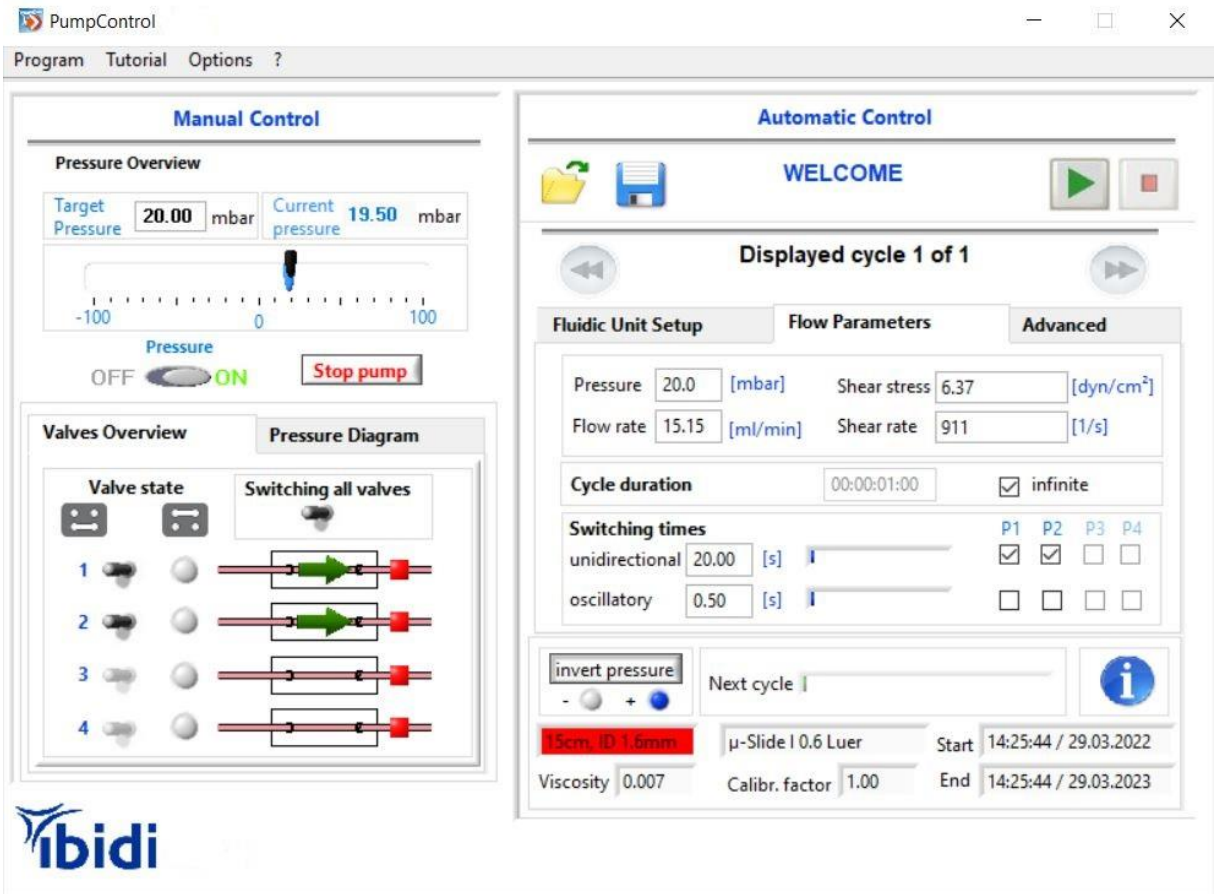
ibidi PumpControl Instruction Manual

Version 1.6.1.1



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ibidi PumpControl

Instruction Manual

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1. General Information

The ibidi PumpControl software is designed to control the ibidi Pump. Other devices are not supported, and the program will only start in demo mode.
The PumpControl software controls the outgoing pressure [mbar] that will be applied to the Fluidic Unit(s) and the switching operation of the valves. Shear stress and flow rate are calculated according to the selected Perfusion Set and µ-Slide.

1.1 System Requirements

To run PumpControl on a computer, the following components are required:

- Operating system: Windows 10, 64-bit
- Free USB port
- Hard disk space for PumpControl ca. 250 MB
- Working storage (RAM): min. 4 GB
- Processor (CPU): min. Intel Pentium Dual Core

1.2 Installation

The installation package can be downloaded from the ibidi website.

Save the complete installer package on your computer and follow these steps:

- Log in as administrator, if necessary.
- If the software doesn't start automatically, run 'setup.exe' and follow the setup routine.
- Setup the pump as described in the ibidi Pump System Instructions. Connect the pump to the computer via the supplied USB cable.
- Restart the computer and start PumpControl.
- Choose one of the options in the Fluidic Unit starter window (shown in Figure 1).

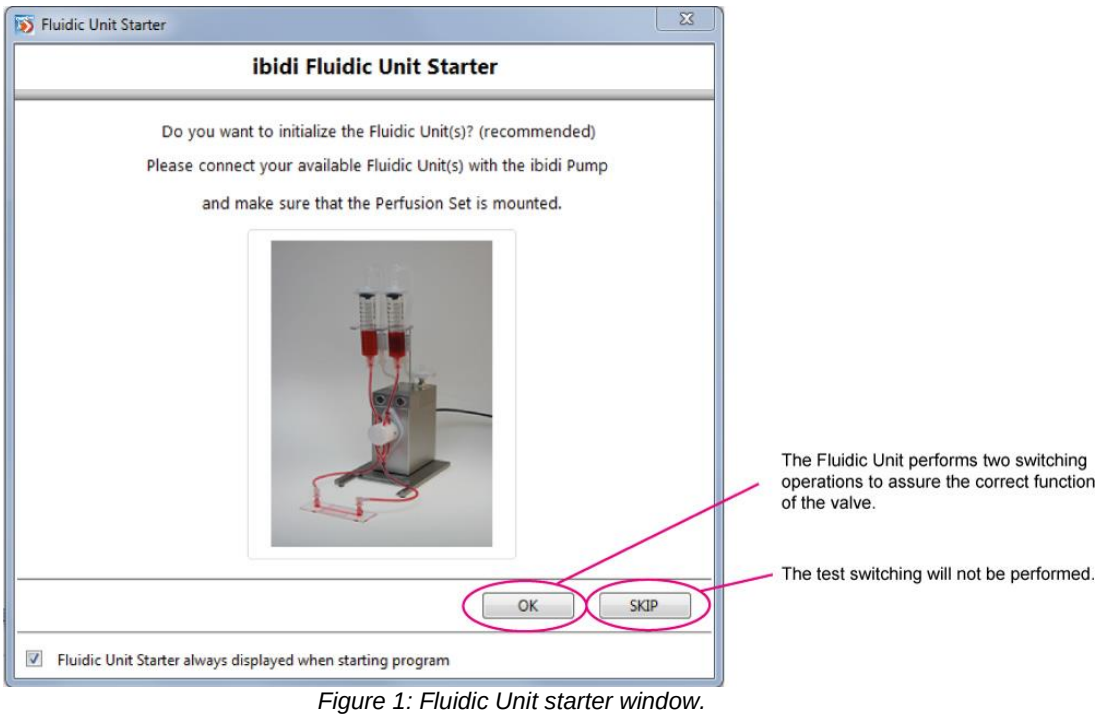


Figure 1: Fluidic Unit starter window.

- If the Pump is recognized by the computer, the main window opens in standard operating mode (not in demo mode!). If the demo mode is activated (red text in Figure 3), switch back to standard mode by clicking on the option 'Demomode' in the program menu (Figure 2):

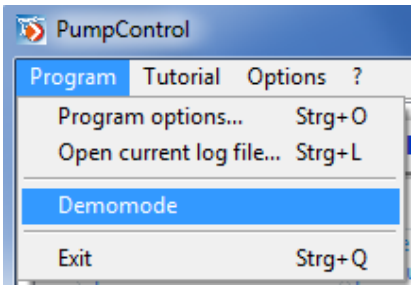


Figure 2: Changing from Demo mode to standard mode and back.

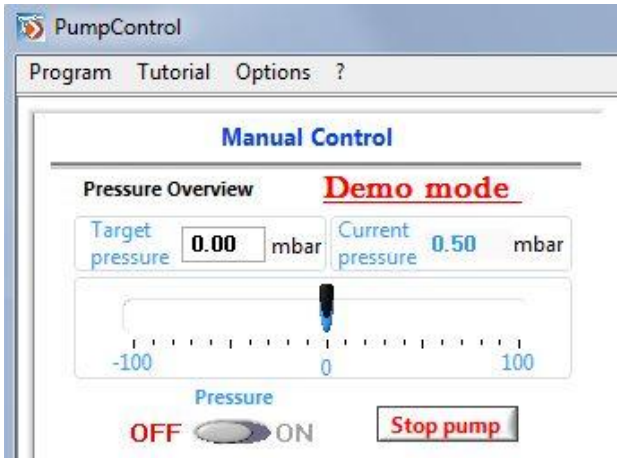


Figure 3: Indication of the demo mode in the main window.

- The program is now ready to control the operations of the ibidi Pump.

1.3 Computer Settings

Note! If you install the software on your own computer, you must set the power saving options and update options so that the program's operation is not disturbed or stopped.

These settings must be adjusted:

- Power options
  - Put the computer to sleep: never
  - Advanced settings, turn of hard disk: 0 minutes
  - Advanced settings, allow wake timers: disabled
  - Advanced settings, USB selective suspend setting: disabled
- Windows update properties
  - General/startup type: disabled
  - General/service status: Stop

If one of these settings is not correct and any of these instances should stop the program, this may cause damage to the Fluidic Unit and/or Pump.

2. PumpControl Software

The PumpControl software controls the following parameters:

- Air pressure (-100mbar….+100mbar)
- Cycle duration (1s….infinite)
- Switching intervals
- Valve switching/valve state
- Setup of advanced assays with multiple cycles and loops

On the left of the PumpControl window you can control these parameters separately by hand (manual control). On the right of the window, more complex setups with multiple cycles and loops can be programmed (automatic control).

Note! Be sure to disconnect the computer from the internet when running a program/experiment. Even if update functions are disabled, Windows might interfere and shut down the computer while the software is running. This may cause damage to the Fluidic Unit and/or Pump.

2.1 Manual Control

On the left of the PumpControl panel, you'll find all manual controls and displays for the actual state of the pump, as shown in Figure 4. Manual controls are mainly used for calibration and equilibrating of liquid levels.

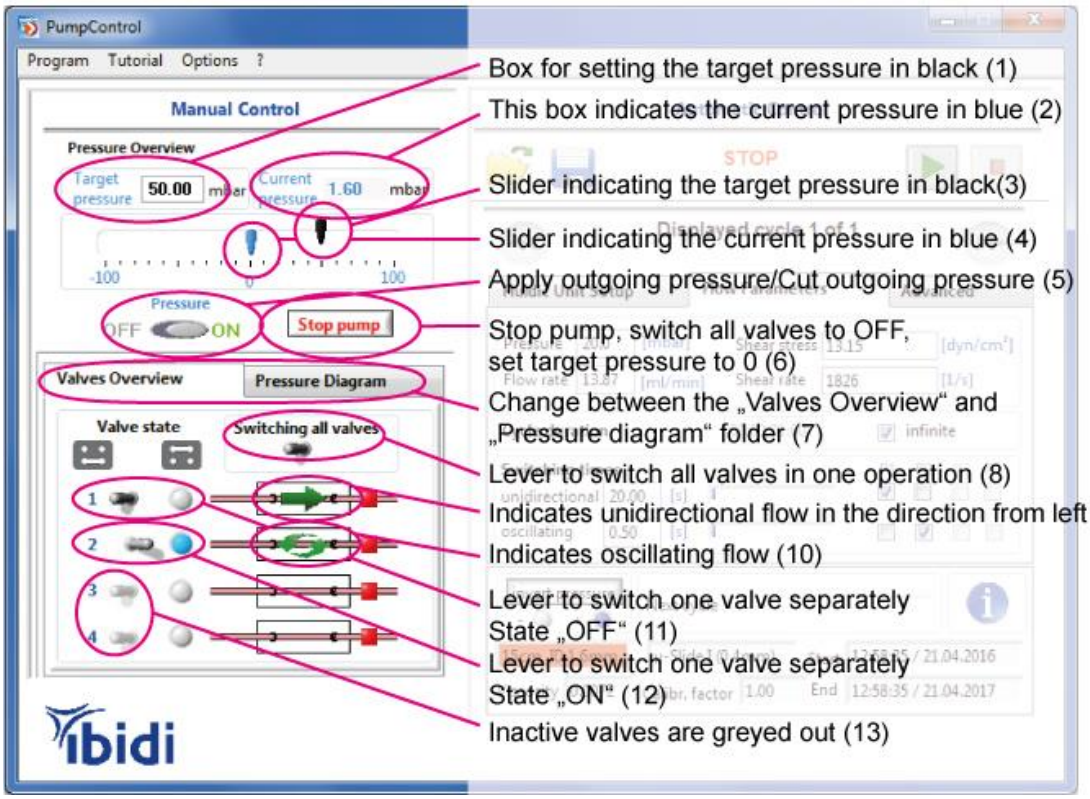


Figure 4: Manual control of the ibidi Pump with the control buttons on the left of the PumpControl software window.

2.1.1 Setting the Output Pressure

Positive or negative pressure is set either by manually moving the black arrow (control 3 in Figure 4), or by entering the pressure numerically (control 1). Values from -100 mbar to +100 mbar are valid. Optimal results are achieved when working between 95 mbar and 5 mbar.

The '**Pressure**' button (control 5) indicates the state of the pump's output pressure. By clicking on this button, the pump switches between the two states:

a) Pressure 'OFF' = the Fluidic Unit is connected to ambient air pressure. The liquid movement will be stopped, but the valves keep their positions, and the target pressure is still shown in the box (control 1).
b) Pressure 'ON' = the preset pressure is applied to the connected Fluidic Unit(s). The medium will be moved through the system.

The '**Stop Pump'** button (control 6) switches the pressure OFF, additionally resets all valve states to their default positions and sets the target pressure to '0'.

2.1.2 Valve Overview and Pressure Diagram Register

(a) (b)

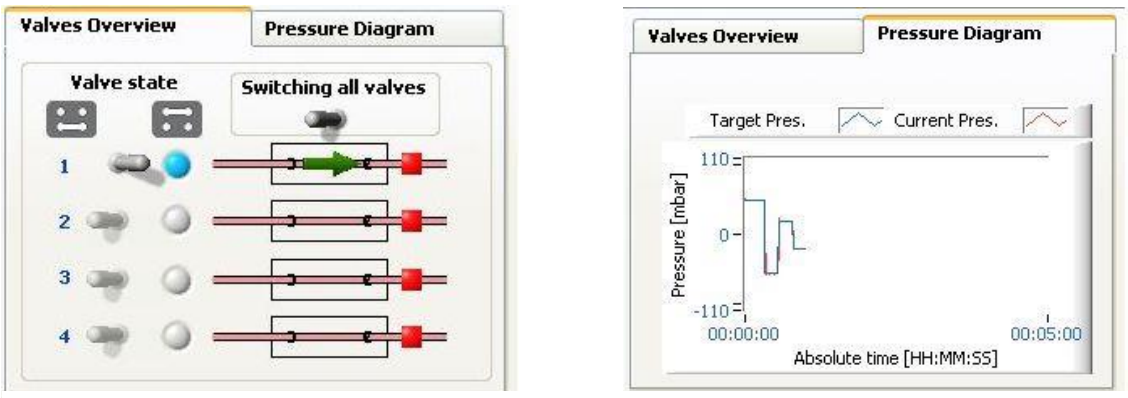


Figure 5: (a) Valve overview and (b) pressure diagram.

Control 7 in Figure 4 shows the position of the two registers, where you can switch between 'Valves Overview' and the 'Pressure Diagram'.

2.1.2.1 Valves Overview

In this tab, you'll find the state of all the connected Fluidic Units valves, and the actual flow direction will be simultaneously indicated. There are 3 types of flow indications:

 Unidirectional flow with positive pressure (from left to right)

 Unidirectional flow with negative pressure (from right to left)

 Oscillating flow

The valve controls (control 11 and 12 in Figure 4) can be switched individually. The two states are shown below:

-  = Valve is in State 2 (Blue LED on pump is ON.)

-  = Valve is in State 1* (Blue LED on pump is OFF.)
  *This is the default position.

There is also a master switch, which acts simultaneously on all active valves. When using this control (8), all active valves will change their current state. Please note that depending on the actual state, one valve might change from State 1 to State 2, while another might switch from State 2 to State 1.

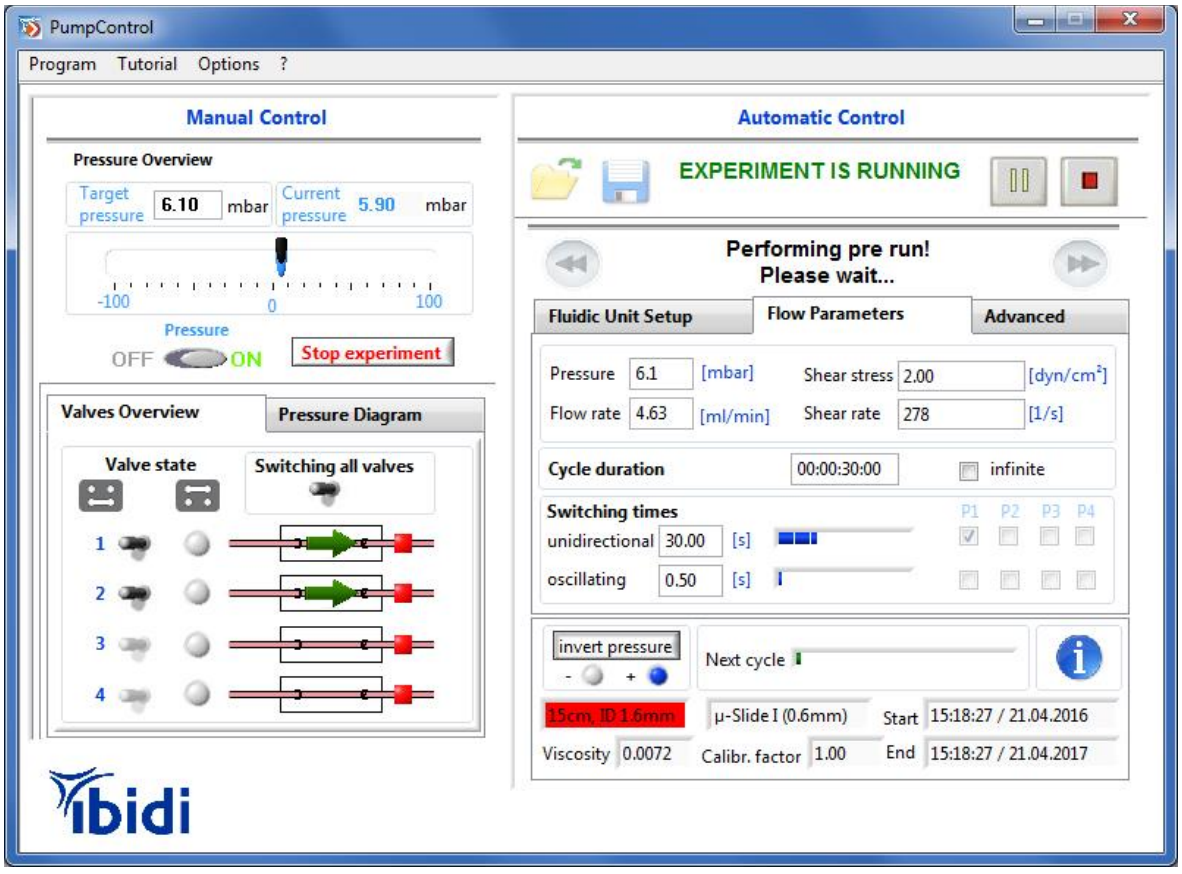
2.1.2.2 Pressure Diagram

In this tab, you'll find a graph that illustrates the recorded course of the air pressure at the pump output. Please note that this small graph only displays the last 5 recorded minutes of your experiment. To view the whole process, you need to click on the graph, then a new window will open. Please refer to section 4 to learn more about this.

Please keep in mind that the valves will only switch correctly if the tubing is inserted properly. Please refer to the ibidi Pump System manual for correct tube handling.

2.2 Automatic Control

The right side of the PumpControl panel is made for running automated flow assays. It's designed to make the experiment setup easy.



The automatic control was developed to perform exact cycles over seconds, minutes, days, or even weeks. With this tool, the shear stress, which is applied to the cells in the µ-Slide, can either be set by choosing the air pressure, the flow rate, or the shear rate, or by directly entering the shear stress. In any of these cases, the program will automatically calculate the

remaining values. Please note that the value of the viscosity must be adapted to your medium, in order to achieve a correct relation between the flow rate and the shear rate. The next sections will explain the automatic control.

Symbols of the Automatic Control:

	➔ Starts the scheduler with the current settings.
	➔ Pauses the scheduler. Please note that the pressure will be switched OFF to ensure that the medium inside the fluidic unit(s) does not move.
	➔ Stops the scheduler immediately. All valves will be switched OFF and the pump will stop.
	➔ Switches to the next cycle.
	➔ Switches to the previous cycle.
	➔ Displays the current settings of your experiment. Please note that this is only a summary of your settings. Parameters cannot be changed.
	➔ Saves your cycle settings to a file (*.wof).
	➔ Loads a '*.wof' file to your scheduler. Please note that, not only the 'Flow Settings', but also the 'Fluidic Unit Setup', will automatically be imported to the scheduler.
	➔ Adds another cycle to the scheduler.
	➔ Removes the current cycle from the scheduler.
	➔ Removes all programmed cycles from the scheduler.
	➔ Repeats all cycles. When you activate this button, you can select the number of sequences. The maximum number is limited to 100,000.
	➔ The 'Experiment Builder' allows you to build loops from the defined cycles.
	➔ In the 'Cycle Creator', the parameters of your settings can be changed in a table overview.

3. Detailed Instructions of the ‘Automatic Control’

With the Automatic Control functions of PumpControl you can create a schedule that has all necessary settings with just a few clicks.
The Automatic Control window consists of two sections: The one, where you can define your parameters and the one, which shows you the actual settings.

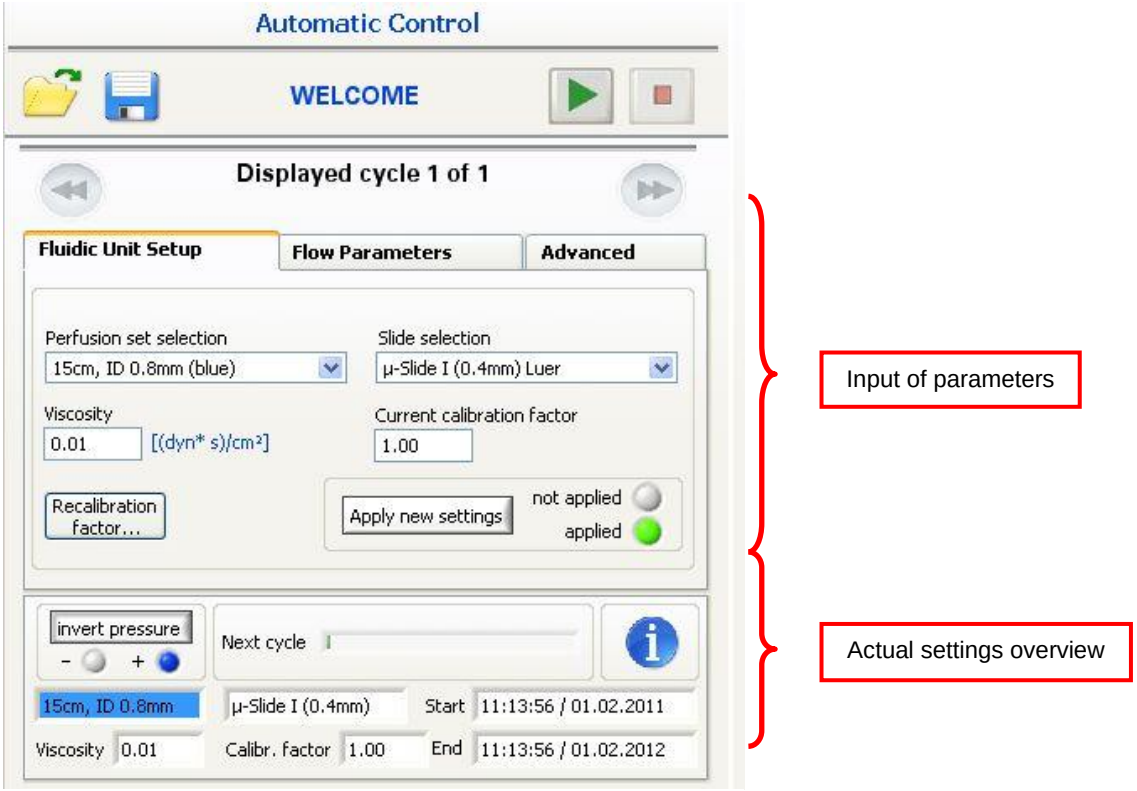


Figure 6: Automatic control panel.

In the ‘Input’ section you will find three registers for defining the setup: ‘Fluidic Unit Setup’, ‘Flow Parameters’, and ‘Advanced’.

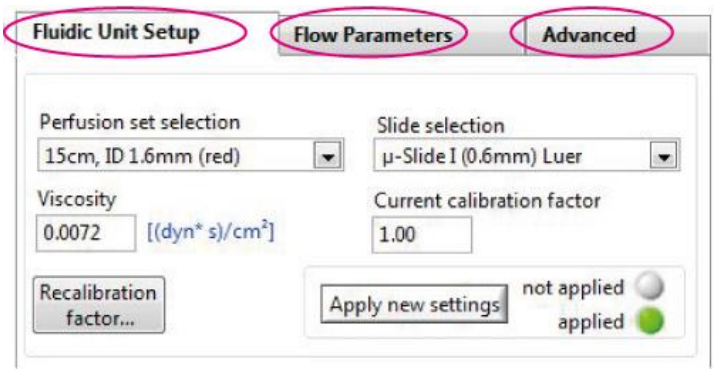


Figure 7: Three register tabs for defining the parameters.

The ibidi Pump and PumpControl runs whether or not you define your Fluidic Unit setup. However, if you want to make use of the automated calculation of the shear stress or the flow rate, you have to set the information about the viscosity of your medium, the selected Perfusion Set (control 1 in Figure 8) and µ-Slide (control 2 in Figure 8) in the register tab ‘Fluidic Unit Setup’.

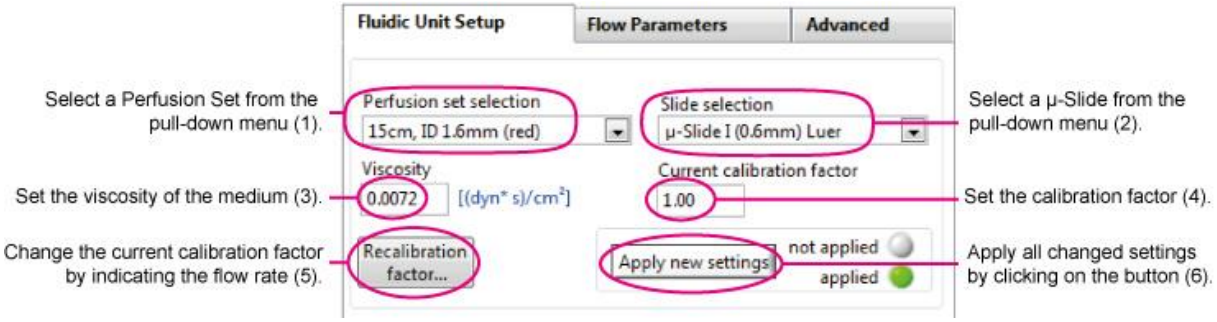


Figure 8: Fluidic Unit setup.

Please note: The 'Apply new settings' button needs to be pressed after every change.

After entering your settings, you need to confirm the change by pressing the 'Apply new settings' button (control 6 in Figure 8). If any parameters such as µ-Slide, Perfusion Set, viscosity, or the recalibration factor have been changed, the 'Apply new settings' button will start blinking. The blinking stops when these changes are confirmed. This will mean that the software is now using this new set up for the internal calculations.

3.1 Flow Parameters

3.1.1 Definition of Flow Rate, Shear Stress, or Shear Rate

After selecting the viscosity, Perfusion Set, and µ-Slide, you can define the desired flow parameters, such as the flow rate (control 1 in Figure 9), the shear stress (control 2), or the shear rate (control 3). You can only define one parameter. The scheduler will automatically calculate the required pressure of your inputs. Please select the register tab 'Flow Parameters' to choose these settings.

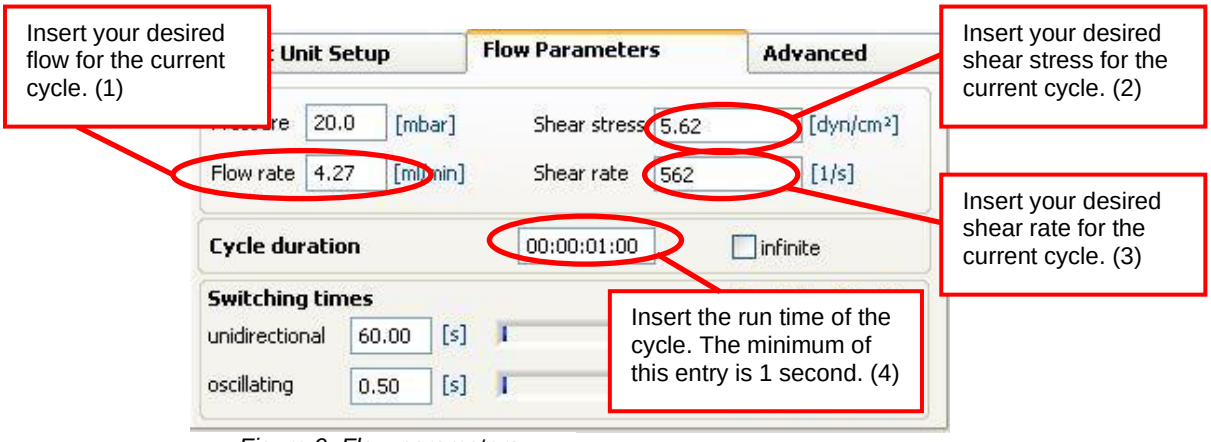


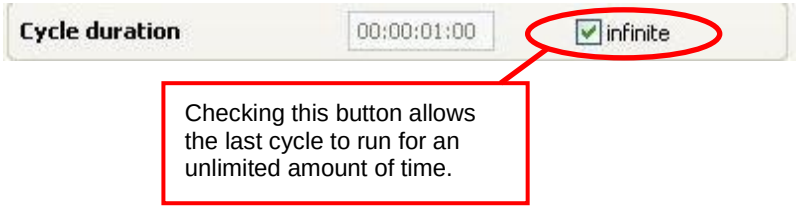
Figure 9: Flow parameters.

You can also insert a pressure amount and observe the changes in flow rate, shear stress, and shear rate.

Please note that based on the calculated flow, the switching time will change. However, the cycle time can still be modified manually after that calculation

3.1.2 Cycle Duration

The cycle duration defines the length of a cycle, with its defined flow parameters. Since the cycle parameters also include the switching time, the cycle duration must be a multiple whole number of the switching interval. The cycle duration must be set for every cycle. You can find the cycle duration in the respective box (control 4 in Figure 9). The unit for this setting is [DD:HH:MM:SS]. If you wish to run the cycle in an endless loop, you need to check the box 'infinite'. Once the 'infinite' check box is active, the creation of additional cycles is no longer available. Please note that only the last cycle has the option to be infinite.



3.1.3 Active Pump Ports

The software automatically detects all connected Fluidic Units (active pump ports). This can be verified either by controlling the checked boxes, shown in Figure 10, control 3. It can also be verified on the manual control panel, in the valve overview. Active valve switches (control 11-13 in Figure 4) are shown in full color, inactive valve switches are greyed out.

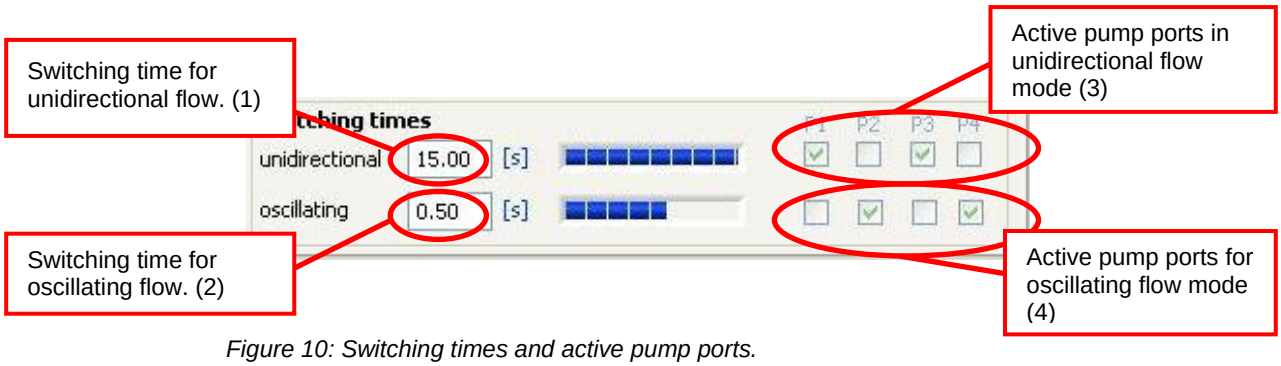


Figure 10: Switching times and active pump ports.

3.1.4 Switching Times

There are two different switching times: unidirectional and oscillating. Which switching time applies to the Fluidic Units can be controlled by checking the respective boxes (control 3 and 4 in Figure 10).

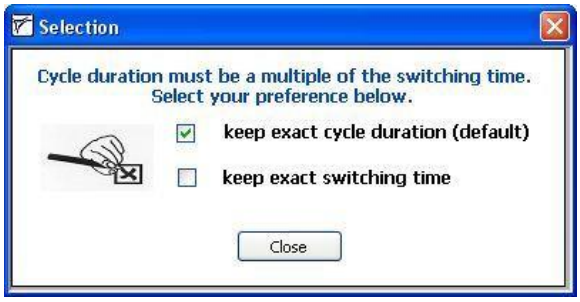
3.1.4.1 Unidirectional Switching Time

The unidirectional switching time is needed in every experiment, at least for one Fluidic Unit. In order to prevent the reservoirs from running dry, the valves of the Fluidic Unit(s) need to be switched before the liquid level reaches the bottom of the syringe. This time depends on the flow rate and is called 'switching time'.
After each switching operation, the flow direction of the medium in the reservoirs is inverted (not in the slide!). In the standard case, this switching time is calculated from the flow rate. If you want to define the switching time yourself, you can insert the value in the box. There are three aspects to consider when choosing an appropriate switching time:

1. During the time interval, the reservoir must not run dry.

2. The vertical height difference between the liquid levels in the reservoirs never should not be too high, in order to prevent pressure variations due to the hydrostatic pressure variations. This is important when working with low pressure (5–10 mbar).
3. Also, the switching time should not be too short, to ensure that fresh medium can be brought to the cells.

If you change the switching time (unidirectional), make sure that the cycle duration is a multiple of the switching time. Otherwise, a new window will open, as shown below.



The default setting keeps the exact cycle duration. This option will adjust the switching time. The other setting keeps the exact switching time. When you choose this option, the cycle duration will be adjusted.

3.1.4.2 Oscillating Switching Time

The PumpControl software also supports oscillating flow assays that can simulate turbulent flow at the cell position. This type of experiment requires you to change your setup, and this is described in the ibidi Pump instructions.
The main Fluidic Unit must be set in the unidirectional switching mode. Up to three Fluidic Units can be run in the oscillating switching mode. The oscillating switching time does not influence the inversion of flow in the reservoirs. This must be controlled by the unidirectional switching time of the main Fluidic Unit.

3.2 Start the Scheduler (Automatic Control)

Before you start the Scheduler program, make sure the reservoirs contain the same amount of liquid (e.g., 5 ml in 10 ml reservoirs, or 1 ml in 2 ml reservoirs).

If you've followed the previous instructions, you are ready to run your experiment.

By pressing the 'Play' button (), the automatic flow process will start. A pop-up window will appear in the monitor. If you want to disable the function, uncheck the according checkbox.

The 'Play' button will then change into a 'Pause' (), allowing you to pause the current experiment at a specific point, and restart it later.

While the schedule is running, we recommend that you observe the liquid levels in the reservoirs so that you can control the flow rate. If for any reason the flow is blocked, or the Fluidic Unit doesn't work properly, the reservoirs might run dry and/or the cells in the µ-Slide might not be supplied with the medium. Please refer to the trouble shooting section 6 to solve this problem.

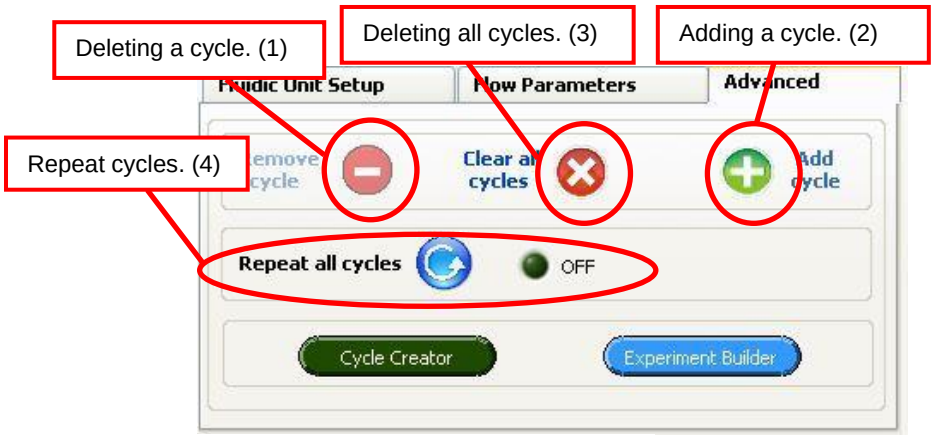


Figure 11: Adding and deleting cycles.

3.2.1 Add More Cycles to Your Scheduler

By following steps 3.1.1 to 3.1.4, you have input all of the necessary information needed to set up a cycle, and start an experiment. However, in some cases you might want to run successive cycles, with different settings, to perform your experiment. For that, you can add a cycle by clicking on the 'Add cycle' button (control 2 in Figure 11) on the register tab 'Advanced'. Afterwards, you'll have a copy of the first cycle that can be modified as described in sections 3.1.1 to 3.1.4. You can add as many cycles as you need. To switch between the cycles, you have to click on the 'next cycle' button, or the 'previous cycle' button, shown in Figure 12, controls 1 and 2.

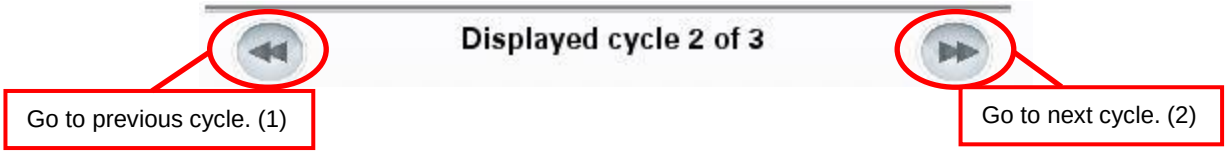


Figure 12: Switching between different cycles.

3.2.2 Clear All Cycles

On the register tab 'Advanced', you'll find a button labelled 'Clear all cycles' (control 1 in Figure 13). This function allows you to clear all programmed cycles at once.

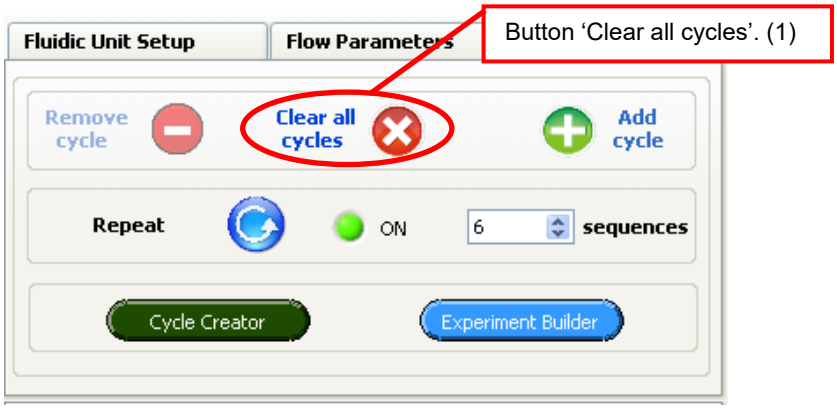


Figure 13: Clear all cycles.

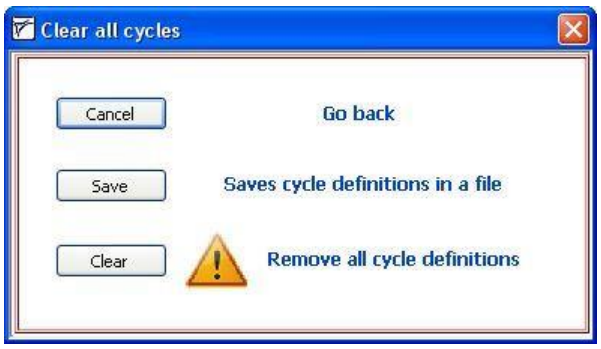


Figure 14: Clear all cycles (options).

After clicking on this button, a safety query will pop up (Figure 14). In this dialog box, you can choose from three options:

1. 'Cancel' → you will return to your main program.
2. 'Save' → you will save all current cycle definition sets into a file.
3. 'Clear' → all your cycle definition sets will be deleted.

3.2.3 Repeat All Cycles

If you want to repeat one cycle, or a series of cycles, you can build loops. For this action, you'll find a button in the 'Advanced' tab, labelled 'Repeat all cycles' (control 4 in Figure 11). By activating this button, you can choose the number of sequences you'd like to run from the set of cycles you've already created.

In the example below, you have previously created three cycles, as shown in Figure 15. If you decide to 'Repeat all cycles' six times, as indicated in Figure 6, you will end up running 18 cycles, in the order of 1➔ 2 ➔ 3 ➔ 1 ➔ 2 ➔ 3, and so on.

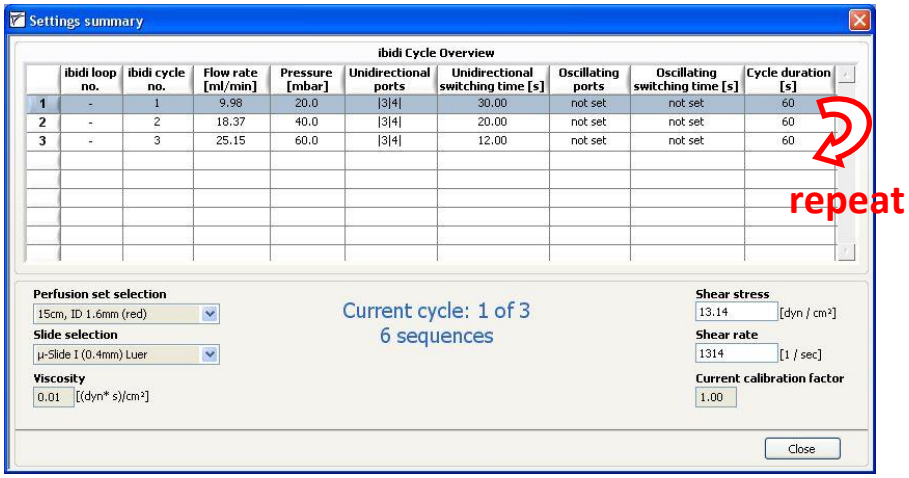
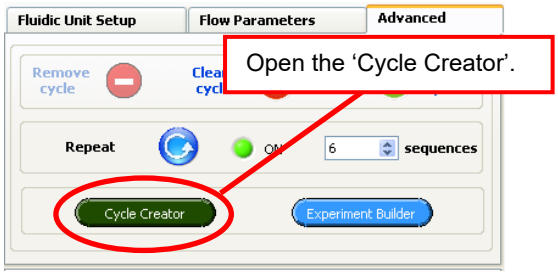


Figure 15: Set of cycles.

If you want to check the status of your experiment, you can always open the 'information' box (control 2 in Figure 29 on page 26) to see how many sequences have been generated, and which cycle is currently being executed. This overview function is described in more detail in section 4.2.

3.2.4 Cycle Creator

The ‘Cycle Creator’ function is designed to help create sets of cycles without having to click through the different register tabs. Once you’ve entered the ‘Cycle Creator’, a table will appear and you will be prompted to enter the following parameters for the ibidi Pump, and the connected Fluidic Unit(s):



1. The flow rate, which will result in a certain air pressure
2. the cycle duration,
3. the active pump ports, and also
4. the switching time of the individual cycle.

You can also modify the ‘Fluidic Unit Setup’, for example the Perfusion Set and µ-Slide. These parameters will be valid for all cycles that are being created.

The ‘Cycle Creator’ will automatically list all the cycles that are currently programmed into the PumpControl session.

In Figure 16, you can see an example of some entries in the ‘Cycle Creator’ function.
To run an experiment, you need to define at least one cycle. However, you can create an experiment containing 100 cycles or more; each of them can, and most likely, will have different flow settings.

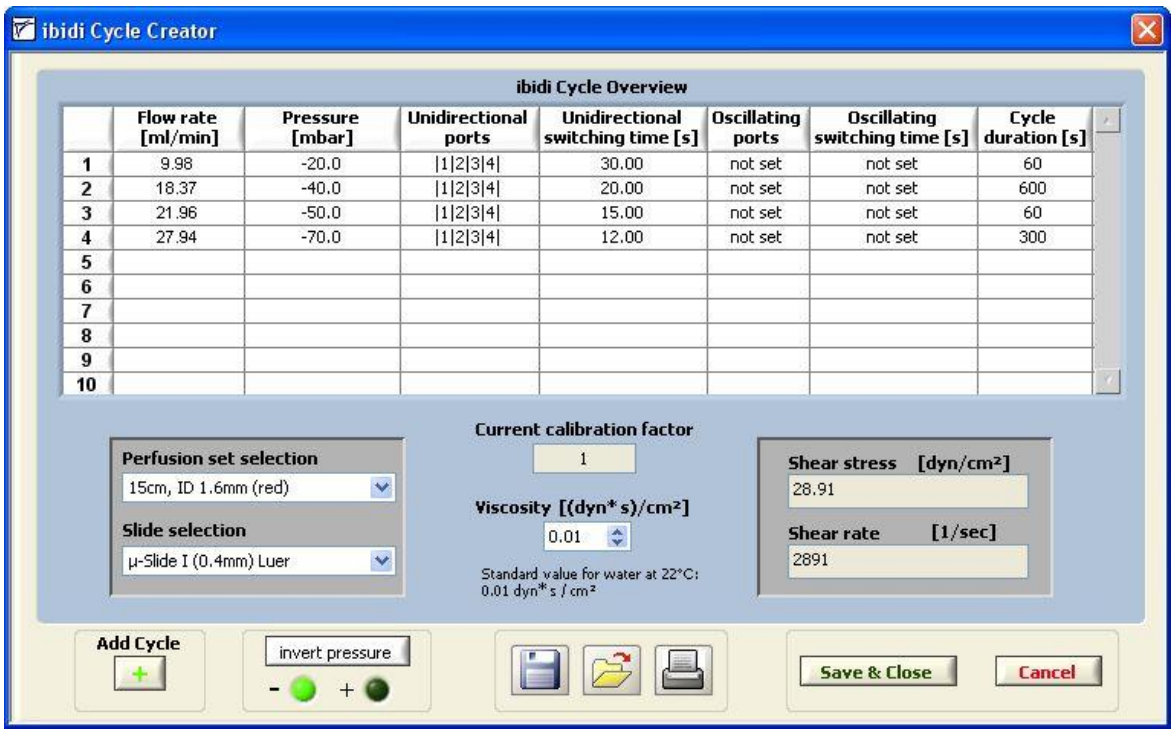


Figure 16: Set of cycles in the 'Cycle Creator' table.

Example:

Your cells need to be adapted to specific flow conditions. We recommend starting with a low flow rate and then increasing the shear stress over time:

- First cycle: Low flow for 30 minutes and a shear stress of 2 dyn/cm²
- Second cycle: Shear stress of 5 dyn/cm² and cycle duration of 30 minutes
- Third cycle: Long cycle duration of 3 days and final shear stress of 10 dyn/cm²

These different flow rates can be realized by defining the three cycles. Afterwards, you can still change the order of the list, which will then become the order for the schedule.

Add a Cycle to Your Overview

To create a new cycle, you can either right-click in the list and choose ‘Add Cycle…’, or press the ‘Add Cycle’ button, as shown in Figure 17.

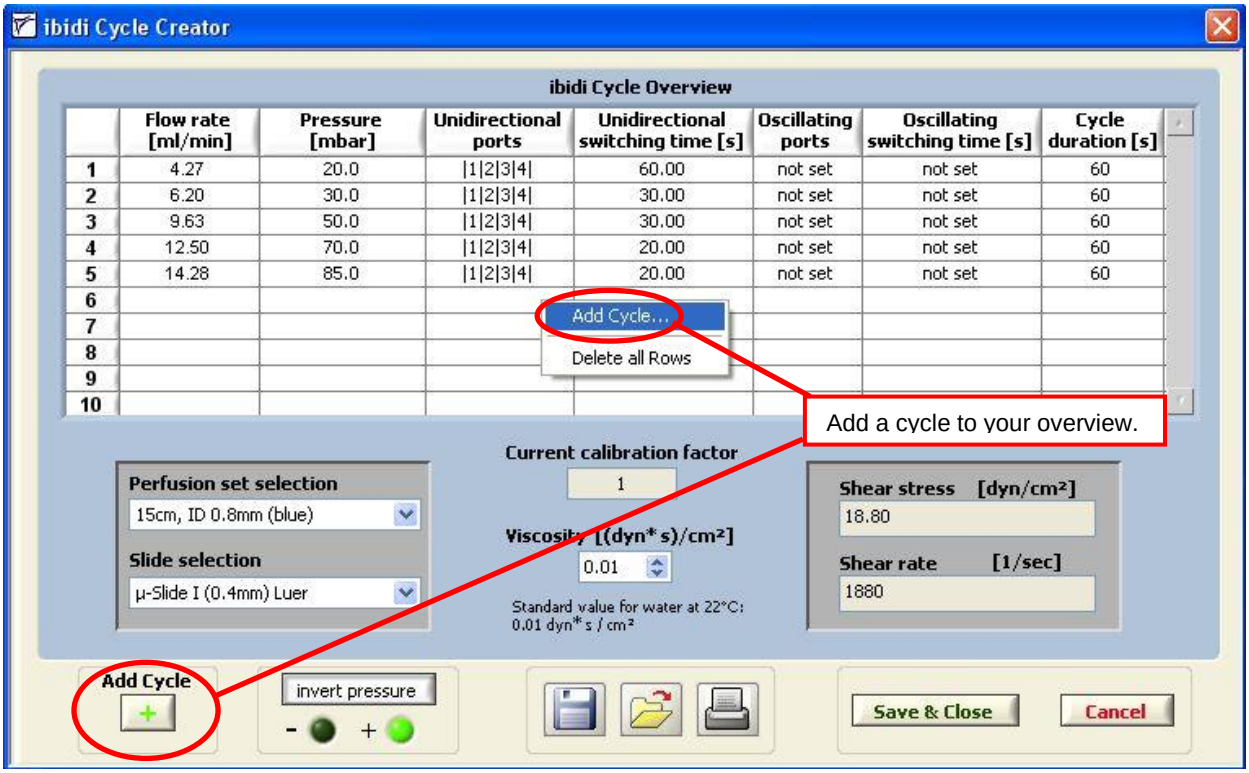
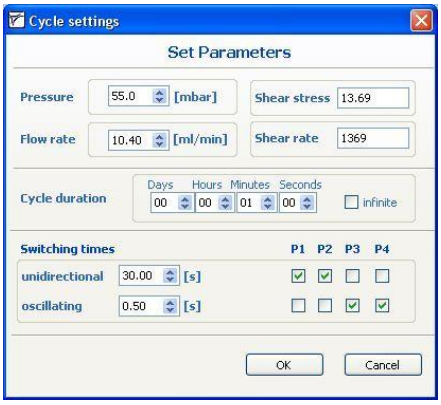


Figure 17: Create a new cycle.

A new dialog box will open where all the required parameters can be set (see picture below, ‘Cycle settings’). If you have more than one Fluidic Unit, you are able to select between unidirectional and oscillating flow. For more details, please refer to the ibidi Pump manual.

Once the ‘Cycle settings’ window is open, the following parameters can be entered:

1. Pressure
2. Flow rate
3. Shear stress
4. Shear rate
5. Cycle duration
6. Pump ports (P1–P4)
7. Switching times



Please note that, depending on the flow rate, the cycle time of the unidirectional switched valves will be internally recalculated. You will still be able to manually modify this value.

After confirming your selection with the ‘OK’ button, the cycle is added to the overview.

3.2.4.1 Modifying Cycles

By right clicking on a row that has an existing cycle set in the table, you can modify the entries, as shown in Figure 18. Here are the following options:

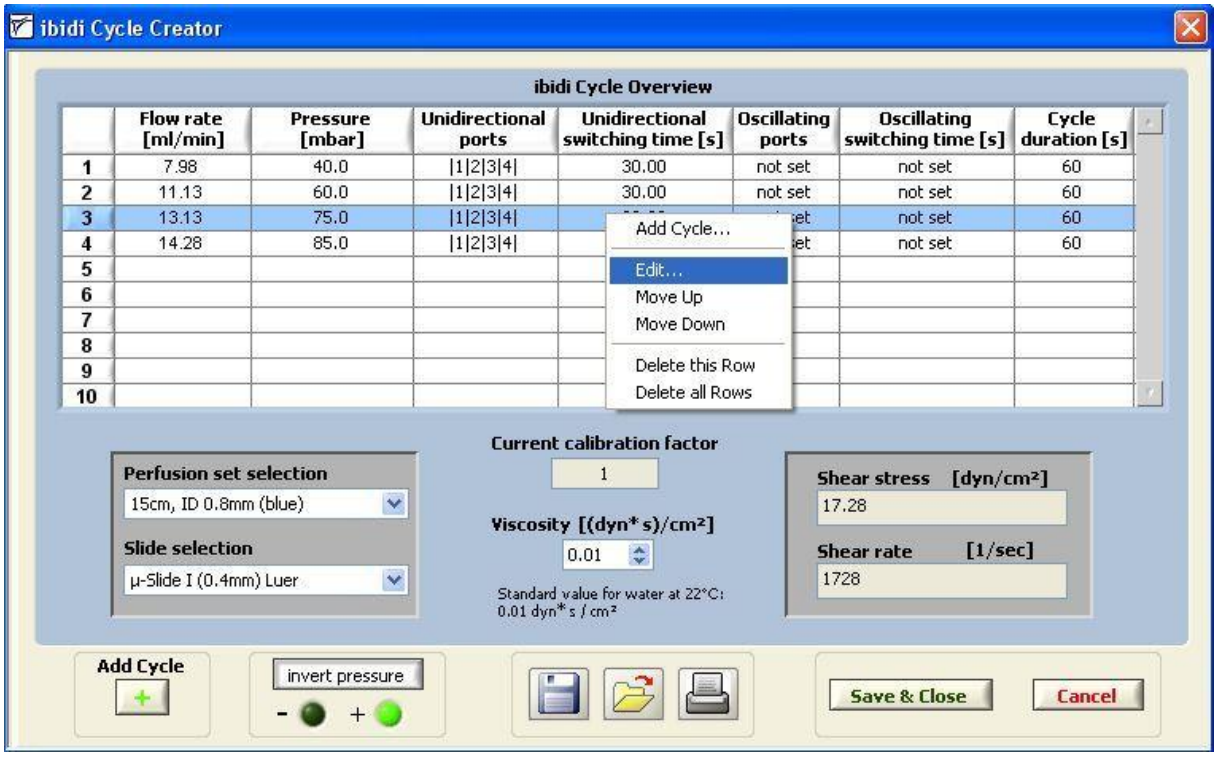
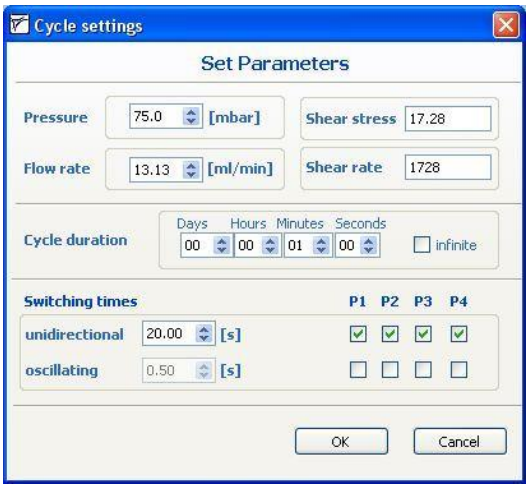


Figure 18: Modifying Entries in the 'Cycle Creator'

- Add another cycle to the list of cycles ➔ ‘Add cycle…’
- Edit the cycle ➔ ‘Edit…’
- Move a cycle up or down ➔ ‘move up’ / ‘move down’
- Delete a cycle ➔ ‘Delete this row’
- Delete all cycles ➔ ‘Delete all rows’

If you choose to edit a cycle, the ‘Cycle settings’ dialog box will appear:



3.2.4.2 Additional Functions

The ‘Cycle Creator’ includes most of the functions available in the PumpControl main menu. Figure 19 shows the overview with a brief description.

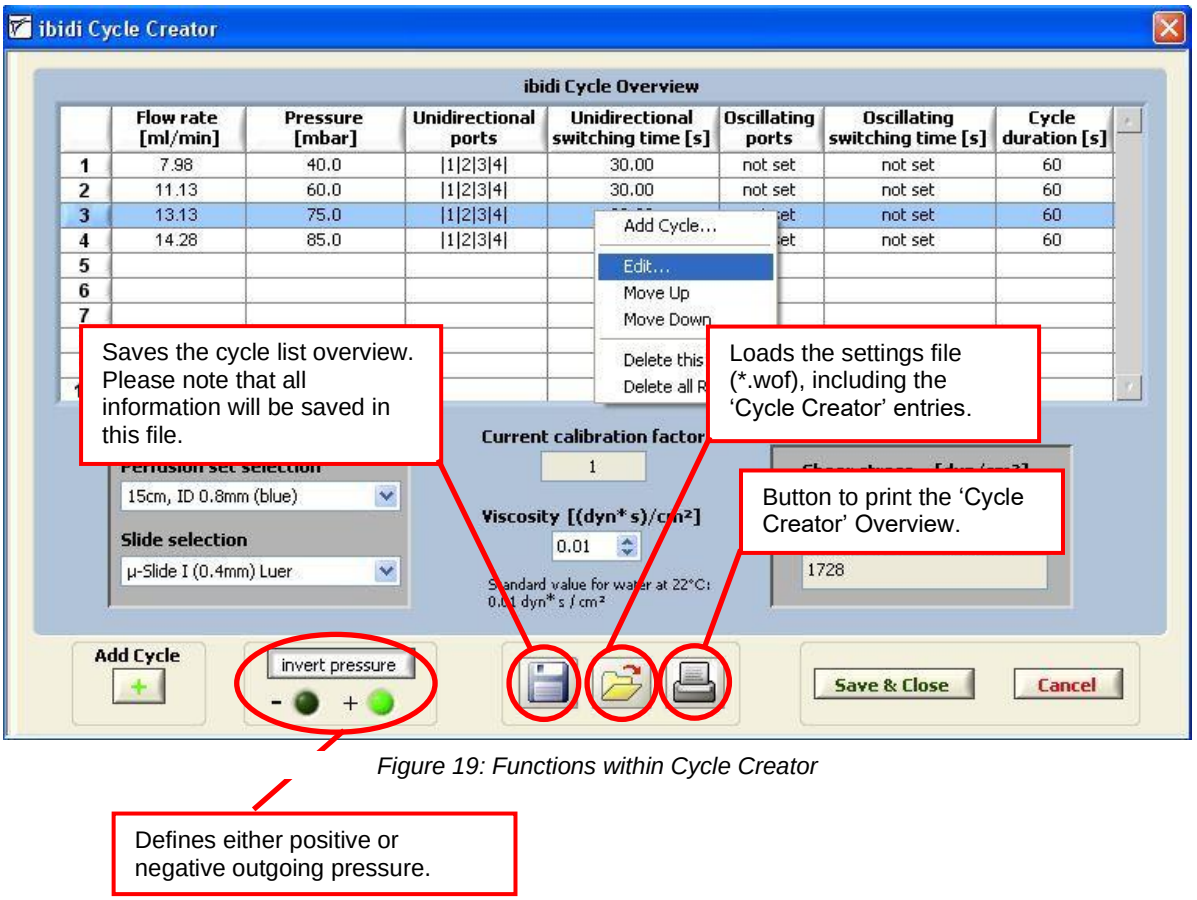
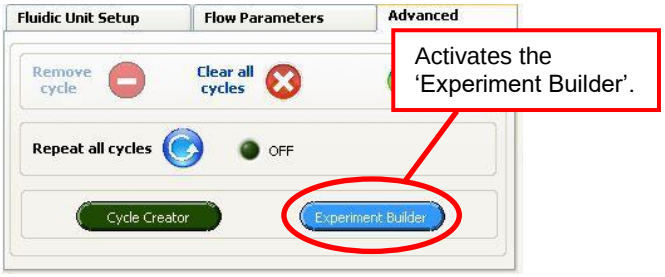


Figure 19: Functions within Cycle Creator

3.3 ibidi Experiment Builder

In addition to the Cycle Creator, we offer a function called the 'Experiment Builder'. With this tool, you can insert loops into your cycle schedule. You can loop any cycle you have already programmed into the Cycle overview.



Before you start the ibidi 'Experiment Builder', you need to have created at least one complete cycle set. If you want to use more than one set, you should first arrange your list in the desired order. This is done because all cycles are initially executed once (e.g. if you have 3 cycle sets, as shown in Figure 20, you will always start your experiment by executing cycles 1 to 3, before starting the first loop).

Once you have arranged your cycle list, you can click on the 'Experiment Builder' button, as illustrated above. A new window will open and all the programmed cycles will appear in the 'Cycle Overview' table, as shown in Figure 20.

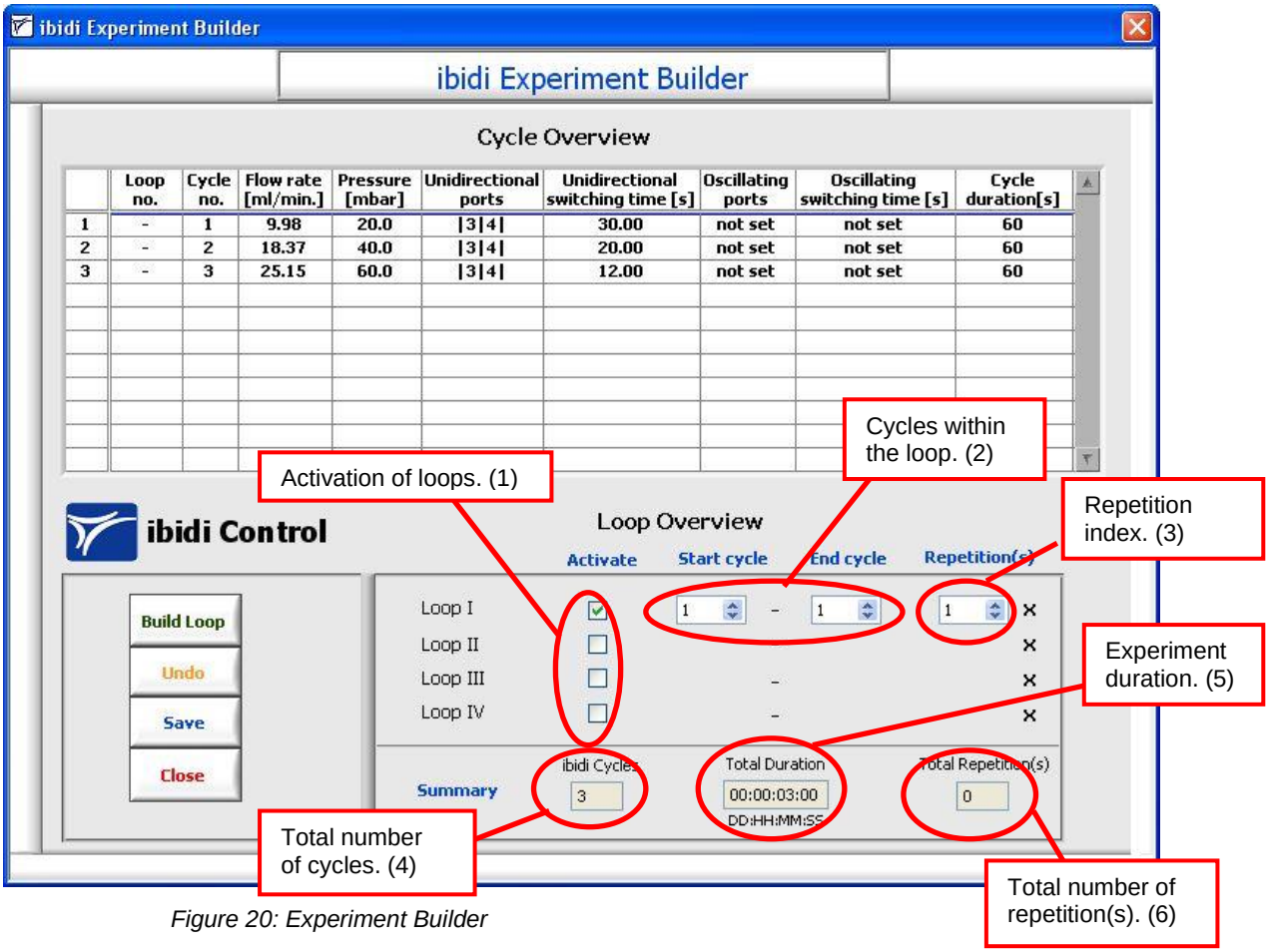


Figure 20: Experiment Builder

There are several functions shown in this window:

1. Activate up to four loops (Loop I → IV) (control 1, in Figure 20).
2. Choose the start and the end cycle (control 2).
3. Choose the number of runs per loop (control 3).

4. Transfer the loop content to the Cycle overview by pressing the ‘Build Loop’ button.
5. Undo this operation with the ‘Undo’ button.
6. Store the new cycle list so that the cycles will be executed by pressing the ‘Save’ button.
7. Afterwards, you can close the window with the button ‘Close’.

Additionally, you’ll find the indicators 4 and 5, as shown in Figure 20. These show the total number of cycles of your experiment, and also the total duration. Indicator 6 displays the repetition(s) of the loops.

Example:

Step 1-3:

- 3 loops are activated.
- Start and end cycles are entered.
- The number of repetition(s) is selected.

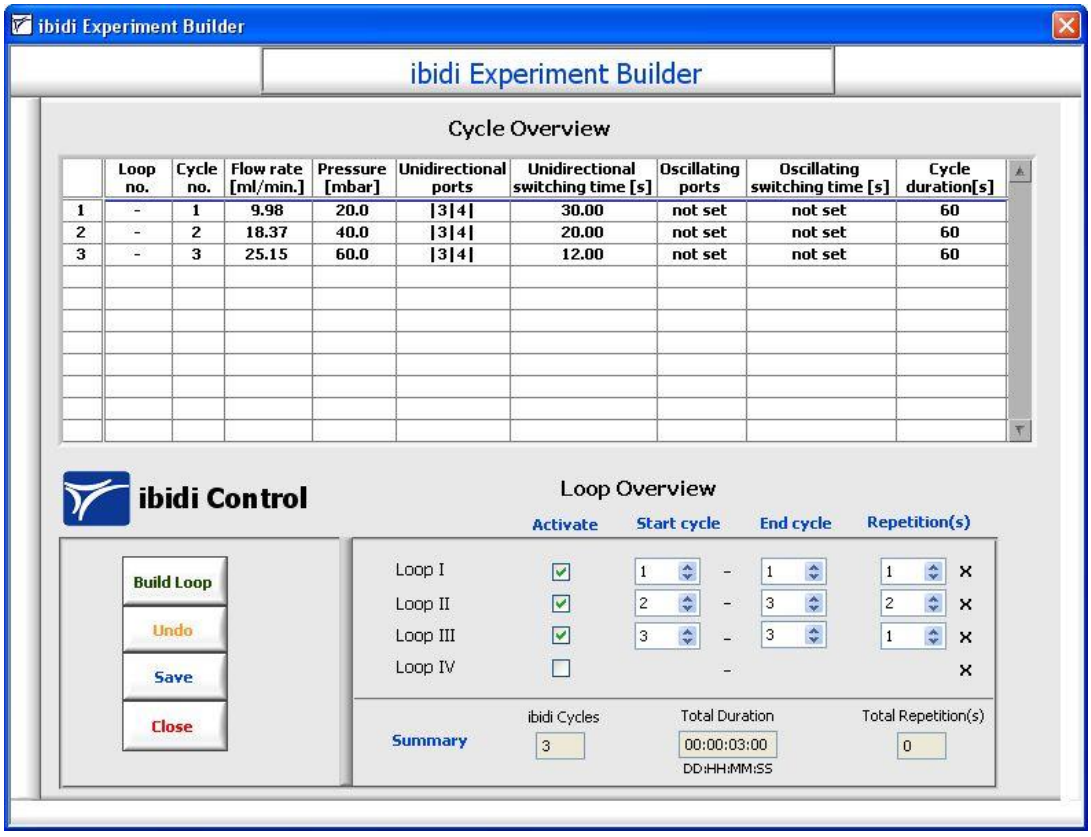


Figure 21: Experiment Builder Steps 1-3

Step 4:

- The loops are transferred to the Cycle Overview list with the ‘Build Loop’ button.

Please note: the individually programmed cycles from the main menu of PumpControl will always appear, and be executed, one time at the beginning of the experiment. These cycles are characterized with (-) and are called the ‘basic cycles’.

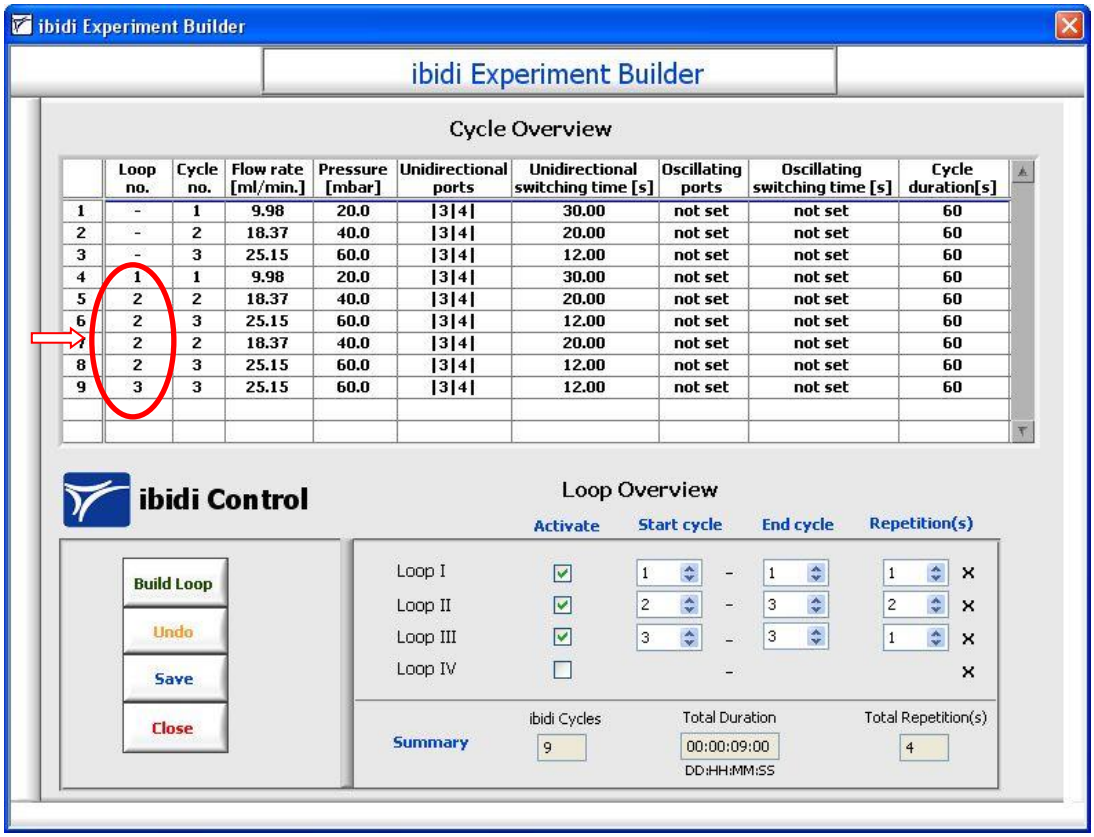


Figure 22: Experiment Builder Step 4

Step 5:

- It is possible to erase the created cycle loop list.
- When you press the ‘Undo’ button, all loops in the Cycle Overview will be erased.
- After this, you can create new loops.

Step 6:

- When you press the ‘Save’ button, after Steps 1-4, the new cycle list will be transferred to the main PumpControl menu. You can view the cycle list in the Cycle Overview table (see Figure 23) or by selecting the information box, as shown in section 4.2 (control 2 in Figure 25).

	Loop no.	Cycle no.	Flow rate [ml/min.]	Pressure [mbar]	Unidirectional ports	Unidirectional switching time [s]	Oscillating ports	Oscillating switching time [s]	Cycle duration[s]
1	-	1			|1|2|3|4|	60.00	not set	not set	60
2	-	2			|1|2|3|4|	30.00	not set	not set	60
3	-	3			|1|2|3|4|	30.00	not set	not set	60
4	1	1	4.27	20.0		60.00	not set	not set	60
5	2	2	7.98	40.0		30.00	not set	not set	60
6	2	3	11.13	60.0	|1|2|	30.00	not set	not set	60
7	2	2	7.98	40.0	|1|2|	30.00	not set	not set	60
8	2	3	11.13	60.0	|1|2|3|4|	30.00	not set	not set	60
9	3	3	11.13	60.0	|1|2|3|	00	not set	not set	60

Figure 23: Cycle Table of the Built Cycles

Step 7:

- You can exit your ibidi 'Experiment Builder' by pressing the 'Close' button. Once you've successfully exited this operation, you'll see the message shown below in Figure 19.

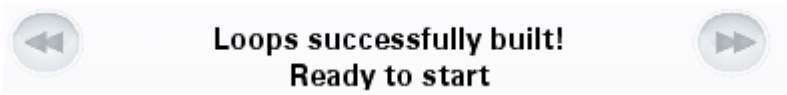


Figure 24: Loops Main Menu Information

3.4 Overview of the Actual Flow Settings and Experiment Status

At the bottom of the 'Automatic Control' panel, in **Fehler! Verweisquelle konnte nicht gefunden werden.**, you'll find the overview and information sections like indicated in Figure 25.

In the middle of Figure 25, you'll find the horizontal progress bar, indicating the start of the next cycle. On the lower right-hand side, the experiment start and finishing times are shown. Next to this, the 'Fluidic Unit Setup' is displayed and shows the selected µ-Slide, the Perfusion Set, the viscosity, and the calibration factor.

In this panel, you'll also find two functional buttons: 'invert pressure' and 'i'nformation. With the 'invert pressure' button (control 1 in Figure 25), you can invert the airflow by switching between the positive and negative air pressure. Activating the 'i'nformation button (control 2 in Figure 25), allows you to reach an overview table that lists the cycles you've created. In that menu, the active cycle is highlighted. More details can be found in section 4.2.

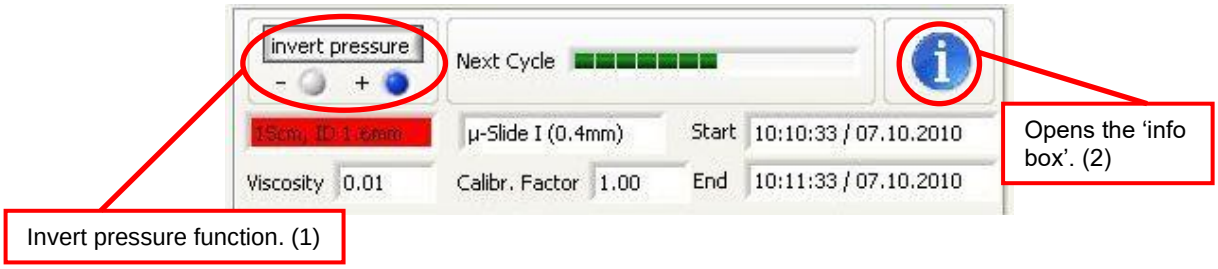


Figure 25: Overview and Information

3.5 Recalibration of the Fluidic System

Recalibration of the Fluidic System
As previously explained, PumpControl automatically calculates the flow rate and the shear stress from a given air pressure. This is achieved by internal calibration tables. For most applications, the automatically generated values are sufficient. However, you have to keep in mind that the calibration conditions (using distilled water at 20 °C) might differ too much from you own settings. In this case, it is necessary to calibrate your system in order to correct your specific pressure-flow relation. Calibration is easily done using the 'Recalibration factor …' function. We recommend calibrating your system each time you change your setup, especially when you rely on very precise shear stress values. Recalibration is also needed when you observe a strong deviation from the expected flow rate.

This function is reached by clicking on the 'Recalibration factor…' button, after which the 'Recalibration dialog' box will appear (see Figure 26).

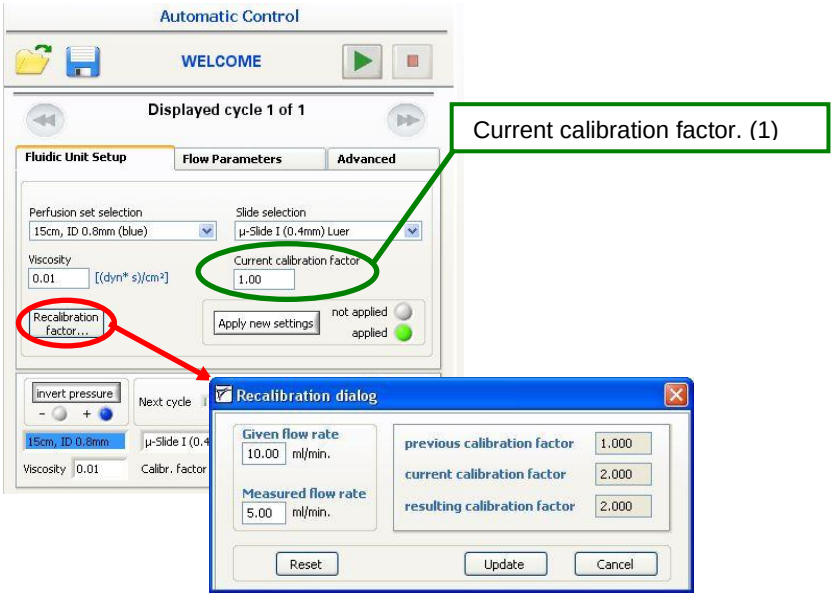


Figure 26: Recalibration Factor

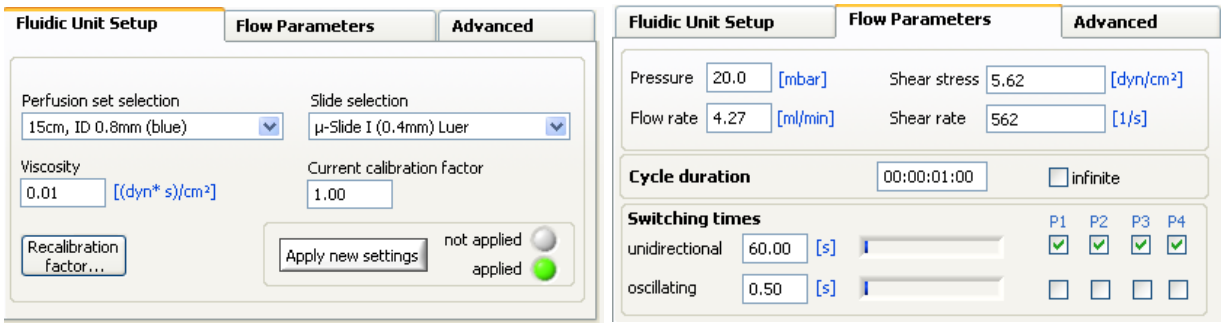
The current calibration factor is indicated in the register tab as control (1) in Figure 26. The default value is 1.00 (for water at 20°C).

3.5.1 How to Run a Recalibration?

Below, you will find an example of a recalibration. Please follow the next steps to perform your own calibration:

Step1:

- Create a complete cycle, including the 'Fluidic Unit Setup' and the 'Flow Parameters', as shown in the figure above.
- Set up a perfusion experiment without any air bubbles. Use the same condition (tubing, µ-Slide, medium and temperature) as will be used for your experiment.
- Equilibrate the reservoirs.



Step 2:

- Measure the time the medium takes to flow 2 ml. If you filled the reservoirs with 10 ml of medium and equilibrated it at around 5 ml, we recommend measuring the time the medium needs to run from 4 ml to 6 ml. It is sufficient to use the markers on the reservoirs to achieve the accuracy of the system.

Now you can calculate the flow by inserting the time you measured into the formula below:

$$\Phi\left[\frac{ml}{\min}\right] = \frac{2ml \cdot 60\frac{\sec}{\min}}{t[\sec]}$$

Step 3:

- Now you can use this value to update the PumpControl software in the 'Recalibration factor…' menu. To do so, click on the 'Recalibration factor…' button and insert the preset flow rate (control 1 in Figure 27) and also the measured one (control 2). You will end up with a new calibration factor (indicator 3, in Figure 27), which will correct the flow rate, the shear stress, and the shear rate.

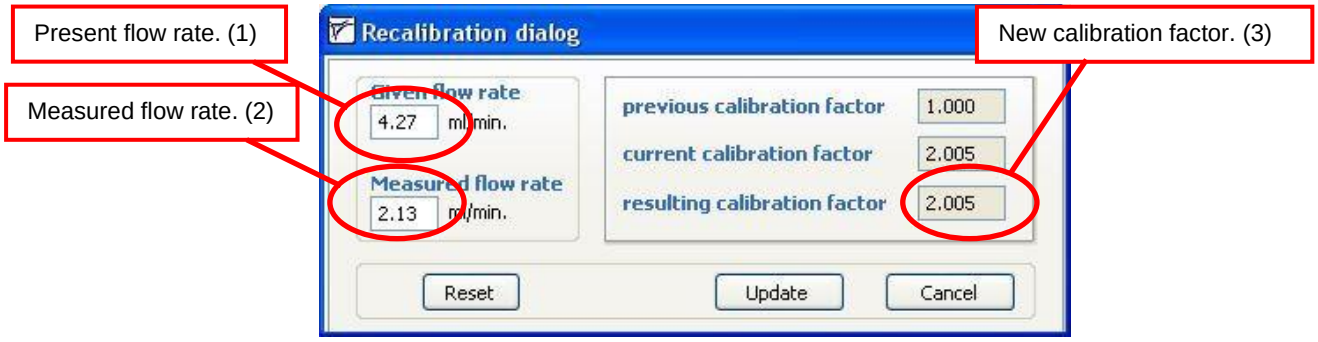


Figure 27 Recalibration Dialog

Explanation of the 'Recalibration dialog' window:

- The default value for the 'Given flow rate' is 1.00 ml/min. This value has to be set to the expected value.
- Initially, the 'previous calibration factor' is the default value, or your 'current calibration factor'. Please note that if you have calibrated your system before, and you have already changed the calibration factor, this value will differ from the default.
- The 'current calibration factor' is the ratio from the 'Given flow rate' to 'Measured flow rate'. In our example it is '2'.
- The 'resulting calibration factor' is the product of the 'previous calibration factor' and the 'current calibration factor'. Here it is also '2'. Again, if you had a 'previous calibration factor' that is not '1', the 'resulting calibration factor' is:

(resulting calibration factor) = (previous calibration factor) × (current calibration factor)

With this method, we can account for the previous calibrations, and you are able to iteratively find your optimal calibration. Normally, only one calibration operation is necessary, due to the precision of the system.

When you now confirm your new calibration, by clicking on the 'Update' button, the window closes and the 'resulting calibration factor' will automatically be set as your 'Current recalibration factor'.

Next, you need to accept the new settings by pressing the blinking 'Apply new settings' button. Afterwards, this value is stored in the system. In our example, you will find this as *Calibr. Factor = 2.00* in Figure 28.

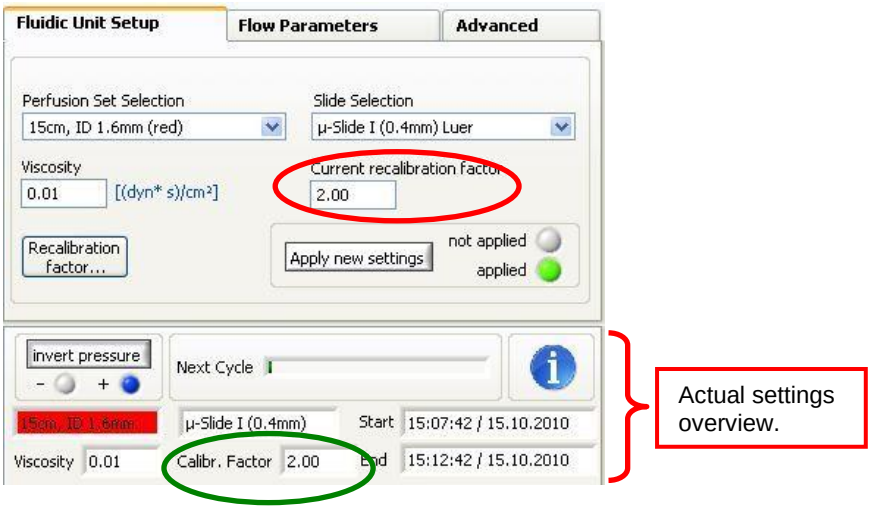
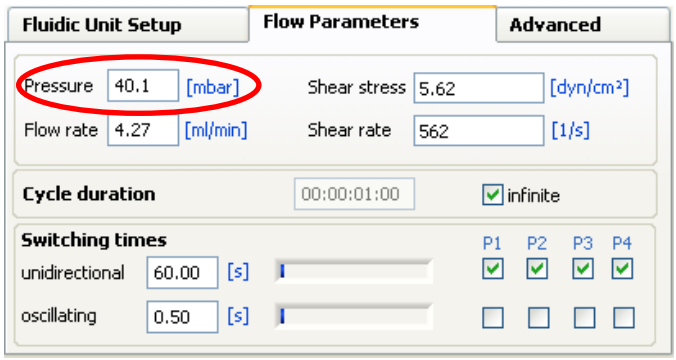


Figure 28 Applied Settings

Once the ‘Current recalibration factor’ has been changed, the air pressure will be adapted. The pressure is the only parameter that will be modified, as shown in the figure below:



If you want to undo your manual calibration, you can use the ‘Reset’ button in the ‘Recalibration dialog’ window and confirm with the ‘Update’ button. This will restore the default values in the system.

4. Control During an Active Scheduler

While the Scheduler is running, the functions are limited in the ibidi Pump. Most importantly, you can still stop the pump immediately by clicking on the 'Stop' button (controls 1 and 2 in Figure 29). You can also find several indicators (3) to (8) that display the current status of the experiment, as seen in the figure below.

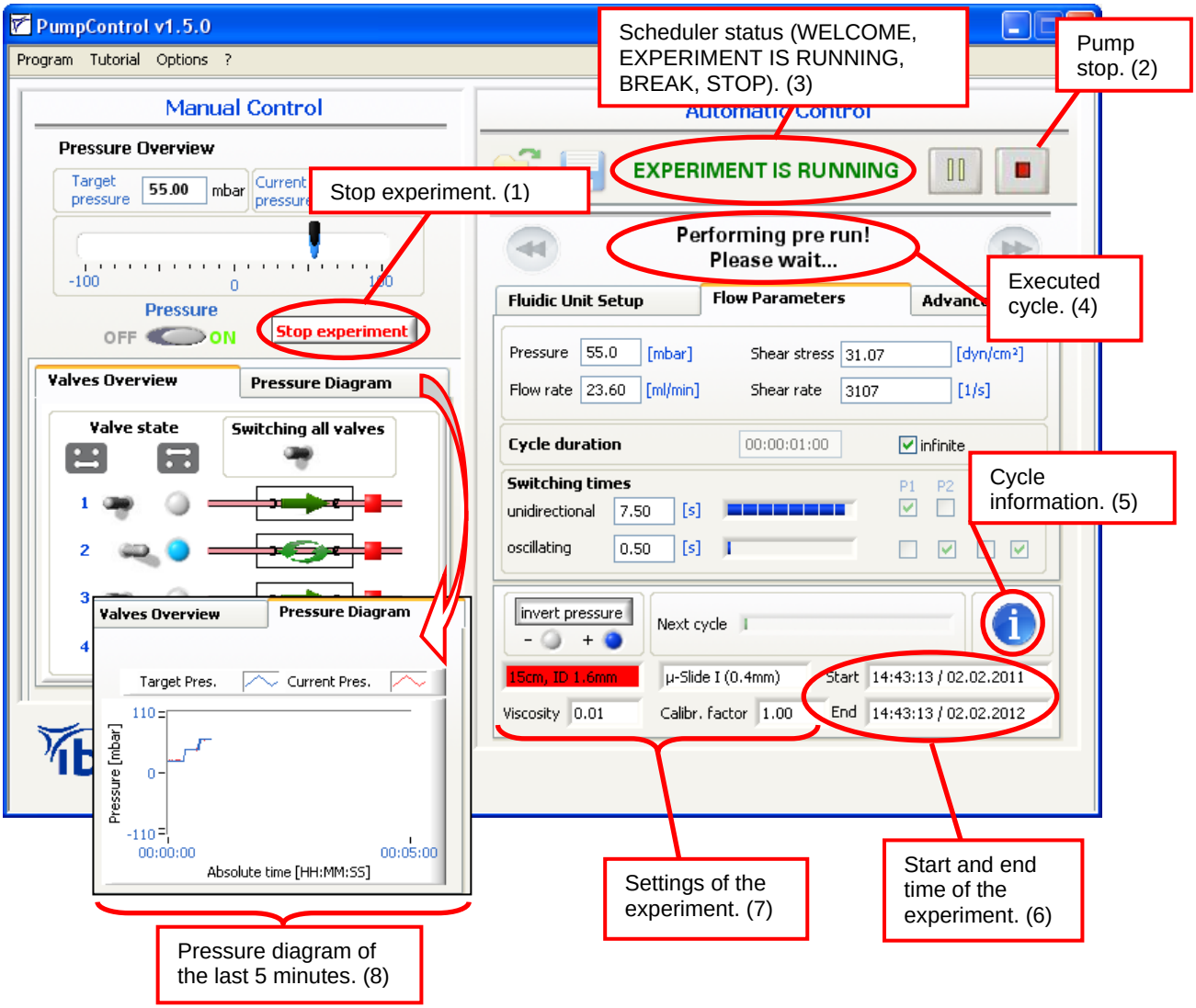


Figure 29: Pump Control During the Course of the Experiment

Pre- and Post-Run

In order to have the liquid levels in the reservoirs equilibrated, PumpControl performs a pre- and post-run at the beginning and end of each cycle. These two runs apply the same conditions to the Fluidic Unit with only half of the switching time. As a result, the minima and maxima of the liquid levels in both reservoirs should be the same.

Please note that the ibidi Pump has no feedback loop for the liquid levels. Therefore, we recommend checking them after starting the experiment, so that the reservoirs don't run dry.

4.1 Working with the Pressure Diagram

The small graph, on the left side of PumpControl menu, only shows the course of the last 5 minutes of your experiment. To see the entire recorded data of your experiment, click on the small diagram to extend the graph (see Figure 30).

Figure 30: Extended Graph Window

In the 'Extended graph' window, you have the opportunity to work with the data:

- You can save the graph into a bitmap or as ascii file. You can also zoom in and out of the graph.

4.2 Settings Summary / Info Box

The 'i'nfo button (control 5, in Figure 29) can be opened at any time to view a summary of the Scheduler's current status. This might be useful during longer experiments, to see which cycle and cycle parameters are being executed.

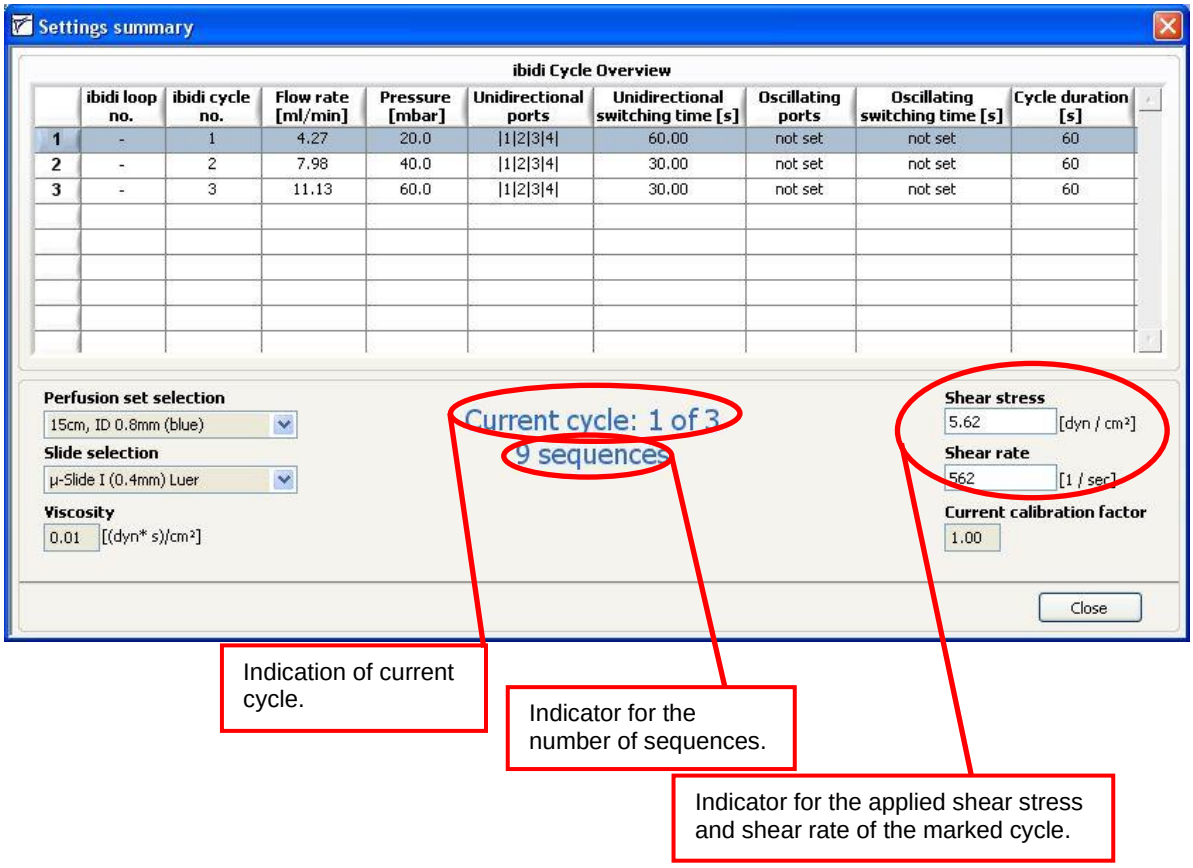


Figure 31: Settings Summary

In the above table, you will find all of the flow parameters:

- Flow rate
- Air pressure
- Pump ports for unidirectional or oscillating flow
- Switching time for unidirectional or oscillating flow, and
- the cycle duration

Below the table, you'll see the setup of your experiment:

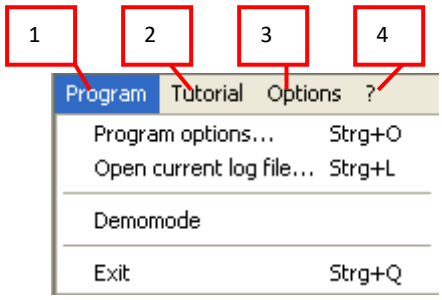
- Selected µ-Slide
- Selected Perfusion Set
- Viscosity, and
- Calibration factor

If you select a cycle from the list, you will find two indicators displaying:

- Shear stress, and
- Shear rate

You can also find the number of stored cycles and the cycle currently being executed. Additionally, the number of outstanding sequences is also displayed.

5. PumpControl Navigation Bar



The four different points are described in the following sections.

5.1 Program

5.1.1

5.1.2 Program Options

To modify the settings for PumpControl, go to 'Program' in the navigation bar and select 'Program options…', or use the shortcut 'Strg'+'O'.

5.1.2.1 General

PumpControl gives you the opportunity to log the data and parameters of your experiment. This can be useful in reproducing any expected, but also the unwanted effects which occurred during your experiment. This data can help you debug a failed experiment. You can turn the logging setting to ON or OFF and also set the recording interval (see Figure 32).

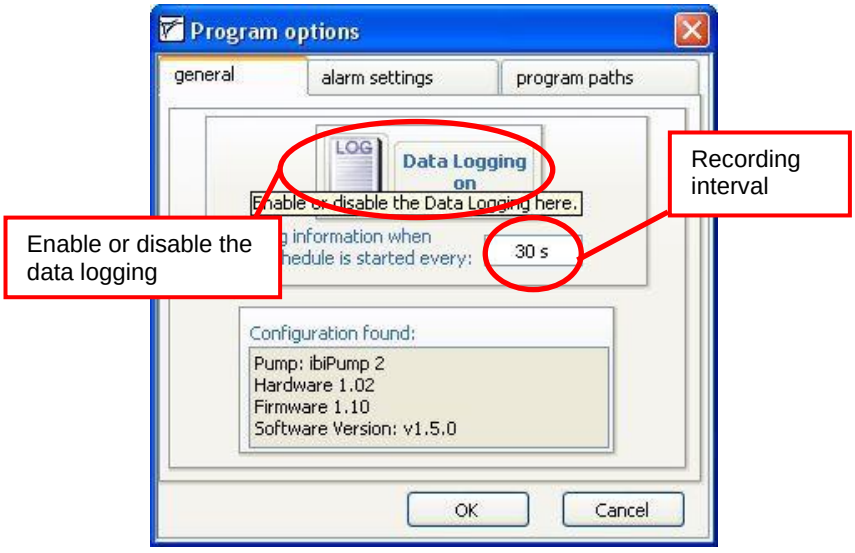


Figure 32: Program Options

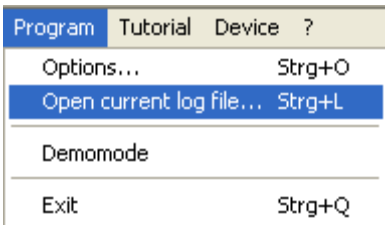
The path of your log file, and all other paths, can be set in the register tab 'Program Paths' (see section 5.1.2.3). By default, the log files are stored in 'C:\Users\[user]\Documents\PumpControl_v1.6.1\log'. Log files are text files (.txt) that can be viewed with any text program ('notepad', 'WordPad', etc…).

The log file contains all the information about the state of the ibidi Pump. It is composed of the following elements:

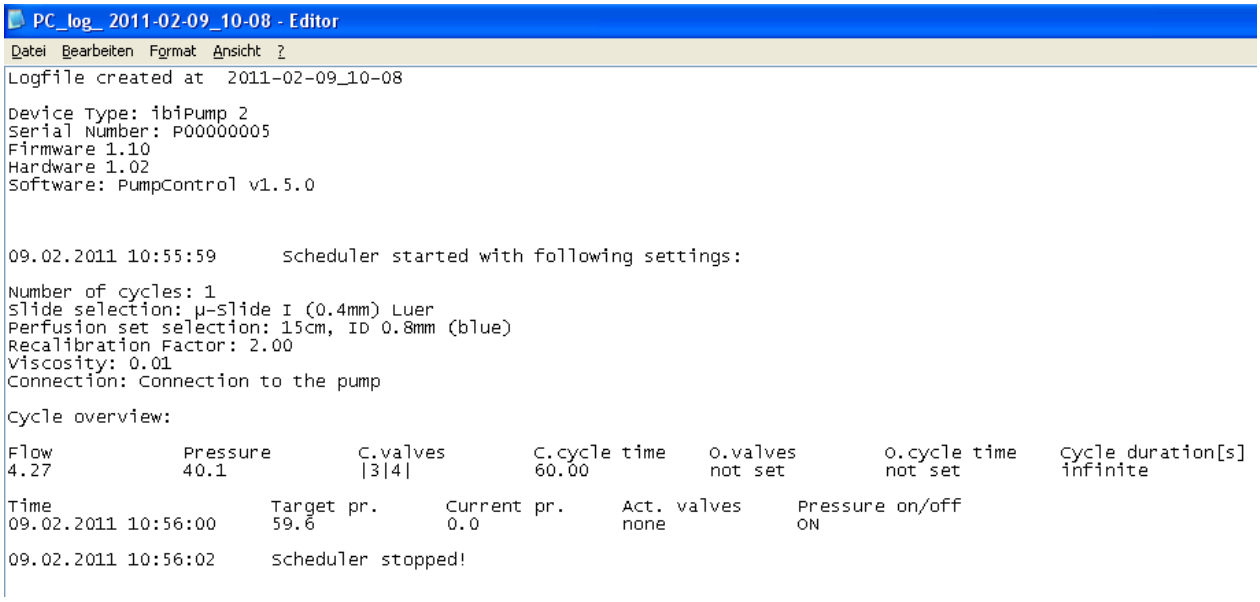
Time	Target Pressure	Current Pressure	Act. Valves	Pressure on/off
Date + Time	55.5 mbar	55.4 mbar	1|2|	ON

The time stamp of the logged data.	The target pressure.	The output pressure.	Active pump ports / Connected Fluidic Units.	Pump status: Pressure On/Off.

The current log file can also be opened by selecting ‘Program’ → ‘Open current log file…’, or by using the shortcut ‘Strg’+’L’.

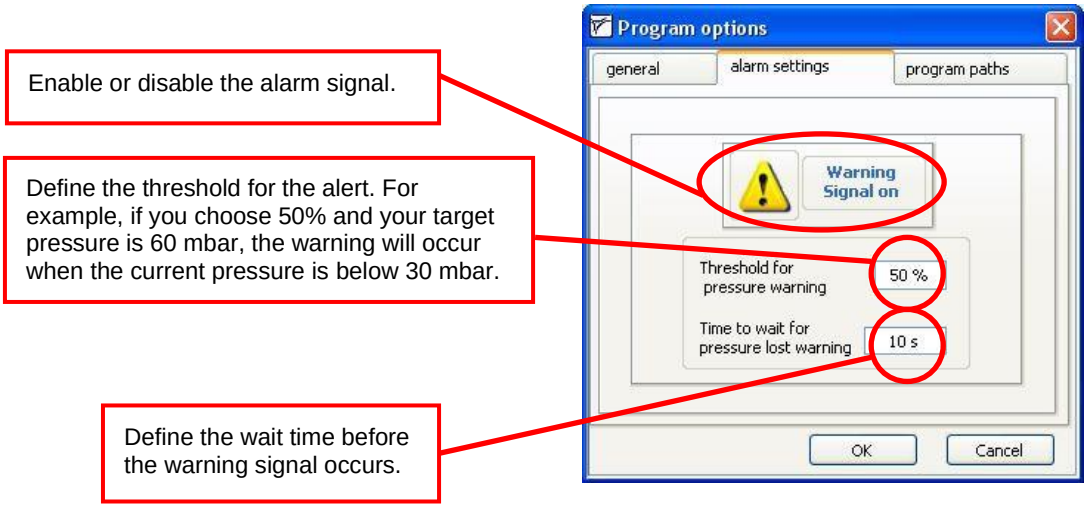


The screenshot below shows you an extract of the log file.

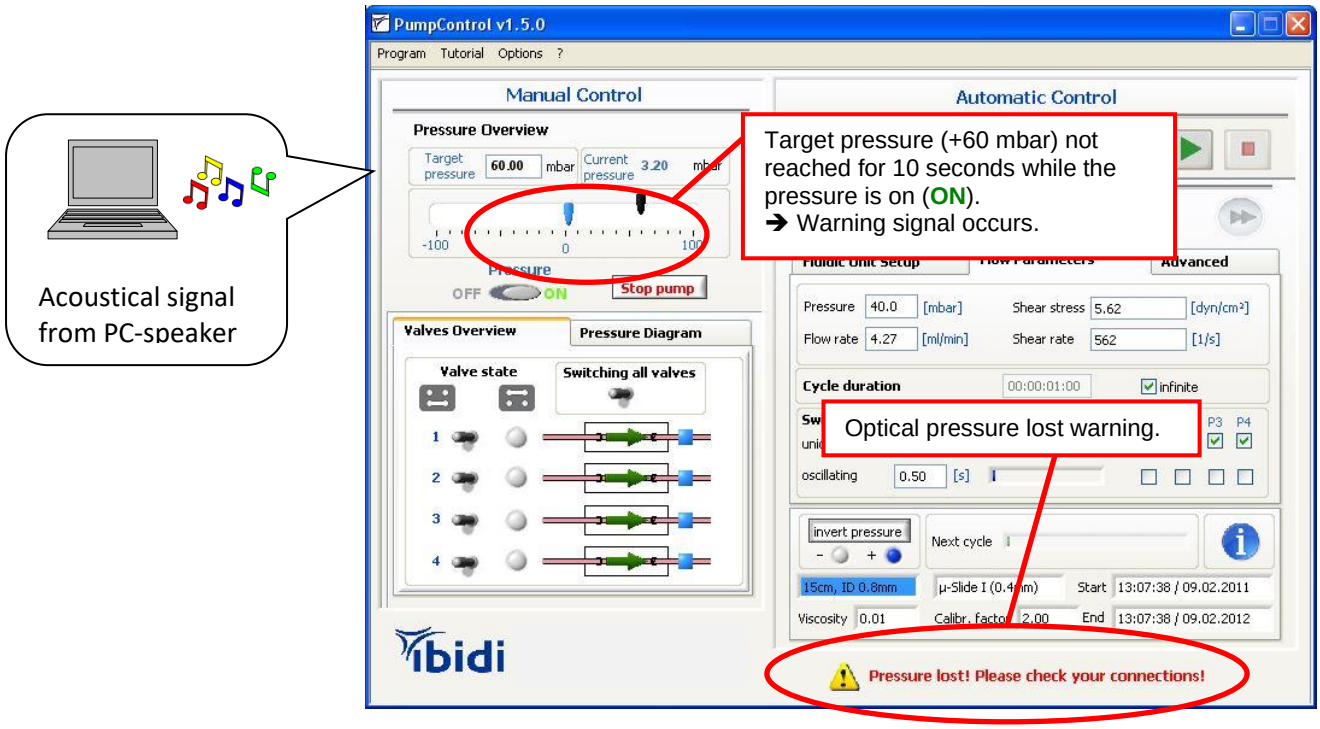


5.1.2.2 Alarm Settings

PumpControl offers an alarm function, with an optical and acoustical warning, if the pump should lose pressure below a certain threshold. This warning is also included in the log file. This feature can be very useful for finding out when, and why, the experiment did not finish as expected.



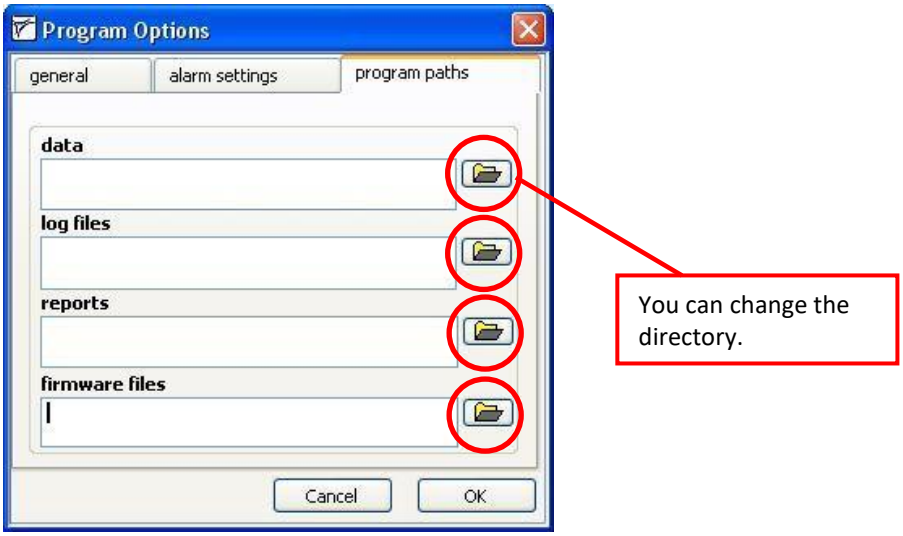
Example:



The most common problem is a communication failure between PC and pump:
'No connection to the pump!' Please check your cable connection(s).

5.1.2.3 Program Paths

The third register tab, in ‘Program Options’, is ‘program paths’. Here you’ll find the four paths where different types of files are stored.



The following files are found under these paths:

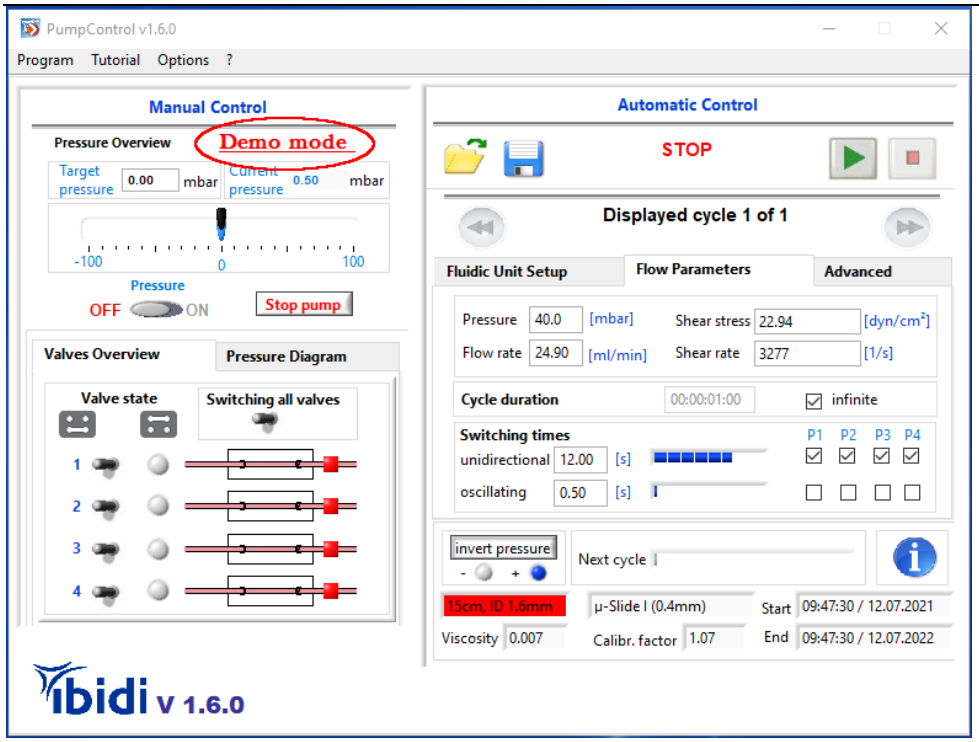
- Data
  - Calibration files
  - Instructions
  - Movies for the PumpControl v1.5.0 (still up to date for v1.6.1)
  - USB driver
  - Software driver
- Log files
  - Log files, as described in section 5.1.2.1.
- Report files
  - Graphical recording of the preset and the output pressure, as described in section 4 on page 26.
- Firmware
  - This path is currently unused.

5.1.3 Open Current Log File…

Please refer to section 5.1.2.1 on page 29 (General).

5.1.4 Demo Mode

You can also use the PumpControl software, even if the pump is not connected to the computer. In this case, the software starts in ‘Demo mode’.



Please do not use the 'Demo mode' when you perform a cell experiment.

When the program is in 'Demo mode', there is no communication with the pump. This usually happens when the USB cable is not connected between the devices (PC➔ pump). If you unclick 'Demo mode', you might see a message as shown in **Fehler! Verweisquelle konnte nicht gefunden werden.**.

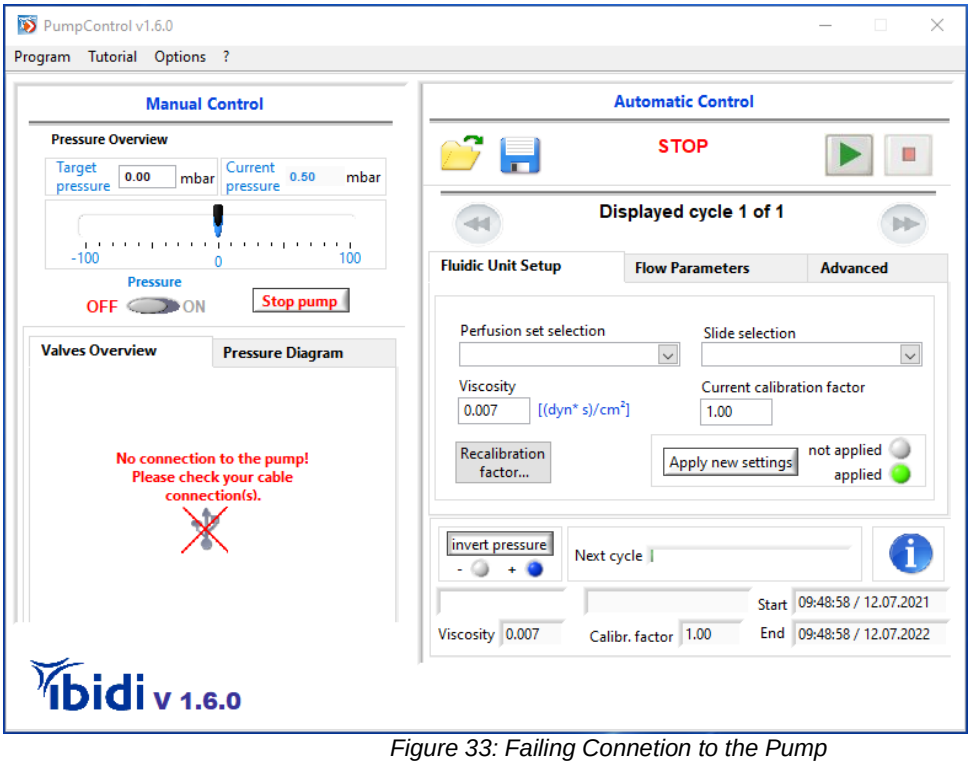


Figure 33: Failing Connetion to the Pump

'No connection to the pump!' Please check your cable connection(s).

5.1.5 Exit

Close PumpControl and save all important parameters.

5.2 Tutorial

5.2.1 Load Demo Setups

5.2.1.1 Demo Experiment

To get started using PumpControl, we've created a tutorial program called 'Demo Experiment'. It shows you some of the typical settings in a sample experiment.

You can access this tutorial by selecting 'Tutorial' ➔ 'Load demo setups' from the menu bar. There, you have the option to load an ibidi demo file. In this file, all cycles are defined and you can immediately start the demo experiment.

Before the settings are applied to the system, the 'Fluidic Unit' is initialized.

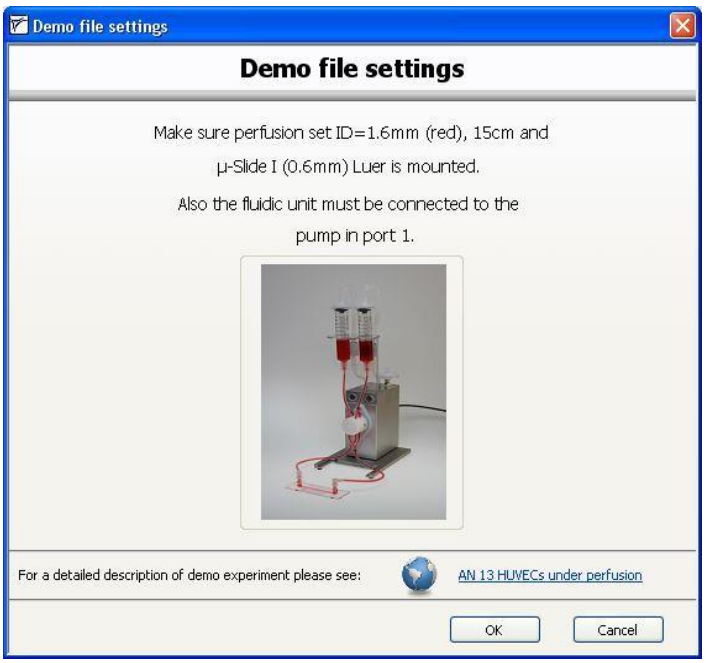


Figure 34: Demo Experiment

Please make sure that if you use this option, you've already installed the red Perfusion Set with the µ-Slide I (0.6mm), as shown in Figure 34.

For a detailed description, please see *Application Note 13 'ECs under Perfusion'*.

5.2.1.2 Remove Air Bubbles

If you find air bubbles in the tubes and/or in the µ-Slide you can remove them by running a flow through the Perfusion Set. You can do this by selecting 'Tutorial' ➔ 'Load demo setups' ➔ 'Remove air bubbles' from the menu bar. There is a defined cycle in this file that creates an appropriate flow through the system to remove the bubbles.

Please make sure that if you use this option you have installed the red Perfusion Set with the middle connector, as shown in Figure 35.

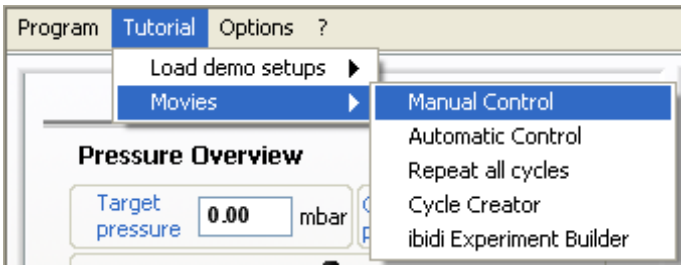
Please be careful that you are not using slides with seeded cells, as they might be washed out.

For a detailed description, please see *Application Note 13 'ECs under Perfusion'*.

Figure 35 Air Bubble Removal Settings

5.2.2 Movies

In the PumpControl tutorial, you will find a library, containing some movies that show how to use PumpControl v1.5.0. They are applicable for version 1.6.1 as well. If you select 'Tutorial' ➔ 'Movies' you can find a list of movies about the basic and advanced functions.



5.3 Options

5.3.1 Settings

Under the menu option, you can find four settings that relate to the following functions in the program.

- Fluidic Unit Starter
- Equilibrated dialog
- Software update
- Firmware update

Here, you can enable or disable the ‘Fluidic Unit Starter’ and the ‘Equilibrated dialog’. You can also check for PumpControl software and firmware updates. For the last two options, you must ensure that your computer is connected to the Internet.

5.3.2 Set Device Parameter…

In the ‘Options’ section of the menu bar, you’ll also find a selection called ‘Set device parameter…’. This function is for the manufacturer only. If you encounter any problems with the ibidi Pump, or with PumpControl, please contact info@ibidi.de.

5.3.3 Manual

You can open the PumpControl instruction manual with this menu option.

5.3.4 About…

In this window, you can find information about the ibidi Pump.

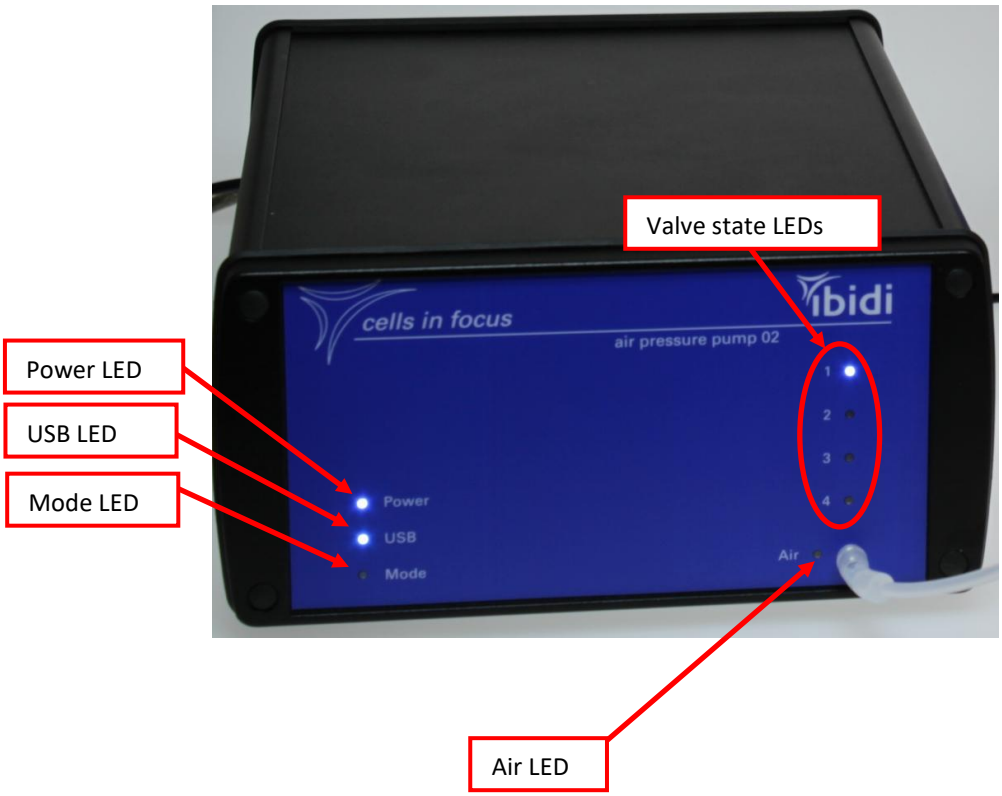
6. Troubleshooting

If you encounter any problems with the ibidi Pump System, please check the table below for some troubleshooting solutions:

Problem	Possible Solution
○ No output pressure	The pressure button is OFF.
○ No continuous flow in the µ-Slide.	The Perfusion Set is not mounted correctly. Refer to the ibidi Pump instruction manual for further instructions.
○ No connection to the pump.	Make sure all electrical connections are plugged in properly. Wait a few seconds and try again. Make sure that all drivers are properly installed, then ➔ Restart PumpControl.
○ Error message occurs.	Sometimes the pump needs an internal restart. To do that, disconnect the power cable and reconnect it afterwards to restart the pump manually.
○ Calibration file not found.	In this case you need set the 'Data path'. In the pump control menu, click on program ➔ options ➔ program path ➔ data (see section 5.1.2.3). On the menu's right side, there's a button for selecting the folder. Choose the right path, (by default, PumpControl is installed in C:\programs\PumpControl v.1.6.x\data), then restart PumpControl. If the problem still remains, please contact us at: info@ibidi.de
○ Reservoirs running dry.	Please check if the Perfusion Set is correctly mounted. Refer to the ibidi Pump instruction manual for further explanations. If the flow rate is not as expected, please recalibrate the system (refer to section 3.5 and follow the instructions).
○ Valves are not switching.	Please make sure that the Perfusion Set is mounted correctly.
○ No communication between PC and pump.	It's possible that the .NET framework software is not yet installed on your PC. In this case, you'll need to install the driver for the software. (see section 1.2. ➔ installation point 5 ➔ Software driver.)

6.1 Troubleshooting Using the Control LEDs

Control LEDs on the Pump:



Power LED

The ‘Power’ LED is on when you connect the power supply.

USB LED

The ‘USB’ LED is on when you connect the pump to the PC with a USB cable, and when the USB drivers are installed. If the drivers are not installed, the light will remain OFF.
To install the USB drivers manually, you need to go to the Windows start menu and choose ‘Programs’ → PumpControl v1.6.1’ → ‘data, where you can find the driver installer (CDM v2.12.06 WHQL Certified.exe).

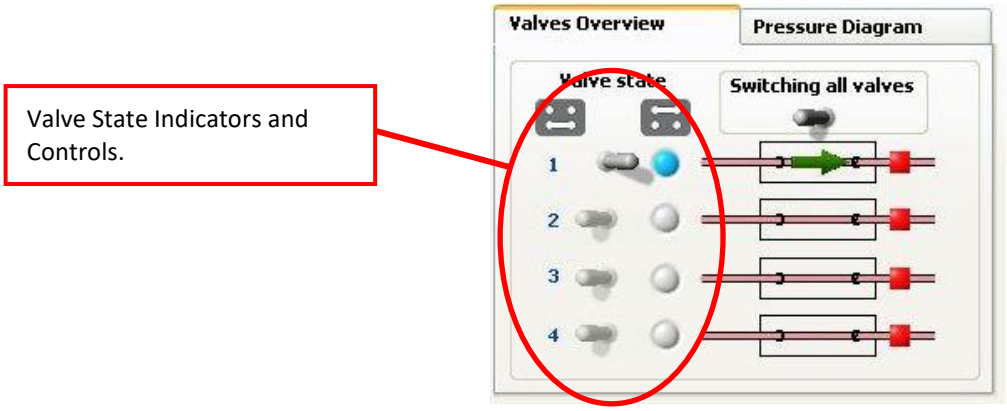
Please double click the file and the system will automatically install the drivers.
Afterwards, the USB light should be illuminated.

Mode LED

When the 'Mode' LED is blinking, the firmware is initializing. The LED is blinking when the pump is connected to the power supply system. The blinking lasts for approximately 5 seconds.

Valve State LEDs

There are four LEDs on the pump to indicate which Fluidic Unit is being switched.
The software is using the same indicators.



Air LED

The 'Air' LED is illuminated when there is pressure at the outlet.

- Pressure ON ➔ LED ON.
- Pressure OFF ➔ LED OFF.

Please contact us for further troubleshooting questions, and to report any bugs you find to **info@ibidi.com**