PH01 Perfusion Cannula Manual

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Multi Channel Systems

MCS GmbH

Aspenhaustrasse 21

72770 Reutlingen

Germany

Fon	+49-7121-9 09 25- 0

Fax	+49-7121-9 09 25-11

info@multichannelsystems.com

www.multichannelsystems.com

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1 Important Information and Instructions

1.1 Operator's Obligations

The operator is obliged to allow only persons to work on the device, who

- are familiar with the safety at work and accident prevention regulations and have been instructed how to use the device;
- are professionally qualified or have specialist knowledge and training and have received instruction in the use of the device;
- have read and understood the chapter on safety and the warning instructions in this manual and confirmed this with their signature.

It must be monitored at regular intervals that the operating personnel are working safely.

Personnel still undergoing training may only work on the device under the supervision of an experienced person.

1.2 Guarantee and Liability

The *General conditions of sale and delivery* of Multi Channel System MCS GmbH always apply. The operator will receive these no later than on conclusion of the contract.

Guarantee and liability claims in the event of injury or material damage are excluded when they are the result of one of the following.

- Improper use of the device
- Improper installation, commissioning, operation or maintenance of the device
- Operating the device when the safety and protective devices are defective and/or inoperable
- Non-observance of the instructions in the manual with regard to transport, storage, installation, commissioning, operation or maintenance of the device
- Unauthorized structural alterations to the device
- Unauthorized modifications to the system settings
- Inadequate monitoring of device components subject to wear
- Improperly executed and unauthorized repairs
- Unauthorized opening of the device or its components
- Catastrophic events due to the effect of foreign bodies or Acts of God

1.3 Important Safety Advice

Warning: Make sure to read the following advice prior to install or to use the device and the software. If you do not fulfill all requirements stated below, this may lead to malfunctions or breakage of connected hardware, or even fatal injuries.

Warning: Obey always the rules of local regulations and laws. Only qualified personnel should be allowed to perform laboratory work. Work according to good laboratory practice to obtain best results and to minimize risks.

The product has been built to the state of the art and in accordance with recognized safety engineering rules. The device may only

- be used for its **intended purpose**;
- be used when in a **perfect condition**.
- Improper use could lead to serious, even fatal injuries to the user or third parties and damage to the device itself or other material damage.

Warning: The device and the software are **not** intended for medical uses and **must not** be used on humans.

Malfunctions which could impair safety should be rectified immediately.

Requirements for the installation and operation

- The heating element produces heat and can get hot during operation.
- Do not touch the perfusion cannula during operation.
- Do not use the perfusion cannula for perfusion with flammable or aggressive (corrosive) liquids.
- Do not store flammable materials nearby during operation.
- Check in regular intervals that the perfusion cannula does not overheat.

2 Installation and Operation

2.1 Welcome to the PH01 — Perfusion Cannula with Heating Element and Sensor

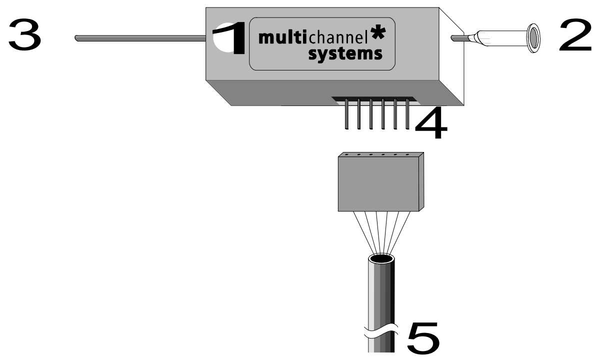
Cell lines and tissue slices are placed and cultivated directly in the MEA culture chamber, on the microelectrode array. All MEA systems can be upgraded with an enhanced perfusion system (indicated by the code –E), featuring a **perfusion cannula PH01 with programmable fluid temperature** and a two-channel temperature controller TC02 for controlling both the MEA culture chamber temperature and the fluid temperature in parallel. A perfusion system is required especially for recordings from acute slices.

The perfusion cannula with heating element and sensor PH01 and a temperature controller TC01/TC02 is also useful for general perfusion purposes, whenever the fluid stream needs to be temperature controlled.

The PH01 is to be used only for the temperature-control of **non-corrosive** liquids up to a temperature of **50 °C**. The programmable fluid temperature is controlled by a temperature controller TC01/02. The PH01 is equipped with a standard platinum temperature sensor (PT 100).

Bath or test solutions can be applied at flow rates from **500 µl to 4.5 ml per minute**. The PH01 can be connected to any tubing that fits to the perfusion inlet (**luer lock**) and outlet (**1 mm** OD stainless steel cannula). A titanium wire inserted into the cannula results in a **turbulent flow** that ensures that the temperature of the liquid is constant across the flow stream.

For setting up a complete perfusion system, a **pump** and a perfusion port for **aspirating** the liquid from the culture chamber are required. Standard setups for MEA recording use a **peristaltic** pump or **syringe** pump, and a standard metal cannula with **beveled tip** for aspiration.



1 PH01
2 Perfusion inlet (luer lock)
3 Perfusion outlet (1 mm OD)
4 TC01/02 connector
5 Heating element cable (connected to TC01/02)

2.2 Setting Up and Connecting the PH01

Warning: Do not operate the PH01 unless it is completely filled with liquid. Otherwise, overheating may irreversibly damage the PH01.

Warning: Do not start the perfusion until you have double-checked that the perfusion lines are set up properly and that the inflow and outflow rate are matching. Spilled liquid may irreversibly damage electronic instruments.

If the setpoint temperature is changed, the controller will need a short time to respond.

Please note that there will always be an intrinsic **offset** between the setpoint and the actual temperature, at the tip of the cannula as well as when the liquid arrives in the culture chamber. The offset at the tip depends mainly on the flow rate, see Flow Rate Dependent Temperature Offset.

The temperature in the culture chamber depends on several parameters such as the tubing diameter, length, and material (if a tube is used for guiding the liquid into the chamber), the type of pump used, the flow rate, and so on. The user has to determine empirically the offset of the individual setup once before starting the experiment.

The tube that leads into the perfusion chamber should be as short as possible to avoid that the buffer cools down in the line. You can also carefully bend the metal cannula itself, so that the outlet can be positioned directly in the chamber.

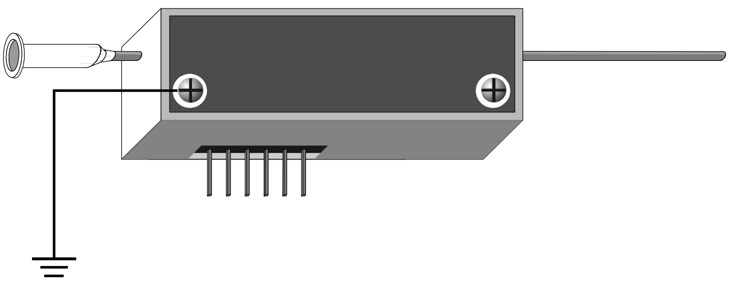
The flow rate should be constant during the experiment to ensure a stable temperature control. Make sure that the ambient temperature is kept constant and shield the setup from air drafts.

You can fix the PH01 directly onto the MEA1060 amplifier (or next to the perfusion chamber), for example with tape.

1. Insert the into the perfusion inlet of the culture chamber (or carefully bend the cannula) so that the liquid can flow into the culture chamber.

2. Connect the (luer lock) perfusion inlet of the to the perfusion tubing (connected to the pump) and tightly secure it.

3. Connect one of the screws on the 's rear side to the ground of the amplifier/setup. Inside the PH01, the cannula is connected to ground via a 10 µF capacitor. This reduces noise to a minimum and avoids DC offset currents.



4. Make sure the temperature controller is switched off. Connect the to one channel of a temperature controller TC01/02.

5. Fix the position of the perfusion cannula (for example, with tape).

6. Set up the aspiration system.

7. Set the inflow rate to the optimum operating range (500 µl/min to 4500 µl/min). Make sure the programmed outflow rate of the aspiration system is identical (or slightly higher) than the inflow rate. For example, you can use a double-head peristaltic pump, and use one channel of the pump for the input and the other for the output. An alternative would be a push-pull pump that holds paired syringes, with one syringe for the inflow and one for the outflow.

8. Carefully double-check the setup. Make sure all perfusion lines are tight and set up properly.

9. Switch on the pump. Again, check that the outflow rate matches the inflow rate.

10. Switch on the temperature controller.

11. Check the temperature of the flow stream. Adjust the setpoint temperature accordingly. See also chapter Temperature Control.

2.3 Flow Rate Dependent Temperature Offset

The following temperature curve shows the deviation of the setpoint and the actual liquid temperature, depending on the flow rate. This gives you a hint on how to adjust the setpoint temperature. For example, if you have a flow rate higher than 4000 µl/min, you will need to set the setpoint temperature to 37.7 °C if you want to achieve an actual temperature of 36.7 °C at the cannula tip. Please note that the actual temperature needs to be verified empirically for each perfusion setup.

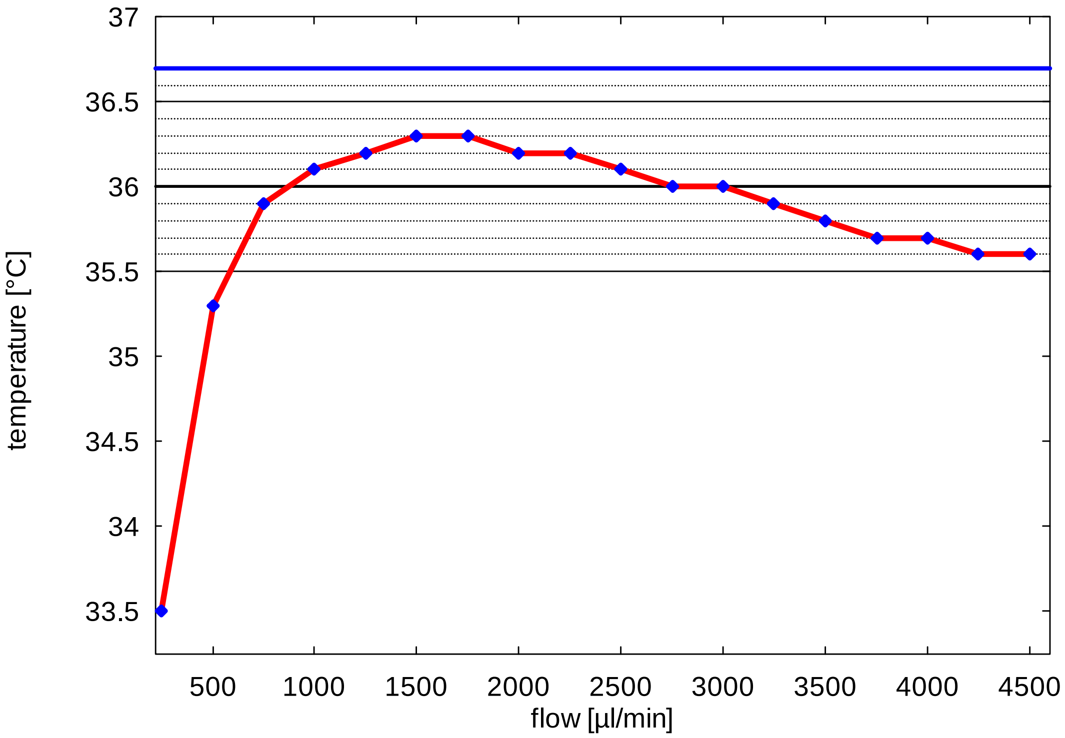


Fig. 1 Actual temperature of the fluid stream, measured at the cannula tip for different flow rates with a setpoint temperature = 36.7 °C and an ambient temperature = 24 °C.

Note: For **flow rates > 1000 µl/min and < 2500 µl/min**, the temperature at the outlet is 36.2°C (± 0.1°C) with a given setpoint of 36.7°C.

For **flow rates lower than 1000 µl/min**, the difference between setpoint and actual temperature is due to the temperature loss along the cannula itself, which depends on the ambient temperature.

2.4 Air Drafts and Ambient Temperature

→ Ensure that the setup is **shielded** from air drafts. Even the smallest degree of turbulences in the air — such as when a person walks by — can affect the PH01's temperature control and thus the outlet liquid temperature as well.

→ Ensure that the ambient temperature remains as **constant** as possible.

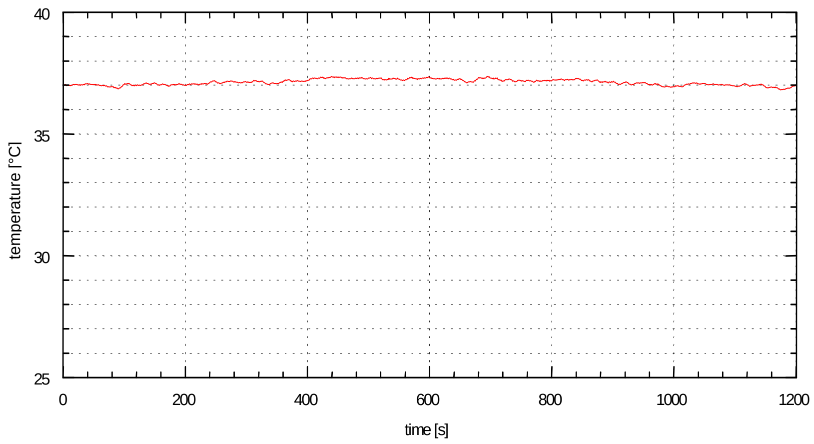
2.5 Pump Types

The differences between **peristaltic** and **syringe** pumps are shown here for comparison.

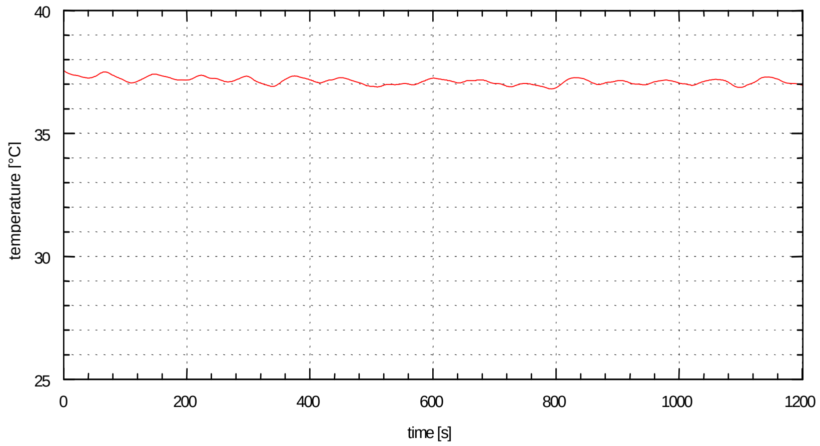
Both types of pumps deliver the same average outflow temperature (37 °C).
The offsets are 0.18 °C for the peristaltic pump and 0.35 °C for the syringe pump
(with a flow rate = 1000 µl/min).

The two illustrations below show typical long-term properties (20 min) for both pump types at a flow rate of 1000 µl/min.

Temperature kinetics using a peristaltic pump



Temperature kinetics using a syringe pump



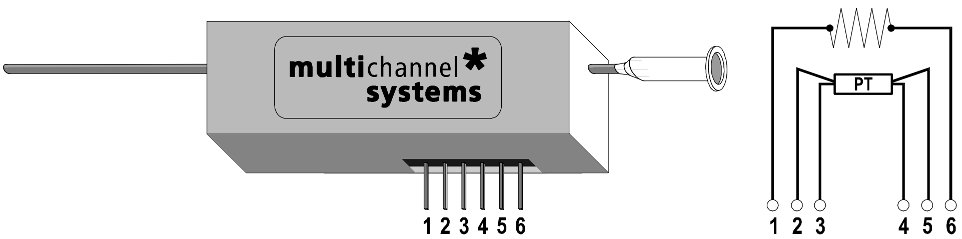
2.6 Flow Stream

A **titanium wire** inserted into the cannula ensures a **turbulent** flow. In turbulent flow, there are no discrete layers of flowing liquid. The momentum of the fluid overcomes the viscous shear forces, and there is extensive and continual mixing across the flow stream. This ensures that the temperature of the liquid is constant across the flow stream. Especially **flow rates higher than 2500 µl/min** would be critical without the wire, because the heat transmission to the inner layers is impaired by the laminar flow.

The titanium wire can be removed by the user to increase the inner diameter and thus the maximum flow speed through the cannula. This is not recommended, as removal of the wire results in a **laminar** fluid flow. In laminar flow, the fluid moves in layers, with one layer sliding smoothly over the other. There is no mixing of fluid from layer to layer, since viscous shear forces damp out relative motions between layers. As a result, the separate layers show different temperatures. The inner layers move much faster than the outer layers. As the temperature sensor is on the outside of the flow stream, the controller will stop the heating if the actual temperature of the outer layer reaches the setpoint temperature. In other words, the inner layers will be considerably colder than the setpoint temperature.

3 Appendix

3.1 Pin Assignment



Pin 1, 6	Heating
Pin 2, 5	Temperature sensor power supply
Pin 3, 4	Temperature sensor probe