditions do not guarantee optimal performance of the ibidi Pump.

Installation and Connecting Cables

Connecting the ibidi pump to the external power supply

WARNING! Do only operate the ibidi pump with the delivered external power supply (Sinpro M/N:SPU26-106, 14V). Do only use the delivered cables and plugs. If not doing so you risk electric shock and fire.

Connect the external power supply to the electrical socket. Then connect the external power supply to the ibidi Pump.

CAUTION Ensure that the external power supply is well accessible. The pump has to be installed in a way that it does not hinder the access to the external power supply.

Connect the pump to the Notebook by using the delivered network cable.

Maintenance and Operation

Pay attention to the instrument operating environment and always keep it clean so that the instrument can be used in a stabilized condition over a long period. Do not place anything heavy on the instrument.

Cleaning the ibidi Pump

Unplug the ibidi Pump and remove the external power supply from the electrical socket. Only use dry cloth or cloth wetted with water for cleaning of the instrument.

CAUTION Do not use ethanol or other types of organic solvents to clean the ibidi Pump as they may remove the instrument paint.

Transporting the ibidi pump

Unplug the ibidi Pump and remove the external power supply from the electrical socket. Carry the instrument carefully and avoid mechanical shocks.

CAUTION The weight of the ibidi Pump is approx. 3 kg. Dropping the instrument while moving it, may cause personal injury or damage.

Functional Disorder

In case of a functional disorder unplug the ibidi Pump and wait for five minutes, then connect the instrument again. If there is still a functional disorder unplug the device, remove the external power supply from the electrical socket and contact the ibidi service.

Repairing the ibidi pump

Do not try to repair the instrument by yourself. Do contact the ibidi service for repairing the instrument.

CAUTION The manual opening of the ibidi Pump is not allowed. Manual opening pose a risk of personal injury or damage to the instrument. Contact ibidi service personnel if you need to open the instrument.

Waste Treatment

Waste treatment is your own responsibility. You must hand it to a company specialized in waste recovery. Do not dispose the ibidi Pump in a litter bin or at a public waste disposable site. For detailed information please contact the ibidi service.

1 Working principle

The ibidi Pump and the ibidi Fluidic Unit are designed to work together to create a flow – unidirectional, oscillating, or pulsating – of medium within the channel of the ibidi slides. The working principle of the pump is explained in the following chart which points out the correlation between air pressure, flow rate and shear stress.

1. The pump generates a constant pressure (mbar) which pumps the liquid from one reservoir to the other and back.

2. The applied pressure results in a certain flow rate (ml/min). This rate is dependent on the pressure input, the viscosity of the medium, and the flow resistance of the perfusion system (tubing and slide).

3. The specific flow rate (ml/min) causes a wall shear stress (dyn/cm²) to which the cells are exposed.

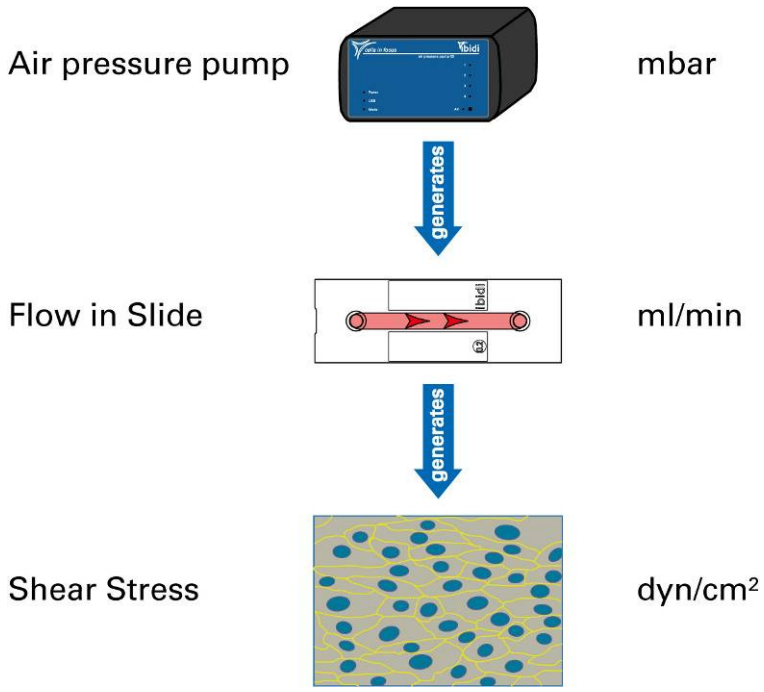


Figure 1: Principle of flow and shear stress generation. For recalibration and viscosity influences, please see section 5.1. Keep in mind that cells can only sense wall shear stress and medium conditions but do not sense applied pressure or flow rate.

In order to have minimal consumption of medium, the liquid is pumped back and forth between two media reservoirs of the Fluidic Unit. The setup is shown in Figure 2 and Figure 3.

The figures show that to create an unidirectional flow, two four way valves, labeled (V1) and (V2), are integrated in the Fluidic Unit which are switched synchronously via the pump. The pump itself is controlled by the PumpControl software on the computer.

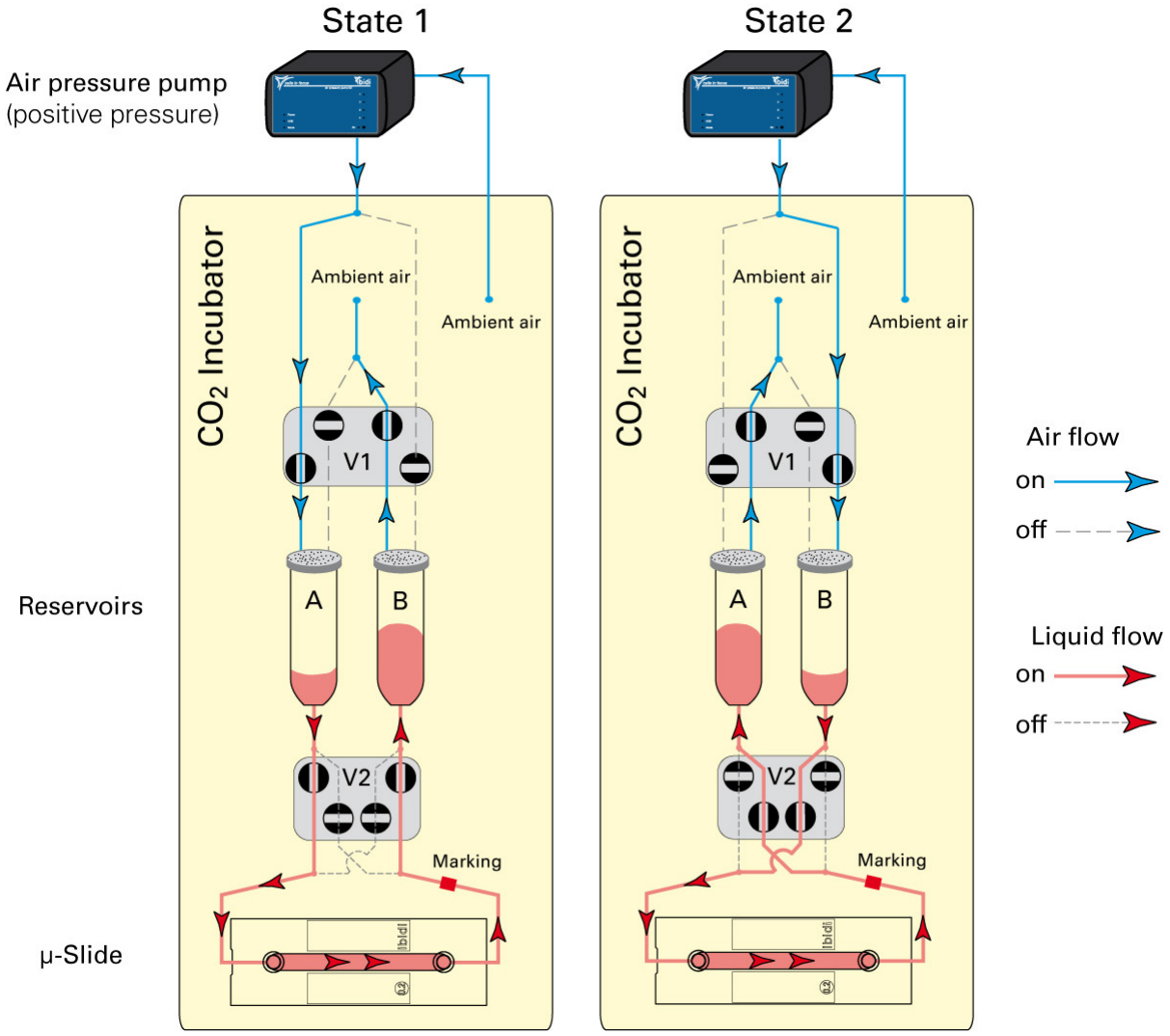


Figure 2: Working principle of the ibidi Pump system using positive pressure

The Fluidic Unit is switched between two states whereas the pump is only supplying a constant positive or negative air pressure. The use of negative or positive air pressure is described in more detail in section 5.3. When applying pressure, an unidirectional and constant flow of medium is created within the channel of the ibidi µ-Slides. In State 1, the valve (V1) is set such that the air is pushed from the pump into reservoir (A). In this position valve (V1) allows air from reservoir (B) to flow out. This creates a flow from reservoir (A) to reservoir (B). In order to have a controlled flow across the slide, valve (V2) is needed. Valve (V2) combined with the fourfold branching of the silicone tubes (called Perfusion Set)

function as a fluidic rectifier. The valve (V2) is always in a state that one of the two branches of the tube coming from the reservoirs is open and the other is pinched off. In State 1, the flow is running from reservoir (A) through the tube branch which is open to the left unmarked tube connecting the µ-Slide as indicated Figure 2. The branch, which is connected to the marked side of the slide, is pinched off. This way the medium is pressed through the µ-Slide from left to right. On the right side the tube branching and the pinch valve (V2) are set such that the open tube is connected to reservoir (B).

In State 2 of Figure 2, valve (V1) is set such that the pressure, which is remaining constant, is directed to the reservoir (B). The liquid is exiting reservoir (B) into reservoir (A). The pinch valve (V2) is now in the opposite state and pinching off the first branch leaving the second open. Again, this results in the medium flowing from the unmarked to the marked tube from left to right. As valve (V2) is in the opposite state, the liquid can now flow into the reservoir (A). This way we create unidirectional flow.

Switching between State 1 and State 2 generates a continuous flow of medium through the slide. Sterility is maintained by the use of air filters within the pump and at the point between the Fluidic Unit and the ambient atmosphere.

Please keep in mind that it is beneficial to supply CO_2 rich air to your medium. Therefore, the rear pump port should be connected to the incubator.

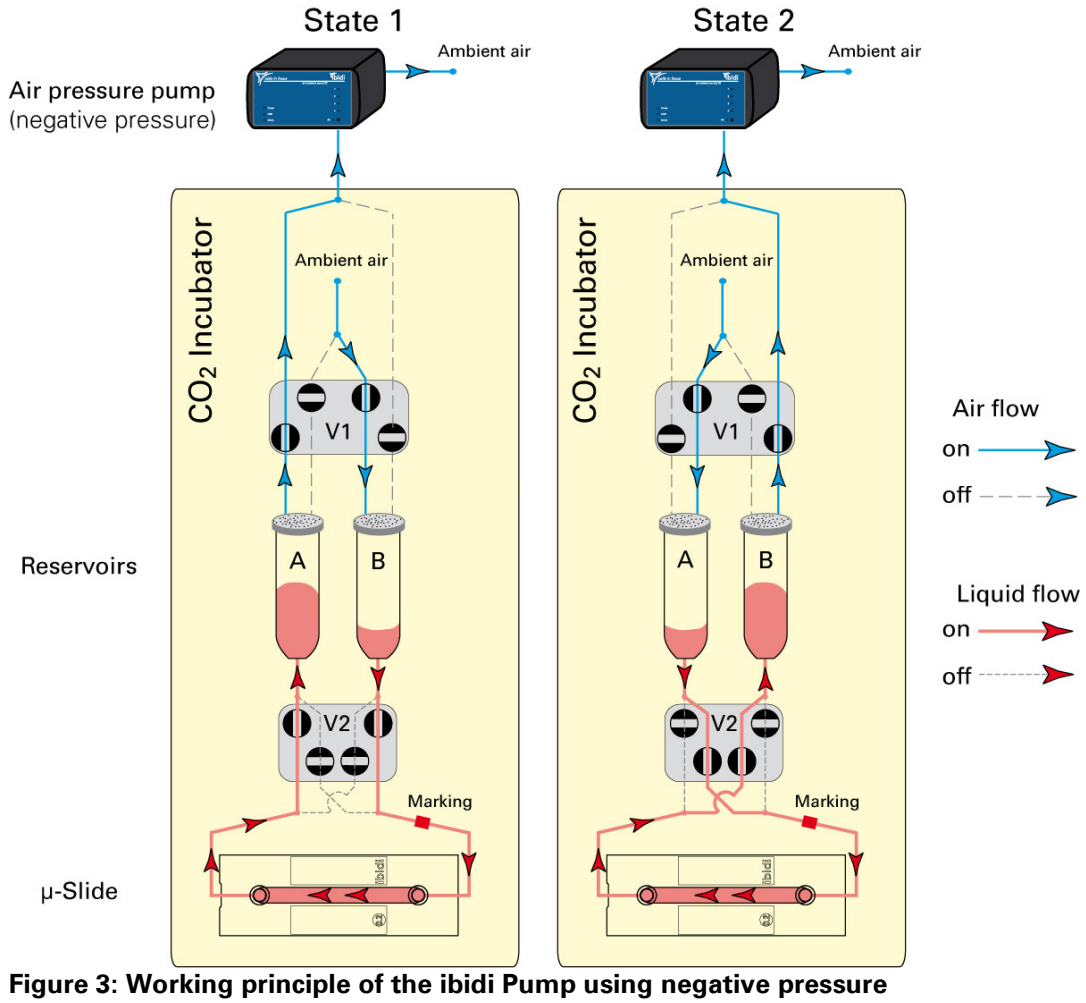


Figure 3: Working principle of the ibidi Pump using negative pressure

If the system is run with negative pressure, the pump system principle remains the same. However, the flow reverses the direction as shown in Figure 3. The use of permanent negative pressure is not recommended.

2 Equipment

Before you start setting up your experiment, please make sure that you have all items listed below

2.1 A basic setup includes the following parts

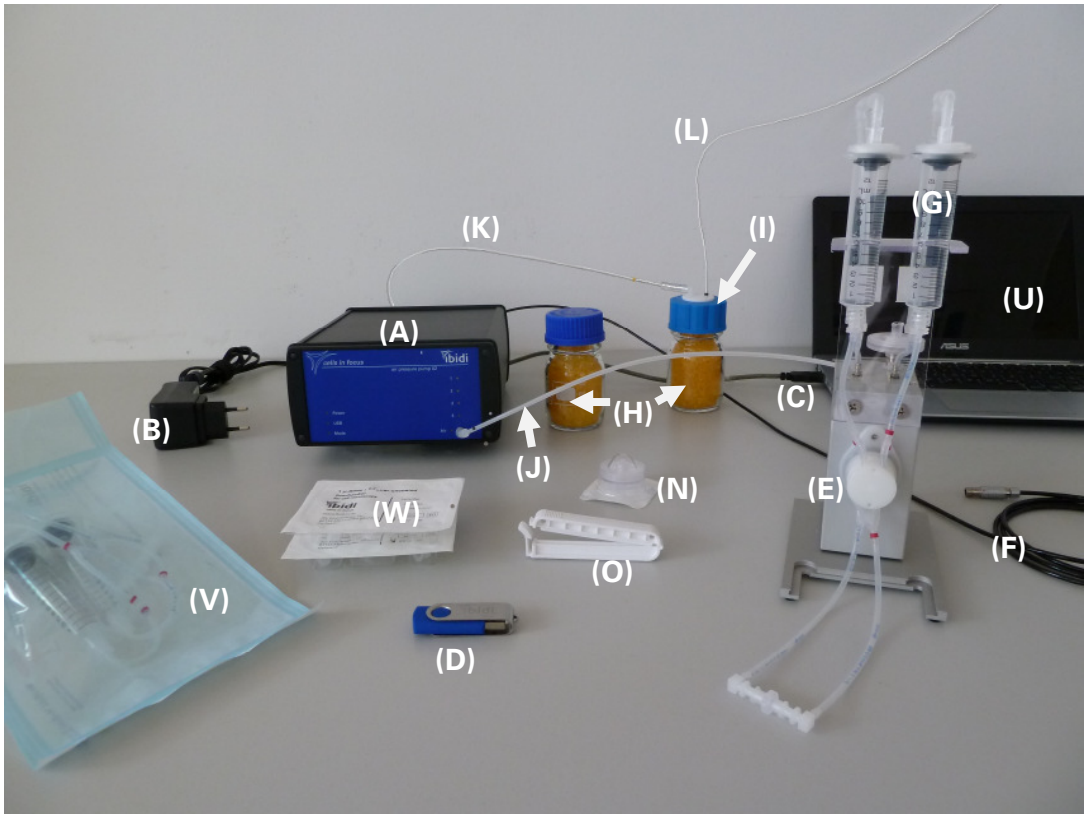


Figure 4: Basic equipment set with one ibidi Pump and one Fluidic Unit

(A) 1 ibidi Pump
(B) 1 External Power Supply (country specific) for the ibidi Pump
(C) 1 USB cable to connect the pump to your PC
(D) 1 USB thumb drive with the latest PumpControl software
(E) 1 to 4 Fluidic Unit(s)
(F) 1 to 4 Electric cable(s) to connect Fluidic Unit and Pump (length 2 m)
(G) 1 to 4 Non-sterile Perfusion Set(s)
(H) 2 Drying bottles filled with orange Silica beads
(I) 1 Connection cap for the drying bottle
(J) 1 Air pressure tubing (2 m) to connect the pump to the Fluidic Unit (positive pressure)
(K) 1 Short yellow marked air pressure tube (0.6 m); rigid air tube to connect the pump to the drying bottle
(L) 1 Long black marked air pressure tube (2.1 m); rigid air tube to connect the drying bottle to the inside of the incubator.
(M) 1 Filter for the drying bottle (inside the bottle, not shown)
(N) 1 to 4 Sterile replacement filter(s) for the Fluidic Unit(s)
(O) 1 to 4 Hose clip(s)

(P) Air Pressure Splitter Set for 2 Fluidic Units (not shown)
(Q) Air Pressure Splitter Set for 3 Fluidic Units (not shown)
(R) Air Pressure Splitter Set for 4 Fluidic Units (not shown)
(S) Oscillating Flow Kit for 2 Fluidic Units (not shown)
(T) Oscillating Flow Kit for 4 Fluidic Units (not shown)

Optional:

(U) Notebook with preinstalled PumpControl software
(V) Sterile Perfusion Sets
(W) Sterile µ-Slides Luer Type

2.2 Product description

A brief overview is provided in this section, including the nomenclature for later descriptions in this document.

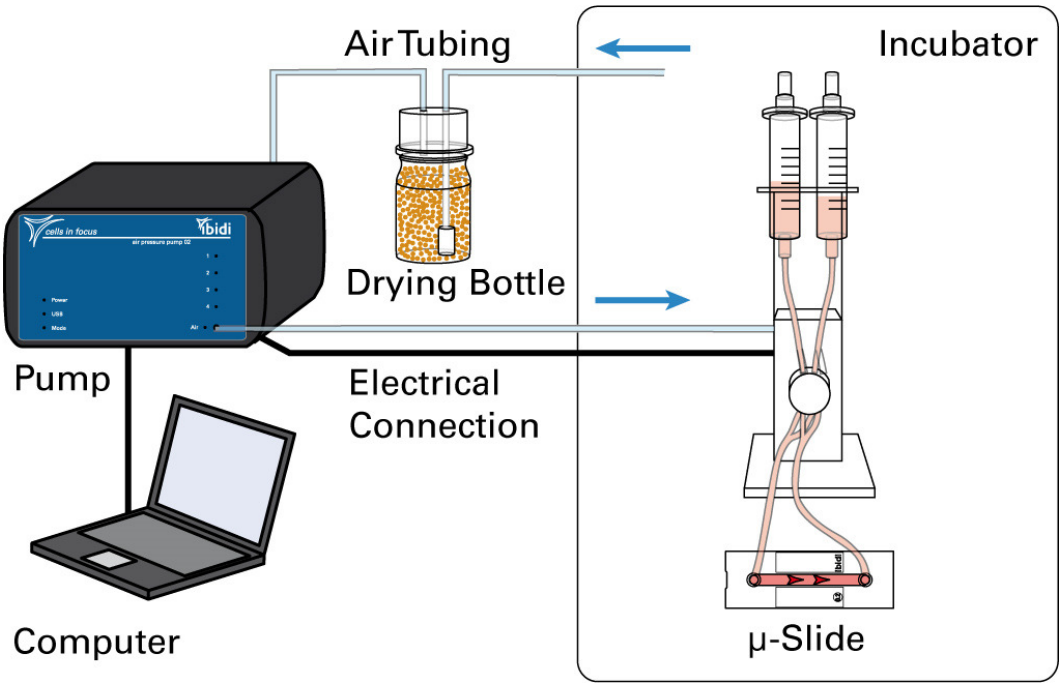
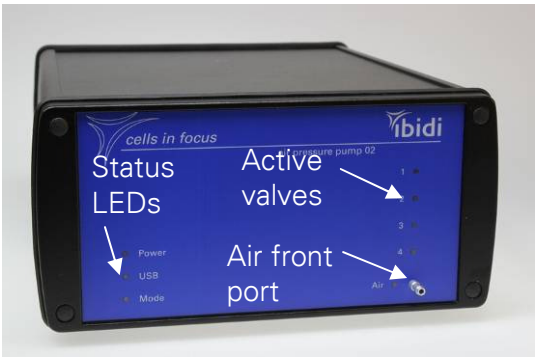


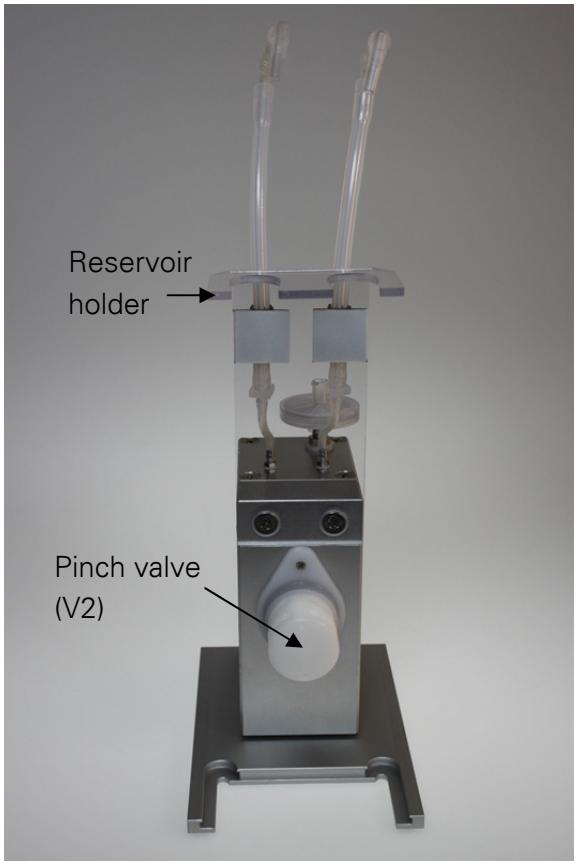
Figure 5: Schematic setup with positive air pressure

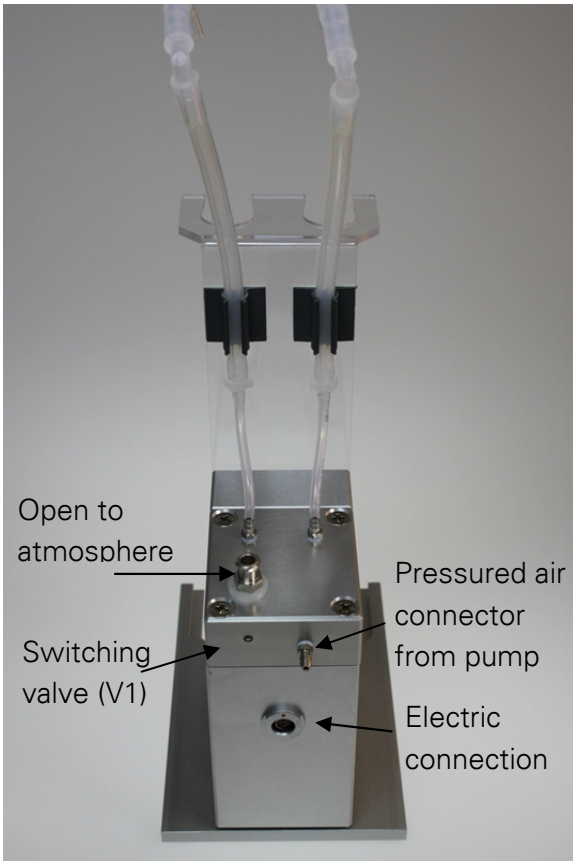


2.2.1 ibidi Pump

The ibidi Pump has the capability to create an air pressure of up to 100 mbar. It is recommended to apply 5 to 95 mbar, which is the most precise working range. The pump also has the capability of setting the air flow direction. With the use of positive pressure it will take in air from the rear port. With the use of negative pressure the pump will expel air from the rear port with the desired pressure. The use of positive or negative pressure or air flow is further described in section 5.3. We recommend using positive pressure for experiments. In addition to the generation of air pressure, the ibidi Pump controls the switching times of the Fluidic Unit(s). Up to four Fluidic Units can be controlled with the pump simultaneously. The pump needs a supply voltage of 14 V DC. The communication to the PC is achieved via an USB interface.

2.2.2 Fluidic Unit





The Fluidic Unit as shown in the pictures above has two active components which are the two switching valves (V1) and (V2). There are two connectors in the rear of the Fluidic Unit, one electric connection for the valve control and another for the pressurized air. Both connect the Fluidic Unit with the ibidi Pump.

2.2.3 Perfusion Sets

The disposable Perfusion Sets are delivered in a sterile package, which is gas permeable. The tubing is color-coded for a simple identification.

The Perfusion Sets are specifically designed to be used with the Fluidic Unit. Nonetheless, the Luer adapters can be connected to any suitable flow chamber with Luer adapters.

Figure 6: Sterile packaged Perfusion Set

Parts of the Perfusion Set: (as shown in Figure 7)

(a) Sterile air filters, modified (0.2 µm, Teflon)

(b) Syringe reservoirs

(c) Silicone tubing

(d) Branched tubes for insertion in pinch valves

(e) Luer adapters to the slide

(f) Middle connector for setup without slide

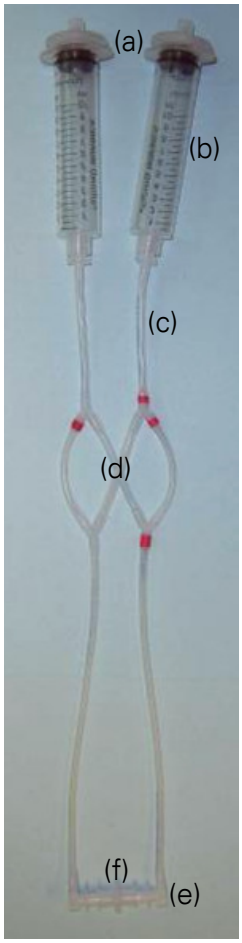


Figure 7: Description of Perfusion Set parts

Sterilization and cleaning:

All parts of the Perfusion Sets can be cleaned and sterilized by different techniques for reuse. Syringes, filters and slides are **not** autoclavable. Please remove them before autoclaving and if necessary replace them with new parts. Replacement reservoirs (Reservoir sets) can also be purchased (#10971 for 10 ml reservoirs, #10972 for 2 ml reservoirs). Best results are achieved with new Perfusion Sets.

	Autoclavable	Ethanol (70 %)	Water
Filters	no	yes	yes
Syringe reservoirs	no	yes	yes
Tubing	yes	yes	yes
PP adapters	yes	yes	yes
µ-Slide	no	yes	yes

Selection of a Perfusion Set

For a pump demo

Please follow all steps in section 4 for a successful first demo run. Selection of Perfusion Set and µ-Slide for the unsterile testing is already included in the demo packaging.

For an experiment

In order to set up a successful experiment, you need to select a suitable Perfusion Set and choose the right µ-Slide for your purpose. Please read this selection carefully. In addition to shear stress, more parameters need to be considered, such as working volume, dead volume, and tube length.

For the following sections, a red Perfusion Set (#10962) with an inner diameter of 1.6 mm and a µ-Slide I Luer is assumed. The installation of the equipment is independent of the combination you choose.

An overview of all available flow rates and shear stress can be found in the following table with guideline values valid for water at 20°C.

MIN values are based on recommended minimal working pressure of 5 mbar. MAX values are based on recommended maximal working pressure of 95 mbar.

Table 1 Perfusion set and µ-Slide selection

	Perfusion Set blue (#10961) Inner diameter: 0.8 mm Total working volume: 11.3 ml		Tube length: 15 cm Dead volume of tubing: 0.5 ml			
	Growth area [cm²]	Channel volume [µl]	Flow rate [ml/min]		Shear stress [dyn/cm²]	
			MIN	MAX	MIN	MAX
µ-Slide I 0.2 Luer	2.5	50	0.83	11.53	4.26	59.14
µ-Slide I 0.4 Luer	2.5	100	1.12	15.30	1.47	20.14
µ-Slide I 0.6 Luer	2.5	150	1.13	15.58	0.68	9.36
µ-Slide I 0.8 Luer	2.5	200	1.15	15.91	0.40	5.53
µ-Slide VI 0.4	0.6	30	1.12	15.25	1.97	26.86
µ-Slide y-shaped	2.8	110	1.08	14.50	2.46	32.98
without slide	-	(75)	1.18	16.51	-	-

	Perfusion Set red (#10962) Inner diameter: 1.6 mm Total working volume: 12.3 ml		Tube length: 15 cm Dead volume of tubing: 1.5 ml			
	Growth area [cm²]	Channel volume [µl]	Flow rate [ml/min]		Shear stress [dyn/cm²]	
			MIN	MAX	MIN	MAX
µ-Slide I 0.2 Luer	2.5	50	1.28	19.11	6.57	98.02
µ-Slide I 0.4 Luer	2.5	100	2.65	33.17	3.49	43.66
µ-Slide I 0.6 Luer	2.5	150	2.74	34.98	1.65	21.02
µ-Slide I 0.8 Luer	2.5	200	2.75	35.14	0.96	12.21
µ-Slide VI 0.4	0.6	30	2.76	35.33	4.86	62.24
µ-Slide y-shaped	2.8	110	2.59	32.12	5.89	73.05
without slide	-	(75)	2.79	35.86	-	-

	Perfusion Set white (#10963) Inner diameter: 0.8 mm Total working volume: 11.7 ml		Tube length: 50 cm Dead volume of tubing: 0.9 ml			
	Growth area [cm²]	Channel volume [µl]	Flow rate [ml/min]		Shear stress [dyn/cm²]	
			MIN	MAX	MIN	MAX
µ-Slide I 0.2 Luer	2.5	50	0.42	7.15	2.15	36.67
µ-Slide I 0.4 Luer	2.5	100	0.57	8.75	0.75	11.52
µ-Slide I 0.6 Luer	2.5	150	0.61	9.40	0.37	5.65
µ-Slide I 0.8 Luer	2.5	200	0.64	10.11	0.22	3.51
µ-Slide VI 0.4	0.6	30	0.63	9.77	1.11	17.21
µ-Slide y-shaped	2.8	110	0.58	8.94	1.32	20.33
without slide	-	(75)	0.61	9.43	-	-

	Perfusion Set yellow/green (#10964)					
	Inner diameter: 1.6 mm			Tube length: 50 cm		
	Total working volume: 13.6 ml			Dead volume of tubing: 2.8 ml		
	Growth area [cm²]	Channel volume [µl]	Flow rate [ml/min]		Shear stress [dyn/cm²]	
			MIN	MAX	MIN	MAX
µ-Slide I 0.2 Luer	2.5	50	1.03	17.00	5.28	87.20
µ-Slide I 0.4 Luer	2.5	100	1.91	25.95	2.51	34.16
µ-Slide I 0.6 Luer	2.5	150	1.98	27.44	1.19	16.49
µ-Slide I 0.8 Luer	2.5	200	2.13	30.25	0.47	10.51
µ-Slide VI 0.4	0.6	30	2.06	28.95	3.63	51.00
µ-Slide y-shaped	2.8	110	1.94	26.60	4.41	60.50
without slide	-	(75)	2.44	36.02	-	-

	Perfusion Set yellow (#10965)					
	Inner diameter: 0.5 mm			Tube length: 15 cm		
	Total working volume: 2.5 ml			Dead volume of tubing: 0.5 ml		
	Growth area [cm²]	Channel volume [µl]	Flow rate [ml/min]		Shear stress [dyn/cm²]	
			MIN	MAX	MIN	MAX
µ-Slide I 0.1 Luer	2.5	25	0.07	1.35	1.42	27.34
µ-Slide III 0.1	0.43	4.5	0.06	1.08	6.40	115.27
µ-Slide VI 0.1	0.17	1.7	0.08	1.44	8.54	153.69

	Perfusion Set black (#10966)					
	Inner diameter: 0.5 mm			Tube length: 50 cm		
	Total working volume: 2.7 ml			Dead volume of tubing: 0.7 ml		
	Growth area [cm²]	Channel volume [µl]	Flow rate [ml/min]		Shear stress [dyn/cm²]	
			MIN	MAX	MIN	MAX
µ-Slide I 0.1 Luer	2.5	25	0.03	0.61	0.61	12.36
µ-Slide III 0.1	0.43	4.5	0.03	0.52	3.20	55.50
µ-Slide VI 0.1	0.17	1.7	0.03	0.62	3.20	66.17

3 Installation

3.1 Basic Installation without cells

This section will explain how to connect all the components to a basic setup. For a basic setup, the following equipment is needed:

- ibidi Pump including power connector and air tubing
- 1 Fluidic Unit including electric cable, Perfusion Set, and µ-Slide
- 1 Drying bottle with air tubes and filter
- 1 Computer

3.1.1 Connecting the ibidi Pump to your PC

1) Power up the ibidi Pump with the power supply which is included in the delivery. To verify that power is connected, check the blue 'Power' LED status on the front panel of the pump. (Figure 8.)
2) Connect the pump to a PC using the included USB cable. The computer will automatically recognize the new hardware. In order to enable the communication between the pump and the PC there are two drivers needed. These drives are automatically installed as part of the PumpControl software. After installation, the blue 'USB' LED will be illuminated.

Figure 8: Status LEDs of ibidi Pump when connected to the PC

If you encounter any installation problems, refer to the Trouble Shooting pages in section 6.1.

3.1.2 Installing the PumpControl software

In order to use the ibidi Pump the ibidi PumpControl software has to be installed. The installation software is included in the shipment. If the setup does not auto-run, install the program manually by executing the setup program from the installation drive. The installation includes both the PumpControl program and the runtime engine from 'National Instruments'. Both programs are installed automatically by executing 'setup.exe'.

When the installation is finished and the PumpControl program is running, you will be able to program and control the ibidi Pump. For detailed description of the PumpControl functionalities, please refer to the PumpControl manual included with the shipment or on http://www.ibidi.com.

If you cannot establish the communication refer to the Trouble Shooting list in section 6.2.

3.1.3 Mounting a new Perfusion Set on the Fluidic Unit

The Fluidic Unit is the device which generates the unidirectional flow through valve switching and serves as a reservoir holder.

Please note: Only unfilled Perfusion Sets should be mounted. In the following pictures the Perfusion Set has been already filled to improve the visibility.

A purchased Fluidic Unit will come with a non-sterile Perfusion Set for demonstration purposes. Please make sure the tubing is mounted correctly when inserting into the valve. This is crucial for proper valve switching and ensures the correct flow direction.

Carefully follow the instructions below for the correct installation of the Perfusion Set, which is also illustrated in Figure 9.

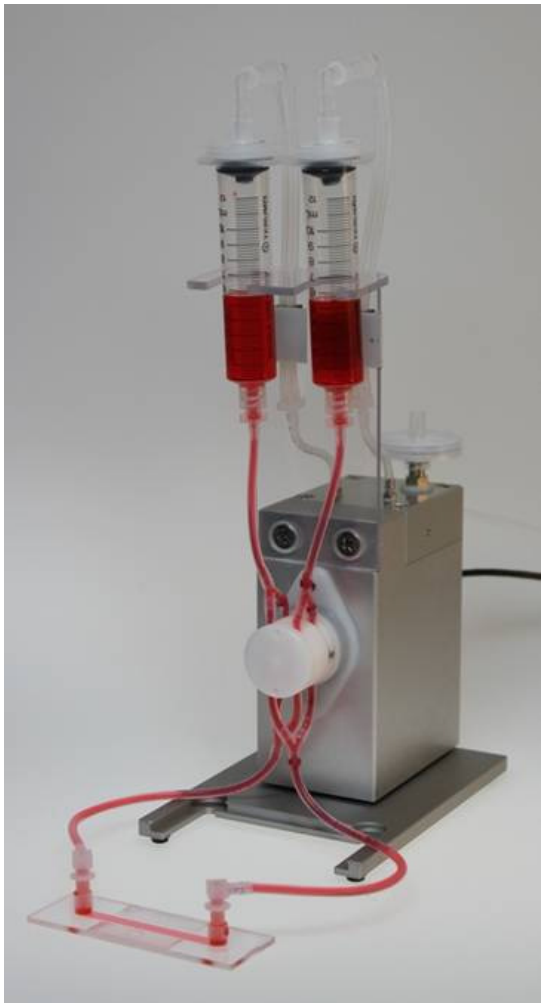


Figure 9: Correctly mounted Perfusion Set

1) Check the connection between the reservoirs and tubing by screwing the adapters tightly into the reservoirs. Please also check the tight fit of the Luer adapters in the middle connector.
2) Insert the reservoirs into the holder. The reservoir connected to the tubing with the extra red marking (reservoir B) has to be inserted on the right side of the valve (front view). The mounting is easier if the reservoirs are squeezed. (Figure 10).

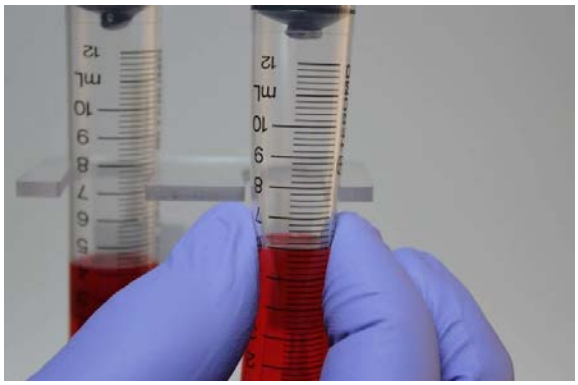


Figure 10: Squeeze reservoirs for insertion into holder

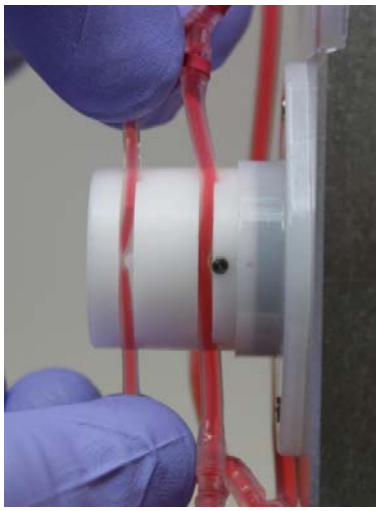


Figure 11: Mounting order of Perfusion Set tubes. Start with the rear right side.

3) Begin with the right side slots of the valve. Insert the two branches of the tubing coming from reservoir B into these slots.
   - Insert the red marked branched tubing into the rear slot.
   - Insert the unmarked branched tubing into the front slot.
   - The setup at this point is shown in Figure 11
4) For the slots on the left side, follow the same procedure:
   - Insert the red marked branched tubing into the rear slot.
   - Insert the unmarked branched tubing into the front slot.
   - The complete correct setup is shown in Figure 13.

It is recommended to stretch the tubing and to move them up and down for easier mounting. Stretch the tubes only between the y-connectors to ensure that you do not disconnect the tubes. The stretching of the tubing is illustrated in Figure 12.

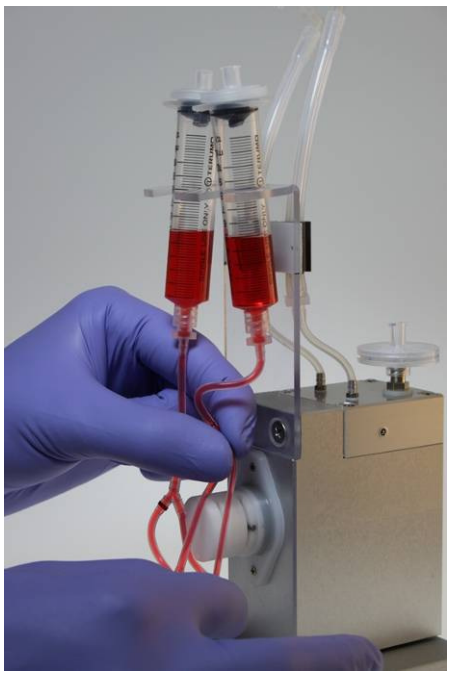


Figure 12: Stretched tubes ease insertion

Verify that the Perfusion Set is mounted correctly. First, check the position of the tubes in the openings of the pinch valve. There is a pinch bolt which is pinching off the front or the rear tubes. Please make sure that the tubes are inserted such that this bolt is pinching off the full diameter of the tubes as shown in Figure 14.

For every mounted Perfusion Set please perform the test procedure described in section 0.

Figure 13: Correct order of Perfusion Set tubing

Please keep in mind that most problems are caused by not correctly mounted tubing.

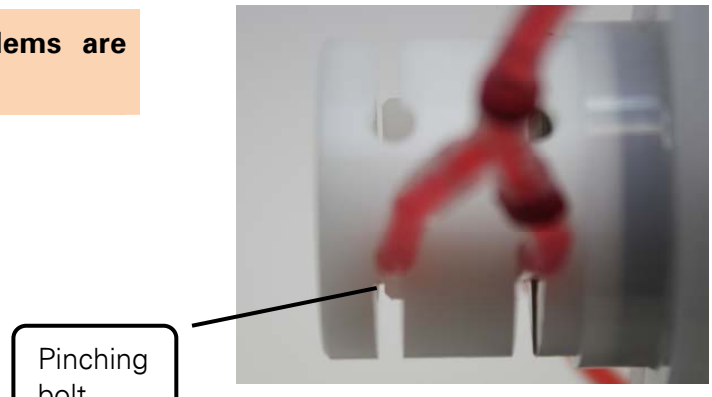


Figure 14: Correct position of tube in pinching valve.

3.1.4 Filling the perfusion Set with medium

To fill the reservoirs with liquid or medium, perform the following steps:

1) Connect the air pressure tubes to the sterile filters on top of the syringes (Figure 15).

2) Make sure that the Elbow Luer connectors are connected by the middle piece and the flow tubing is securely attached to the reservoirs.

3) Pull off one filter from the syringe as shown in Figure 16.

4) Fill in the required amount of medium depending on the Perfusion Set used (see Table 1 on page 23). For the red Perfusion Set (#10962), use 12.3 ml of your medium and distribute it evenly into both reservoirs.

5) Remove the air bubbles from the Perfusion Set either by running an automated cycle or by simply using the pre-defined protocol in the PumpControl software as described in section 3.1.7.

Figure 15: Filter connection

Figure 16: Reservoir filling

Figure 17: Backside of connected Fluidic Unit

3.1.5 Connecting the Fluidic Unit to the Pump

Use the electric cable to connect the Fluidic Unit with the pump. This cable enables the switching of the valves of the Fluidic Unit. The plug for the Fluidic Unit is marked with a red dot which aligns with the red dot on the Fluidic Unit. The opposite side of the electric cable can be placed in any of the ports on the back side of the pump as the pump will automatically recognize which port is connected.

If using positive pressure, connect the Fluidic Unit directly to the pump. To ensure the correct CO_2 amount of the pressured air suck in the athmosphere from the incubator. For this, connect the rear port of the pump to the drying bottle and put the second tubing into the incubator (see figure 5).

If using negative pressure, connect the Fluidic Unit via the drying bottle to the pump.

3.1.6 Drying bottle

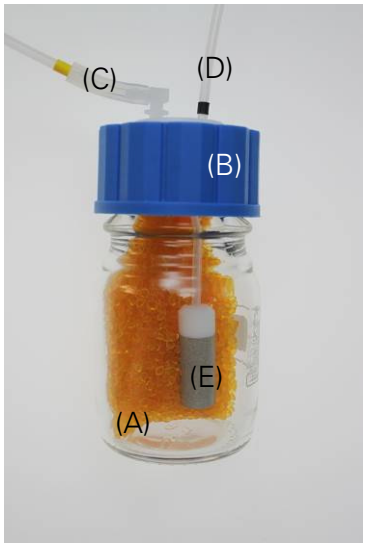


Figure 18: Drying bottle

Setting up the drying bottle

The following parts are needed to correctly set up the drying bottle, as shown in Figure 18:

(A) 1 Glass bottle with orange Silica beads
(B) 1 Connection cap for the drying bottle; with 2 openings, one of them plugged with an Elbow Luer connector
(C) 1 Short yellow marked air pressure tube (0.6 m) for connection to the pump
(D) 1 Long black marked air pressure tube (2.1 m) for connection to the inside of the incubator
(E) 1 Filter for the drying bottle
(F) 1 Additional air pressure tubing (2 m) for positive pressure setup (not shown, see below)

Assembly of the drying bottle:

1) Connect the yellow marked tube to the Elbow Luer connector on the bottle cap.
2) Pass approximately 6 cm of the black marked tube through the remaining opening of the cap until the marker reaches the cap.
3) Slide the filter on the end of the black marked tube.
4) Remove the cap of the glass bottle with the Silica beads and replace it with the cap prepared in steps 1 – 3.

Silica beads have an orange indicator that turns white when saturated with moisture. Silica beads are recoverable when saturated. Please refer to section 7.1 for further instructions.

Connection of the drying bottle to the pump and Fluidic Unit:

The drying bottle is required to protect the pump from the incoming humidity from the incubator. Thus, it has to be placed in the air tubing between incubator and pump.
There are two possibilities to apply the pressure: negative pressure, which means taking air into the pump from the front port and out the rear, or positive pressure, which means taking in air from the rear port and out the front. For several reasons it is recommended to apply a positive pressure. For details please see section 5.3. The setup is different in both cases.

Positive pressure: The air is blown out by the pump into the Fluidic Unit. In this setup the pump is taking air in from the rear port and pumping it out through the front port. To assure that the gas mixture contains enough CO_2 it is crucial to use the air from inside the incubator into the rear port of the pump. However, as water vapor is also transported, the drying bottle has to be integrated there.

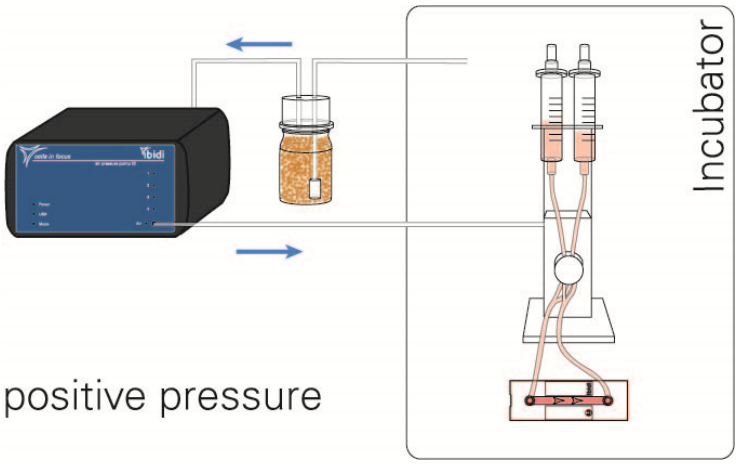


Figure 19: Setup for possitive air pressure

1) Place a sufficient length of the black marked tubing (D) inside the incubator.
2) Connect the yellow marked tubing (C) to the back port of the pump (see section 0).
3) Connect the 2 m tubing (F) to the front port of the pump and to the Fluidic Unit.

Negative pressure: The air is taken in via the Fluidic Unit from inside the incubator into the pump. As during normal operation the Fluidic Unit is placed in an incubator.

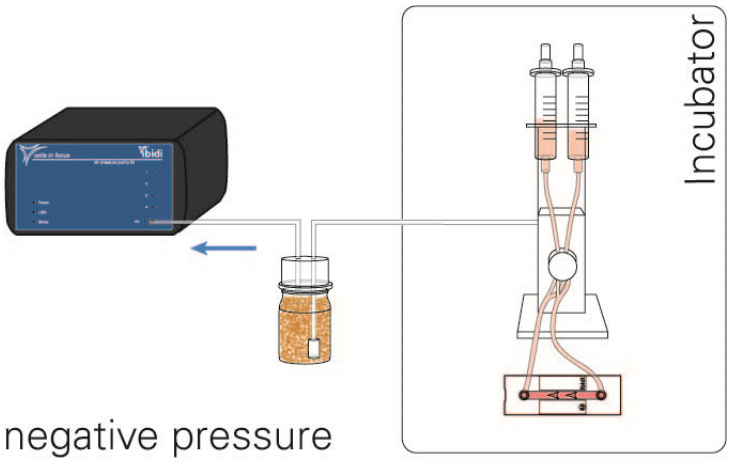


Figure 20: Setup for negative air pressure

1) Connect the black marked tubing (D) to the Fluidic Unit (inside the incubator).
2) Connect the yellow marked tubing (C) to the pump.

3.1.7 Remove air bubbles from the Perfusion Set

To remove the air bubbles, start the pump with the software. For a detailed description of the software please refer to the software manual.

1) Equilibrate the liquid levels of the two reservoirs by applying low pressure (~20 mbar) to the Fluidic Unit using the manual control panel in the PumpControl software.

Control the flow direction in the reservoirs by switching the valves. When the liquid levels are equilibrated, switch of the pressure.

2) Set an automatic cycle with a fast flow rate and let the cycle run for at least 5 minutes. After 5 minutes, check the flow tubing to confirm that all the air bubbles have been removed.

3) Once the tubes are free of bubbles the setup is ready for the connection with the slide.

You can also use a pre-installed setup in the software "Remove air bubbles" (Tutorial → Load demo setups → Remove air bubbles) for 5 min.

3.1.8 Pinch off test

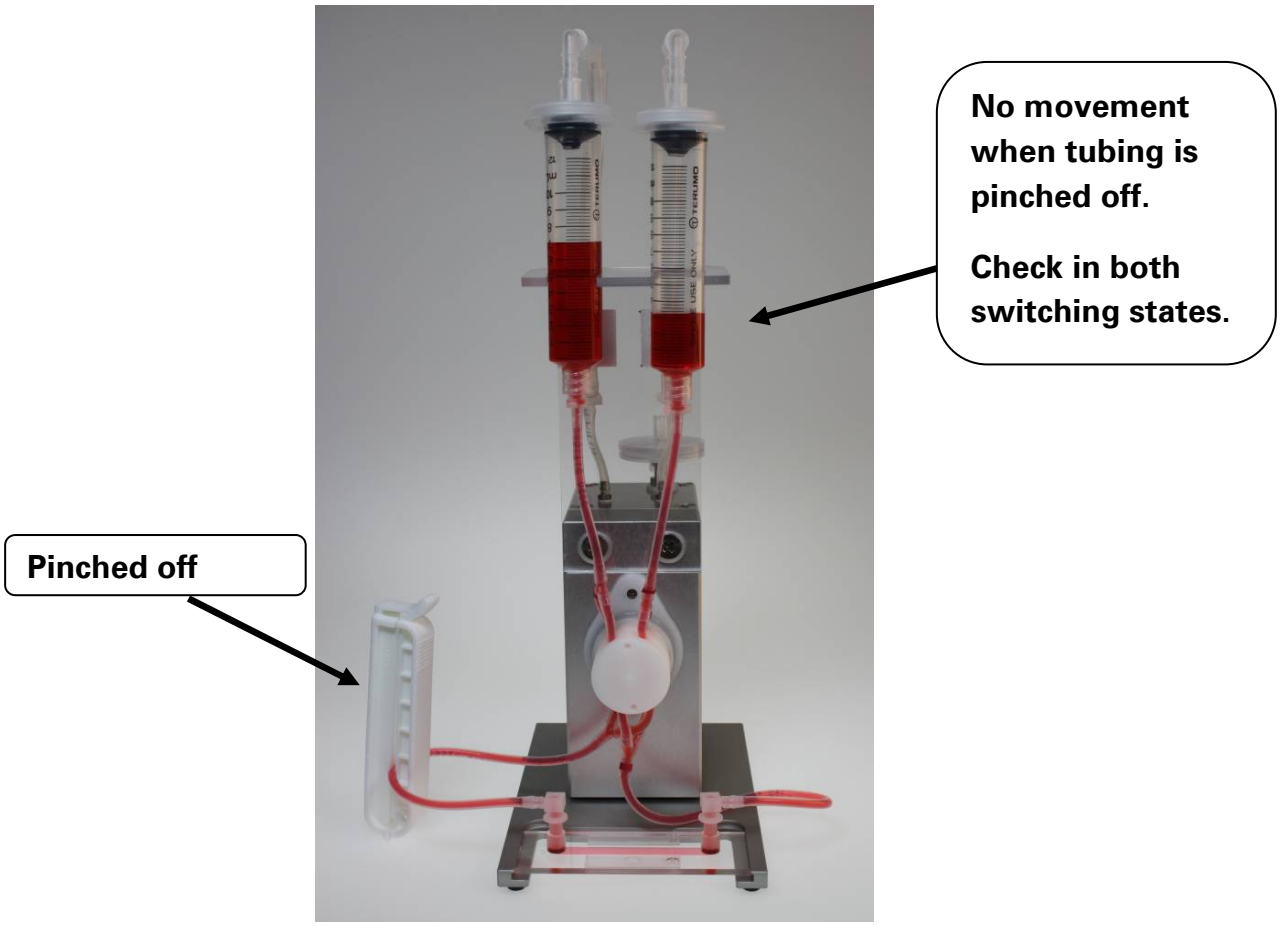


Figure 21: Pinched off tubing for test purposes

For this test the Fluidic Unit has to be connected to the pump and the pump to the computer.

1) Start a perfusion program with a clearly visible flow in the reservoirs (automated cycle).

2) Pinch off the tubing near the slide or near the middle connector in case the slide is not connected yet. See Figure .

3) Observe the movement of the liquid levels in the reservoirs. When the tubing is pinched, liquid should stop flowing in the reservoirs. Make sure you check both switching positions (State 1 and State 2).

→ If there is no movement, the setup is correct, otherwise recheck the mounting of the Perfusion Set.

3.1.9 Connecting the Perfusion Set to the slide

In this section a brief guide is given for setting up the Perfusion Set with liquid and for connecting a slide to the Perfusion Set. For a detailed description of an experiment culturing cells under flow conditions please follow the instructions in Application Note 13 "HUVEC under perfusion".

Three points are crucial when connecting the µ-Slide to the Perfusion Set:

- Avoid air bubbles which can remove the seeded cells from the slide.
- Keep everything sterile.
- Avoid disturbance of the cells, such as strong temperature variations.

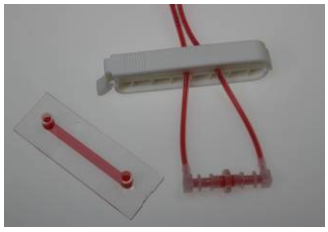


Figure 22: Clamp off tubing before disconnecting the Perfusion Set

Below is a short guide to follow in order to prevent these problems:

1) Once the tubes are free of bubbles, clamp off the Perfusion Set tubes between the valve and slide using the hose clip (Figure 22). This allows the two ends of the Perfusion Set to be disconnected without medium running out.
2) Before connecting the Perfusion Set to the µ-Slide, the reservoirs of the slide must be filled to the top to avoid inclusion of bubbles. This is shown in Figure 23. For a detailed description please refer to Application Note 13 "HUVEC under perfusion".

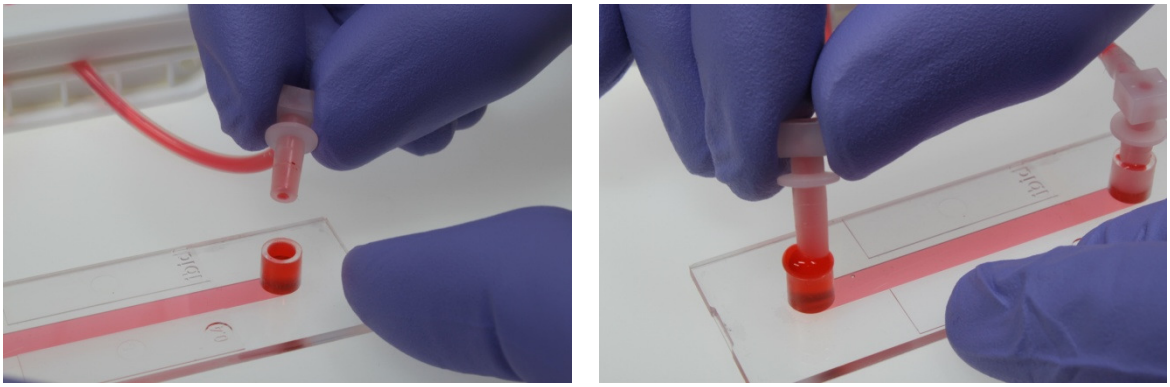


Figure 23: Connecting the µ-Slide

After removing the hose clip the experiment is ready to be started.

3.2 Installation using two or more Fluidic Units

Please read this section if you are planning to use the ibidi Pump with several Fluidic Units. It is very easy to modify the setup based on a setup with only one Fluidic Unit. The only difference is the pressured air tubing. Your shipment includes branched air tubings for the use of two, three and four Fluidic Units. When using positive pressure connect the Fluidic Units with the pump as shown in **Fehler! Verweisquelle konnte nicht gefunden werden.** (example with 4 Fluidic Units). The rear port is connected to the drying bottle as described in section 3.1.6. Then link the electrical cables to the Fluidic Units and the pump. Before you start your experiment verify that all Fluidic Units are set up as described in section 3.1.3.

For a setup with positive air pressure, the drying bottle has to be installed between the rear port of the pump and the incubator. For that the x-fold branched air pressure tube has to be plugged directly into the front pump port.

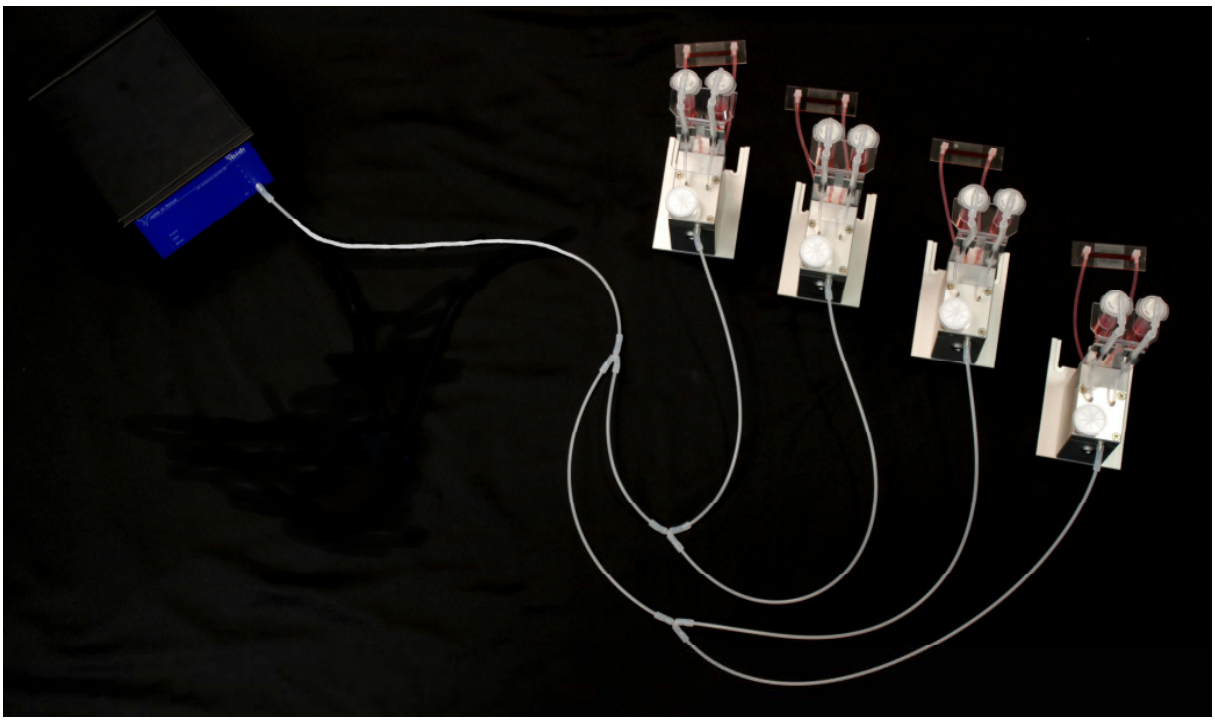


Figure 24: Connection with 4 Fluidic Units

3.3 Setting up an experiment with cells

3.3.1 Using the Fluidic Units inside an incubator

To ensure optimal conditions for cells, the Fluidic Units must be placed inside an incubator. Please note that only the Fluidic Units, not the pump, are to be placed inside the incubator. ibidi offers an incubator that allows up to two Fluidic Units to be inserted. As the incubator is small in size the optimal positioning of the Fluidic Units is such that only one of the delivered shelves of the incubator is inserted just on top of the water container. Please refer to Figure 25 to check the suggested placement.

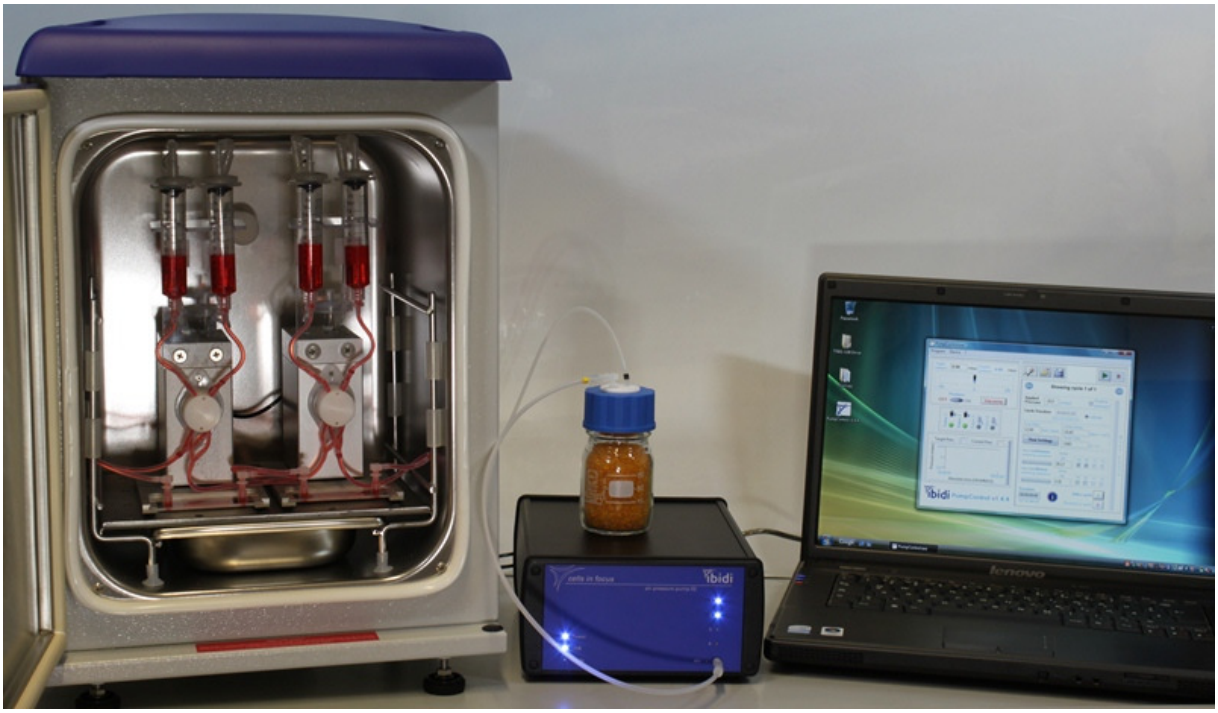


Figure 25: Fluidic Units inside an incubator

Once you have placed the Fluidic Units inside the incubator, the air tubes and the electric cables have to be connected to the ibidi Pump which remains outside. To do this, the incubator has an opening in the back side which is typically covered with a rubber cap. Remove the rubber cap and pass through both the electric cable(s) and the air tubing. To prevent leaking of the heat and CO_2, it is recommended to seal the opening by e.g. a modified rubber cap.

3.3.2 Degassing of slides, tubing, and medium

To avoid air bubbles, the degassing of every plastic part and of the medium used in the experiment is crucial. Put the slides (within the packaging) and the medium for the cell seeding, as well as the Perfusion Set (also within the sterile packaging) inside the incubator one day before starting the experiment. The amount of the needed medium can be filled in a small vessel. The cap has to be unscrewed slightly.

The reason for this procedure is the temperature dependency of gas solubility in water and plastic. At a higher temperature water and plastic can take up less gas than at a lower temperature.

If components have been stored at room temperature, the gas that is solved in plastic and liquids will be released when heated up in the incubator. Air bubbles will then emerge inside the slide and tubing. Therefore, you have to prevent this effect by degassing all the parts involved beforehand.

Each time you take the system out of the incubator, this process will start over. Take care that you work as fast as possible at room temperature. Never leave the Fluidic Unit outside the incubator for more than 15 minutes.

3.3.3 Sterility

All disposable parts are delivered sterile. The Fluidic Unit can be cleaned by wiping with ethanol (70 %).

For degassing, put the packaged slide or Perfusion Set in the incubator. The sterility is maintained if the packaging is not opened. Ensure proper sterile handling while filling the reservoirs, seeding and pre-cultivating cells as it is described in the Application Note 13. When you work sterile, you can set up the experiment without antibiotics.

Taking pictures on the microscope does not affect the sterility either. Only the air pressure tubing and the electric cable are disconnected and the Fluidic Unit can be taken to the microscope. As the tubing system is not opened, sterility is not affected.

4 Pump Demo Instructions

4.1 Goals of the demo

The pump demo is intended to familiarize the users on the proper setup of the system for first time use with cells in the lab. As the setup is complex, it is highly recommend that the users follow the instructions of the demo experiment in Application Note 13, "HUVEC under perfusion". If the lab does not work with HUVEC cells, any other adherent cell or cell line like MDCK or HT-1080 will work.

- Always degas slides, perfusion sets and medium (see section 3.3.2).
- Always perform the "pinch off test" for controlling the insertion of the tubing (see section 0).
- Always work as fast as possible when cells or tubing are outside the incubator.

4.2 Material

The ibidi pump system demo includes the following parts:

- Complete ibidi Perfusion Demo System
    - Air Pressure Pump
    - Fluidic Unit
    - Unsterile Perfusion Set for initial testing
        - 1 Perfusion Set 15 cm, ID 1.6 mm (red) (unsterile)
        - 1 µ-Slide I 0.6 Luer, ibiTreat (unsterile)
- Computer with software (PumpControl) installed
- ibidi Pump Demo Set – (for use with cells, purchase number 10982)
    - 1 Perfusion Set of choice
    - 5 Sterile µ-Slides of choice

Furthermore you will need the following components to perform cell experiments (not provided by ibidi):

- Colored water or buffer for unsterile testing
- Sterile Medium
- Cells (~2.5 x 10^5 per slide)

4.3 Demo experiment parameters

The demo experiment will set the PumpControl software to the following setup:

- 1 Fluidic Unit
- 1 µ-Slide I 0.6 Luer, ibiTreat
- 1 Perfusion Set 15 cm, ID 1.6 mm (red)

The flow rate profile is programmed to create a stepwise increase of the shear stress as shown in Figure 26.

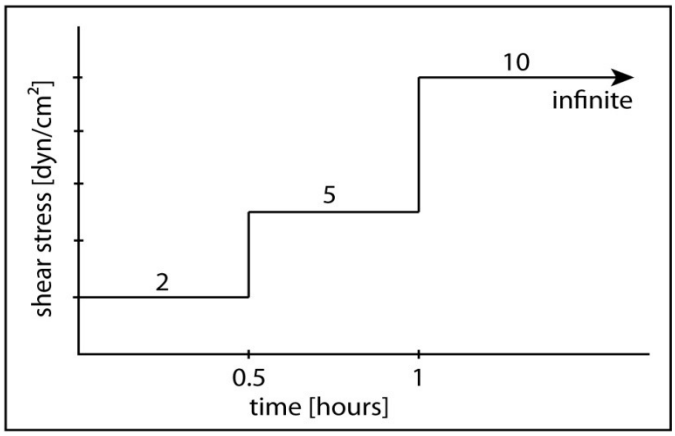


Figure 26 Shear stress profile of demo experiment

4.4 Example demo schedule

Below is an example of how the pump demo schedule should be organized. A detailed description for the technical setup can be found in section 3. All protocol steps concerning the cell culture are described in Application Note 13 "HUVEC under perfusion".

Day One:

- When demo pump is received, check shipment for completeness.
- Install the system on a work bench.
- Attach the non-sterile red perfusion set to the Fluidic Unit. Fill 6.15 ml (half the volume of recommended total working volume) of buffer with food coloring or medium into each reservoir and get accustomed to the software.
- Perform "Remove air bubbles" (Tutorial→Load demo setups→Remove air bubbles) for 5 minutes.
- Connect an unsterile Slide and perform "Demo experiment" (Tutorial→Load demo setups→Demo experiment). See Figure 26 for shear stress profile. Let it run for at least 3 hours and confirm that the flow is running and the reservoirs do not run dry.
- Prepare perfusion demo kit (sterile items) for next days experiment. Place two sterile µ-Slides I Luer (one for backup), one sterile Perfusion Set and a small centrifuge tube with ~30 ml culture medium (cap slightly opened) into the incubator. Allow them to degass overnight.

Day Two:

- Seed the cells in the morning by following the instructions found in Application Note 13. Fill the reservoirs of the the µ-Slide with cell free medium (60 µl each) as soon as the cells have attached.
- Mount a sterile Perfusion Set as shown in section 3.1.3.
- Fill the sterile, mounted Perfusion Set with the degassed medium (6.15 ml into each reservoir) and place the Fluidic Unit in the incubator with connection to the pump. Load the "Remove air bubbles" setting and let it run until you are ready to connect the µ-Slide.
- The slide can be connected to the tubing immediately after cell attachment. Do not cultivate cells for more then some hours at static conditions. Connect the Perfusion Set to the slide. Load Tutorial→Load demo setups→Demo experiment and start the experiment.

Day Three:

- Check the cells on the microscope.
- If the cells look healthy, the setup was done correctly then the system can be adapted to the parameters for the desired experiment.

5 Technical Details

5.1 Flow calibration and viscosity adjustment

The PumpControl software is programmed to automatically calculate the flow rate and the shear stress once the air pressure, the perfusion set and the specific slide are selected. The automatic calculation is based on internal calibration tables.

Please keep in mind that the calibration is done with the recommended working volume for each Perfusion Set of pure water and at a room temperature of 20°C. If the viscosity of the medium deviates from 0.01 dyn·s/cm², the automatic calibration will not be correct for the experiment. Another factor that may affect calibration are large air bubbles in the Perfusion Set. These will cause the system to yield different flow rates and shear stresses.

Positive or negative air pressure will not influence the calibration.

If the automatically calculated flow rate of the PumpControl software does not fit the desired observations, the setup can be manually calibrated as described below. It is normal to see differences up to 10 % due deviations in viscosity and temperature.

5.1.1 Flow rate measurement

To set a specific calibration, follow the steps below:

1. Set up a perfusion experiment using the tubing, slides and medium that have been equilibrated to the conditions required for the experiment.
2. Equilibrate the fluid level in the reservoirs.
3. Start the system and measure the time t (in seconds) which the medium needs to flow 2 ml[1] by a stopwatch. By filling the reservoirs with the recommended working volume (see Table 1); it is equilibrated around 5 ml. The time should be measured for how long it takes the liquid to run from 4 ml to 6 ml. It is sufficient to use the markers on the reservoirs to reach the accuracy of the system.
4. Conduct at least four measurements and calculate the mean value.

To calculate the flow rate [ml/min], insert the time that was measured in the formula below:

$$\Phi[\frac{ml}{min}] = \frac{2\,ml \cdot 60[\frac{s}{min}]}{t\,[s]}$$

[1] For Perfusion Sets yellow (#10965) and black (#10966) use only 1 ml.

As the flow rate is strongly influenced by temperature and media components, it is recommend to perform this measurement under later experimental conditions.

5.1.2 Flow calibration in the software

The measured flow rate can be used to update the PumpControl software in the 'Recalibration factor' menu. To do so, click on the 'Recalibration factor' button and insert the given flow rate and the one that was measured. The software will then provide the correctly displayed flow rate, shear stress and shear rate.

The recalibration factor influences the relationship between pressure and resulting flow rate (see Figure 27).

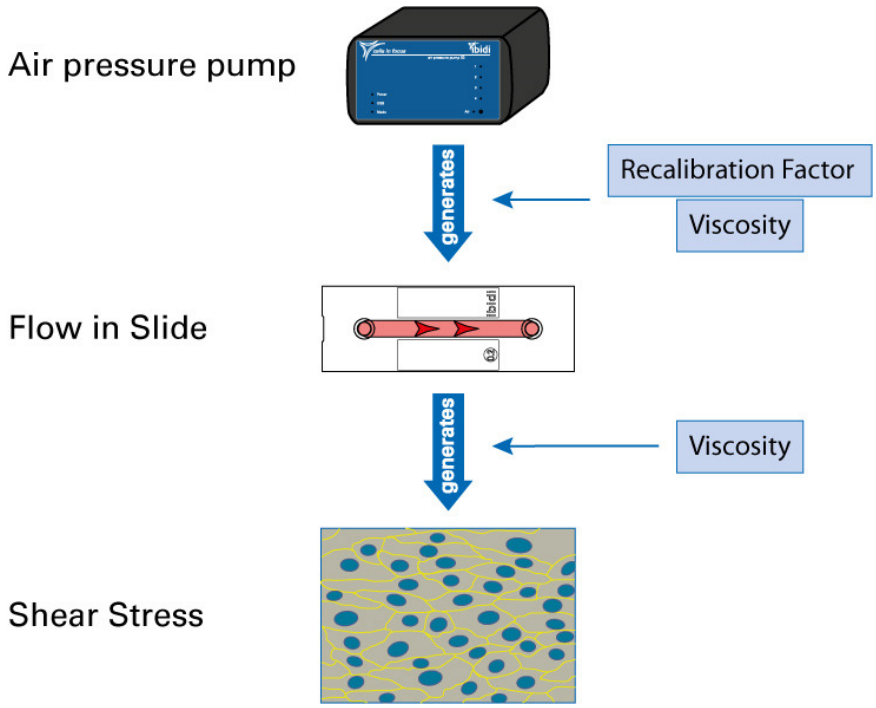


Figure 27: Relation between air pressure, flow rate, and shear stress

5.1.3 Viscosity adjustment

The viscosity influences the system at two points: The relationship between pressure and flow rate and the correlation between flow rate and shear stress (see Figure 27). For an exact calculation of the shear stress that the cells are exposed to, it is crucial to know the viscosity of the perfusing medium. This information can be found by asking the medium supplier or by measuring the medium with a viscosimeter.

Please keep in mind, that the viscosity is highly temperature dependent!

Water has a viscosity 1 mPa·s at 20°C, but only 0.69 mPa·s at 37°C (difference of 30 %!). A detailed graph for the viscosity of water is shown in the appendix on page VII.

5.2 Flow characteristics

The ibidi convention of the flow direction within the µ-Slide channel is based on the correct mounting of the Perfusion Set as described in section 3.1.3.

Applying positive pressure, the unmarked tube connecting the µ-Slide is the source of the flow.

Applying negative pressure inverts the situation and the marked tube is the source of the flow as described in section 0.

Due to the geometry of the setup, the flow within the tubes and the µ-Slide channel is always laminar independent of the flow rate and the flow type, i.e. continuous, oscillating or pulsating conditions.

5.2.1 Continuous unidirectional flow

The normal condition under which the Fluidic Unit is operating is to create a continuous and unidirectional flow within the µ-Slide channel. The working principle is explained in the beginning of the manual in section 0.

5.2.2 Oscillating flow

Some experiments require an oscillating flow to simulate turbulences in vessels for example. These conditions are achieved by oscillatory switching of the flow direction with frequencies around 2 Hz[2].

To perform an oscillating flow assay with the perfusion system, at least two Fluidic Units are needed. Also, there are some minor modifications that need to be done to the air tubing in order to change the functionality of the Fluidic Units. With the correct setup one Fluidic Unit will act as the 'master' Fluidic Unit, which is responsible for the air pressure inside the reservoirs. The second unit known as the 'slave' Fluidic Unit switches the flow direction.

To learn more about how to set up oscillating flow assays, please read section A.1 in the appendix.

5.2.3 Pulsating flow

Pulsating flow is also achievable with the ibidi Pump system. Again, two Fluidic Units are necessary. Please contact ibidi,GmbH for detailed instructions.

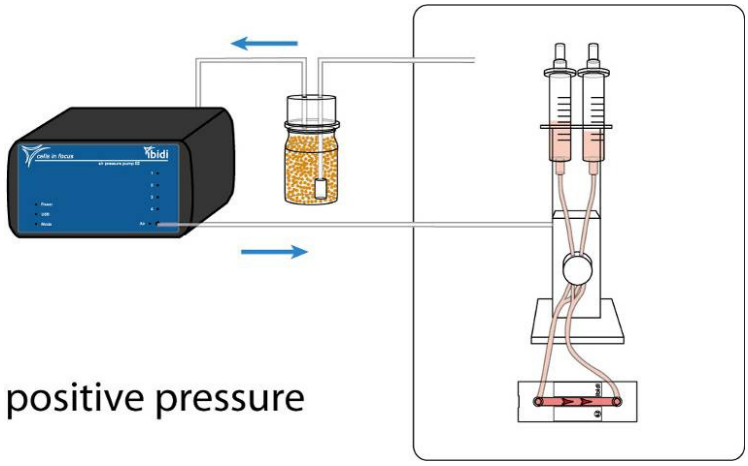
[2] Tomonori Hosoya et al., **"***Differential responses of the Nrf2-Keap1 system to laminar and oscillatory shear stresses in endothelial cells."* J. Biol. Chem Volume 280 (2005) pages 27244-27250

5.3 Positive and negative air pressure

With the ibidi air pressure pump, it is possible to use positive or negative pressure. The difference between the two is explained below.

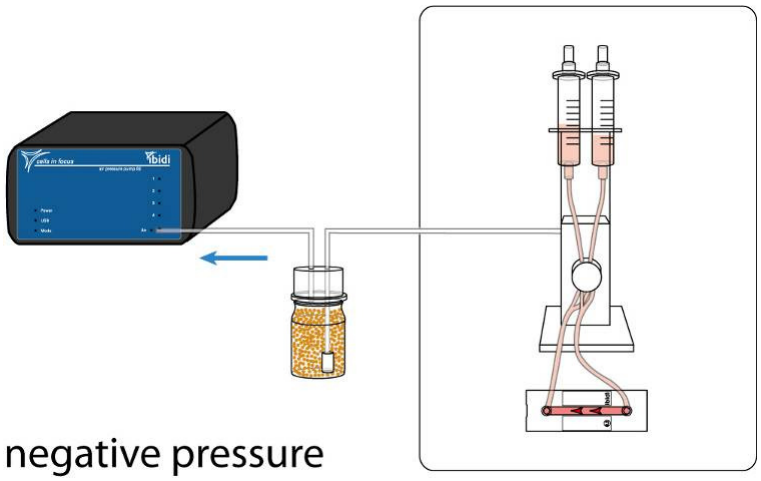
5.3.1 Positive air pressure

To produce positive air pressure, air is taken into the ibidi Pump from the rear pump port. With positive pressure, the air which is used to pump the medium back and forth from one reservoir to the other is filtered ambient air. Because ambient air has a low concentration of CO_2 this may not be the best atmosphere to have in contact with the medium which is supplying the cells in the µ-Slide. If the cells need a larger amount of CO_2 it is recommended to connect an air tube – which is part of the setup – to the inlet at the back of the pump and place the open end inside an incubator. When using this setup, remember to use the drying bottle. This will prevent condensation in the pump from the warm and humid air.



5.3.2 Negative air pressure

Using negative air pressure the pump aspirates air through the Fluidic Unit. As the Fluidic Unit is incubated, the air coming in contact with the medium and cells is the humid and CO_2 rich atmosphere from inside the incubator. Due to the humidity of the air, the drying bottle has to be integrated between the Fluidic Unit and the pump.



5.3.3 Advantages

When deciding on which pressure to use, there are a couple of aspects to consider. ibidi recommends using positive pressure.

When using positive air pressure, an 'overpressure' is created within the system. As a result, air or the medium is more likely to be pressed out of the system then to be taken into it, making the system less receptive for contamination or inclusion of air bubbles. Additionally the air supply is a dry air which will keep the sterility filters dry and not affect their performance. On the other hand, the supply of dry air increases the evaporation of medium.

With negative air pressure, the ambient air which enters the reservoirs comes directly from inside the incubator and is very humid therefore reducing evaporation. However, the humid air that is taken in through the filters can cause them to become damp and possibly blocked.

The table below explains the differences between positive or negative pressure. ibidi **recommends using positive pressure**.

	Positive Pressure (recommended)	**Negative Pressure**
Contamination	Less sensitive	More sensitive
CO_2	To reach the desired CO_2 level, gas from inside the incubator has to be connected to the pump and needs to be dried.	The incubator's atmosphere is directly used.
Air bubbles	Less sensitive	More sensitive
Performance of Perfusion Set filters	Dry air is pumped through the filters. Filters stay dry and performance loss is not likely.	Humid air is pumped through the filters. Permeability loss of filters may occur.
Physiology	*In vivo* there is positive pressure	Can barely be found *in vivo*
Medium evaporation	Higher	Lower

6 Trouble shooting

6.1 ibidi Pump is not recognized by the PC

6.1.1 Using PumpControl v1.5.0 or higher

If you have installed the latest PumpControl version you should not experience problems regarding the drivers. All required drivers are installed automatically with the software. In order to be able to run PumpControl v1.5.0 or higher you need a firmware version of your pump of v1.02 or higher.

6.1.2 Using PumpControl v1.4.4 or lower

If the installation did not work automatically and the communication between the pump and the PC is not established, you can install the required drives manually. To do so, follow the instruction during the hardware installation and specify the location of the folder for the USB drivers which is on the PumpControl installation thumb drive under 'USB driver'. If you still receive an error message, you must go to the hardware manager of your PC and look for the 'Ports'. There you will find the non-functional component, which is the ibidi Pump. Click on it and check the driver. Install the driver which is included on the PumpControl installation thumb drive. Please remember that you have to install two drivers; one for the 'USB serial converter' and one for the 'USB serial port'.

6.2 ibidi Pump is not communicating with the PumpControl software

If the PumpControl does not communicate with the pump, check that both the 'Power' and the 'USB' LED lights are illuminated on the front of the pump. If both LEDs are illuminated, rerun the PumpControl program. The program should not start in 'Demo Mode'. If, after rerunning the program, the communication between the pump and your computer still does not work, then you are most likely missing the '.NET' drivers. You can either download these drivers through the internet or from the ibidi web page:

http://www.ibidi.de/software/dotnetfx.zip

After extraction and installation of these drivers you should have the communication to the ibidi Pump setup.

6.3 Pressure kickback after pressure switch off

If using the rear port of the pump to take in CO_2 rich air from the incubator into the pump, you might experience air pressure kickback when switching the system off. This kickback is caused by a vacuum build up in the system. The vacuum can only build up if the tubing system is pinched and the air supply hindered. You can solve this problem by ensuring that the air tubes are not being squeezed and rechecking the setup of the drying bottle. If the tubing looks fine, remove it at the rear port of the pump. If the problem remains, please contact ibidi for assistance.

6.4 No flow – or stopping after 1 ml

Problem: No flow is observed or only a small amount (~1 ml) flows for a short time and then stops.

Possible reason	Solution
Clogged filters do not permit air to pass the outlet filter. If the filters get in contact with medium or water, the gas permeability decreases or the filter is blocked entirely.	Filters that become wet with water can be dried at 55 °C for a few hours. Filters that are contaminated with medium are clogged with substances from the medium. These can be washed by connecting them to a syringe with a Luer lock connector and purging with ethanol followed by water. Dry the filters at 55°C. If the flow resistance remains affected replace them with new ones.

6.5 Flow rate is too high

Problem: The flow rate indicated by the software doesn't match the flow rate you measured with a stop watch. It is two or more times higher. Values of up to 10 % are normal due to differences of viscosity and temperature.

Possible reason	Solution
The liquid is not flowing through the whole loop of the tubing where the slide is connected. There is a "short cut" between the two reservoirs, such that the liquid is going directly from the source reservoir to the switching valve and then to the sink reservoir. This is caused by incorrect inserted tubing as indicated in Figure 28.	Check the correct fit of the tubing by clamping off the tubing near the slide. When completely blocked no flow should be observed in each of the two switching states. If the liquid is moving you have to rearrange the tubing. For the procedure please see section 0

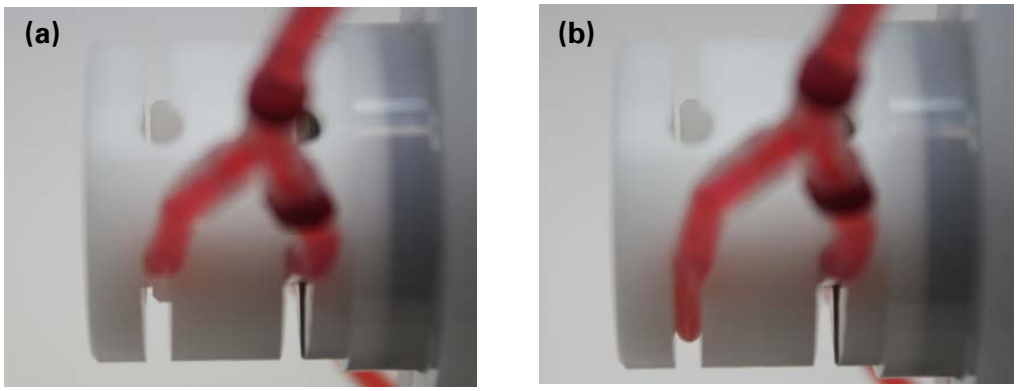


Figure 28: (a) Correct placement of tubing; (b) incorrectly mounted Perfusion Set

6.6 Evaporation

Problem: In long term experiments you may observe evaporation of the medium. Do not allow the medium concentration to get too high. Values up to 5 % volume loss per day are normal.

Possible reason	Solution
Due to the air stream in the reservoirs, a slight evaporation of medium is observed.	Depending on the requirements of your cells, exchange the medium after a few days or refill the evaporated amount of volume with sterile water.

6.7 Air bubbles

6.7.1 Air bubbles before starting the flow

Problem: You observe air bubbles in your tubing system or slide before you start the flow.

Possible reasons	Solution
The air bubbles were introduced while filling the tubing, the slide or while connecting the slide to the tubing.	To assure that there are no air bubbles left in the tubing, start an automatic cycle in PumpControl with a high flow rate. For this purpose you can load the "remove air bubble" settings in the tutorial menu in the software.
The air bubbles are created when connecting the slide to the Luer adapters.	Make sure the reservoirs of the slide are filled to the top with no evident air bubbles sitting on the surface. Next, when pulling out the Luer adapter from the middle piece, hold the Luer adapter upwards. This will cause the air bubbles to rise to the middle piece and not stay in the Luer adapter.

6.7.2 Air bubbles emerging after a few hours

Problem: Air bubbles start to emerge and accumulate somewhere in your tubing system after you start the flow.

Possible reason	Solution
Media, tubing and slides are not gas and temperature equilibrated.	Equilibrate everything inside the incubator one day before starting the experiment. See section 3.3.2 for details.
Loose or leaky adapters in combination with negative pressure can suck small air bubbles into the system.	Make sure all adapters and connectors fit tightly. Work with positive pressure to avoid this.

6.8 Cells are detaching

6.8.1 Cells detach before flow

Problem: Your cells look unhealthy and are not well attached to the surface before you connect them to the Perfusion Set.

Possible reason	Solution
The cells don't have enough medium with nutrients. Especially in low channels (100 or 200 µm) as the volume of medium is very small. If you culture the cells for more than a few hours in the channel slide, the cells may run out of required nutrients.	Never cultivate cells in low channels for more than a few hours. If, for some reason, you have to maintain the culture in the channel for a longer time, refresh the medium in the channel in short intervals.

6.8.2 Cells detach when connecting the slide to the tubing

Problem: You check your cells after the connection to the tubing and they look detached and accumulated in clusters.

Possible reason	Solution
The cells are under too much stress.	Try to work as fast and cautiously as possible with the cells. Never put the slide directly on a cold metal surface.
The cells are not healthy. Primary cells are very different in their fitness.	Try another lot or passage of cells.

6.8.3 Cells detach in flow

Problem: Cells detach after starting the flow experiment.

Possible reason	Solution
Shear stress is too high.	Try a lower flow rate.
Shear stress is applied too fast.	Get the cells accustomed to the flow step by step. Begin with a very low flow rate.
Cells are not healthy.	Try another lot or passage of cells.
Cell number in the slide is too low. Cells cannot stick together and are washed off the surface.	Seed more cells. Before starting the flow cells should be almost confluent.
Coating is not stable. Coating is washed away by flow.	Check the coating with fluorescence staining before and after applying the flow. Try alternative coatings or ibiTreat.

6.9 Flow direction in the channel is changing

Problem: You observe a changing flow direction in the slide even if you have set unidirectional flow.

Possible reason	Solution
Perfusion Set is not mounted correctly.	Check correct tube insertion.

7 Maintenance

The ibidi Pump system is almost maintenance free. There are only two parts which have to be checked from time to time.

7.1 Silica gel from the drying bottle

The Silica beads are coated with an orange indicator which turns white when saturated with moisture. The Silica gel can be used until all orange color is gone.

For recovery, place the Silica beads in a glass Petri dish. Place the Petri dish into a drying oven at 120°C for at least 8 hours. The beads will turn orange once the Silica has been recovered. After the beads cool off to room temperature they can be placed back in the drying bottle for use.

7.2 Filters of the Fluidic Unit

The filter of the Fluidic Unit prevents the inside from particles or dust. It needs to be exchanged when the pores of the filter are blocked. We recommend exchanging the filter every 6 months if the system is used consistently. For this, use a 0.2 mm Teflon air filter with a diameter of 30 mm and a male Luer Lock slip (e.g. Sartorius Minisart 16596 HY).

Appendix

A.1 Instructions for oscillating flow experiments

In order to apply oscillating flow to the µ-Slides you need at least two Fluidic Units (one 'master' and one 'slave' Fluidic Unit) to separate the switching times of the two valves of the Fluidic Unit as simultaneous switching results in unidirectional flow. The basic principle is such that the master Fluidic Unit has a long switching time t_{master}. During t_{master} the master Fluidic Unit supplies an unchanged air flow to the reservoirs of the slave Fluidic Unit. Now the switching time of the slave Fluidic Unit t_{slave} can be set as a fraction of the t_{master} so that the flow direction is reversed t_{master} / t_{slave} times before the master Fluidic Unit switches the air flow to the reservoirs. As a result you have a setup were one Fluidic Unit creates a unidirectional flow and one which supplies oscillating flow within the µ-Slide channel. Since only one controlling master Fluidic Unit is needed but the pump can control up to four Fluidic Units, you can extend the setup to operate with up to 3 oscillating slave Fluidic Units.

A.1.1 Setting up the Fluidic Units

You will also need air tubing splitters (Figure 29), besides the two Fluidic Units.

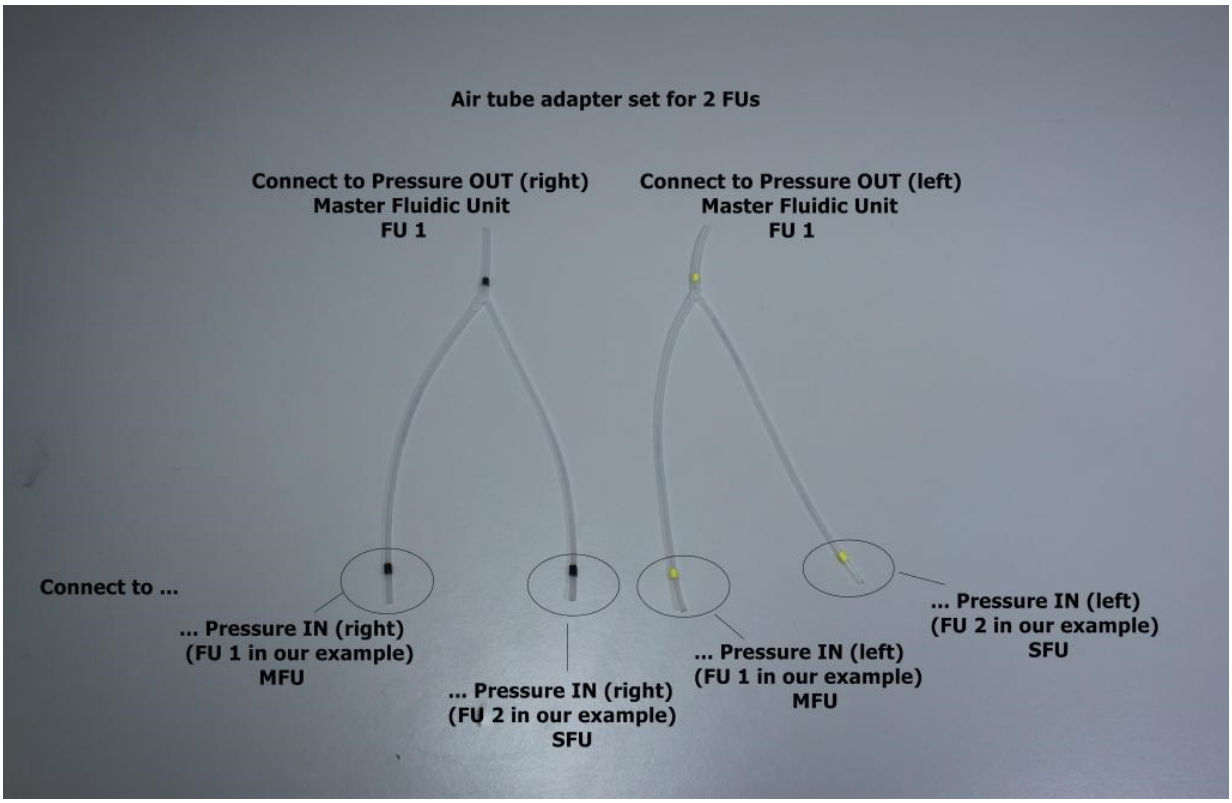


Figure 29: Air pressure splitter for 2 Fluidic Units

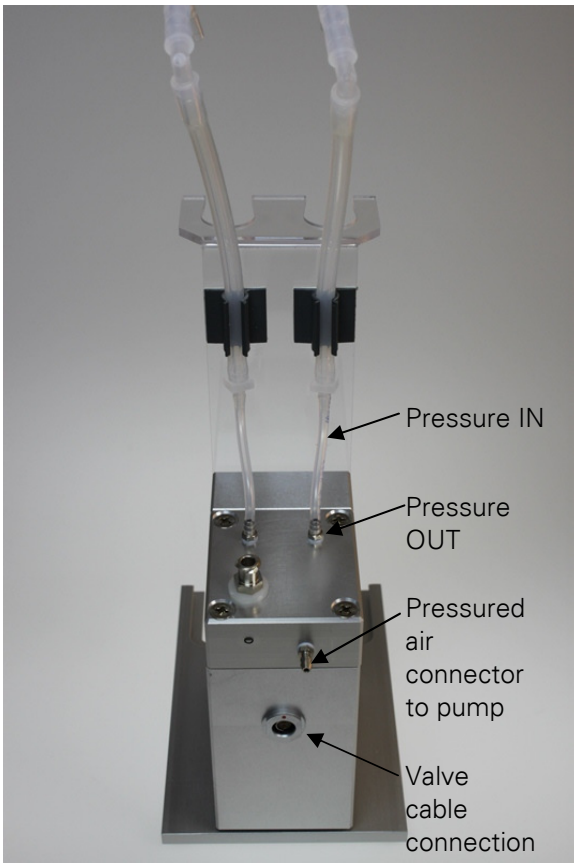


Figure 30: Fluidic Unit connectors

Please follow the steps below for correct installation and compare with Figure 30:

1) Connect the air pressure tube of the pump to the master Fluidic Unit.
2) Use one of the splitters, for example, the yellow marked to connect the left 'Pressure OUT' port of the Master Fluidic Unit to
   a) the left 'Pressure IN' port of the master Fluidic Unit and
   b) the left 'Pressure IN' port of the slave Fluidic Unit.
3) Repeat step 2) with the black marked air splitters and the right side of the master and slave Fluidic Unit.
4) Connect the pump and the two Fluidic Units with the electric cables. In the presented case we use 'Port 1' for the master Fluidic Unit and 'Port 2' for the slave Unit.

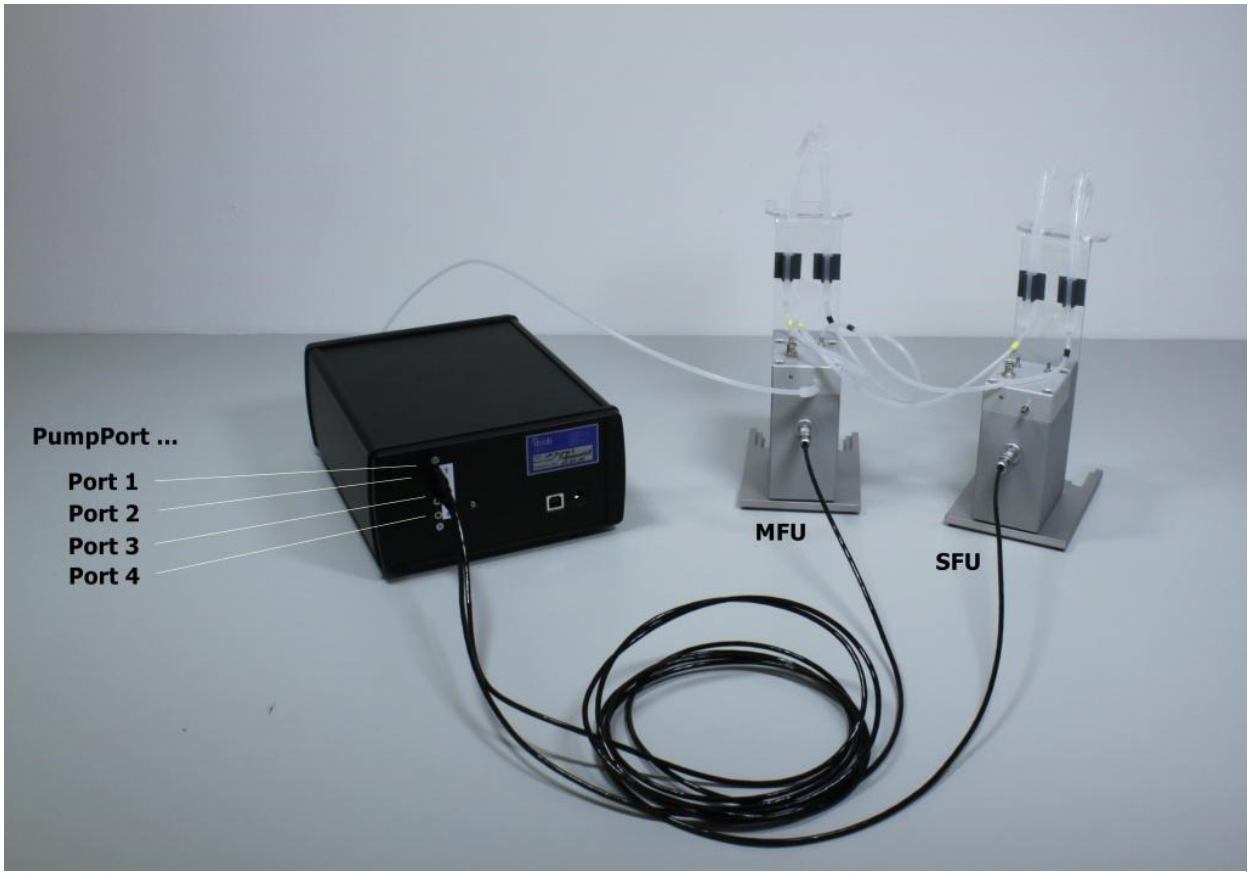


Figure 31: Connected master (MFU) and slave Fluidic Unit (SFU)

The setup should like like Figure 31

A.1.2 Oscillating experiment with 4 Fluidic Units

To extend an experiment to one master Unit and 3 oscillating slave Fluidic Units, a corresponding air pressure splitter is needed. The connection is according to the setup with two Fluidic Units. Figure 32 shows an example of the required air pressure tubing.

The connection is according to section A.1.1 with a simple extension for the additional Fluidic Units. The connection to the air pressure tubing is shown in Figure 33.

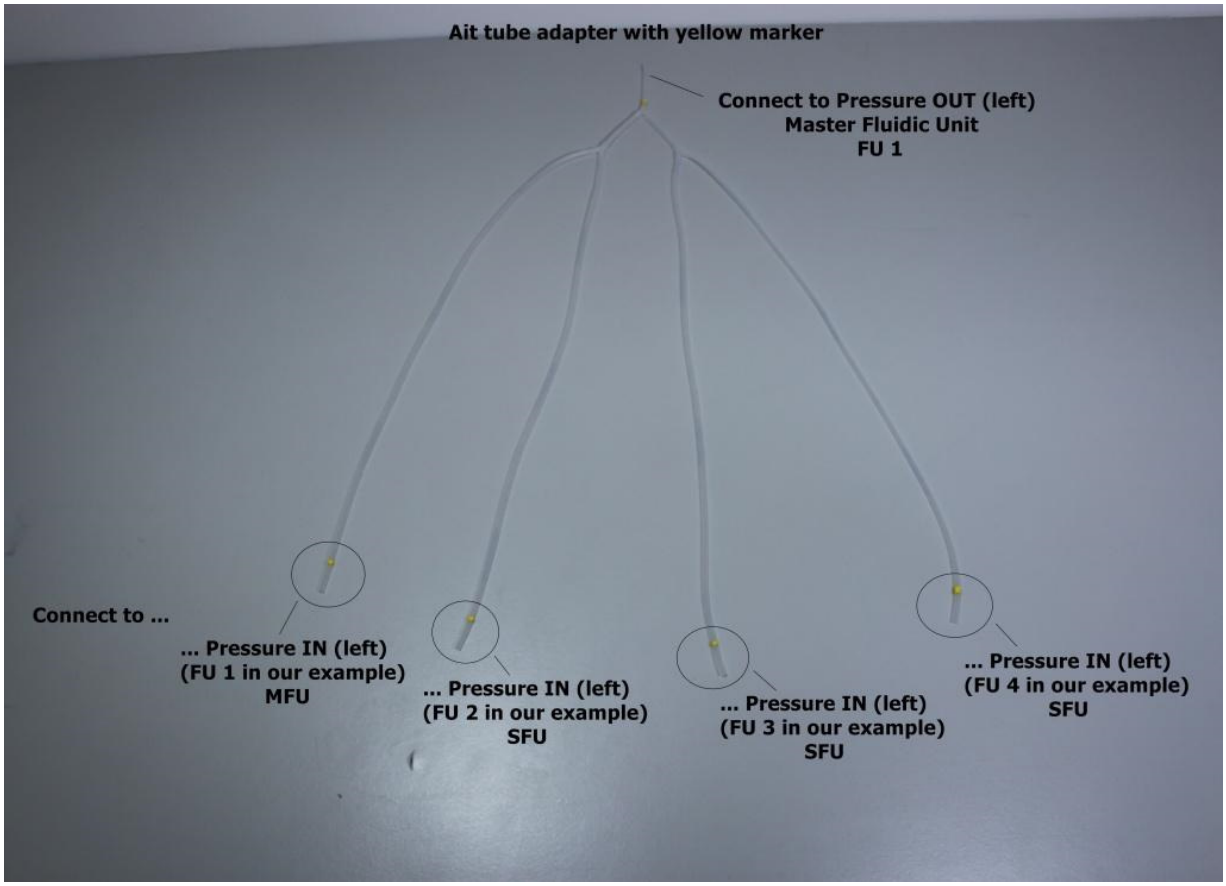


Figure 32: Air tubing for experiment with 4 Fluidic Units: 1 master Fluidic Unit (MFU) and 3 slave Fluidic Units (SFU 1-3).

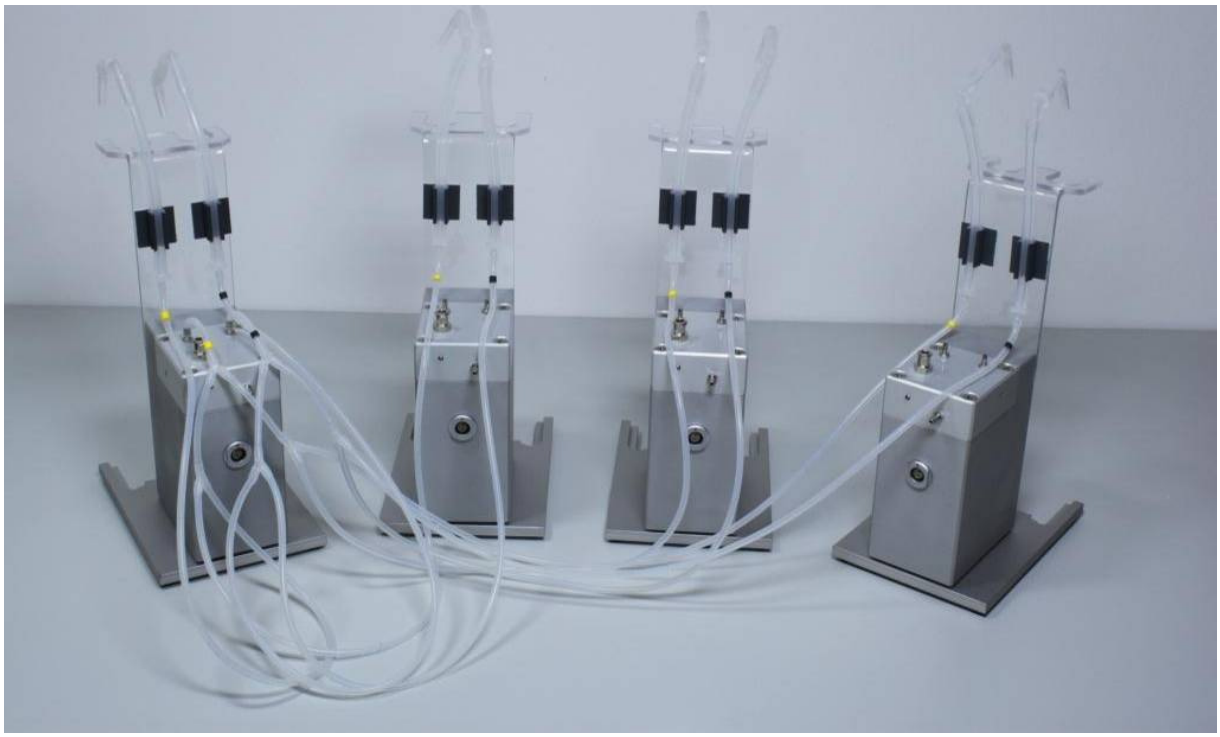


Figure 33: Air pressure connection with a setup using 4 Fluidic Units

A.1.3 Operation mode for oscillation flow experiments

	Possible?	
	Oscillating flow	Continuous flow
Master Fluidic Unit (FU 1)	no	yes
Slave Fluidic Unit (FU 2)	yes	yes
Slave Fluidic Unit (FU 3)	yes	yes
Slave Fluidic Unit (FU 4)	yes	yes

Please be aware that the master Fluidic Unit can only be used for unidirectional flow experiments. All slave Fluidic Units can reverse the flow direction with switching times set by PumpControl.

A.1.4 Settings within PumpControl

As the switching times are different for the master and the slave Fluidic Units the PumpControl program of the ibidi Pump has to be set accordingly. For that you find in the

PumpControl software checkboxes for 'continuous' and 'oscillating' valves and switching operations. Please check Figure 34 how to correctly set the corresponding parameters.

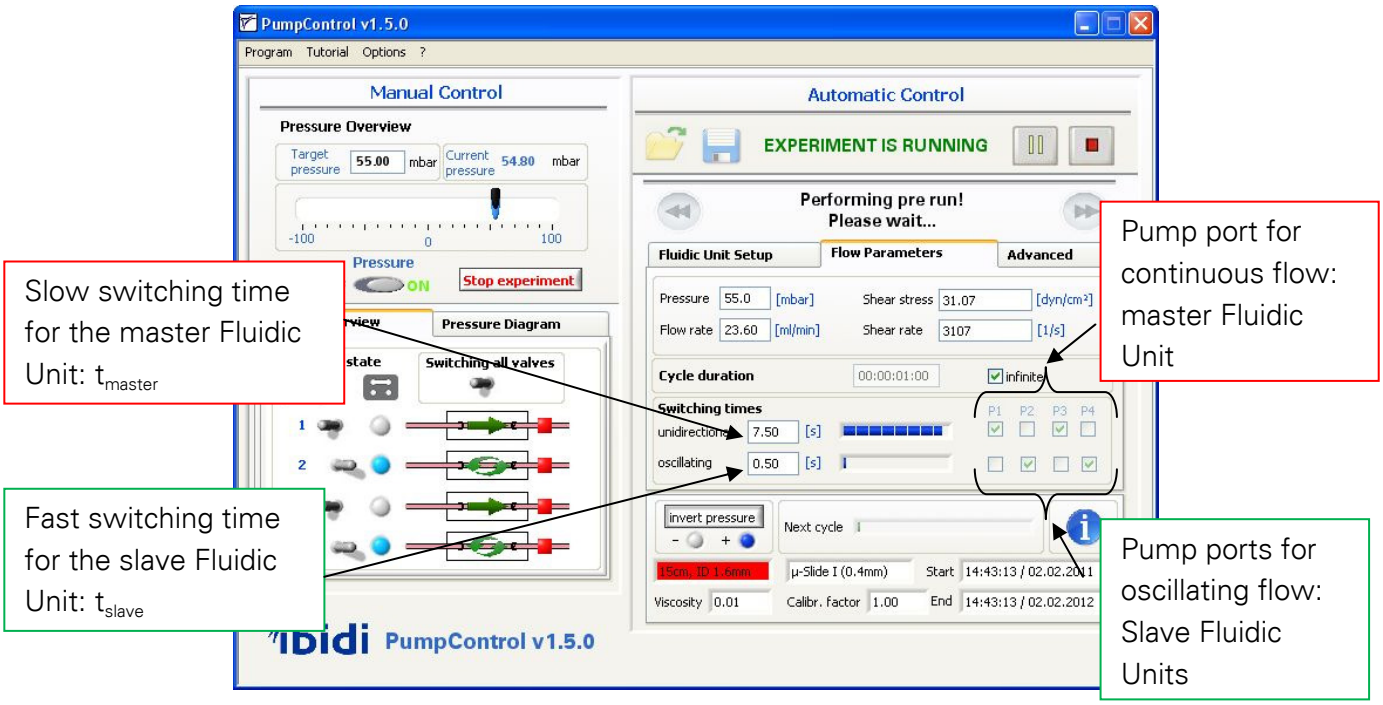


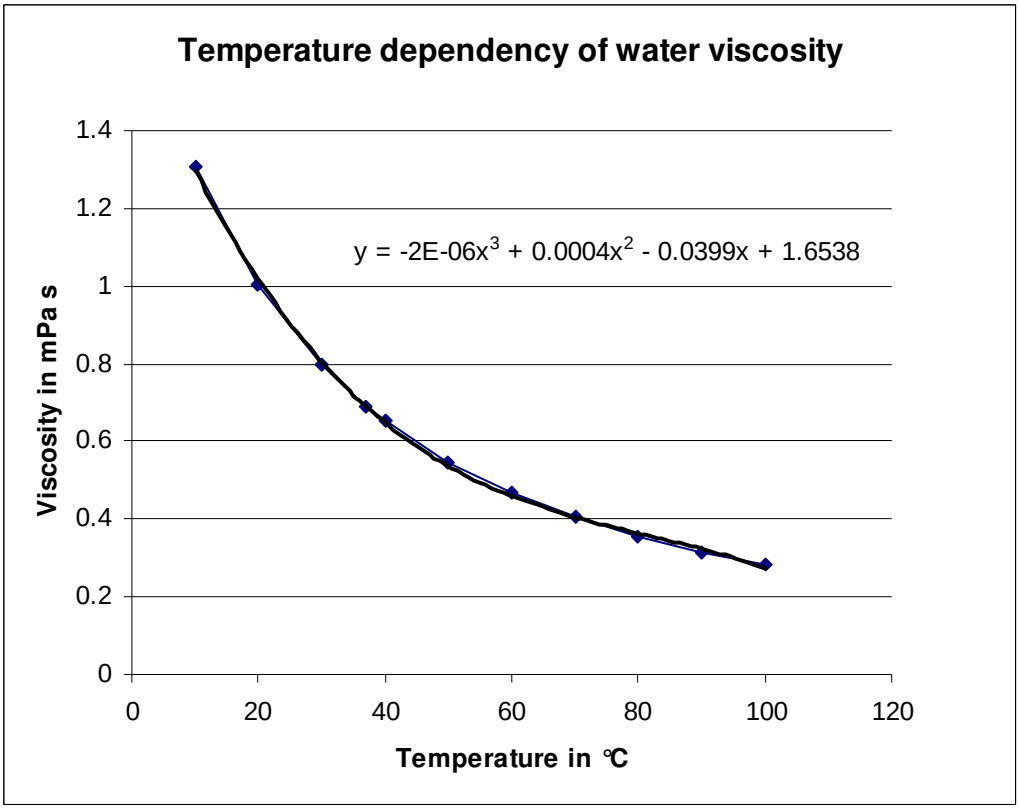
Figure 34: Screen shot of settings for oscilalting experiments

A.1.5 Equilibrating master and slave Fluidic Units

As master and slave Fluidic Unit are connected to the same air pressure and switching time equilibration of the liquid levels of both Fluidic Units cannot be done simultaneously. To stop the liquid movement in the reservoirs of either Fluidic Unit the Perfusion Set has to be clamped off (pinch off test described in section 0) with the supplied hose clamp. As the order is indifferent you can start off equilibrating the liquid levels of the master Fluidic Unit by clamping off the Perfusion Set of the slave unit. Afterwards the same should be done with the slave unit. This step is beneficial as it shows if the Perfusion Set is mounted correctly in both Fluidic Units.

A.2 Viscosity of water

The viscosity of water or medium is strongly temperature dependent.



1 mPa·s = 0.01 dyn·s /cm²