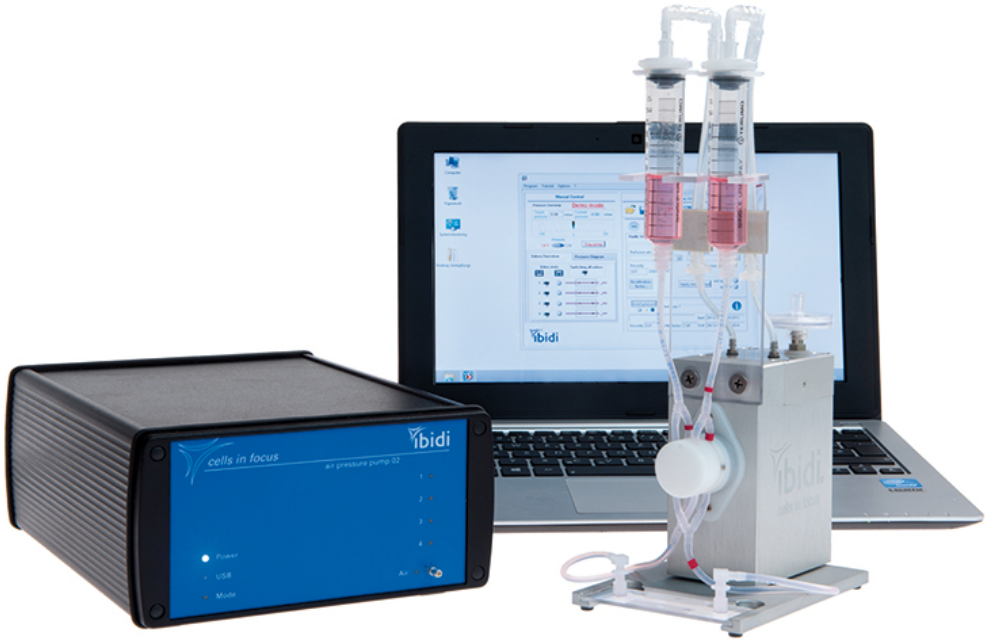
ibidi®
cells in focus

Instruction Manual
ibidi Pump System

Version 2.2



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1 Preamble

1.1 Introduction

This manual is your guide to using the ibidi Pump System for flow experiments with the ibidi Channel Slides. It instructs first–time users how to use the instrument, and serves as a reference for experienced users.

Before using the ibidi Pump System, please read this instruction manual 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. If this manual gets lost, order a replacement from www.ibidi.com.

To ensure operation safety, the ibidi Pump System must only be operated and maintained with the supplied components, and according to the instruction manual.

1.2 Safety Symbols

Note that the signal words **WARNING**, **CAUTION** and **NOTE** have specific meanings in this manual. Do not proceed beyond a signal word until you have performed the indicated actions.

WARNING! A potentially hazardous situation which, if not avoided, could result in serious injury or even death. Warning messages in the text are displayed in a gray shaded box.

CAUTION A potentially hazardous situation which, if not avoided, could result in minor or moderate injury. It is also used to alert against damaging the equipment or the instrument.

NOTE Additional information to help achieve optimal instrument and assay performance.

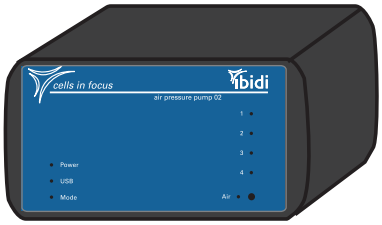
Symbols on the product identification label and back panel of the device:

CE Marking: This symbol indicates the product's compliance with EU legislation.

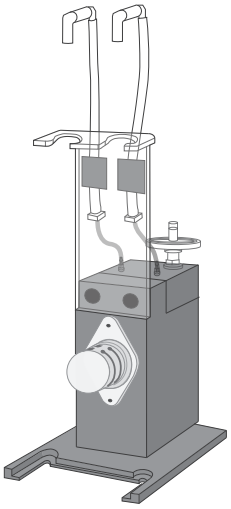
This label is positioned on the back of the device and prompts you to read the manual before using the device.

Product disposal: The symbol indicates that this product must be recycled/disposed of separately from other household waste. See page 14 for details.

1.3 Nomenclature



ibidi Pump



Fluidic Unit

1.4 Specifications

Table 1 – Specifications of the ibidi Pump System

Electrical Specifications Power Supply	
Protection class	I
Ingress protection rating	IP 20
Overvoltage category	II
External power supply	AC 100-240 V, 50/60 Hz, 0.93 A (Sinpro MODEL NO:SPU41A-106)
Input line voltage and current ibidi Pump	DC 14 V, 2.85 A max.
Output voltage and current (to Fluidic Unit)	DC 14 V, 590 mA
Standby current	60 mA
Max. current at max. air flow	1000 mA
Max. current with 4 Fluidic Units at	1475 mA

Operating Conditions of the ibidi Pump System	
Operating area	Enclosed rooms
Environmental operating temperature	15-40°C/59-104°F
Operating humidity ibidi Pump	80% RH up to 31°C/88°F, 30% RH up to 40°C/104°F
Operating humidity Fluidic Unit	$\leq$ 100% (non-condensing)
Operating Altitude	max. 2000 m (atmospheric pressure 800-1060 hPa/11.6-15.4 psi)
Storage Conditions	-5-50°C/23-122°F, humidity <60% relative humidity (RH)

Table 1 – (continued)

Outer Dimensions and Characteristics of the Components	
ibidi Pump	90 × 170 × 230 mm^3
	3000 g/6.6 lbs
Fluidic Unit	85 × 135 × 270 mm^3
	1100 g/2.4 lbs
USB cable	1.8 m
Power supply cable	2.0 m (power supply to wall)
	1.2 m (power supply to device)
Electrical cable (FU to pump)	2.0 m
Air pressure tubing	2.0 m
Air pressure tubing drying bottle	2.1 and 0.6 m

Pressure range of the ibidi Pump	
Total pressure range	0-100 mbar
Recommended pressure range	5-95 mbar

Electrical Input Fluidic Unit	
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)

1.5 Disclaimer

- ibidi shall not be held liable, either directly or indirectly, for any damage incurred as a result of product use.
- The contents of this manual are subject to change without notice for product improvement.
- This manual is considered complete and accurate at publication.
- This manual does not guarantee the validity of any patent rights or other rights.
- If an ibidi software program doesn't function properly, this may be caused by a conflict from another program operating on the computer. In this case, take corrective action by uninstalling the conflicting product(s).
- ibidi is a registered trademark of ibidi GmbH in Germany and other countries.

1.6 Safety Considerations

WARNING!

- Only operate the ibidi Pump System with the supplied components.
- Only use the cables and plugs delivered with the system. The power plug of the control unit must be inserted in an outlet with a ground (earth) contact.
- Do not replace detachable power cables by power cables with inadequate specifications. By violating these instructions you risk electric shock and fire.
- Only use extension cables that have a protective ground wire.
- Do not operate the ibidi Pump System under conditions that pose a risk of explosion, implosion, or the release of gases. Only operate the ibidi Pump System with aqueous solutions.
- Do not operate a damaged ibidi Pump System. If the housing seems damaged or something is rattling inside the controller, contact the ibidi service hotline for repair.

CAUTION

- Ensure that the external power supply is easily accessible. The ibidi Pump System must be installed in a manner such that none of its components hinders access to the external power supply.
- Immediately replace damaged cords, plugs, or cables to avoid risk of personal injury or damage to the instrument.
- Only ibidi technical staff and technical staff instructed by ibidi are permitted to open and service the ibidi Pump System.
- The external power supply should not be brought into contact with moisture. If the housing is damaged, the external power supply should not be used.

- Avoid strong magnetic fields and sources of high frequency. The ibidi Pump System might not function properly when located near a strong magnetic field or high frequency source.
- Avoid vibrations from vacuum pumps, centrifuges, electric motors, processing equipment, and machine tools.
- Avoid dust and corrosive gas. Do not install the ibidi Pump System where it could be exposed to high levels of dust or to outside air or ventilation outlets.
- Install the ibidi Pump System in a horizontal and stable position, such as a table, bench, or desk upon which the instrument is installed.
- Install the ibidi Pump System in a location that enables easy access for maintenance.
- Do not place heavy objects on the instrument.
- The weight of the ibidi Pump is approx. 3 kg. Moving the pump during operation will pose a risk of personal injury or damage to the instrument.
- The ibidi Pump can build pressure up to 100 mbar. Do not unplug the fluidic connections during pump operation. Pressurized liquid could emerge from the tubes and damage surrounding equipment. Excess moisture can cause the external power supply or nearby electrical equipment to short circuit.
- Do not suction in any liquid into the ibidi Pump.

1.7 Regulatory Statement

The ibidi Pump System has been designed, produced and tested in compliance with the European standard DIN EN 61010-1 (IEC 61010-1, ”Safety requirements for electrical equipment for measurement, control and laboratory use”). Furthermore it meets the IEC 61326-1 (”Electrical equipment for measurement, control and laboratory use - EMC requirements”) and CISPR 11 (”International Standard for electromagnetic emissions (disturbances) from Industrial, Scientific and Medical (ISM) Equipment”) standards .

The device carries the CE mark.

The ibidi Pump System meets the Low Voltage Directive 2014/35/EU and the
EMC Directive 2014/30/EC.

1.8 Limited Warranty

Products manufactured by ibidi, unless otherwise specified, are warrantied 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 tubing or fluidic 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, such as cell culture chambers and dishes, tubes, fluidic connectors, reagents etc.

The warranty for all parts supplied and repairs provided under this warranty expires on the warranty expiration date of the original product.

1.9 Transporting the ibidi Pump System

The weight of the ibidi Pump is approx. 3 kg/6.6 lbs. The weight of the Fluidic Unit is approx. 1.1 kg/2.4 lbs. Moving the devices during operation will pose a risk of personal injury or damage to the instrument.

For transport, switch off the ibidi Pump and then disconnect all cables and tubing from the controller and peripheral components. Carry the devices carefully and avoid mechanical shocks.

1.10 Repairing the ibidi Pump System

For inquiries concerning repair service, contact the ibidi service personnel and provide the model name and serial number of your system.

ibidi GmbH
Service Hotline: service@ibidi.com

CAUTION

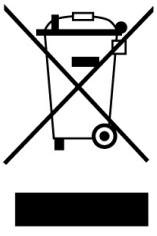
Do not try to repair the ibidi Pump System by yourself. Disassembly of the ibidi Pump System is not allowed. Disassembly poses a risk of personal injury or damage to the devices. Contact ibidi service personnel if there is a need to disassemble a device.

1.11 Waste Disposal – WEEE/RoHS Compliance Statement

The European Union (EU) has enacted two directives, the first on product recycling (Waste Electrical and Electronic Equipment, WEEE) and the second on limiting the use of certain substances (Restriction on the use of Hazardous Substances, RoHS).

1.11.1 EU Directive WEEE

The ibidi Pump System must be disposed of in compliance with the WEEE Directive 2012/19/EC.



This symbol on the product is in accordance with the European Union's Waste Electrical and Electronic Equipment (WEEE) Directive. The symbol indicates that this product must be recycled/disposed of separately from other household waste. It is the end user's responsibility to dispose of this product by taking it to a designated WEEE collection facility for the proper collection and recycling of the waste equipment. The separate collection and recycling of waste equipment will help to conserve natural resources and protect human health and the environment. For more information about recycling, please contact your local environmental office, an electrical/electronic waste disposal company or distributor where you purchased the product.

1.11.2 EU Directive RoHS

Two Categories of products covered by the WEEE Directive are currently exempt from the RoHS Directive – Category 8, medical devices (with the exception of implanted or infected products) and Category 9, monitoring and control instruments.

All of our products fall into either Category 8 or 9, and are currently exempt from the RoHS Directive. Nevertheless, the ibidi Pump System meets the requirements set forth in the RoHS Directive 2011/65/EC.

2 Intended Use of the ibidi Pump System

The ibidi Pump and Fluidic Unit(s) create unidirectional long term flow of medium within a channel slide, e.g. ibidi Channel Slides. This constant flow mimics physiological conditions for cell types, like e.g. endothelial cells of the blood or lymphatic system, which experience constant flow *in vivo*.

The mechanical force generated by fluid flow on cells is called (wall) shear stress ($\frac{dyn \cdot s}{cm^2}$ or $Pa \cdot s$). Under physiological conditions a laminar flow leads to a shear stress on the cell layer on the vessel wall which is proportional to the velocity of the fluid. The shear stress varies among different tissues and organisms.

The ibidi Pump System offers the following advantage for cell culture under flow:

- The ibidi Pump System covers the whole physiological range of shear stresses.
- Precise control of flow conditions with the PumpControl software.
- Defined shear stress calculation in the ibidi Channel Slides.
- Unidirectional flow for long–term studies (up to weeks).
- Oscillatory and pulsatile flow to mimic turbulent flow situations and pulsatile blood flow.
- Easy access to the culture vessel during incubation for imaging on the microscope.
- The setup can be done under sterile conditions.
- Minimization of the medium consumption with a circulating medium flow.
- Mechanical stress on suspended cells is minimized to avoid destruction and non-specific cell–activation.

3 Equipment

The ibidi Pump System consists of the ibidi Pump, the Fluidic Unit(s), and disposable parts, such as Perfusion Sets and Slides.

3.1 Components of the ibidi Pump System

An overview of the different ibidi Pump System versions is given in this section. Table 2 lists all available options of the ibidi Pump System.

Table 2 – Overview of the ibidi Pump System Variants

Cat. No.	Product Name	Description
10902	ibidi Pump System	Complete ibidi Pump System with 1 Fluidic Unit, 1 sterile Perfusion Set and all cables and components needed. The details are listed below.
10906	ibidi Pump System Quad	Complete ibidi Pump System with 1 Fluidic Unit Quad, 2 sterile Perfusion Sets and all cables and components needed. The details are listed below.
10903	Fluidic Unit	Switching valves for various flow assays, suitable for all Perfusion Sets and channel µ–Slides
10904	Fluidic Unit Quad	4 Fluidic Units on a stable plate, switching valves for various flow assays, suitable for all Perfusion Sets and channel µ–Slides

The following parts are included in the ibidi Pump System (#10902 and #10906).

- ibidi Pump
- External Power Supply (country specific) for the ibidi Pump
- USB cable to connect the pump to your PC
- USB flash drive with the latest PumpControl software
- 1 Fluidic Unit with Reservoir Holder (#10902)/1 Fluidic Unit Quad with Reservoir Holders (#10906)
- Fluidic Unit cable(s) to connect Fluidic Unit(s) and Pump (length 2 m); 1 per Fluidic Unit
- Non-sterile Perfusion Set (1 per Fluidic Unit)
- Drying bottles filled with orange Silica beads (2)
- Connection cap for the drying bottle
- Air pressure tubing (2 m)

- Air pressure splitter set to connect the Fluidic Unit Quad (only #10906)
- Short, yellow–marked air pressure tube (0.6 m); rigid air tube to connect the pump to the drying bottle
- Long, black–marked air pressure tube (2.1 m); rigid air tube to connect the drying bottle to the inside of the incubator
- Filter bubbler for the drying bottle
- Sterile replacement filter (1 per Fluidic Unit)
- Hose clip (1 per Fluidic Unit)
- Laptop with pre–installed PumpControl software

The following parts are provided, but are only needed for experiments using more than one Fluidic Unit:

- Air Pressure Splitter Set for 2 Fluidic Units
- Air Pressure Splitter Set for 3 Fluidic Units
- Air Pressure Splitter Set for 4 Fluidic Units
- Oscillatory Flow Kit for 2 Fluidic Units
- Oscillatory Flow Kit for 4 Fluidic Units

The following parts must be ordered separately:

- Sterile Perfusion Sets
- Sterile µ–Slides Luer Type

3.2 ibidi Pump

The ibidi Pump can generate air pressure up to 100 mbar. The most precise working range is 5 to 95 mbar. Additionally, the pump can set the air flow direction. When using positive pressure, the pump will expel air from the front port, and intake it from the rear port. When using negative pressure the pump will take in air from the front port and expel it from the rear port. The use of positive or negative pressure or air flow is detailed in Section 8.2. Optimal conditions are achieved 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 simultaneously with one pump. The pump requires a supply voltage of 14 V DC. Communication with the computer is achieved via a double shielded USB interface (see also Section 10.4.3).

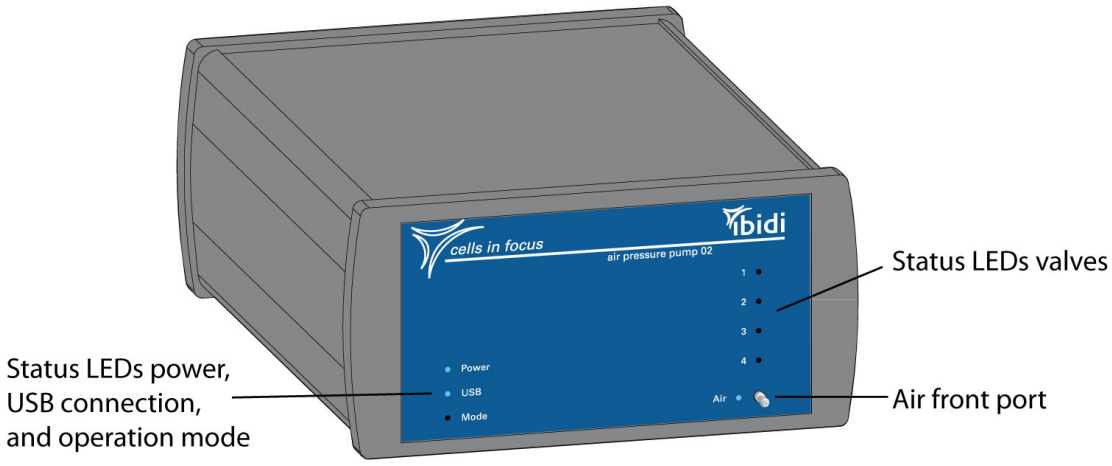


Figure 1 – ibidi Pump front side with air pressure front port and status LEDs.

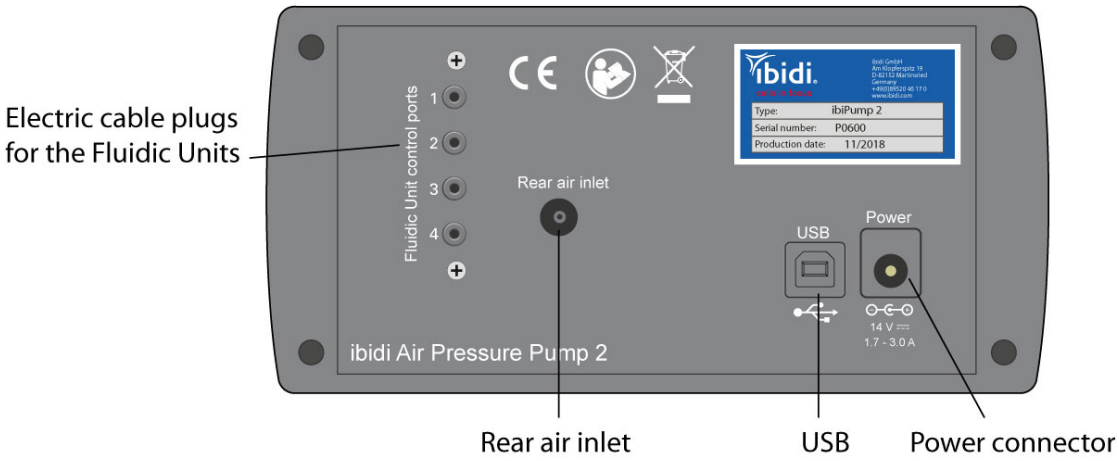


Figure 2 – Rear view of the ibidi Pump with air rear port, connections for the USB, power supply port, and electrical cables ports to the Fluidic Units.

Name	Function
Air front port	Pressurized air connection to Fluidic Unit(s)
USB	USB cable connection to the computer. To setup computer communication with the ibidi Pump, the USB cable must be connected.
Power connector	External power supply (Sinpro MODEL NO:SPU41A-106, 14V)
Air rear port	Pressurized air connection to incubator (positive pressure only)
Fluidic Unit ports	Electric connection to the Fluidic Unit(s)

3.3 Fluidic Unit

The Fluidic Unit holds the Perfusion Set (fluidic reservoirs and tubing), and performs the switching operations to generate the unidirectional constant flow in the flow chamber. The Fluidic Unit's (Figure 3) active components are the two switching valves (V1) and (V2). There are two connectors in the rear of the Fluidic Unit, one electrical connection for the valve control and another for the pressurized air. Both connect the Fluidic Unit to the ibidi Pump.

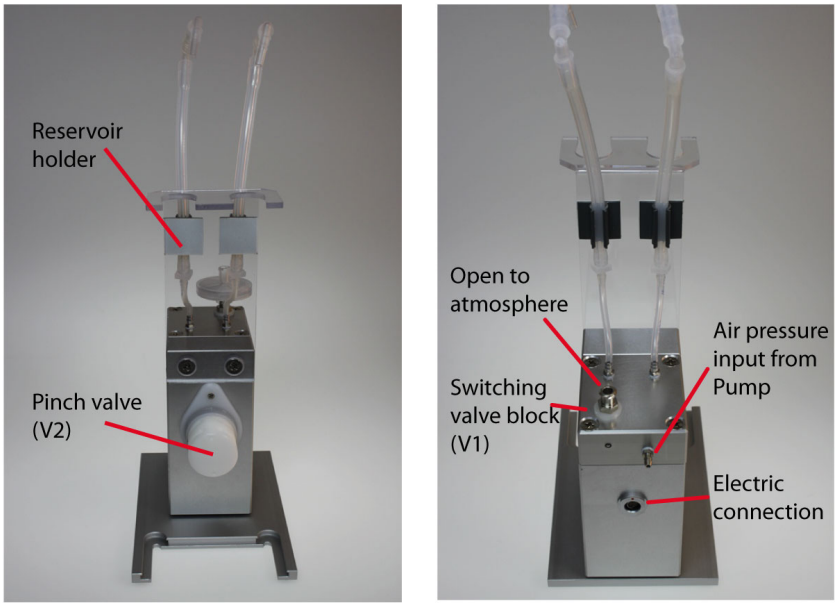


Figure 3 – Front and back view of the Fluidic Unit.

The Fluidic Unit can be equipped with a choice of one of three holder sizes, for the respective syringe reservoir sizes. The Reservoir Holder can easily be exchanged by the user. The standard reservoirs are indicated in Table 6 on page 23.

Table 4 – Overview of Reservoir Holders and compatible Perfusion Sets and Filter/Reservoir Sets

Reservoir Holder	Cat. No.	Compatible Perfusion Sets	Cat. No.
for Fluidic Unit, 10 ml	10976	Perfusion Set RED	10962
for Fluidic Unit Quad, 10 ml	10986	Perfusion Set YELLOW/GREEN	10964
		Perfusion Set BLUE	10961
		Perfusion Set WHITE	10963
		Filter/Reservoir Set, 10 ml	10971
for Fluidic Unit, 2 ml	10977	Perfusion Set YELLOW	10965
for Fluidic Unit Quad, 2 ml	10987	Perfusion Set BLACK	10966
		Filter/Reservoir Set, 2 ml	10972
for Fluidic Unit, 50 ml	10978	Filter/Reservoir Set, 50 ml	10974

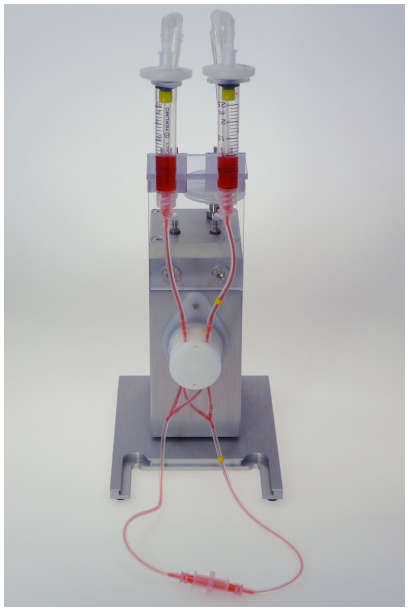


Figure 4 – 2 ml syringe reservoirs

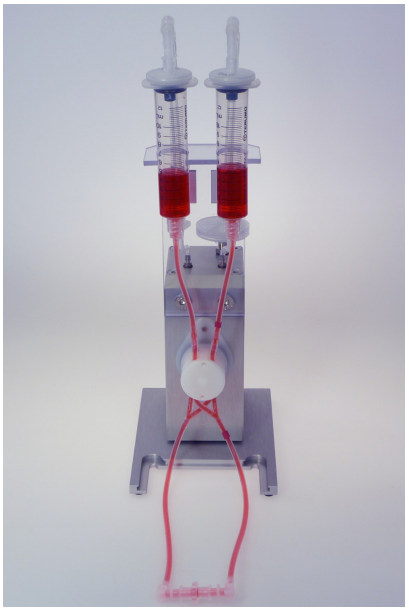


Figure 5 – 10 ml syringe reservoirs

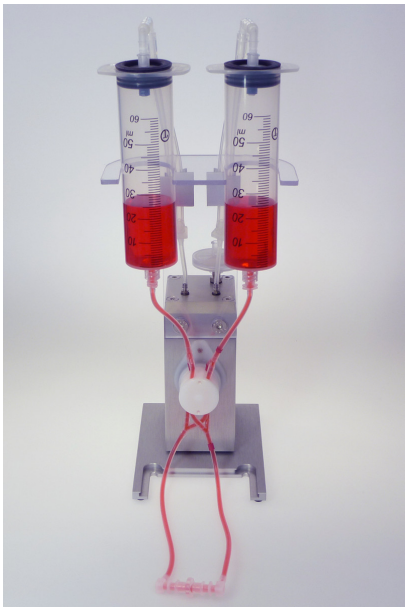


Figure 6 – 50 ml syringe reservoirs

Important!

To clean the Fluidic Unit, wipe the outside with an alcohol soaked paper towel. Never spray disinfectant directly on the unit, which could damage the valve electronics.

3.3.1 The Valve Block

The valve block (V1) is located on top of the aluminum block of the Fluidic Unit. The function of the valve is to guide the air pressure either to the right or left reservoir. The valve block is a magnetic valve. Be careful not to draw any liquid into this valve!

3.3.2 The Pinch Valve

The pinch valve (V2) at the front of the Fluidic Unit squeezes the tubing that is inserted into its slots with a movable bar, pinching off the inserted tubing parts and thus blocking fluid flow. The valve has four slots (two forward and two rear slots), so that four pieces of tubing can be inserted at once. Either the front or the rear tubing parts are closed at a time (two switching states).

Important!

Be aware that the pinch valve can wear out over time. The performance of the valve can be checked as follows: Perform the pinch test and observe the actuation of the bar in the pinch valve. If you see the bar moving inside the valve and the pinch test is ok, the valve works properly. If not, contact ibidi or your local supplier for repair.

3.4 Perfusion Sets

The disposable Perfusion Sets are supplied in a gas–permeable sterile package. The tubing is color–coded for easy identification. The Perfusion Sets are specifically designed for use with the Fluidic Unit. However, the Luer adapters can be connected to any suitable flow chamber with Luer connectors.

Figure 7 – Sterile packaged Perfusion Set

Perfusion Set Parts: (Figure 8)

(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) Female Luer Coupler for setup without slide

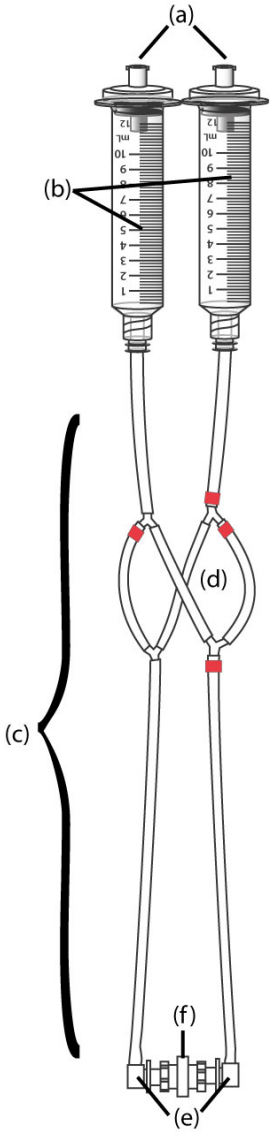


Figure 8 – Description of the Perfusion Set parts.

Perfusion Set Types: The Perfusion Sets are available with multiple inner diameters and tubing lengths.

Table 6 – Characteristics of the Perfusion Sets

Perfusion Set color code	ID Tubing	Tube Length	Total Working Volume	Dead Volume Tubing	Reservoir Size
Perfusion Set RED	1.6 mm	15 cm	12.3 ml	1.5 ml	10 ml
Perfusion Set YELLOW/GREEN	1.6 mm	50 cm	13.6 ml	2.8 ml	10 ml
Perfusion Set BLUE	0.8 mm	15 cm	11.3 ml	0.5 ml	10 ml
Perfusion Set WHITE	0.8 mm	50 cm	11.7 ml	0.9 ml	10 ml
Perfusion Set YELLOW	0.5 mm	15 cm	2.5 ml	0.5 ml	2 ml
Perfusion Set BLACK	0.5 mm	50 cm	2.7 ml	0.5 ml	2 ml

Sterilization and cleaning: All parts of the Perfusion Sets can be cleaned and sterilized by different techniques. Syringes, filters and slides are not autoclavable and must be removed before autoclaving or replaced with new parts. Replacement reservoirs (Filter/Reservoir Sets) are available for purchase (#10971, #10972 and #10974). Best results are achieved when a new Perfusion Set is used for every experiment.

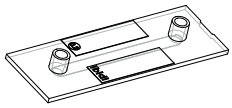
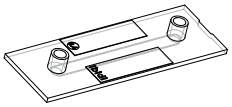
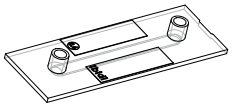
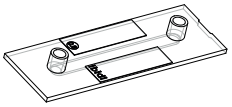
Table 8 – Sterilization Compatibilities of Perfusion Set Parts

	Autoclavable	Ethanol	Ethylene oxide
Filters	no	yes	yes
Syringe reservoirs	no	yes	yes
Tubing	yes	yes	yes
PP adapters	yes	yes	yes
µ–Slide	no	yes	yes

3.5 ibidi µ–Slides

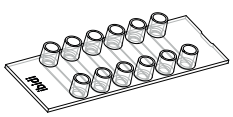
For flow applications, µ–Slides with different coatings and characteristics are available. All ibidi Channel Slides provide female Luer adapters for an easy connection to any flow setup via standard male Luer connectors (see flow accessories on the ibidi website).

The **µ–Slide I Luer** provides a single channel for all types of flow assays. Different versions of channel heights are available.

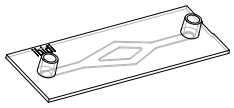





Channel height: 0.2 mm 0.4 mm 0.6 mm 0.8 mm

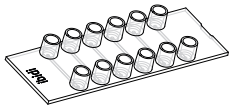
The **µ–Slide VI** $^{0.4}$ provides female Luer adapters and six independent channels for general flow assays.



The **µ–Slide y-shaped** consists of a channel with a bifurcation for flow assays within inhomogeneous fields of shear stress.



The **µ–Slide VI** $^{0.1}$ provides female Luer adapters and six independent micro–channels for general flow assays.



µ–Slide III $^{\mathbf{3in1}}$ offers three inlet channels leading to a middle channel with one outlet.

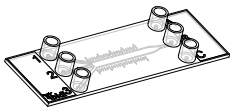


Table 10 – Overview of all available ibidi channel slide geometries with medium volumes and areas.

ibidi Channel Slides to use with 10 ml Perfusion Sets				
	Channel Height	Channel Volume	Growth Area	Coating Area
µ–Slide I $^{0.2}$ Luer	0.2 mm	50 µl	2.5 cm^2	5.2 cm^2
µ–Slide I $^{0.4}$ Luer	0.4 mm	100 µl	2.5 cm^2	5.4 cm^2
µ–Slide I $^{0.6}$ Luer	0.6 mm	150 µl	2.5 cm^2	5.6 cm^2
µ–Slide I $^{0.8}$ Luer	0.8 mm	200 µl	2.5 cm^2	5.8 cm^2
µ–Slide VI $^{0.4}$	0.4 mm	30 µl	0.6 cm^2	1.2 cm^2
µ–Slide y-shaped	0.4 mm	110 µl	2.8 cm^2	5.6 cm^2
µ–Slide III ^{3in1}	0.4 mm	60 µl	1.23 cm^2	3.05 cm^2

ibidi Channel Slide to use with 2 ml Perfusion Sets				
	Channel Height	Channel Volume	Growth Area	Coating Area
µ–Slide VI $^{0.1}$	0.1 mm	1.7 µl	0.17 cm^2	0.34 cm^2
µ–Slide III ^{3in1}	0.4 mm	60 µl	1.23 cm^2	3.05 cm^2

3.6 Slide and Perfusion Set Selection Guide

To set up a successful experiment, select a suitable Perfusion Set and µ–Slide for the specific application. In addition to shear stress, consider parameters, such as working volume, dead volume, and tubing length.
For the first experiment in a demo run, a red Perfusion Set (#10962) with an inner diameter of 1.6 mm and a µ–Slide I $^{0.6}$ Luer is recommended.
An overview of all available flow rates and shear stresses are detailed in the following table. The MIN values are based on the minimal working pressure of 5 mbar, the MAX values are based on the maximal working pressure of 95 mbar.

Perfusion Set Red				
Viscosity: 0.0072 dyn·s/cm^2	Flow rate		Shear stress	
Temperature: 37°C	Min.	Max.	Min.	Max.
µ-Slide I $^{0.2}$ Luer	2.5 ml/min	27.4 ml/min	9.0 dyn/cm^2	98.3 dyn/cm^2
µ-Slide I $^{0.4}$ Luer	5.2 ml/min	46.9 ml/min	4.8 dyn/cm^2	43.2 dyn/cm^2
µ-Slide I $^{0.6}$ Luer	5.4 ml/min	49.4 ml/min	2.3 dyn/cm^2	20.8 dyn/cm^2
µ-Slide I $^{0.8}$ Luer	5.4 ml/min	49.6 ml/min	1.3 dyn/cm^2	12.0 dyn/cm^2
µ-Slide VI $^{0.4}$	5.5 ml/min	46.7 ml/min	6.8 dyn/cm^2	57.6 dyn/cm^2
µ-Slide y-shaped*	5.1 ml/min	42.4 ml/min	8.2 dyn/cm^2	67.4 dyn/cm^2
µ-Slide III ^{3in1}**	2.5 ml/min	27.5 ml/min	4.0 dyn/cm^2	43.7 dyn/cm^2
Without any Slide	5.5 ml/min	52.5 ml/min	–	–

*The indicated values for the µ-Slide y-shaped refer to the single channel area (see Application Note 18.)
**The indicated values for the µ-Slide III ^{3in1} refer to the single channel area (see Application Note 11.)

Perfusion Set Yellow/Green				
Viscosity: 0.0072 dyn·s/cm^2	Flow rate		Shear stress	
Temperature: 37°C	Min.	Max.	Min.	Max.
µ-Slide I $^{0.2}$ Luer	2.0 ml/min	22.7 ml/min	7.2 dyn/cm^2	81.5 dyn/cm^2
µ-Slide I $^{0.4}$ Luer	3.8 ml/min	33.9 ml/min	3.5 dyn/cm^2	31.2 dyn/cm^2
µ-Slide I $^{0.6}$ Luer	3.9 ml/min	36.1 ml/min	1.7 dyn/cm^2	15.2 dyn/cm^2
µ-Slide I $^{0.8}$ Luer	4.2 ml/min	36.3 ml/min	1.0 dyn/cm^2	8.8 dyn/cm^2
µ-Slide VI $^{0.4}$	4.1 ml/min	35.1 ml/min	5.1 dyn/cm^2	43.3 dyn/cm^2
µ-Slide y-shaped*	3.8 ml/min	32.3 ml/min	6.1 dyn/cm^2	51.4 dyn/cm^2
µ-Slide III ^{3in1}**	2.0 ml/min	23.9 ml/min	3.2 dyn/cm^2	38.0 dyn/cm^2
Without any Slide	4.8 ml/min	37.2 ml/min	–	–

*The indicated values for the µ-Slide y-shaped refer to the single channel area (see Application Note 18.)
**The indicated values for the µ-Slide III ^{3in1} refer to the single channel area (see Application Note 11.)

Perfusion Set Blue				
Viscosity: 0.0072 dyn·s/cm^2	Flow rate		Shear stress	
Temperature: 37°C	Min.	Max.	Min.	Max.
µ-Slide I $^{0.2}$ Luer	0.65 ml/min	8.8 ml/min	2.3 dyn/cm^2	31.6 dyn/cm^2
µ-Slide I $^{0.4}$ Luer	0.87 ml/min	10.2 ml/min	0.8 dyn/cm^2	9.4 dyn/cm^2
µ-Slide I $^{0.6}$ Luer	0.88 ml/min	10.7 ml/min	0.37 dyn/cm^2	4.5 dyn/cm^2
µ-Slide I $^{0.8}$ Luer	0.90 ml/min	10.7 ml/min	0.22 dyn/cm^2	2.6 dyn/cm^2

µ-Slide VI $^{0.4}$	0.87 ml/min	10.7 ml/min	1.1 dyn/cm^2	13.1 dyn/cm^2
µ-Slide y-shaped*	0.84 ml/min	10.5 ml/min	1.4 dyn/cm^2	16.7 dyn/cm^2
µ-Slide III ^{3in1}**	0.76 ml/min	9.8 ml/min	1.2 dyn/cm^2	15.6 dyn/cm^2
Without any Slide	0.92 ml/min	10.9 ml/min	–	–

*The indicated values for the µ-Slide y-shaped refer to the single channel area (see Application Note 18.)
**The indicated values for the µ-Slide III ^{3in1} refer to the single channel area (see Application Note 11.)

Perfusion Set White

Viscosity: 0.0072 dyn·s/cm^2	Flow rate		Shear stress	
Temperature: 37°C	Min.	Max.	Min.	Max.
µ-Slide I $^{0.2}$ Luer	0.4 ml/min	4.7 ml/min	1.5 dyn/cm^2	16.8 dyn/cm^2
µ-Slide I $^{0.4}$ Luer	0.4 ml/min	5.4 ml/min	0.4 dyn/cm^2	4.9 dyn/cm^2
µ-Slide I $^{0.6}$ Luer	0.4 ml/min	5.4 ml/min	0.2 dyn/cm^2	2.3 dyn/cm^2
µ-Slide I $^{0.8}$ Luer	0.4 ml/min	5.4 ml/min	0.1 dyn/cm^2	1.3 dyn/cm^2
µ-Slide VI $^{0.4}$	0.4 ml/min	5.4 ml/min	0.5 dyn/cm^2	6.6 dyn/cm^2
µ-Slide y-shaped*	0.4 ml/min	5.4 ml/min	0.65 dyn/cm^2	8.5 dyn/cm^2
µ-Slide III ^{3in1}**	0.4 ml/min	5.4 ml/min	0.65 dyn/cm^2	8.5 dyn/cm^2
Without any Slide	0.4 ml/min	5.4 ml/min	–	–

*The indicated values for the µ-Slide y-shaped refer to the single channel area (see Application Note 18.)
**The indicated values for the µ-Slide III ^{3in1} refer to the single channel area (see Application Note 11.)

Perfusion Set Yellow

Viscosity: 0.0072 dyn·s/cm^2	Flow rate		Shear stress	
Temperature: 37°C	Min.	Max.	Min.	Max.
µ-Slide VI $^{0.1}$	0.15 ml/min	2.1 ml/min	10.0 dyn/cm^2	155.0 dyn/cm^2
µ-Slide III ^{3in1}**	0.25 ml/min	3.5 ml/min	0.4 dyn/cm^2	5.5 dyn/cm^2
Without any Slide	0.25 ml/min	3.7 ml/min	–	–

**The indicated values for the µ-Slide III ^{3in1} refer to the single channel area (see Application Note 11.)

Perfusion Set Black

Viscosity: 0.0072 dyn·s/cm^2	Flow rate		Shear stress	
Temperature: 37°C	Min.	Max.	Min.	Max.
µ-Slide VI $^{0.1}$	0.063 ml/min	1.0 ml/min	4.7 dyn/cm^2	73.0 dyn/cm^2
µ-Slide III ^{3in1}**	0.088	1.4	0.14 dyn/cm^2	2.2 dyn/cm^2
Without any Slide	0.09 ml/min	1.4 ml/min	–	–

**The indicated values for the µ-Slide III ^{3in1} refer to the single channel area (see Application Note 11.)

3.7 Computer with PumpControl Software

The ibidi Pump is controlled by the PumpControl Software that is installed on a laptop or a desktop computer. Using a laptop configured and approved by ibidi will ensure that all settings are correct. We cannot provide troubleshooting for a computer that was not set up by ibidi. The system requirements for the respective software versions are detailed in the download section of the ibidi website.

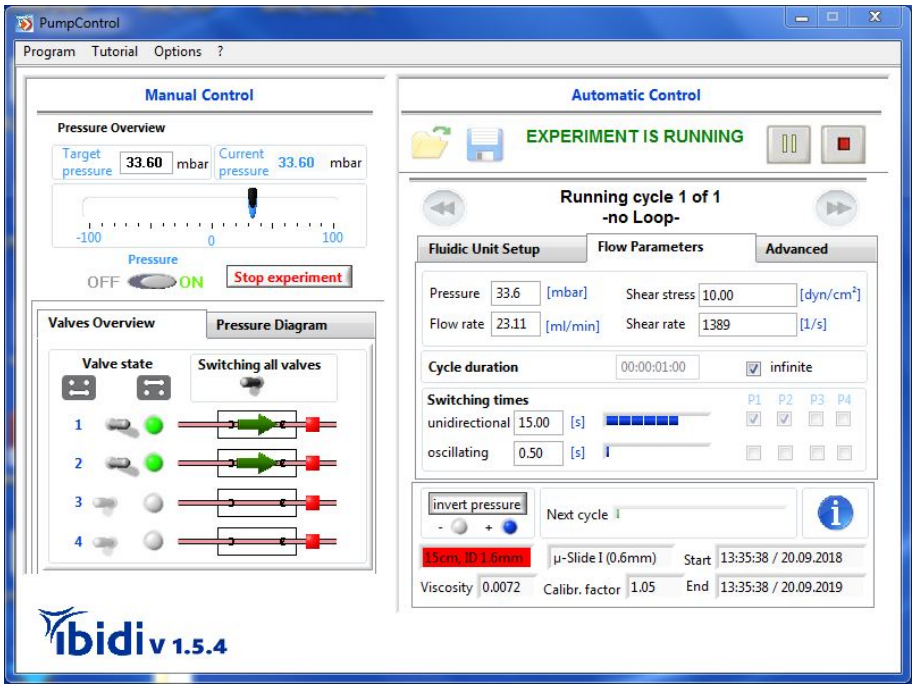


Figure 9 – PumpControl software

4 Quick Start Guide

This section provides an overview for the standard setup components of the ibidi Pump System with one Fluidic Unit in an incubator. All steps are described in detail in Section 5 and 6.

1. Place the pump on the working bench and connect the power supply.
2. Place the computer with installed PumpControl next to the pump and connect the power supply.
3. Connect the computer to the pump via the USB cable.
4. Place the Fluidic Unit inside the incubator.
5. Connect the air front port of the pump to the air pressure input of the Fluidic Unit with the 2 m air pressure tubing.
6. Connect the Fluidic Unit port with the electic connection of the Fluidic Unit with the Fluidic Unit cable.
7. Install the drying bottle according to the instructions on page 32 and connect it to the air rear port.

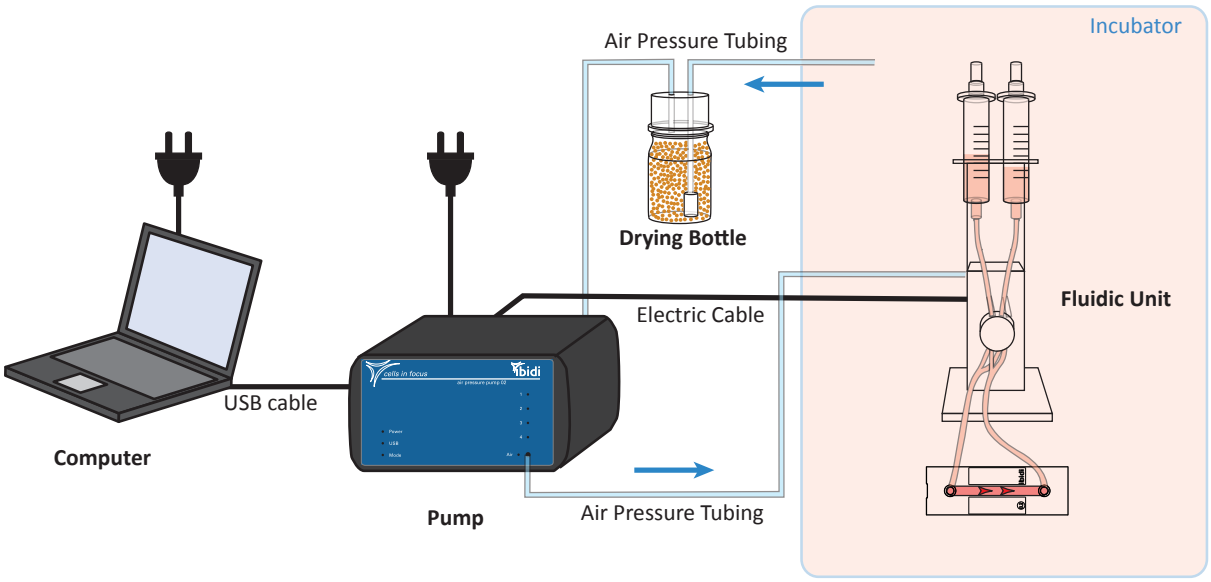


Figure 10 – ibidi Pump System standard setup using positive pressure. The setup using negative pressure is shown in figure 17.

Note!

For training purposes, it is recommended to install the entire system outside the incubator, and practice controlling the flow with deionized water.

5 Basic Setup

This section details how to connect all the components for a basic setup using a 10 ml Perfusion Set. For first–time users of the ibidi Pump System, it is best to set up the system outside the incubator and practice using deionized water.
Before setting up the experiment, make sure that you have all items listed in Section 3.1.

Important!

The ibidi Pump System is intended for use in combination with a cell culture incubator with 37°C, 5%CO_2 and 80-100% humidity!

5.1 General Setup of the Components

The computer and the pump are placed next to the incubator on a stable surface (e.g., work bench). When preparing an experiment with cells, the Fluidic Unit with the mounted Perfusion Set is placed inside the incubator. The pump remains outside and is connected to the Fluidic Unit with the electrical cable and air pressure tubing. The setup using positive pressure, which is recommended [1], is shown in Figure 11.

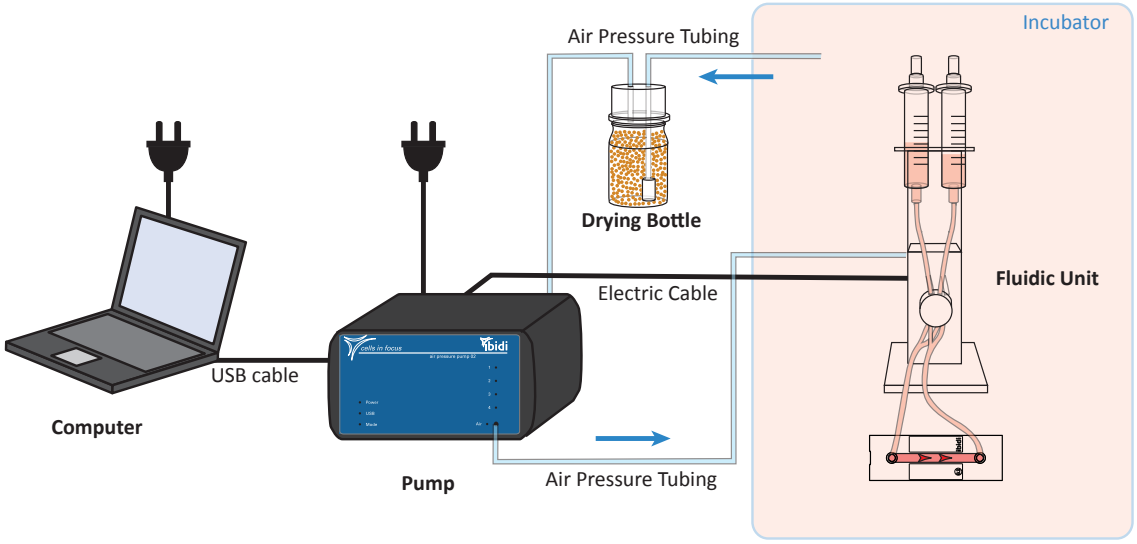


Figure 11 – Positive pressure system setup of one Fluidic Unit inside the incubator. The cables and tubing are inserted through the back port of the incubator.

There are several options for leading the tubing and cable(s) inside the incubator:

- The optimal configuration is through an opening in the back of the incubator. The tubing and cable can be passed through this opening, and then sealed with a suitable modified rubber cap to prevent leakage of heat and CO_2.

[1] The setup with negative pressure is shown in figure 17.

- If the incubator has no back port, lead the cable and tubing through the front door. Most incubators have a rubber seal that is flexible enough to introduce the connections directly. The air pressure tubing is rigid and will not be compressed by the door.

When working with positive pressure, the atmosphere from the incubator is drawn in through the pump's rear port to ensure a saturation of 5% CO_2. To prevent condensation inside the pump, a drying bottle (Section 5.5) is inserted. The setup is shown in figure 11. The modified setup for negative pressure is shown on page 34.

5.2 Installing the PumpControl Software

The PumpControl software comes pre-installed on the laptop that is delivered with the ibidi Pump System. If you need to install the software on a different computer, follow the instructions below.

Important!

The power and update options on a computer running PumpControl must be set so as not to interfere with the software. If you install PumpControl on another computer, set the power and update options as they are set on the laptop delivered with the Pump System. ibidi does not guarantee the function of PumpControl on computers other than the laptop delivered with the system.

The installation software for PumpControl is provided on a USB flash drive. If the setup does not auto–run after connecting the flash drive, click on the "setup.exe" file and the installation will begin. The installation includes both the PumpControl program and the runtime engine from National Instruments GmbH. Both programs will be installed following the two installation routines.
When the installation is finished and the PumpControl program is running, you will be able to program and control the ibidi Pump. The main window of the PumpControl software is shown in Figure 12. Detailed directions are in the PumpControl instruction manual.
If you cannot establish communication, refer to the Troubleshooting list in Section 10.5.

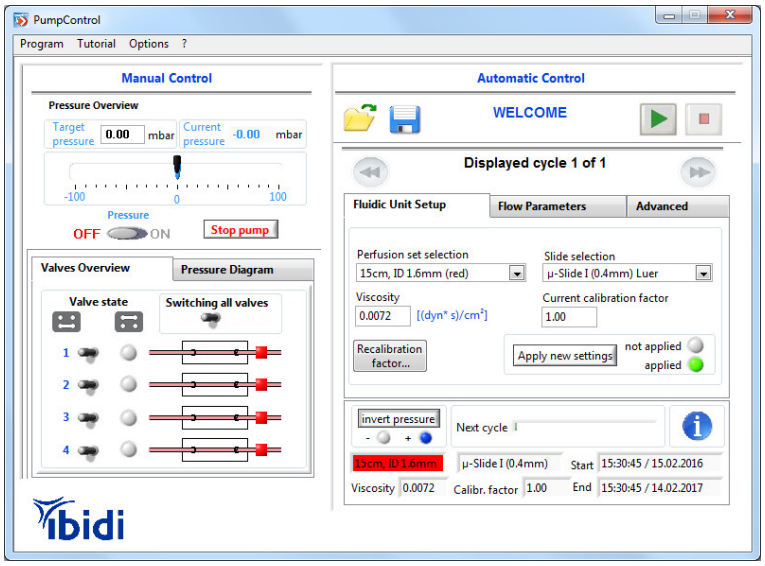


Figure 12 – Main window of the PumpControl software

5.3 Connecting the ibidi Pump to the Computer

The power supply and the USB cable are included. For stability reasons, it is imperative to use the included power supply and USB cable.

1. Power up the ibidi Pump with the power supply. To verify that power is connected, check the blue "Power" LED status on the front panel (Figure 13).
2. Connect the pump to the computer using the USB cable. The computer will automatically recognize the new hardware. To enable communication between the pump and the computer, two drivers are required. The drivers are automatically installed as part of the PumpControl software. After installation, the blue "USB" LED will be illuminated.

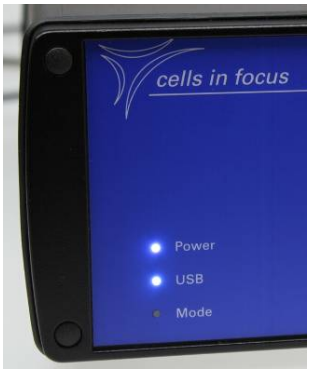


Figure 13 – LEDs on the pump's front panel indicating the connected USB and power supply.

If the LEDs do not light up, refer to the Troubleshooting pages in Section 10.4.

5.4 Connecting the Fluidic Unit to the Pump

While the pump itself is outside the incubator, the Fluidic Unit(s) is placed inside. There are two connections between the pump and the Fluidic Unit. The 2 m cable length is long enough to run the cable/tubing out through the back port or the door of the incubator:

- The Fluidic Unit cable sends switching signals from the pump to the Fluidic Unit.
- The air pressure tubing provides pressurized air to the Fluidic Unit and reservoirs.

The Fluidic Unit cable's plug is marked with a red dot that aligns with the red dot on the back of the Fluidic Unit. The other end of the electric cable is plugged into any of the Fluidic Unit ports on the back of the pump. The pump will automatically recognize which port is connected. If using positive pressure, connect the Fluidic Unit directly to the pump with the 2 m air pressure tubing (Figures 14 and 15). To ensure the correct CO_2 amount is being drawn into the pump, connect the drying bottle to the rear port of the pump and feed the second piece of tubing into the incubator (Section 5.5).

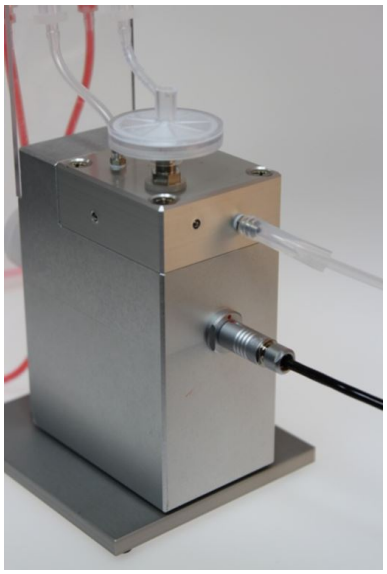


Figure 14 – Rear view of the Fluidic Unit with the air pressure tubing and the electric cable connected.

5.5 Drying Bottle

The drying bottle protects the pump from the moisture from the incubator. It must be inserted into the air line leading from the incubator to the pump. Failure to use the drying bottle will result in condensate inside the pump, which could damage the pump and cause it to malfunction.

5.5.1 Components of the Drying Bottle

The following parts are needed to set up the drying bottle (Figure 15):

(A) Glass bottle with orange Silica beads

(B) Connection cap with two openings. One opening is plugged with an Elbow Luer connector

(C) Short yellow–marked air pressure tube (0.6 m) for connection to the pump

(D) Long black–marked air pressure tube (2.1 m) for connection to the inside of the incubator

(E) Drying bottle filter

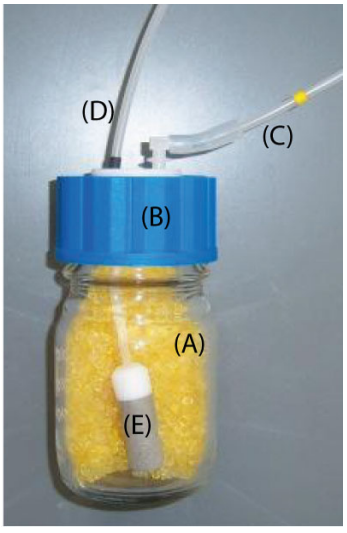


Figure 15 – Components of the Drying Bottle

5.5.2 Assembling the Parts of the Drying Bottle

The drying bottle is supplied with a standard G45 cap that closes tightly. To use the drying bottle replace the stock cap with the connection cap with tubing inserts.

1. Connect the yellow–marked tube to the Elbow Luer connector on the bottle cap.
2. Pass approximately 2 cm of the black–marked tube through the remaining opening in the cap until the marker reaches the cap.
3. Slide the filter on the end of the black marked tube.
4. Remove the stock cap from the drying bottle and replace it with the connection cap.
5. Turn the bottle upside down and push in the black–marked tubing until the filter reaches the bottom of the bottle.

The silica beads have an orange indicator that turns white when saturated with moisture. The silica beads can be dried and reused. Refer to Section 9.2 for detailed instructions.

5.5.3 Connection of the Air Pressure Tubing and the Drying Bottle

The drying bottle protects the pump from humidity coming from the incubator. Therefore, the bottle must be in-line with the tubing that connects the incubator and pump.
There are two options for applying pressure (see Figures 16 and 17). The two setup options are as follows:

- Positive pressure: the pump forces air out of the front port and into the reservoirs.
- Negative pressure: the pump draws in air from the front port, and therefore from the reservoirs.

ibidi recommends the application of positive pressure. For details please see Section 8.2.

Positive pressure: The air is forced out by the pump into the Fluidic Unit. In this setup the air intake is from the rear port and is pumped out through the front port. To ensure that the gas mixture contains enough CO_2 it is crucial to draw in the air from inside the incubator into the rear port of the pump. However, as water vapor is also present in the gas from the incubator, the drying bottle must be integrated between the incubator and pump.

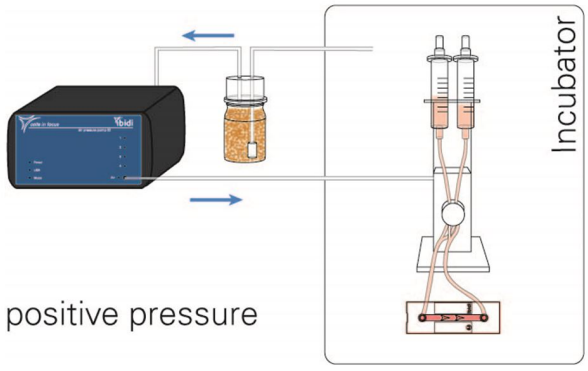


Figure 16 – Setup with positive pressure.

1. Place a sufficient length of the black–marked tubing (D) inside the incubator. Make sure that liquid does not enter the tubing!
2. Connect the yellow–marked tubing (C) to the back port of the pump.
3. Connect the 2 m tubing (F) to the front port of the pump and to the Fluidic Unit (inside the incubator).

Negative pressure: The air is drawn into the pump via the Fluidic Unit from inside the incubator. Because the Fluidic Unit is placed inside the incubator where the air is saturated with CO_2, the drying bottle must be placed between the Fluidic Unit and the front port on the pump.

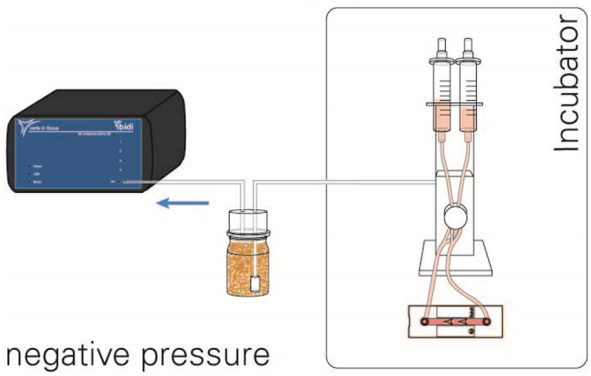


Figure 17 – Setup with negative pressure.

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

6 Setting Up an Experiment With Cells

This section explains all necessary steps to setup an experiment with cells. A more detailed cell culture protocol for performing an experiment with HUVEC under perfusion is provided in Application Note 13 "Endothelial Cells under Perfusion".

Note!

For first time use we recommend practicing handling with a non-sterile Perfusion Set and slide. Each Fluidic Unit is supplied with a non-sterile Perfusion Set.

6.1 Degassing of Slides, Tubing, and Medium

To avoid air bubbles, the degassing of all plastic components and the medium is critical. Place the following parts inside the incubator one day before starting the experiment. Sterility is maintained as long as the packaging is not opened.

- µ-Slides (within the packaging)
- Perfusion Set(s) (within the packaging)
- Cell culture medium for cell seeding (add the volume needed to a small vessel, and loosen the cap slightly)

This procedure is necessary because of the temperature dependency of gas solubility in water and plastic. At higher temperatures, water and plastic can absorb less gas than at lower temperatures. The solubility of O_2, N_2 and CO_2 in water is shown in Figure 18.

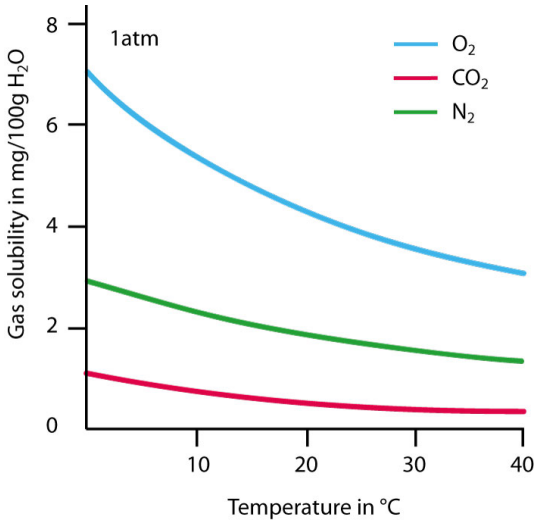


Figure 18 – Solubility of O_2, N_2 and CO_2 in water at 1 atm

If components have been stored at room temperature or in the refrigerator, gases dissolved in the plastic and liquids will be released when heated up in the incubator. Gas bubbles will then appear

inside the slide and tubing. Degassing all plastic components before the experiment will eliminate this effect.

Important!

Each time you take the system out of the incubator, the process of gas absorption begins again. Therefore work quickly at room temperature and never leave the Fluidic Unit outside the incubator for longer than 10 minutes.

6.2 Mounting a Perfusion Set on the Fluidic Unit

The tubing must be inserted correctly into the valve for proper valve switching and flow direction.
To facilitate the proper insertion, the sections of the tubing are marked with colored tabs (Figure 19).
It is best to mount the Perfusion Set on the sterile working bench immediately before filling the Perfusion Set with medium.

To mount the Perfusion Set onto the Fluidic Unit follow these steps:

1. Place the Fluidic Unit and the packaged Perfusion Set in a laminar flow hood.
2. Open the packaging and check the Perfusion Set connections before mounting.
   (a) Verify the connection between the reservoirs and tubing by screwing the adapters tightly into the reservoirs (Figure 20).
   (b) Make sure the Luer adapters in the Female Luer Coupler are secure (Figure 21).

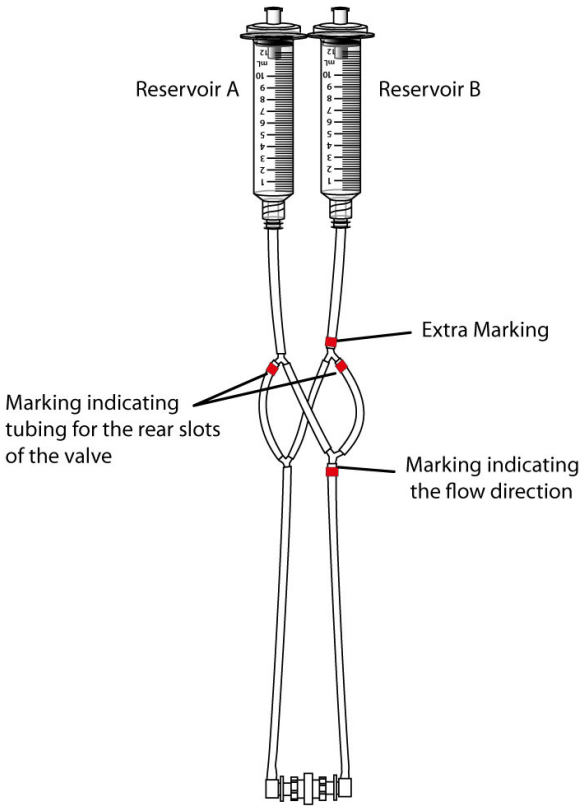


Figure 19 – Colored tabs on the Perfusion Set.

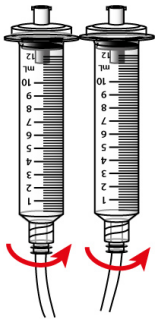


Figure 20 – Screw the adapters tight into the reservoirs.

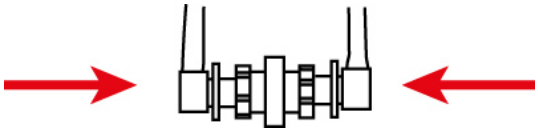


Figure 21 – Secure the Luer adapters in the Female Luer Coupler.

3. Insert the reservoirs into the holder. The reservoir connected to the tubing with the extra red marking (reservoir B) must be inserted into the right side of the holder (viewed from the front). Slightly squeeze the reservoirs for easy insertion (Figure 22).
4. Begin with the valve's **right side** slots. Insert the two sections of the tubing coming from reservoir B into these slots (Figure 23).
5. Perform the same procedure for the slots on the **left side** (Figure 24).

Figure 22 – Squeeze the reservoirs for insertion into the holder.

Figure 23 – Insert the tubing coming from reservoir B into the right side slots. The marked tubing goes in the rear slot.

Figure 24 – Insert the tubing coming from reservoir A into the left side slots. The marked tubing goes in the rear slot.

6. Check the correct position of the tubing in the openings of the pinch valve (Figure 25).

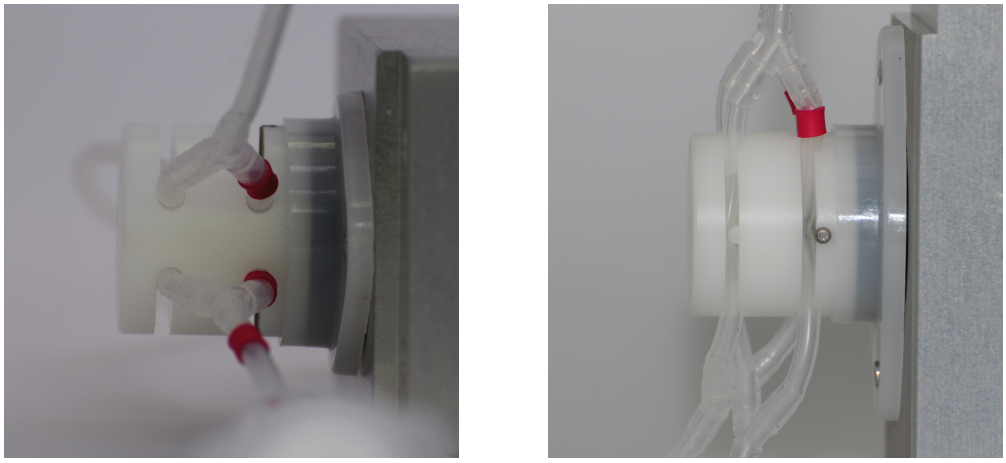


Figure 25 – Top view (left) and side view (right) of properly mounted tubing in the pinch valve.

Handling Tips:

For easy mounting, stretch the tubing and move it up and down. Stretch the tubing only between the y-connectors to ensure the tubing is not disconnected (Figure 26).
Verify that the Perfusion Set is mounted correctly. Check that the tubes are inserted such that the pinching bolt is closing the full diameter of the tubes by performing the pinch test with each mounted Perfusion Set (Section 6.5).

Important!

The pinch valve must never come in contact with liquid. If medium or any other liquid comes in contact with the pinch valve, proper function is no longer guaranteed.

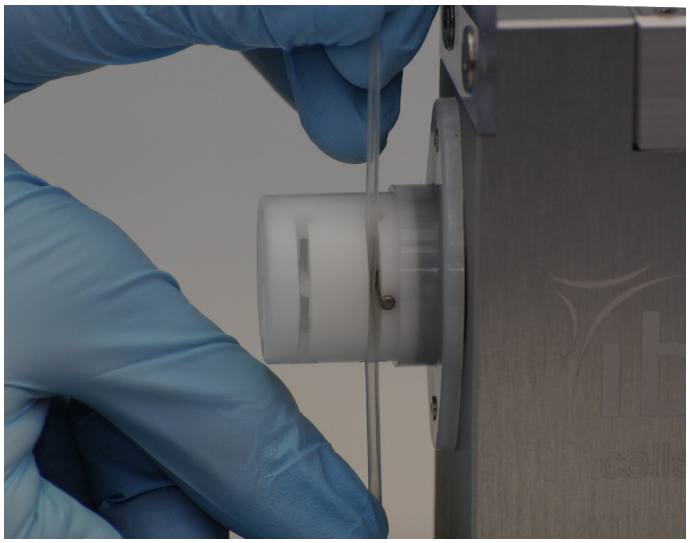


Figure 26 – Move the tubing up and down while stretching for easy placement into the valve slots.

6.3 Filling the Perfusion Set with Medium

To maintain sterility inside the Perfusion Set, do not disconnect any tubing adapter or reservoir filter outside the laminar flow hood.

1. The Fluidic Unit with the mounted Perfusion Set must be placed in a laminar flow hood.
2. First connect the air pressure tubing to the reservoir filters (Figure 27) and then pull off both filters from the syringe.
3. Fill in the required amount of medium appropriate for the Perfusion Set being used (Figure 28). The correct amount of medium is indicated in table 6 on page 23.
4. Put the filters back on the syringes and place the Fluidic Unit back in the incubator.
5. Proceed with removing the air bubbles (Section 6.4).

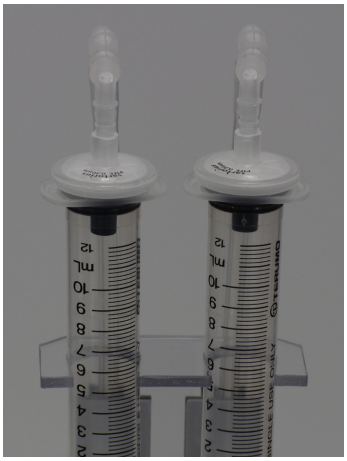


Figure 27 – Connect the filters of the Perfusion Set to the Fluidic Unit tubing.

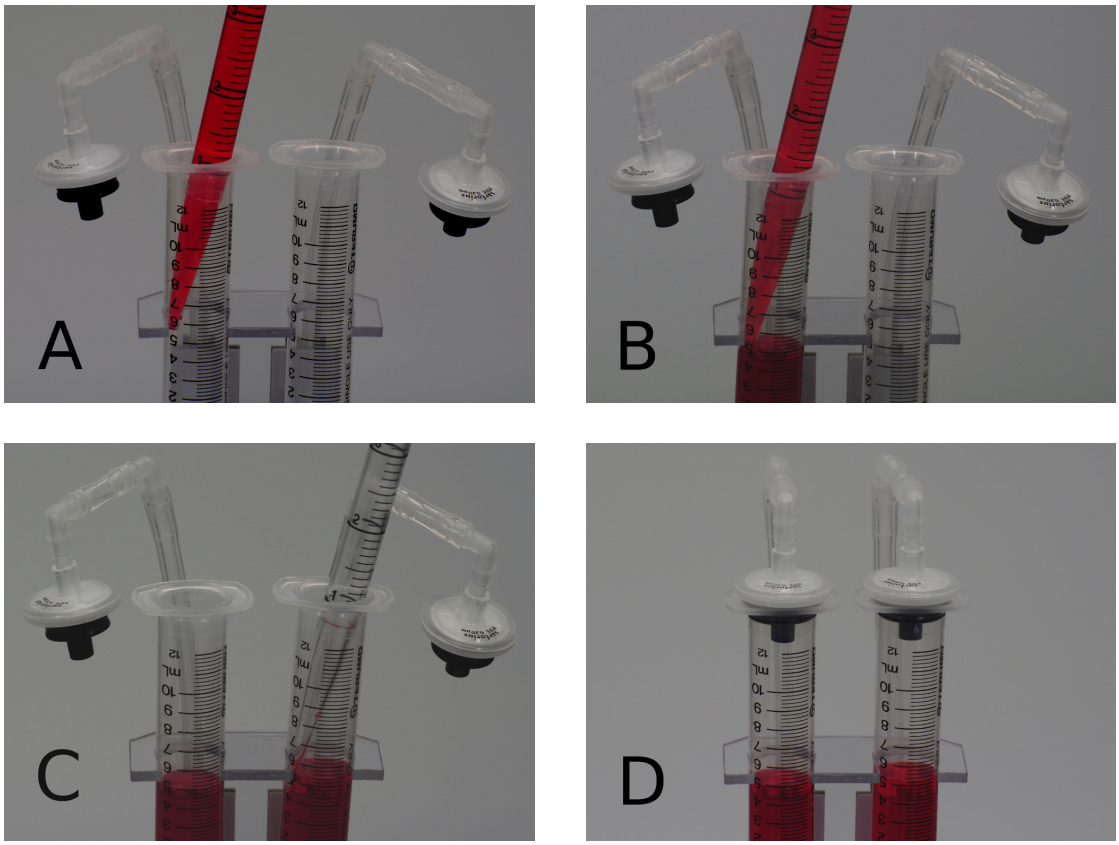


Figure 28 – Fill the syringe reservoirs with medium to equal levels and connect the filters to the reservoirs again.

6.4 Remove Air Bubbles from the Perfusion Set

Once the system is set up and the Perfusion Set contains medium, air bubbles remaining in the branched tubing arms must be removed. To protect the cells from being flushed out, it is critical to remove all air bubbles from the Perfusion Set before connecting it to a slide containing cells.
To remove air bubbles, start the pump with the PumpControl software. Refer to the software manual for detailed instructions.

1. Equilibrate the liquid levels of the two reservoirs using the manual control panel in the PumpControl software.
2. Set an automatic cycle with a high pressure (50-80 mbar) and let the cycle run for at least 5 minutes. Then check the flow tubing to confirm that all the air bubbles have been removed.

Handling Tip!

If working with one Perfusion Set connected to port 1 of the pump, you can load a pre-installed routine in the software "Remove air bubbles" (Tutorial → Load demo setups → Remove air bubbles) and let it run for at least 5 minutes.

6.5 Pinch–Test

Important!

The pinch–test must be performed with each newly inserted Perfusion Set. This test makes sure that the tubing is inserted correctly into the pinch valve.

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

2. Pinch the tubing in the lower loop near the Female Luer Coupler (Figure 29).

3. Observe the movement of the liquid levels in the reservoirs. While the tubing is pinched, the liquid should stop moving. Make sure you check both switching positions (State 1 and State 2). → If there is no movement, the setup is correct, otherwise check the mounting of the Perfusion Set.

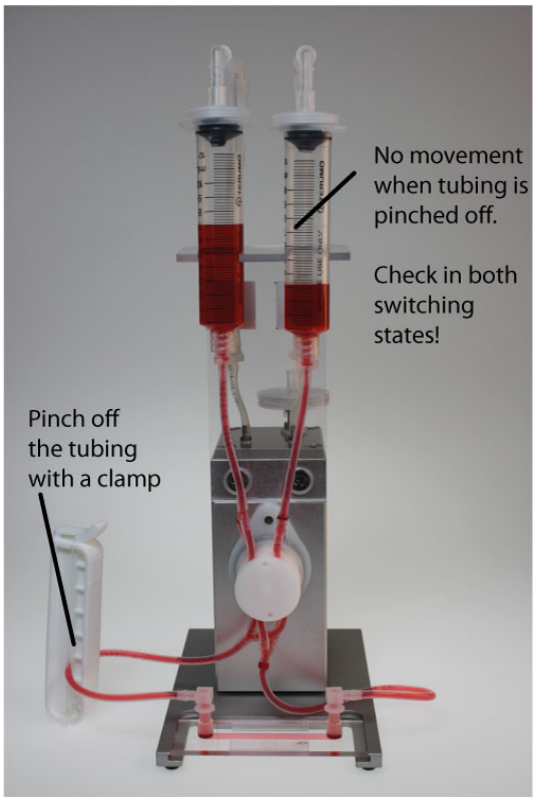


Figure 29 – Pinch–test for checking the correct insertion of the tubing into the valve.

Does the liquid move (switching state 1)?: □Yes □No
Does the liquid move (switching state 2)?: □Yes □No
If the answers are "No" in both cases, the insertion is correct.

If the liquid is moving in one or both of the positions, check the insertion of the tubing in the pinch valve. Stretch the tubing and move it up and down for proper placement in the valve's slot (Figure 26). Also check for correct positioning of the tubing (Figure 25). If this action does not correct the problem, contact ibidi or your local distributor.

6.6 Calibration of the Flow Rate

The PumpControl software provides an automatic calculation of pressure, flow rate and shear stress, once the Perfusion Set, the slide and the viscosity of the medium are set. One of the parameters (pressure, flow rate and shear stress/shear rate) is chosen, and the other parameters are calculated automatically, according to the relation below (calibration curve and shear stress calculation).

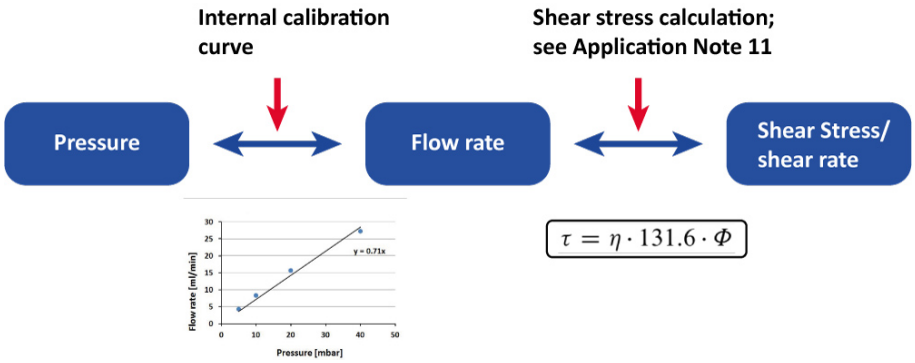


Figure 30 – The correlations among pressure, flow rate and shear stress in the ibidi Pump System.

Because of manufacturing tolerances, setup variations, and temperature fluctuations, the values for each Perfusion Set can differ from the values given in the software. To obtain the required experimental flow rate, we highly recommend performing a system calibration.

6.6.1 Connect the Calibration Slide to the Perfusion Set

Before calibrating the system, a sterile µ–Slide (the same type as in your experiment) without cells must be connected to the Perfusion Set.

1. Check the tubing for air bubbles and use the hose clip to clamp both tubing arms of the Perfusion Set tubes directly beneath the valve (Figure 31). This action allows the two ends of the Perfusion Set to be disconnected without spilling the medium.

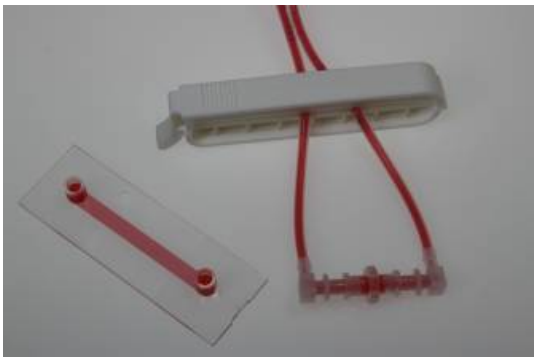


Figure 31 – Clamp the tubing before opening the Female Luer Coupler of the Perfusion Set.

2. Before connecting the Perfusion Set to the µ–Slide, the reservoirs of the slide must be filled to the top to avoid trapping bubbles in the reservoir (Figure 32). For a detailed description, refer to Application Note 13, “Endothelial Cells Under Perfusion”.

3. Remove the hose clip to start the flow rate measurement.

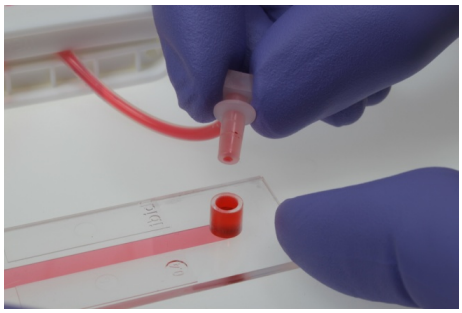


Figure 32 – Fill the reservoir before connecting the Luer adapter.

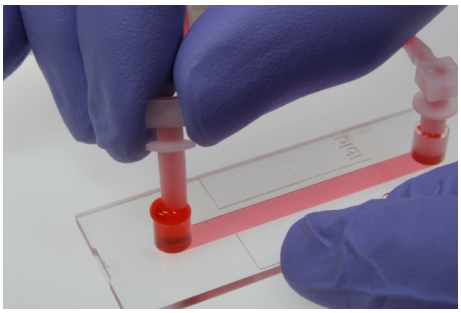


Figure 33 – Connect the second Luer adapter to the µ–Slide.

6.6.2 Flow Rate Measurement

Use a stop watch to measure and calibrate the flow rate.

Important!

The system must be calibrated with the same slide and flow rate you want to use in your experiment.

The measurement is explained with the following example:

Parameter	Example setup
Slide	µ–Slide I 0.6 Luer
Perfusion Set	RED (15 cm, ID 1.6 mm)
Shear stress	10 dyn/cm^2
Flow rate required to obtain 10 dyn/cm^2	23.8 ml/min
Calibration factor (default)	1.0
Viscosity of medium	0.007 dyn*s/cm^2

The table shows that for a shear stress of 10 dyn/cm^2 in this setup, a flow rate of 23.8 ml/min is required (see Application Note 11). To make sure the flow rate is exactly 23.8 ml/min, measure it manually as follows:

1. Set up the perfusion experiment with the Perfusion Set RED and medium inside a cell culture incubator (5% CO_2, 37°C).
2. Connect µ–Slide I 0.6 Luer (Section 6.6.1).
3. Open the PumpControl software. In the "Fluidic Unit Setup" tab, choose the RED Perfusion Set and "µ–Slide I 0.6 Luer" from the drop-down menu.
4. Insert a viscosity of 0.007 dyn*s/cm^2 in the box, then click the "Apply Settings" button.
5. In the "Flow Parameters" tab, enter the shear stress (10 dyn/cm^2) in the respective box. The flow rate (23.8 ml/min) and pressure (33.1 mbar) are now calculated automatically.
6. Equilibrate the fluid levels to 5 ml in both reservoirs.

7. Run the program in the manual control mode, applying 33.1 mbar. Take care to switch the valve before the reservoir runs dry.
8. Measure the time t (in seconds) required for the medium to flow from the 6 ml mark down to the 4 ml mark on the syringe (2 ml volume) with a stopwatch.

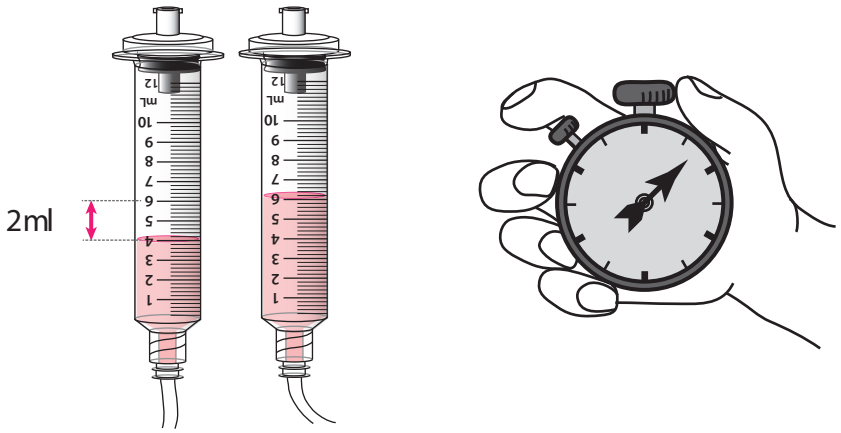


Figure 34 – Measure the time required for the medium to flow from the 6 ml mark down to the 4 ml mark (2 ml volume) with a stopwatch.

9. Conduct at least four time measurements and calculate the mean value. If necessary, perform more measurements to optimize your measurement error.
10. To calculate the flow rate [ml/min], insert the time that was measured (mean value in seconds) in the formula below.

$$\boxed{\Phi\left[\frac{ml}{min}\right] = \frac{2ml \cdot 60\frac{s}{min}}{t[s]}} \quad (1)$$

Example experiment: The mean value of the measurements is 5.7 seconds. This means the actual flow rate is 21.05 ml/min versus 23.8 ml/min as predicted in the software.

Important!

The flow rate is temperature dependent. Perform this measurement under experimental conditions (e.g. at 37°C in the incubator).

6.6.3 Flow Calibration in the Software

Now use the measured flow rate to update the PumpControl software in the "Recalibration factor" menu.

1. Click on the "Recalibration factor" button to open the dialog (Figure 35).
2. Insert the given flow rate and the one that was measured of yourself in the recalibration dialog (Figure 35). Then press "OK".

The software will now compensate the difference in measured and expected flow rate by means of a multiplicative calibration factor.

Example experiment:	
Given flow rate	23.8 ml/min
Measured flow rate	21.05 ml/min
Resulting calibration factor	1.131

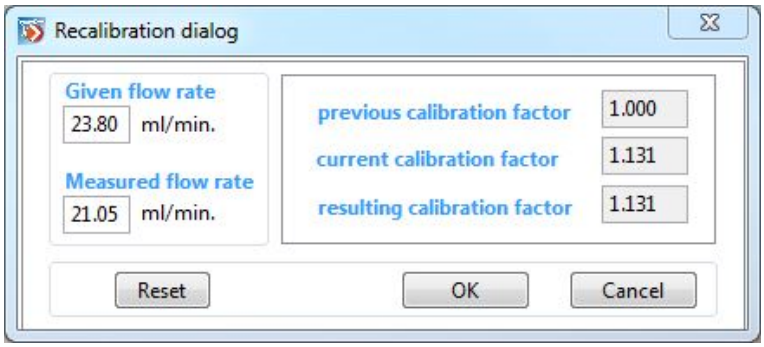


Figure 35 – Recalibration dialog in the PumpControl software.

The recalibration factor affects the relationship between pressure and resulting flow rate (Figure 36).

Figure 36 – Influence of the calibration factor and viscosity on the parameters of the PumpControl software.

Handling Tip

If you are re-using the Perfusion Sets, e.g., after autoclaving the tubings and adapters, note the calibration factor for the next experiment. When starting a new experiment you can immediately enter the calibration factor into the corresponding box.

For special setups, like a custom slide, several slides connected serially or slides which are not implemented in the software, the calibration is mandatory (Section 7.2).

6.7 Connect the Cell-Seeded Slide to the Perfusion Set

Note!

After the calibration remove the cell-free slide and re-connect the female Luer middle connector. Once again remove the air bubbles and then connect the slide with the cells.

Three points are imperative when connecting the µ–Slide to the Perfusion Set:

- Avoid air bubbles, which can remove seeded cells from the slide.
- Maintain sterility by proper sterile handling while seeding and pre-cultivating cells, as well as filling the reservoirs.
- Avoid disturbance of the cells, such as strong temperature variations or fast flow pulses.

The connection procedure is the same as shown in Section 6.6.1. For a detailed description, refer to Application Note 13, "Endothelial Cells Under Perfusion".

6.8 Start the Flow

After connecting the slide to the tubing, check the cells under the microscope. It is crucial that the cell layer is confluent and well adherent when it is exposed to shear stress. If the cells are stressed it may be better to let them recover before initiating flow.
To start flow, replace the whole assembly (Fluidic Unit and slide) in the incubator and connect the Fluidic Unit to the pump (air pressure tubing and electric cable). Start the flow by switching on the air pressure pump with the ibidi Pump Control software (see Application Note 13 and PumpControl instructions).

6.9 Observing Cells at the Microscope

To observe your cells on the microscope stop the pump when the levels in the reservoirs are equilibrated. Then detach the air pressure tubing and the electric cable of the Fluidic Unit. Take the Fluidic Unit with the connected µ-Slide to the microscope, put it beside the specimen holder, and place the slide into the holder.
After the observation, place the Fluidic Unit and slide back in the incubator and restart the flow.

Note!

This procedure does not affect sterility! Take care not to disconnect any adapter or the filters on top of the Fluidic Unit reservoirs!
Make sure that the Fluidic Unit does not stay longer than 10 minutes outside the incubator to avoid cooling effects, such as e.g. stress on the cells or air bubble generation!

6.10 Medium Exchange

A medium exchange is necessary when the nutrients in the medium are used up by the cells (pH) or if evaporation exceeds a certain level (e.g. 5%). When a medium exchange is necessary will depend on your setup.

When changing the medium, never let the tubing dry out! Otherwise air bubbles may form in the tubing.
There are two options to exchange medium: First you can exchange only the medium in the reservoirs, which is typically done for endothelial cells which need a certain proportion of pre-conditioned medium. Second, if you wish to perform a complete exchange, you must push out the used medium from the tubing with fresh medium.
In principal, to perform a medium exchange you need to follow these steps (values in brackets apply to the 2 ml reservoirs):

1. Stop the pump when the medium is equilibrated at 5 ml (1 ml).
2. Disconnect the Fluidic Unit (air pressure tubing and electric cable) and move it to a sterile flow hood.
3. Perform the medium exchange (see the two options below).
4. Transfer the Fluidic Unit back to the incubator, reconnect it and start the program again.

6.10.1 Medium exchange with left-over conditioned medium (option 1):

1. Carefully remove the medium in the reservoirs with a pipette. Make sure that you do not remove any medium from the tubing! Otherwise air bubbles will be introduced.
2. Refill both reservoirs to the 5 ml (1 ml) mark.

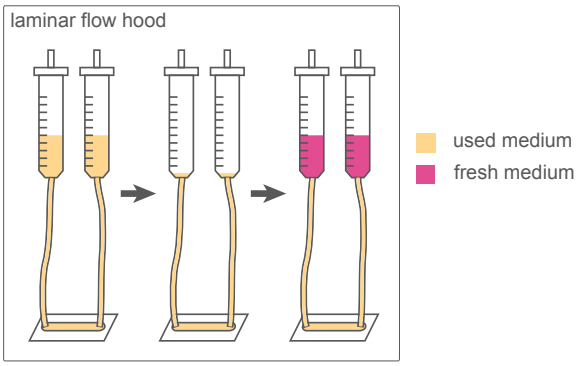


Figure 37 – Medium exchange with a left-over of used medium in the tubing.

6.10.2 Complete medium exchange (option 2):

1. Carefully remove the medium in the reservoirs with a pipette. Make sure that you do not remove any medium from the tubing!
2. Fill one reservoir to the 5 ml (1 ml) mark with fresh medium and await until the medium equilibrates at $\sim$ 2.5 ml (0.5 ml).
3. Remove the used medium from the reservoir.
4. Fill both reservoirs to the 5 ml (1 ml) mark with fresh medium.

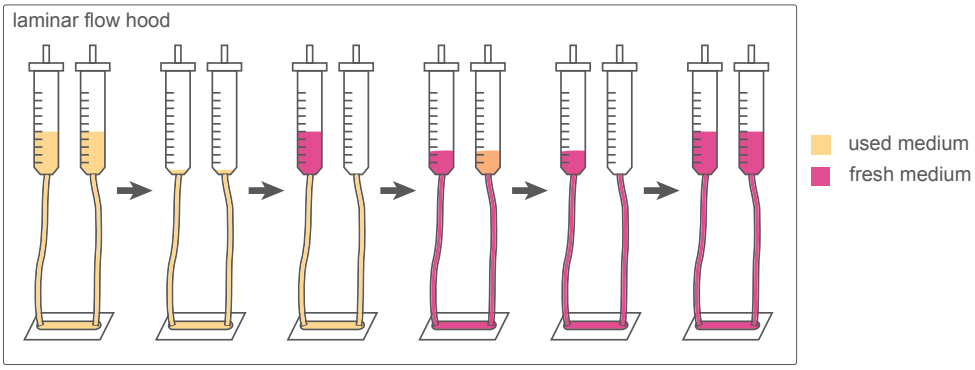


Figure 38 – Complete medium exchange.

7 Installation of Special Setups

7.1 Working With Two or More Fluidic Units

This section describes using the ibidi Pump with a parallel setup of up to four Fluidic Units, working with **positive pressure**.

7.1.1 Installation of Two or More Fluidic Units

The setup of the Pump System is identical to the one Fluidic Unit setup, except for the pressured air tubing. Branched air tubing for use with two, three, or four Fluidic Units is supplied with the pump.

- Connect the Fluidic Units with the pump via the Splitter Sets as shown in Figure 39 (example with 4 Fluidic Units).
- Connect all Fluidic Unit cables to the Fluidic Units and the pump.
- The drying bottle must be installed between the rear port of the pump and the incubator (Section 5.5.3).
- Mount the Perfusion Sets as explained in Section 6.2.

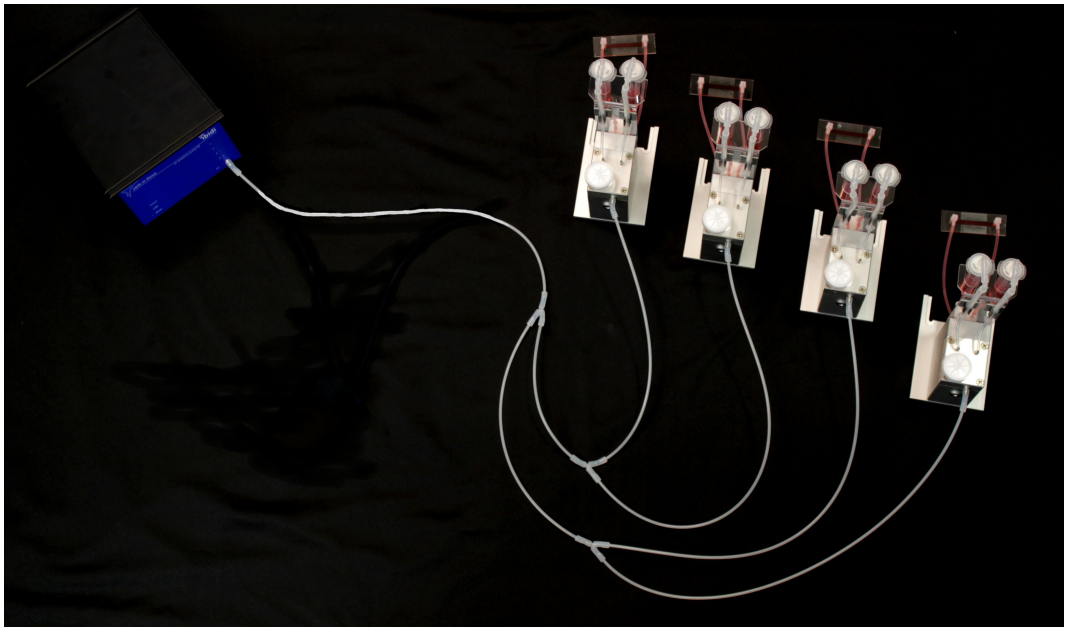


Figure 39 – Connection of the ibidi Pump and four Fluidic Units via the Splitter Set for 4 Fluidic Units.

7.1.2 Flow Calibration Of Two or More Fluidic Units

To calibrate a system running with more than one Fluidic Unit, we recommend measuring the flow rate of each Fluidic Unit separately. The flow calibration procedure is outlined in Section 6.6.
Prepare your Fluidic Units and Perfusion Sets as follows:

1. Install the system as described in Section 7.1.1.
2. Pinch off the tubing of all Perfusion Sets that are not to be calibrated (3 if you are working with 4 Fluidic Units). It is sufficient to pinch one of the tubing branches, as shown in the pinch test (Figure 29).
3. Expel the air bubbles from the remaining Perfusion Set by means of PumpControl manual control (6.4).
4. Perform the Pinch Test (Section 6.5).
5. Equilibrate the liquid levels.
6. Measure the flow rate as described in Section 6.6.
7. Note the flow rate in your documentation.
8. Perform step 2 to 7 for each Fluidic Unit.
9. Finally, calculate a mean flow rate for all Fluidic Units. For reproducible results, the variation of the measured flow rates should not be higher than 10% between Fluidic Units.
10. Insert the mean value in the box "measured flow rate" as explained in Section 6.6.3.

7.2 Calibration of the Flow Rate with Several Slides in Serial Connection

A calibration before starting an experiment with serially connected slides is mandatory. When connecting several slides to one Fluidic Unit, flow resistance is increased, leading to decreased flow rate with significant differences to the standard setup.
The calibration procedure is the same as described in Section 6.6. See Application Note 25 "Serial Connection of Flow Chambers" for instructions on how to connect several slides serially.

Note!

When connecting several slides serially the flow rate (and thus the shear stress) is the same in all slides!

7.3 Instructions for Oscillatory Flow Experiments

For oscillatory flow applications, at least two Fluidic Units are required (one "master" and one "slave") to separate the switching events of the two valves. In oscillatory flow experiments, the master Fluidic Unit has a long switching time t_{master} for controlling the liquid levels in the reservoirs. During t_{master} a constant air flow is supplied to one reservoir of both master and slave Fluidic Unit. The master Fluidic Unit switches before the reservoir runs dry. The switching time of the slave Fluidic Unit t_{slave} can be set to a fraction of t_{master}. The flow direction of the slave Fluidic Unit is reversed each time it switches the valve.
One master Fluidic Unit can control multiple slave Fluidic Units, so with a single pump one can run an experiment with up to three oscillatory slave Fluidic Units. The master Fluidic Unit can only be used for unidirectional flow experiments. All slave Fluidic Units can apply oscillatory or unidirectional flow.

Table 21 – Possible setups of oscillatory flow depending on the number of available Fluidic Units

Number of Fluidic Units	Possible setups
2 Fluidic Units	1 master, 1 slave
3 Fluidic Units	1 master, 2 slaves
4 Fluidic Units	2 masters, 2 slaves
or	1 master, 3 slaves

Important!

The lifespan of Perfusion Set's silicone tubing is dependent on the number of switching events. The material fatigues after 500 000-1 000 000 pinching cycles. To prolong the lifespan of the tubing, change the position of the tubing inside the valve slightly.
For best results, do not re-use the Perfusion Sets, because material fatigue is compounded by use.

7.3.1 Setting up Oscillatory Flow with Two Fluidic Units

In addition to the two Fluidic Units and Perfusion Sets, the respective air tubing splitters for two Fluidic Units are required (Figure 40).

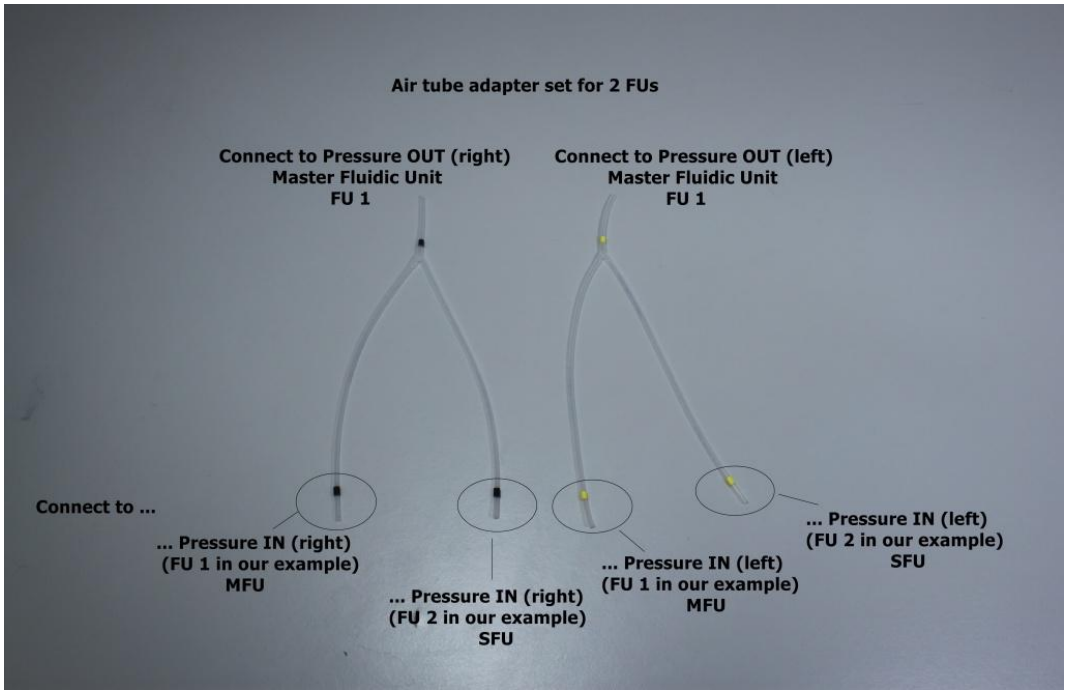


Figure 40 – Air pressure splitter, for oscillatory flow for two Fluidic Units.

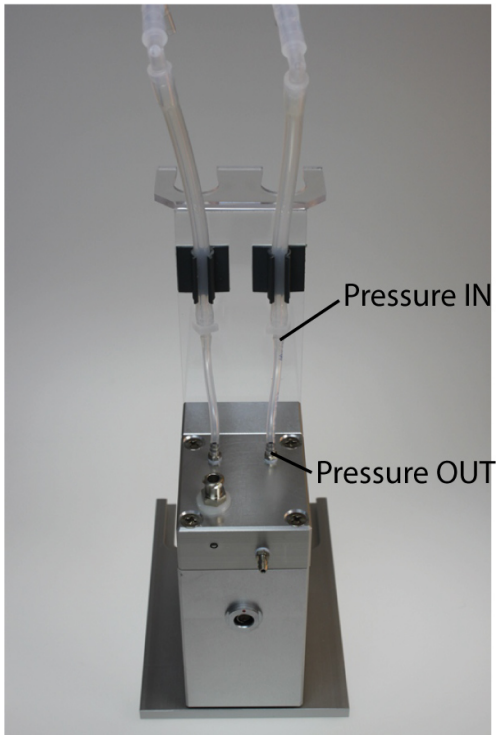


Figure 41 – Parts of the Fluidic Unit where the air splitter sets are connected.

Follow the steps below for correct installation and compare with Figure 41.

1. Connect the air pressure tube (2 m) of the pump to the master Fluidic Unit.
2. Use the yellow–marked splitters to connect the left “Pressure OUT” port of the Master Fluidic Unit to the following:
   (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 this example, “Port 1” for the master Fluidic Unit and “Port 2” for the slave Unit are used (Figure 42).
5. Mount the Perfusion Sets on both Fluidic Units as in the standard setup (Section 6.2) and calibrate the flow rate separately as described in Section 7.1.2, both under unidirectional flow. The master Fluidic Unit generates unidirectional flow, whereas the slave Fluidic Unit generates oscillatory flow. If no unidirectional flow is needed, mount an empty Perfusion Set or insert some tubing pieces into the valve to protect the pinch valve.

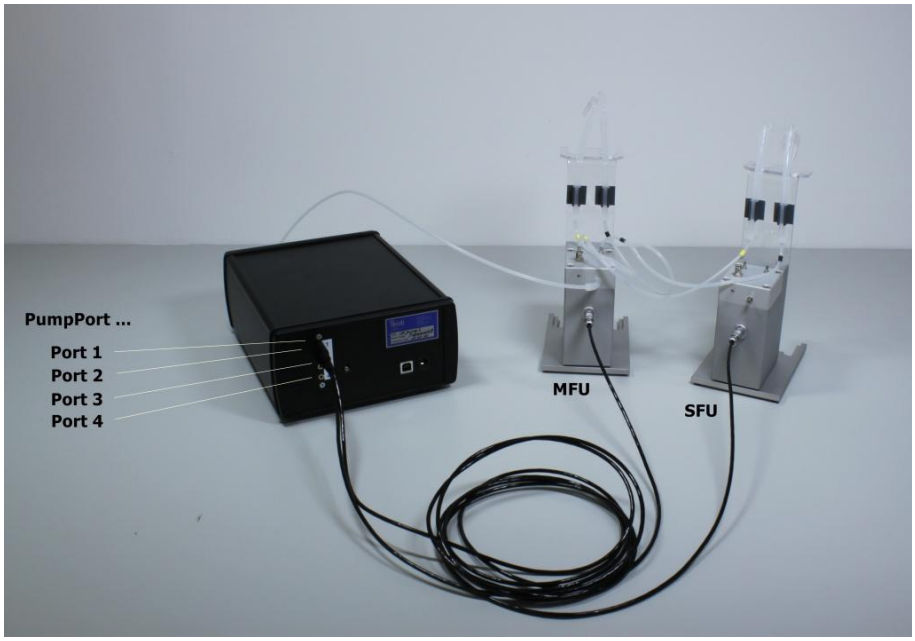


Figure 42 – Oscillatory flow setup with two Fluidic Units (one master and one slave).

7.3.2 Oscillatory Experiment with Four Fluidic Units

For an experiment using one master Fluidic Unit and three oscillatory slave Fluidic Units, a corresponding air pressure splitter is required (Figure 43).

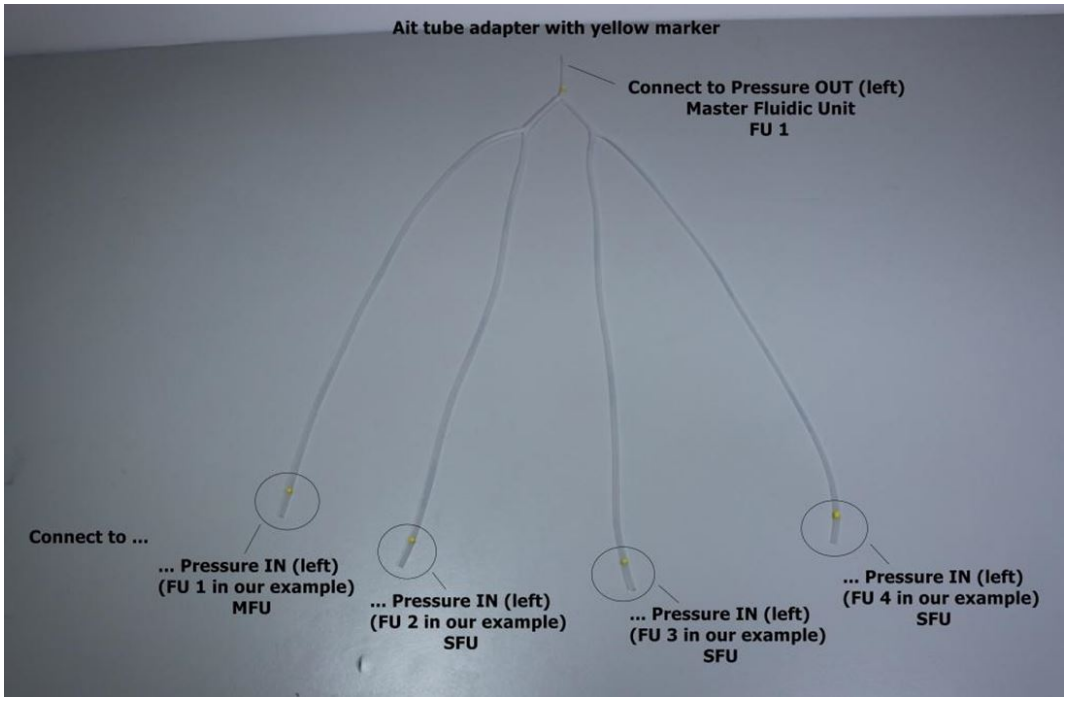


Figure 43 – Air pressure splitter, for oscillatory flow for four Fluidic Units.

The setup is similar to the setup with two Fluidic Units. The air pressure is distributed by the master Fluidic Unit to the slave Fluidic Units by splitting the air pressure from the master Fluidic Unit's "Pressure OUT" to the "Pressure IN" of master and slave Fluidic Units (Figure 44).

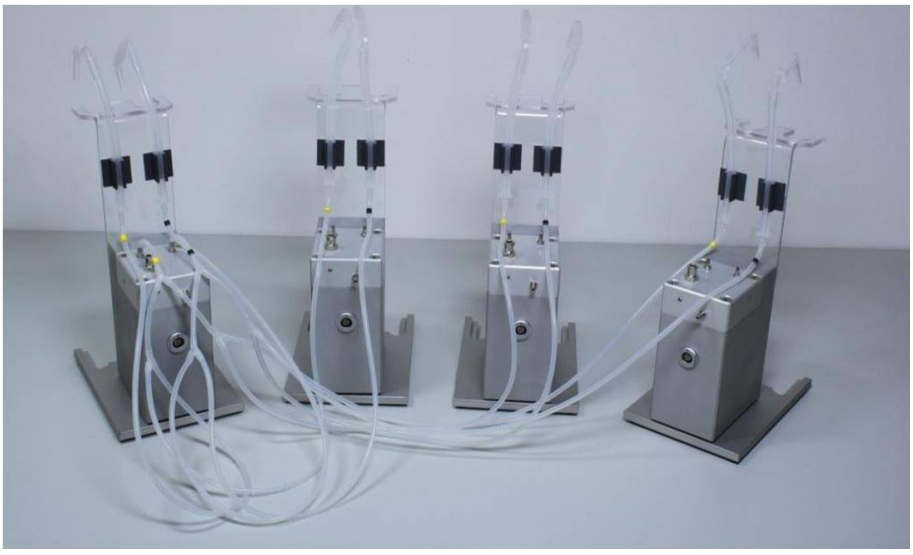


Figure 44 – Oscillatory flow setup with four Fluidic Units (one master and three slaves).

7.3.3 Settings within the PumpControl Software

Because the switching times are different for the master and the slave Fluidic Units, the PumpControl software must be programmed accordingly. Set the checkboxes for “unidirectional” and “oscillatory” valves. Figure 45 shows how to correctly set the corresponding parameters.

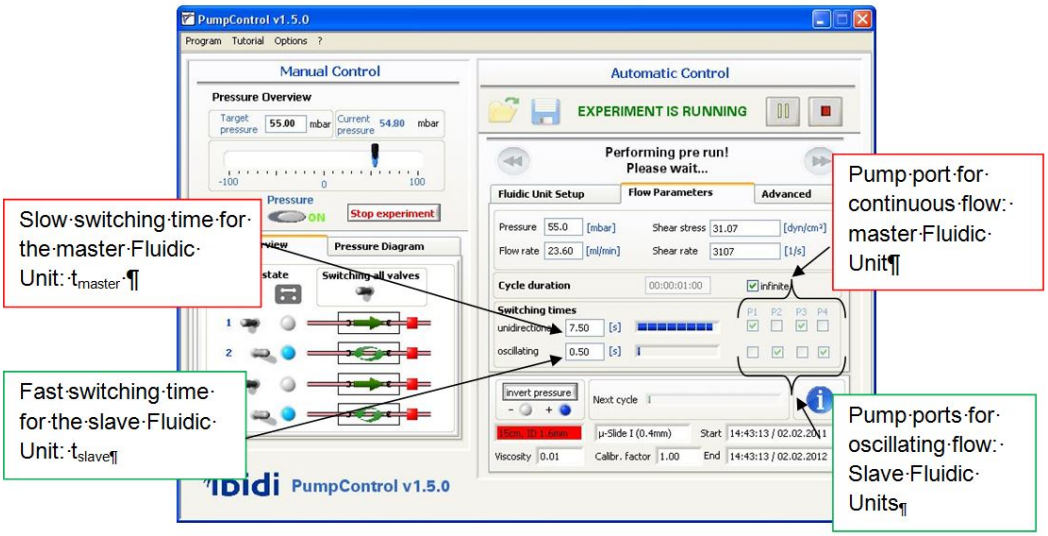


Figure 45 – Settings of the PumpControl Software, when applying oscillatory flow to two master and two slave Fluidic Units.

7.3.4 Equilibrating the Master and Slave Fluidic Units

Because the master and slave Fluidic Unit(s) are connected to the same air pressure and switching time, equilibration of the liquid levels must be performed separately. T stop the liquid movement in the reservoirs of either Fluidic Unit, the Perfusion Set must be clamped with a hose clip. Adjust the reservoir liquid levels to 5 ml each and go on to the next Fluidic Unit.

Note!

Do not forget to perform a pinch–test with each Fluidic Unit (Section 6.5)!

8 Technical Details

8.1 Working Principle of the ibidi Pump

The ibidi Pump and the ibidi Fluidic Unit work together to create a unidirectional, oscillatory, or pulsatile flow of medium within ibidi channel slides. The working principle of the pump is explained in the following figure, which details air pressure, flow rate and shear stress correlations.

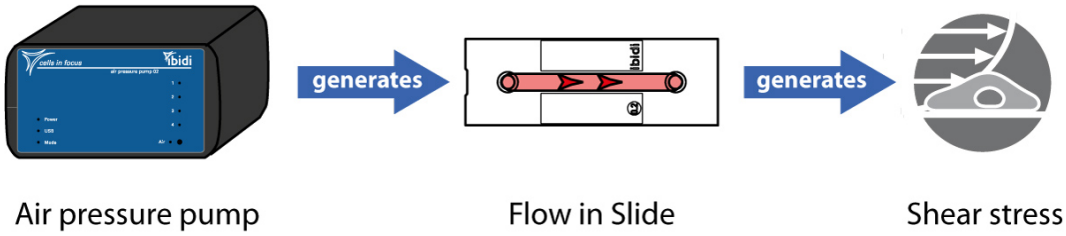


Figure 46 – Working principle of flow and shear stress generation.

1. The pump generates a constant pressure (mbar) that pumps the liquid from one reservoir to the other.
2. The applied pressure results in a specific flow rate (ml/min) that 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) produces a wall shear stress (dyn/cm^2) to which the cells are exposed.

The pump supplies a constant air pressure to the reservoirs of the Fluidic Unit, which generates a constant flow of medium within the ibidi channel slides. Before the reservoir runs dry, the liquid is pumped back and forth between the two media reservoirs of the Fluidic Unit. To create a unidirectional flow, two valves, labeled (V1) and (V2), are integrated in the Fluidic Unit which are switched simultaneously between two states (Figure 47).

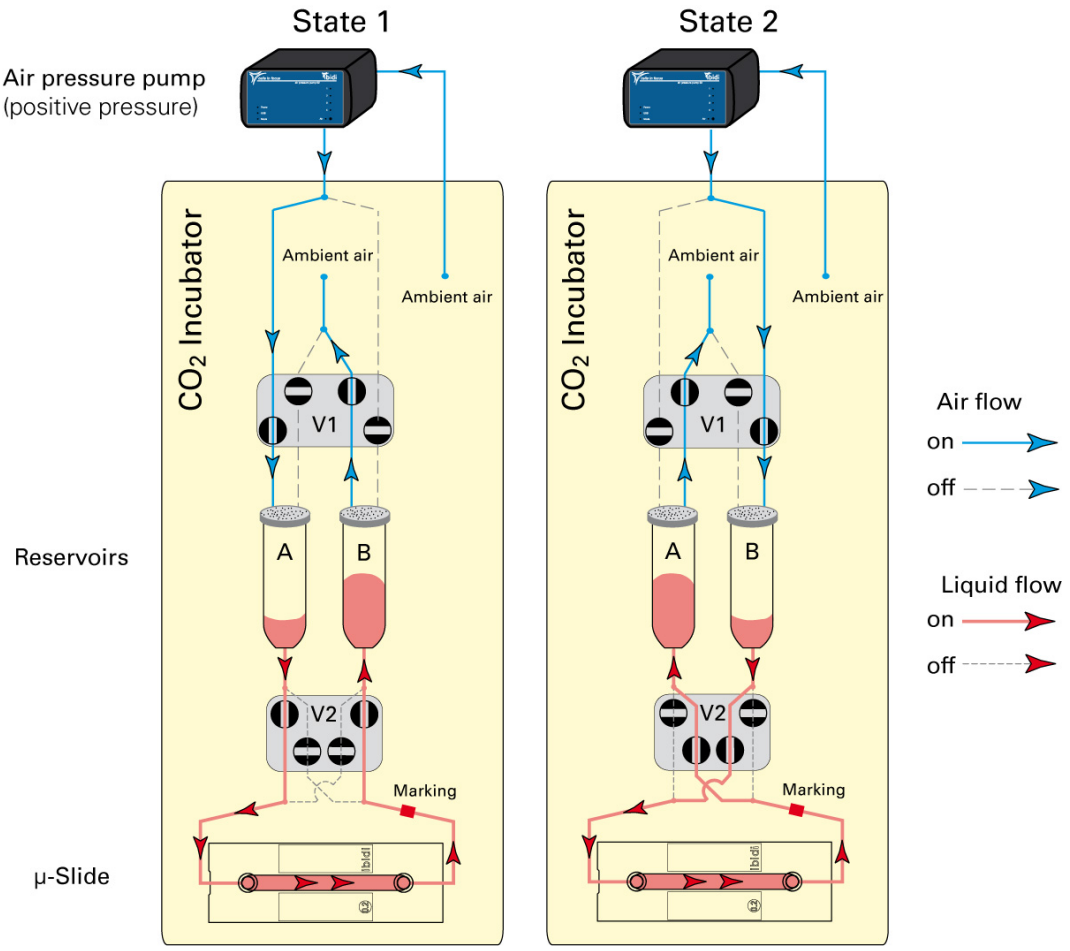


Figure 47 – Working principle of the valves creating a unidirectional flow, using **positive** pressure.

Example with positive pressure In State 1, the valve (V1) is set such that the pressurized air is applied to reservoir (A), while the outlet of reservoir (B) is opened to the atmosphere. This creates a flow from reservoir (A) to reservoir (B). Valve (V2) squeezes the two tubing sections in the front slots, forcing the liquid to flow through the lower loop of the Perfusion Set. The channel is perfused from left to right.
In State 2 valve (V1) is set such that the pressurized air is applied to reservoir (B) while the outlet of reservoir (A) is opened to the atmosphere. The apparent flow direction is inverted to a flow from reservoir (B) to reservoir (A). Valve (V2) also changes state, and now pinches the two tubing sections in the rear slots, again forcing the liquid to flow through the lower loop. The crossed geometry of the Perfusion Set again directs the liquid to the channels left inlet, resulting in a perfusion from left to right.
Switching between State 1 and State 2 generates a continuous unidirectional flow of medium through the slide. Sterility is maintained by the use of air filters on top of the reservoirs. Note, that it is beneficial to supply CO_2 rich air to the medium in order to properly buffer it. Therefore, the rear pump port should be connected to the incubator.
If the system is run with negative pressure, the principle remains the same, however, the flow direction is reversed (right to left).

8.2 Positive Versus Negative Air Pressure

The ibidi Pump System can be set up using positive or negative pressure. Although, in most cases best results are achieved using positive pressure, there are instances in which negative pressure is appropriate for an experiment. The pros and cons of positive and negative pressure are discussed in this section.

Positive Air Pressure

When using positive air pressure, air is drawn into the ibidi Pump from the rear port. The air 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 for the medium that is supplied to the cells in the µ–Slide. If the cells need a greater amount of CO_2, 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, make sure to use the drying bottle to prevent condensation in the pump from the warm and humid air.

Negative Air Pressure

When using negative air pressure, the pump aspirates air through the Fluidic Unit. Because the Fluidic Unit is placed inside an incubator, the air coming in contact with the medium and cells is humid and rich in CO2. The drying bottle must be integrated between the Fluidic Unit and the pump to protect the pump from condensation.
When deciding which kind of pressure to use, one should consider the following pros and cons. When using positive air pressure, an overpressure is created within the system. As a result, air (and also medium) is more likely to be pressed out of the system rather than drawn in. Therefore the system is less vulnerable to contamination or gas bubble formation. Additionally the air supply is dry, which will keep the sterility filters on the Perfusion Sets dry, ensuring optimal performance. However, the setup needs additional tubing compared to the negative pressure setup.
With negative air pressure, the ambient air that enters the reservoirs comes directly from inside the incubator and consists of the correct CO_2 concentration. However, the humid air that is drawn into the filters could cause them to become damp and possibly blocked.
The table below shows an overview of the differences between positive and negative 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 rear port.	The incubator's atmosphere is directly applied on top of the liquid level in the reservoirs.
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	More *in vivo*–like	Can barely be found *in vivo*

8.3 Flow Characteristics

Because of the geometry of the setup, the flow within the tubing and the µ–Slide channel is laminar, independent of the flow rate and type (i.e., continuous, oscillatory or pulsatile).
Working with positive pressure, the source of flow is from the tubing on the left side (front view). Applying negative pressure reverses the direction.

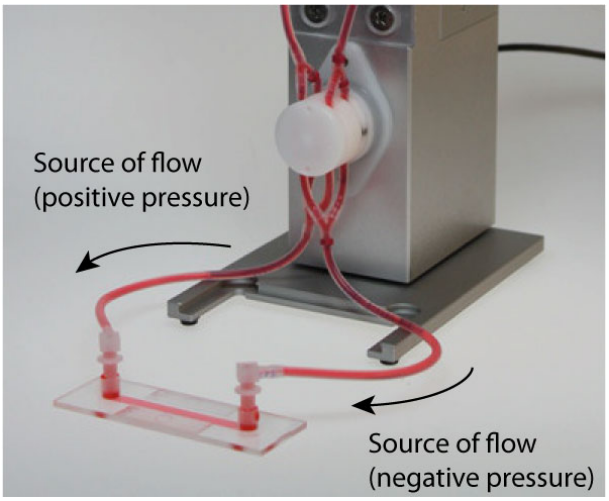


Figure 48 – Flow direction with positive and negative pressure

Continuous Unidirectional Flow

The normal Fluidic Unit operation creates a continuous and unidirectional flow within the µ–Slide channel. The working principle is detailed in Section 8.1.

Oscillatory Flow

Some experiments require an oscillatory flow for simulating turbulences in vessels. These conditions are achieved by oscillatory switching of the flow direction with frequencies of approximately 2 Hz. To perform an oscillatory flow assay, at least two Fluidic Units are required. Additionally, minor tubing modifications are required to change the functionality of the Fluidic Units. With the correct setup, one Fluidic Unit will act as the "master", which is responsible for the air pressure inside the reservoirs. The slave Fluidic Unit switches the flow direction. For more information about how to set up oscillatory flow assays, see Section 7.3.

Pulsatile Flow

To achieve pulsatile flow, two Fluidic Units are required. Contact ibidi GmbH for detailed instructions.

8.4 Viscosity

The viscosity influences the system in two ways: the relationship between pressure and flow rate, and the dependence of shear stress on flow rate (Figure 36). For an exact calculation of the shear stress that the cells are being exposed to, the viscosity of the perfusion medium must be known. This information can be obtained from the supplier or by measuring the medium with a viscometer.
The viscosity of water is 1 mPa s at 20°C; however, it is only 0.69 mPa s at 37°C (30 % difference!). The viscosity of water in relation to temperature is shown in Figure 49 (1 mPa s = 0.01 dyn s /cm^2).

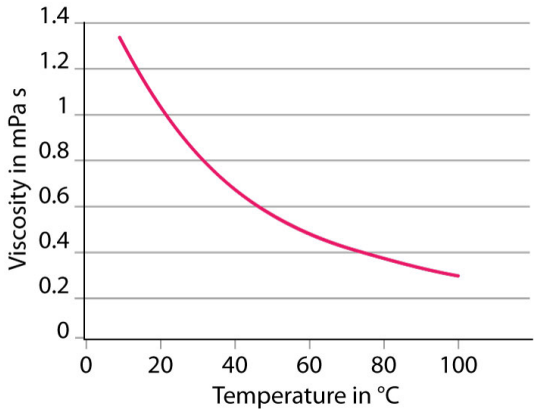


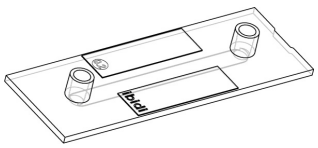
Figure 49 – Viscosity of water as a function of temperature.

8.5 Shear Stress Calculations in ibidi Channel Slides

The wall shear stress in a µ–Slide depends on the flow rate and the viscosity of the perfusion medium. Use the following calculations to determine the flow rates for the corresponding shear stress. Detailed information is provided in Application Note 11 "Shear Stress and Shear Rates".

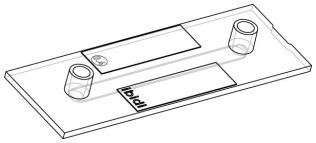
$$\Phi = flowrate \quad \tau = shearstress \quad \eta = viscosity$$

µ–Slide I$^{0.2}$ Luer



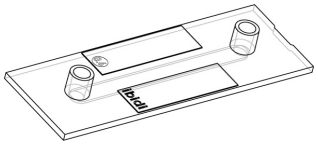
$$\tau \left[\frac{dyn}{cm^2}\right] = \eta \left[\frac{dyn \cdot s}{cm^2}\right] \cdot 512.9 \cdot \Phi \left[\frac{ml}{min}\right]$$

µ–Slide I$^{0.4}$ Luer

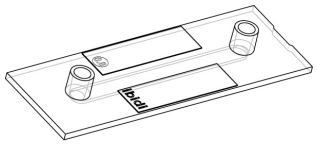
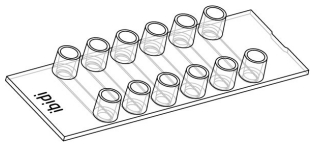
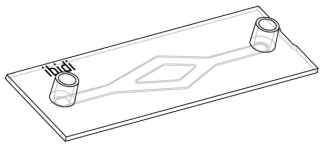
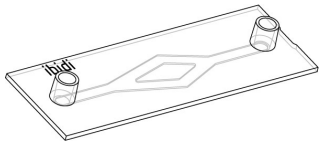
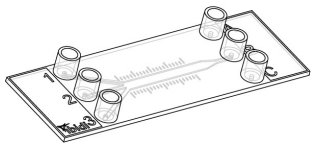
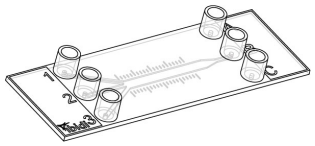
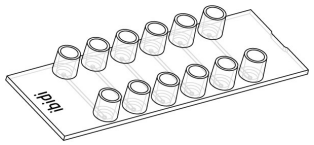


$$\tau \left[\frac{dyn}{cm^2}\right] = \eta \left[\frac{dyn \cdot s}{cm^2}\right] \cdot 131.6 \cdot \Phi \left[\frac{ml}{min}\right]$$

µ–Slide I$^{0.6}$ Luer



$$\tau \left[\frac{dyn}{cm^2}\right] = \eta \left[\frac{dyn \cdot s}{cm^2}\right] \cdot 60.1 \cdot \Phi \left[\frac{ml}{min}\right]$$

µ–Slide I $^{0.8}$ Luer		$\tau\left[\frac{dyn}{cm^2}\right] = \eta\left[\frac{dyn \cdot s}{cm^2}\right] \cdot 34.7 \cdot \Phi\left[\frac{ml}{min}\right]$
µ–Slide VI $^{0.4}$		$\tau\left[\frac{dyn}{cm^2}\right] = \eta\left[\frac{dyn \cdot s}{cm^2}\right] \cdot 176.1 \cdot \Phi\left[\frac{ml}{min}\right]$
µ–Slide y-shaped (single channel)		$\tau\left[\frac{dyn}{cm^2}\right] = \eta\left[\frac{dyn \cdot s}{cm^2}\right] \cdot 227.4 \cdot \Phi\left[\frac{ml}{min}\right]$
µ–Slide y-shaped (branched channel)		$\tau\left[\frac{dyn}{cm^2}\right] = \eta\left[\frac{dyn \cdot s}{cm^2}\right] \cdot 113.7 \cdot \Phi\left[\frac{ml}{min}\right]$
µ–Slide III ^{3in1} (1 mm channels)		$\tau\left[\frac{dyn}{cm^2}\right] = \eta\left[\frac{dyn \cdot s}{cm^2}\right] \cdot 774.1 \cdot \Phi\left[\frac{ml}{min}\right]$
µ–Slide III ^{3in1} (3 mm channel)		$\tau\left[\frac{dyn}{cm^2}\right] = \eta\left[\frac{dyn \cdot s}{cm^2}\right] \cdot 227.4 \cdot \Phi\left[\frac{ml}{min}\right]$
µ–Slide VI $^{0.1}$		$\tau\left[\frac{dyn}{cm^2}\right] = \eta\left[\frac{dyn \cdot s}{cm^2}\right] \cdot 10.7 \cdot \Phi\left[\frac{\mu l}{min}\right]$

Important!

Note that the starting point for most experiments is the shear stress. Depending on the channel geometry and the viscosity of the medium, the relation between flow rate and shear stress is linear, detailed in the formula in Application Note 11. Thus, after defining the shear stress, calculate the required flow rate and make sure, that the correct flow rate is applied to the slide, by measuring the flow rate with a stop watch as described in Section 6.6.2.

8.6 Working with Non-Implemented Flow Channels

This section describes the procedure for working with flow channels that are not implemented in the software. It details how to generate a defined shear stress by means of an example.
Knowledge of how to calculate the wall shear stress in the geometry of the experimental slide is required.

Principle:

1. Define the shear stress needed for the experiment.
2. Calculate the flow rate required to obtain this shear stress in the specific slide geometry.
3. Measure a calibration curve with the slide and the ibidi Pump System (with Fluidic Unit and Perfusion Set), showing the dependence between flow rate and pressure.
4. Determine the pressure needed to obtain the correct flow rate.
5. Apply this pressure with the pump, ignoring the values of flow rate and shear stress displayed in the PumpControl software.
6. Adjust the switching time.

Example:

The sticky-Slide channels are not available in the drop–down menu of the software. The formulas to generate the shear stress are in the sticky-Slide instructions. For this example, the procedure detailed is for the sticky–Slide $^{0.4}$ Luer.

$$\tau \left[\frac{dyn}{cm^2}\right] = \eta \left[\frac{dyn \cdot s}{cm^2}\right] \cdot 104.7 \cdot \Phi \left[\frac{ml}{min}\right]$$

1. Define the shear stress needed for the experiment: 10 dyn/cm^2
2. Calculate the flow rate required in the sticky-Slide I $^{0.4}$ Luer to generate the desiredshear stress using the formulas above. The viscosity of medium at 37°C is 0.0072 dyn s/cm^2.

$$\Phi \left[\frac{ml}{min}\right] = \frac{\tau \left[\frac{dyn}{cm^2}\right]}{\eta \left[\frac{dyn \cdot s}{cm^2}\right] \cdot 104.7} = \frac{10}{0.0072 \cdot 104.7} = 13.3\, ml/min$$

3. Measure a calibration curve with the ibidi Pump System. Measure the respective flow rates you obtain with pressure values from 5 mbar to 95 mbar, using the setup that was chosen (Slide and Perfusion Set).

   Apply the respective pressure and switch the valves manually. Measure the time required for the medium to flow from the 6 ml to the 4 ml mark on the syringe (around equilibrated medium levels).

   Display the values in a graph and calculate a trend line.

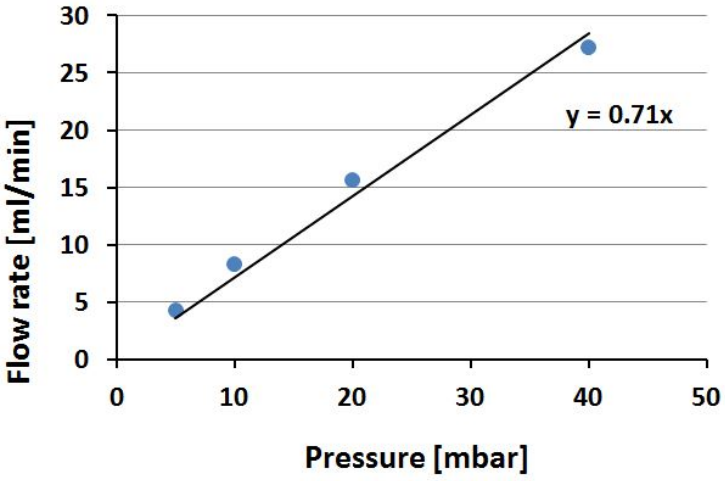


Figure 50 – Example of a calibration curve showing the relation between flow rate and pressure.

4. This calibration curve enables the prediction of the pressure needed to generate the desired flow rate (p in mbar, Φ in ml/min).

$$p = \frac{\Phi}{0.71} = \frac{13.3}{0.71} = 18.7$$

5. In the PumpControl Software, choose the Perfusion Set being used from the drop down menu and choose the option "without any slide" from the slide drop down menu. Apply the pressure of 18.7 mbar to the Fluidic Unit and ignore the flow rate value that is indicated in the software.

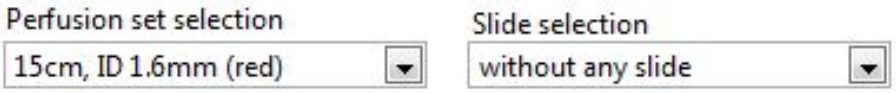


Figure 51 – Selection of Perfusion set red and Slide selection "without any slide"

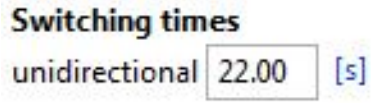
Figure 52 – Inserting 18.7 mbar in the pressure box. The flow rate is 13.3 ml/min, as indicated in the calibration curve. The automatically calculated flow rate must be ignored.

Always check the flow rate when running an experiment. Because of small manufacture tolerances of the slides and tubing, the values vary slightly. Section 6.6 describes how to calibrate the system.

6. The PumpControl Software does not know the correct flow rate, and therefore, the program is not able to calculate the right switching time. Insert the switching time manually to avoid having the reservoirs run dry.

$$Switchingtime = \frac{60\ s/min \cdot 5\ ml}{\Phi}$$
$$= \frac{60\ s/min \cdot 5\ ml}{13.3 ml/min} = 22.5 s$$

Insert the value of 22 s in the box “Switching times, unidirectional” (slightly rounded down).



9 Maintenance

Although the ibidi Pump system requires minimal maintenance, there are some parts that will have to be checked occasionally.

9.1 Disinfection and Cleaning

Pump Controller Unplug the external power supply cord from the ibidi Pump and electrical outlet. Use a dry or damp wipe to clean the pump.
CAUTION Only use water or 70% ethanol/2–propanol to clean the pump. Other organic solvents could remove the instrument paint.

Fluidic Unit To disinfect the Fluidic Unit before placing it in an incubator, wipe it with a moistened cloth with 70% ethanol/2-propanol.
CAUTION Do not spray the Fluidic Unit directly. Don't wet it with any kind of liquid.

9.2 Silica Beads from the Drying Bottle

The silica beads are coated with an orange indicator that turns white when saturated with moisture. The silica beads can be used until the beads turn white. To regenerate the beads, place them 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 all moisture has been removed. After they cool to room temperature they can be returned to the drying bottle for use.

9.3 Replacement Filters for Perfusion Sets

The filters on the Perfusion Sets may become clogged if they come in contact with the medium. If the pores of the filter are blocked, the correct flow rate cannot be obtained. If this happens during an experiment, immediately replace the filter with a new one. Replacement parts are available through ibidi (Filter/Reservoir Sets, #10971, #10972, #10974).

9.4 Fluidic Unit Filters

The Fluidic Unit filter protects the unit's internal components from particles and dust. Change the filter when the pores are blocked. For best performance, change the filter every 6 months, if the system is in regular use. Use a 0.2 mm Teflon air filter with a 26 mm diameter and a male Luer Lock slip (e.g. Sartorius Minisart®HY 16596——-HYK).

9.5 Fluidic Unit Pinch Valves

The pinch valves have a defined life time that is dependent on the number of switching cycles. If the pinch valves are do not function properly, contact ibidi or your local distributor for replacement or repair.

10 Troubleshooting

10.1 Air Bubbles

Air bubbles are a common problem in any type of perfusion setup. Air bubbles can be avoided by following the precautions listed in this section.

10.1.1 Air Bubbles When Connecting the Slide

Problem: Air bubbles emerge in the tubing system or slide directly after connecting the Slide to the Perfusion Set.

Possible Cause	Solution
Air bubbles were introduced while filling the tubing.	To remove air bubbles in the tubing, start an automatic cycle in PumpControl with a high flow rate or load the "Remove air bubble" settings in the tutorial menu in the software.
Air bubbles were created when connecting the Slide to the Perfusion Set.	Fill the slide reservoirs to the top and remove air bubbles on the surface. When pulling out the Luer adapter from the Female Luer Coupler, hold the male Luer adapter upward so that the bubbles rise to the Female Luer Coupler instead of flushing into the Luer adapter. This procedure is shown in Section 6.7.

10.1.2 Air Bubbles Emerging After a Few Hours

Problem: Air bubbles emerge and accumulate somewhere in the tubing system after the flow starts.

Possible Cause	Solution
Medium, tubing and slides were not degassed and equilibrated at the proper temperature.	Equilibrate the system parts inside the incubator one day before starting the experiment (see Section 6.1).
Loose or leaky adapters in combination with negative pressure draw in small air bubbles into the system.	Make sure all adapters and connectors fit tightly and use positive pressure.
The humidity in the incubator is too low. When working in a heated chamber without humidification, evaporation promotes air bubble formation.	Use an incubator with at least 80% humidity.
Temperature changes along the tubing or over time.	Keep the temperature stable.

10.2 Cells are Detaching

There are multiple parameters that influence cell attachment. Monitor the cells and make a note of the time point when the cells start to appear distressed.

10.2.1 Cells Detach Before Starting the Flow

Problem: The cells look unhealthy and do not attach to the slide surface before connecting them to the Perfusion Set.

Possible Cause	Solution
The cells lack medium with nutrients. In low channels (100 or 200 µm), the volume of medium is very low compared to cell number. This effect may appear after only a few hours.	Cultivate cells in low channels for only few hours. If the cells need to be in the channel for more than a few hours, refresh the medium in the channel in short intervals or place the slide on a rocking plate.
The cell culture surface was not suitable for the specific cells.	Make sure the cells adhere to the surface under standard conditions (e.g. in a µ–Dish). If using a protein coating, make sure the concentration of the coating solution is sufficient for the channel slide. A protein coating protocol is available in Application Note 08.
The cells were not healthy.	Try another lot or passage of cells. Primary cells especially are very variable in their fitness.
Evaporation in the slide led to increased medium concentration.	Place the slide in an extra humidity chamber (Petri dish with wet paper towel).

10.2.2 Cells Detach When Connecting the Slide to the Perfusion Set

Problem: After connecting the Perfusion Set, the cells look detached or accumulated in clusters.

Possible Cause	Solution
The cells were detached by too much flow during the connecting step.	Every movement of the medium results in a shear stress. High flow rates can detach even healthy cells.
The cells were stressed by the abrupt temperature change from being placed on the metal surface of the sterile working bench.	Avoid placing slides directly on metal surfaces. For best results, place slides on the ibidi µ–Slide Rack, or a Petri dish.
The cells were stressed, because the connection step took too long.	Connecting the slides is time critical. Work as quickly as possible and make sure to have everything on hand before the connection step.
The cells were not healthy.	Try another lot or passage of cells. Primary cells especially are very variable in their fitness.

10.2.3 Cells detach under Flow Conditions

Problem: Cells detach after starting the flow experiment.

Possible Cause	Solution
The shear stress was too high.	Decrease the flow rate.
The shear stress was applied too fast.	Allow the cells to become accustomed to flow by starting with a very low flow rate and gradually increasing it.
Cells were not healthy.	Try another lot or passage of cells.

The cell number in the slide was too low. Cells could not form a confluent layer and were detached from the surface.	Seed more cells. Before starting the flow, the cells should be nearly confluent.
The coating was not stable and was washed away by the flow.	Check the coating with fluorescence staining before and after applying the flow. Try alternative coatings or the ibiTreat surface.
There was too much evaporation of medium, which increased its concentration.	Check if the volume of the medium decreased. Increase the humidity in the incubator.
The CO_2 concentration in the incubator was too low, and the medium was not equilibrated to a neutral pH.	Make sure the CO_2 supply is sufficient and equilibrate the pH of the medium.

10.3 Clogged Filters

The reservoir filters maintain the sterility of the medium. The air stream from the pump passes through their pores and therefore it is crucial that the filters remain unclogged.
If medium contacts the filter and clogs its pores, flow will be decreased. Usually this happens with only one filter and results in an imbalance in the medium levels (Section 10.11).
Replace clogged filters immediately to avoid complications and damages. The filters cannot be washed or regenerated! Replacement filters with a pore size of 0.2 µm can be purchased from general laboratory suppliers (Sartorius, Minisart®SRP15 17573——-K).

10.4 Pump is not Recognized by the Computer

To control the pump with a computer, the PumpControl software is required. Each pump version requires a specific version of the software. All versions of the PumpControl software and drivers can be downloaded from the ibidi website. To check which firmware version the pump has, click on the question mark button "?" in the task menu and then click on "About...". A new dialog box opens, that reveals the version and serial number information.

10.4.1 Using PumpControl v1.5.0 or Higher

If the latest PumpControl version has been installed, there should not be any problems with the drivers. All required drivers are installed automatically with the software. To run PumpControl v1.5.0 or higher, the firmware version of the pump must be v1.02 or higher.

10.4.2 Using PumpControl v1.4.4 or Lower

If the software did not automatically install and there is no communication between the pump and the computer, install the required drivers manually. Follow the instruction during the hardware installation and specify the location of the folder for the USB drivers which are on the PumpControl installation thumb drive under "USB driver". If an error message occurs, go to the computer's hardware manager and look under "Ports". Click on the non–functional component, which is the ibidi Pump and check the driver. Install the driver that is included on the PumpControl installation thumb drive. Note that installation of two drivers is necessary; one for the "USB serial converter" and one for the "USB serial port".

10.4.3 Pressure Lost Error

Problem: The program stops and the Pressure Lost error message appears.

Possible Cause: The USB cable or the power supply could have an unstable connection.

Solution: Check which type of USB cable and power supply is being used. Generally, any USB cable is suitable, but when encountering connection problems make sure to use a double shielded USB cable (e.g. Tripp Lite USB 2.0, model UR022-006).

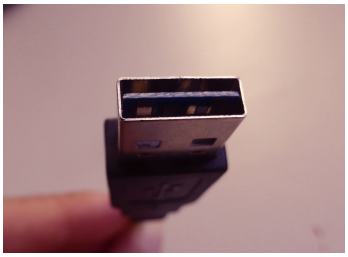


Figure 53 – Front view of the screened USB cable. The plug is formed by the outer metal casing and a thin plastic sheet at the inside.

The power supply (Sinpro MODEL NO:SPU41A-106) should have the following characteristics:
INPUT: AC 100-240 V, 47-63 Hz
OUTPUT: DC 14 V, 2.85 A max.

10.5 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 the computer still does not work, then the ".NET" drivers are likely missing. Either download these drivers through the internet or from the ibidi web page (in the supporting material section). After extraction and installation of these drivers, communication with the ibidi Pump should function.

10.6 Pressure Kickback After Pressure Switch Off

When using positive pressure, the rear port of the pump is connected to the drying bottle, which is connected to the incubator to take in CO_2–rich air. An air pressure kickback when switching the system off could result from a vacuum building up in the system when the tubing system is pinched or clogged, and the air supply is hindered. Make sure that the air tubes are not being squeezed and check the setup of the drying bottle. If the tubing looks fine, remove it from the rear port. If the problem persists, contact ibidi for assistance.

10.7 Flow Rate is Too Low or Absent

Problem: The pump is applying the correct pressure to the Fluidic Unit, but the flow rate is low or there is no flow visible in the Perfusion Set.

Possible Cause	Solution
There was a blockage in the tubing leading from the pump front port to the Fluidic Unit	Change the air pressure tubing.
There was a blockage in the valve block of the Fluidic Unit (valve 1).	Contact ibidi to replace the valve block.
Clogged filters were restricting air passage. When filters come in contact with medium or water, air flow is decreased or ceases.	Filters that become wet with water can be dried at 55°C for a few hours. Filters that are contaminated with medium must be replaced (Section 9.3).

When applying low pressure, an air bubble in the tubing can decrease or even stop the flow.	Check the tubing for air bubbles. If necessary, disconnect the slide and remove all air bubbles from the tubing by setting the pump to a high pressure.

10.8 Flow Rate is too High

Problem: The flow rate measured with a stop watch is much higher than indicated by the software. Variations of up to 20 % are normal caused by differences of viscosity and manufacture tolerances of the tubing and slides.

Possible reason	Solution
The tubing was not inserted correctly into the pinch valve (valve 2) and produced a "by–pass" that leads directly from the source reservoir to the sink reservoir, leading to a high flow rate.	Check that the tubing is correctly mounted by clamping the tubing near the slide. The pinch test is described in Section 6.5.
Wrong calibration factor	Check the flow rate again with a stop watch and input the calibration factor in the software. This procedure is detailed in Section 6.6.

10.9 Evaporation

Problem: Long term experiments could result in medium evaporation. The amount of volume loss per day depends on the humidity in the incubator and the volume flow rate. Do not exceed a volume loss of more than 10% of the total volume.

Possible Cause	Solution
The air stream in the reservoirs results in a slight evaporation of medium, which is normal.	Depending on the requirements of the cells, exchange the medium after a few days or refill the evaporated amount of volume with sterile water.
The humidity in the incubator is not high enough.	Check the humidity of the incubator with a hygrometer. Small and low priced hygrometers are available in electronic gear shops. The minimum recommended humidity is 80 % rel. humidity.

10.10 Flow Direction in the Channel is Changing

Problem: The flow direction in the slide changes even though it is set to unidirectional flow.

Possible Cause	Solution
Perfusion Set was not mounted correctly.	Check that the tube is inserted properly.

10.11 Imbalanced Medium

If the medium is running at different flow rates in the two switching states (running from left to right or running from right to left), perform the following tests to isolate the issue.

Note!

A slight imbalance is normal due to variance from fluid dynamics and in the valve switching events, and is tolerated, as long as the reservoirs do not run dry.

Use a Perfusion Set with clean, dry filters. When the filters have been used before and have come in contact with medium, the filters are clogged. If the integrity of the filters is in question, replace them (Section 9.3).
Note: Use positive pressure for this procedure.

1. Mount the Perfusion Set on the Fluidic Unit.
2. Fill the Perfusion Set with medium to equal levels in both reservoirs (the 5 ml mark for 10 ml Perfusion Sets, or the 1 ml mark in the 2 ml Perfusion Sets).
3. Remove the air bubbles from the Perfusion Set (Section 6.4).
4. Perform the pinch test (Section 6.5) and note the movement of the liquid.
   - Does the liquid move in the first switching state (running from left to right)?
     □Yes □No
   - Does the liquid move in the second switching state (running from right to left)?
     □Yes □No
   - If both answers are "No", then go to step 5.
   - If one or both answers are "Yes", the pinch valve is not operating correctly. Adjust the tubing in the pinch valve by stretching the tubing and moving it up and down (Figure 26). Perform the pinch test again. If adjusting the tubing does not fix the issue, the problem might by the pinch valve. Contact info@ibidi.com for repair.
5. Load the "Remove air bubbles" setup from the "Tutorial" menu. It does not matter which Perfusion Set is being used. Make sure the liquid levels are equilibrated. Alternatively, start the program that was running when the imbalance problem occured.
6. Start the program and observe the liquid levels. Is there an imbalance emerging over time?
   □Yes □No
   - If "No", the problem was fixed by the correct insertion of the tubing.
   - If "Yes", proceed to step 7.
7. Remove the tubing connection from the Perfusion Set and switch the filters. Reconnect to the tubing. Does the imbalance switch to the other side? For example, an observed imbalance with an excess of medium on the right side changed to the left side after switching the filters.
   - □ If the imbalance changed to the other side, one filter is clogged. Replace the filters and restart at step 5.
   - □ If the imbalance stayed on the same side, proceed to step 8.

8. Cross the tubing on top of the reservoirs (Figure 54) and see, if the problem switches to the other side. For example, the imbalance of excess medium on the right side switched to the left side after crossing the tubing.

Figure 54 – Cross the tubing on top of the reservoirs to test if the problem is with the Perfusion Set or in the valve block (valve 1).

- ☐ If the imbalance remained on the same side, there might be a blockage in the branched tubing of the Perfusion Set. Substitute the Perfusion Set and try again.
- ☐ If the imbalance switched to the other side, the problem is located in–between the pump and the Perfusion Set filters. The Perfusion Set and the filters are functioning properly. Proceed to step 9.

9. Keep the tubing above the reservoirs crossed, and cross the tubing on the valve block (Figure 55).

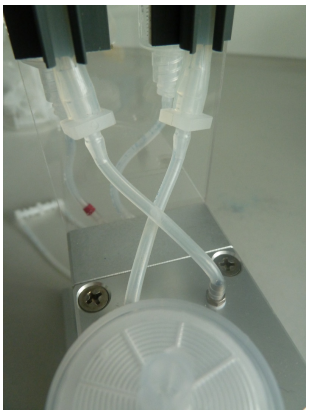


Figure 55 – Crossing the tubing above the valve block (valve 1).

- ☐ If the imbalance switched to the other side, there might be a blockage in the tubing from the valve block to the reservoirs. Check the tubing for particles or debris blocking the air flow and remove the blockage if possible. If the problem persists, contact info@ibidi.com and describe the problem in detail. Refer to the tests that are described in this troubleshooting section.
- ☐ If the imbalance remained on the same side, the problem is inside the valve block. Contact ibidi at info@ibidi.com to have the valve block repaired.

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