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MEA Application Note:
Acute Hippocampal Slices on Perforated MEAs

Imprint

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1 Introduction

1.1 About this Application Note

The intention of the MEA Application Notes is to show users how to set up experiments with a MEA-System based on typical applications that are used worldwide.

The documents have been written by or with the support of experienced MEA users who like to share their experience with new users.

This application note includes several suggestions on how to work with the perforated MEAs on acute hippocampal slices. For instructions about the preparation of acute hippocampal slices, please refer to the MEA Application Note MEA Applications Hippocampus.

1.2 Concept of perforated MEAs

A downside of acute slice recordings on MEAs in contrast to for example an interface chamber is that recordings are done from the cells at the bottom of the slice. These cells get less oxygen and nutrients from the perfused ACSF solution, and therefore are likely to give smaller signals and might eventually die first. Perforated MEAs present a solution to this problem, as they allow a perfusion of the tissue from both sides at the same time, thereby optimizing the oxygen supply of the acute slice.

1.3 Advantages and Disadvantages of pMEAs

Slices can be recorded also with planar arrays and slice grids to hold the slice in place, but the pMEAs provide better contact between slice and tissue, and improved oxygenation. Perforated MEAs are for example especially advantageous if:

- Spontaneous activity is relevant.
- Long term survival of the slice is necessary (> 4-5 h).
- Imaging from the top should be performed, and a slice grid would be in the way.
- Substances should be applied to the bottom cells of the tissue faster than by passive diffusion

However, the additional perfusion cycle also introduces additional noise sources and is technically more demanding than regular perfusion on non-perforated arrays. Also, the imaging possibilities with inverted microscopes are restricted on perforated arrays.

Note: It is recommended to establish slice recordings first on regular MEAs, before moving to pMEAs.

1.4 Acknowledgements

Multi Channel Systems would like to thank all MEA users who shared their experience and knowledge with us. The concept of sucking ACSF solution through the slice presented in chapter 4.2 was originally conceived by Dr. Jonathan Levenson from the company Galena. Perfusion from both sides of the slice was established in the lab of Prof. Ulich Egert in Freiburg.

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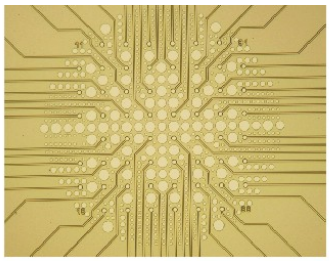
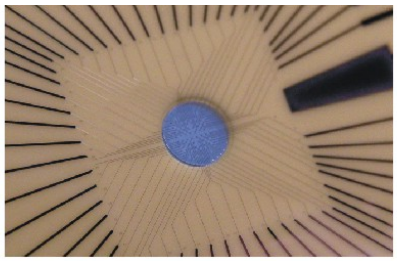
2 Material

2.1 Perforated MEAs

Perforated MEAs (pMEA) are identical in size and function to the regular glass MEAs. However, the electrodes are integrated into a thin polyimide foil instead of a glass substrate. This thin foil is then fixed on a ceramic or glass wafer for mechanical stability and easier handling. In the middle of the wafer, under the electrode field, there is a hole that makes it possible to access the electrode field from below. The area around the electrodes is perforated, to allow a perfusion of the tissue from both sides.

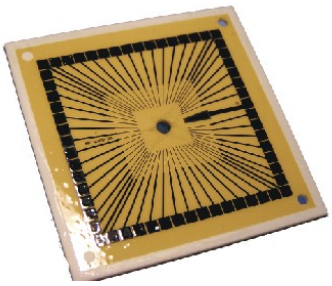
Electrode field with perforation

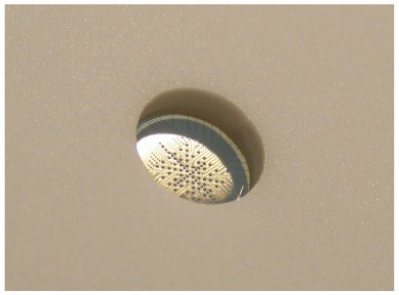




Electrode area from upper side

Perforated MEA





Electrode area from lower side

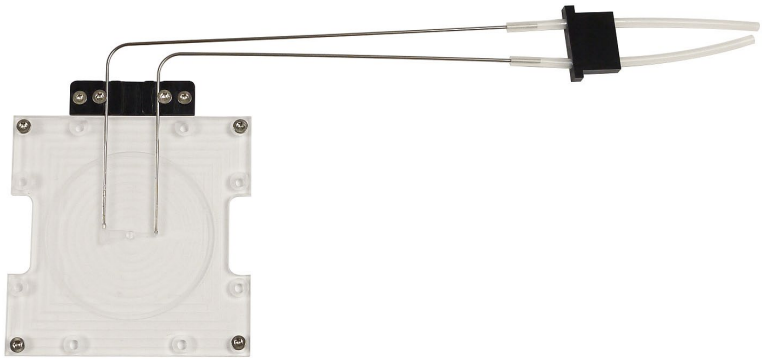
Perforated MEAs have electrodes with 30 µm diameter and an internal reference electrode, they are available with 100 µm a 200 µm electrode spacing, with 60 or 120 channels.

2.2 Perfusion Ground Plate MEA-PGP

The perfusion ground plate MEA-PGP is essential to work with perforated MEAs on MEA1060-Systems. It replaces the standard ground plate of the MEA1060 amplifier. Exchanging the ground plates can be done in only a few minutes. Please note that there are **different types of the MEA-PGP** for different amplifier types (UP, INV and BC). It is also important to keep in mind that the **MEA-PGP does not contain a heating unit**, like the regular ground plate. Therefore, it is recommended to adjust the perfusion heating accordingly, and to use a temperature controlled perfusion also from underneath. The MEA-PGP contains a sealed chamber with a perfusion in- and outlet, which is connected to the underside of the perforated MEA when the MEA1060 amplifier is closed. A replaceable O-ring seals the compartment underneath the pMEA.

2.3 Perfusion Element PE

Just like the PGP for the MEA1060 amplifiers, the Perfusion Element PE is needed to work with pMEAs and a MEA2100 or MEA2100-Mini-System. It replaces the standard heating plate, the exchange can be done in only a few minutes. Please note that there are **different types of PEs** for different amplifier types (MEA2100-60/120, MEA2100-2x60, MEA2100-Mini). There are also differences based on the serial number of your device, so please check carefully on the MCS website, or contact MCS support or your local representative.



It is also important to keep in mind that the **MEA-PGP does not contain a heating unit**, like the regular ground plate. Therefore, it is recommended to adjust the perfusion heating accordingly, and to use a temperature controlled perfusion also from underneath. The MEA-PGP contains a sealed chamber with a perfusion in- and outlet, which is connected to the underside of the perforated MEA when the MEA2100 amplifier is closed. A replaceable O-ring seals the compartment underneath the pMEA.

2.4 Peristaltic Perfusion System PPS2

Any standard peristaltic pump can be used to perfuse the slice preparation through the MEA-PGP or PE. If you are using a pump with two channels, it is possible to use the same pump for perfusion from above and from underneath. Multi Channel Systems MCS GmbH recommends using the peristaltic perfusion system PPS2 with software control. Without the need for different tubing diameters, the volume flow of both channels can be adjusted independently in ml/min.

2.5 Controlled Vacuum Pump CVP

The pressure control unit CVP is a vacuum pump with a pressure sensor and a waste bottle. A sensor measures the pressure in the compartment attached to the waste bottle, and can regulate the suction to maintain a constant negative pressure. With this unit, it is possible to precisely control the suction applied to the slice and to keep the negative pressure stable during the whole recording period. The accuracy of the pressure control is **0.1 mbar**, the maximum negative pressure is **200 mbar** below atmospheric pressure.

The CVP is mandatory for the double perfusion described in chapter **4.3**.

3 Methods

3.1 Preparation of the Slice

For a suggested method to prepare acute hippocampal slices, please see the MEA Application Note Acute Hippocampus Slice**.** The best results with pMEAs are obtained if the perforated area is completely **covered with tissue**. If holes on the pMEA remain uncovered, the suction dissipates. On the other hand, if the slice is much larger than the perforated area, the tissue areas outside the perforation might move in the perfusion flow, as the tissue is only held down in the perforated area. This can also cause artefacts. Hence, it is recommended to **trim the slice** to match the size of the perforated area.

3.2 Preparations for Recording

Note: We recommend the perfusion cannula with temperature control (PH01) for optimal environmental conditions. Please keep in mind that the MEA-PGP does not contain a heating element. Temperature must be controlled by heating the perfusion solution. If you are using a double perfusion (see chapter **4.3**), we recommend using two PH01 and a TC02 temperature controller.

For setting up your recording software (Multi Channel Experimenter, MC_Rack, LTP-Director) and connecting and programming the stimulator, please see the respective user manuals.

We recommend the following preparations and tests before you start the experiment:

1. Test all cable connections.
2. Define your experiment in the software and test it before use.
3. Clean the MEA contacts with a soft tissue and pure alcohol or isopropanol.
4. Define your stimulation pattern and test it with the test model probe.
5. Start carbogen aeration 15 min before mounting the slice.

   Start the perfusion 5 min before mounting the slice at a low flow rate (2-5 ml/min, perfusion from above) to fill all tubing with oxygenated ACSF.

3.3 Mounting the pMEA on the Amplifier

1. Make sure the O-ring on the MEA-PGP/PE is in place. In case it doesn't fit properly, spread a few drops of ACSF on the O-ring and try again.
2. Place the pMEA on the MEA-PGP/PE and close the amplifier. Fill the chamber below the MEA with oxygenated ACSF through the bottom perfusion In and Out ports, until you see liquid rising through the perforation holes into the pMEA. Try to get rid of all bubbles underneath the MEA.
3. Test the perfusion. Adjust the grounding and shielding to avoid noise.
4. Set the temperature for the PH01 on the temperature controller or in the software TCX-Control. There is usually an **offset** between the set temperature and the actual temperature close to the slice. This offset depends heavily on the perfusion rate and the room temperature. Determine the actual offset between the set temperature on the PH01 and the chamber temperature for a given flow rate and room temperature with a thermometer in the chamber. It's usually 2-3 °C. Adjust the set temperature accordingly.

Note: The offset might change with outside condition. Keep an eye on things like air condition, fans, open windows, extremely hot days and such.

5. Start the perfusion 5-10 min before mounting the slice at a low flow rate (2-5 ml/min, perfusion from above) to fill all tubing with oxygenated ACSF.

3.4 Mounting the Slice onto the pMEA

The recommended procedure described in the following instructions positions the slice on the perforated area in the center of the pMEA. After the verification of the correct position, the suction can be applied to keep the slice in place.

Important: Do not touch the slice directly. The slice should not be folded to avoid damage to the tissue. Be careful not to touch the MEA surface with the transfer pipette to avoid damage to the electrodes.

1. Place the slice with a transfer pipette in the ACSF filled MEA; center it roughly on the recording area.
2. Remove the ACSF solution with a 1 mL pipette until the slice sits on the surface of the MEA and covers the holes of the perforation.
3. Position the slice by gently pushing it with a pipette tip from the sides into place. The CA1 region should cover the recording area.
4. Immediately cover the slice with a few drops of ACSF. The buffer should be pipetted onto the slice carefully right from the top, rather than from the side, to avoid the slice from floating. Avoid falling drops, as they can damage the tissue.
5. Confirm the position of the slice and gently apply the suction by any of the methods described in chapter four.

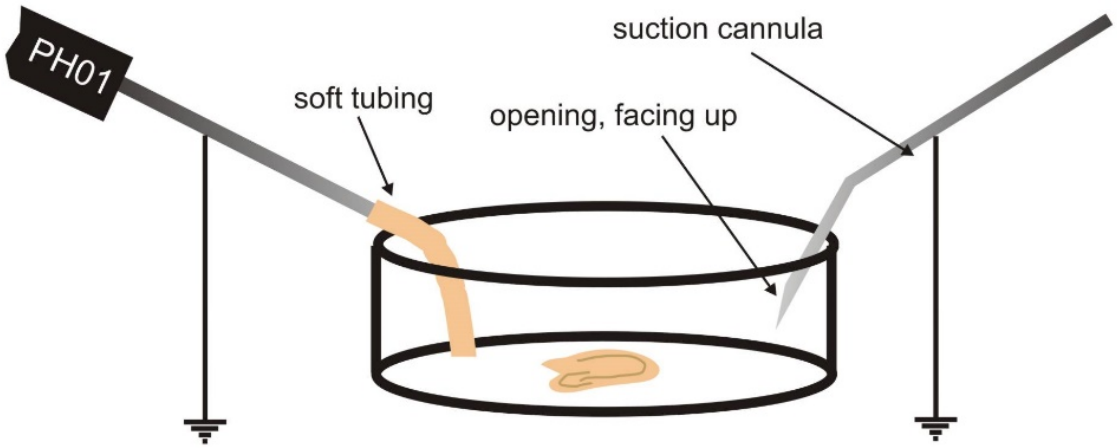
Start regular perfusion from above. Flow rate should be 1 – 5 ml/min.

3.5 Perfusion and Noise

Low frequency fluctuations are most often caused by the perfusion. Shortly switch off the pump to see whether the fluctuations disappear if the pump is off. **50 Hz noise** can also be caused by the perfusion, but is independent of the pump running or not.

Perfusion in and out should contain a piece of **metal** that can be connected to the amplifiers ground to remove 50 Hz noise. The easiest way is to use a bend cannula for suction. The opening of the cannula should be positioned in a way that it always sucks air and liquid at the same time, possibly resulting in a constant slurping noise. This prevents the fluid level from going up and down, which also causes noise.

See a suggested perfusion setup below.



If you experience low frequency noise from the perfusion, try to optimize the suction as described above. Additionally, a **droplet isolator** can be used to interrupt the fluid flow between pump and recording chamber. The PPS2 pump from MCS is already equipped with droplet isolators. A 10 Hz high pass filter in the software can also be used to remove low frequency fluctuations in the baseline, if the amplitude is not larger than maybe 200 µV.

50 Hz noise can be caused by the perfusion or by external noise sources. Remove perfusion in and out from the bath. If the noise persists, check for **external noise sources**, like microscope lamp, power supplies close by and so on. If the noise is caused by the perfusion, check the grounding of the perfusion in and out. If the magnetic perfusion holders (MPH) from MCS are used, the perfusion should already be grounded via the magnet.

If you are unsure about grounding, use a Multimeter to check the connection between any part of the setup and the system ground. Ground connectors are on the back of the USB-ME data acquisition systems and on the back of the interface board of the MEA2100- or MEA2100-Mini-System.

Additional noise issues might arise from **bubbles** in the bottom perfusion chamber. Make sure to fill the bottom perfusion chamber as completely as possible with ACSF before recording. Flushing the bottom chamber to get rid of bubbles is possible, but complicated without losing the slice.

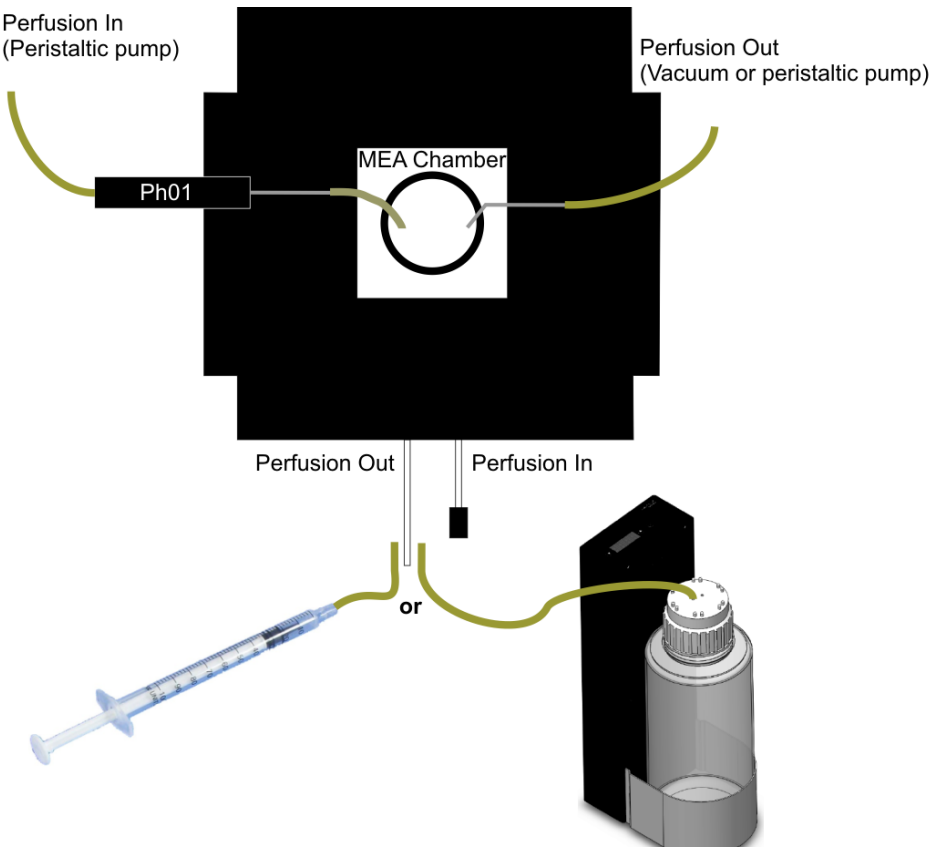
4 Possible Configurations for Working with pMEAs

This chapter describes some options to work with perforated MEAs. These methods differ in their complexity and the additional equipment needed. The appropriate method should be chosen based on the requirements of the experiment. If you are planning to do just short LTP experiments, manually applied suction might be enough. If long term survival of the slice is vital, double perfusion is probably the best choice. If drug delivery to the bottom of the slice is important, perfusion of ACSF trough the slice should be considered.

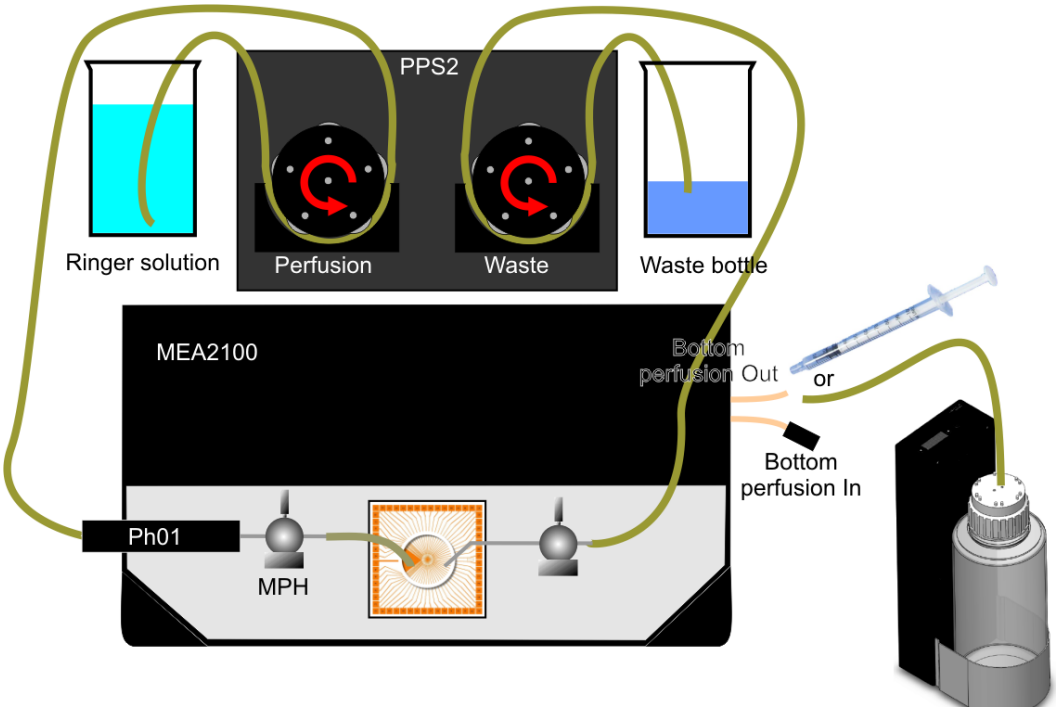
4.1 Suction only

Close the Perfusion In port of the MEA-PGP/PE and attach a 1ml syringe (or smaller, if available) to the Perfusion Out port. After mounting the slice (see chapter **3.4**), gently suck in about 20-50 µl with the syringe. It is better **not to start with the syringe at position 0 ml**, but to have 100-200 µl of ACSF already in. Do not apply more suction, or the slice will be sucked into the holes. Most likely, the negative pressure will dissipate over time, but often the adhesion of the slice to the MEA surface is good enough by then to keep it in place anyway.

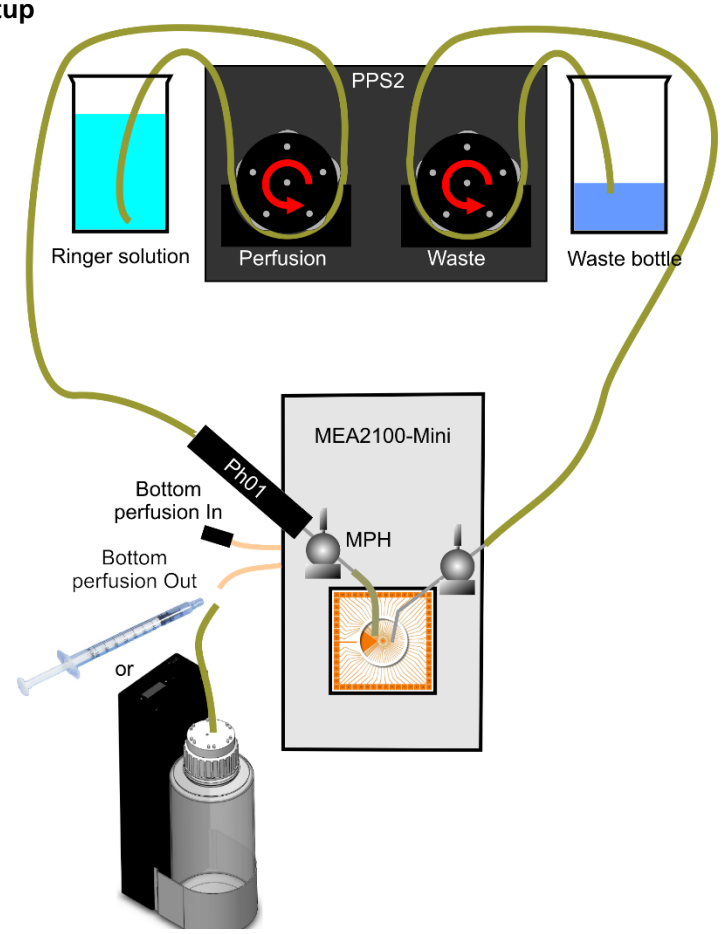
MEA1060 Setup



MEA2100 Setup



MEA-2100-Mini Setup



For precise and continuous suction, it is recommended to replace the syringe with the **Controlled Vacuum Pump CVP**. Set the CVP to a pressure of 15-30 mbar. The CVP will keep the negative pressure stable as long as needed. It is likely that this will result in some ACSF being sucked trough the slice (see chapter **4.2** below). It is recommended to use a valve in the tubing to the CVP, as the CVP starts working immediately when switched on.

The CVP works best if the tubing to the MEA-System is short, and completely filled with ACSF. Air bubbles in the tubing are compressible, and act as pressure buffers between the pressure sensor in the bottle, and the chamber underneath the pMEA. Ideally, place the CVP as close to the setup as possible, to keep the tubing short, and put it **on the same level or above** the MEA-System. This prevents ACSF from passively flowing to the waste bottle

Note: This simple method is intended primarily for short term experiments. It only holds the slice in place and ensures a good contact but does not provide better oxygen supply.

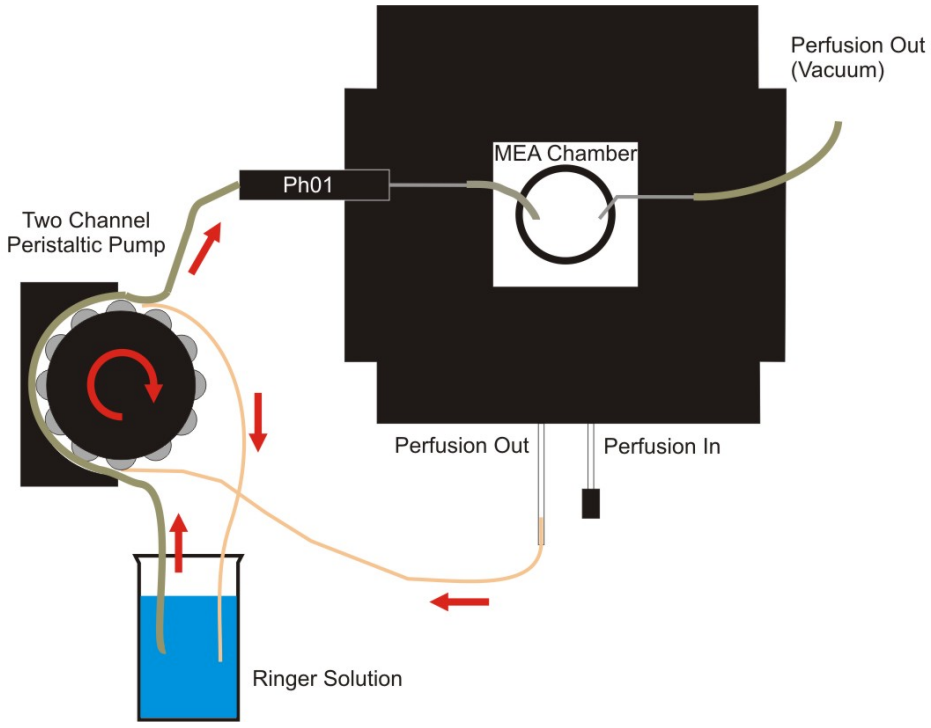
4.2 Perfusion through the Slice

Perfusion through the slice means that the influx of ACSF is still from the top, as usual, but a small amount of the solution is sucked trough the slice from underneath. The rest of the solution is removed from above. This method has three advantages:

- the slice is kept in place
- better oxygen supply for cells at the bottom of the slice
- compounds added to the perfusion solution have a much higher chance of effectively reaching the cells throughout the slice in the applied concentration

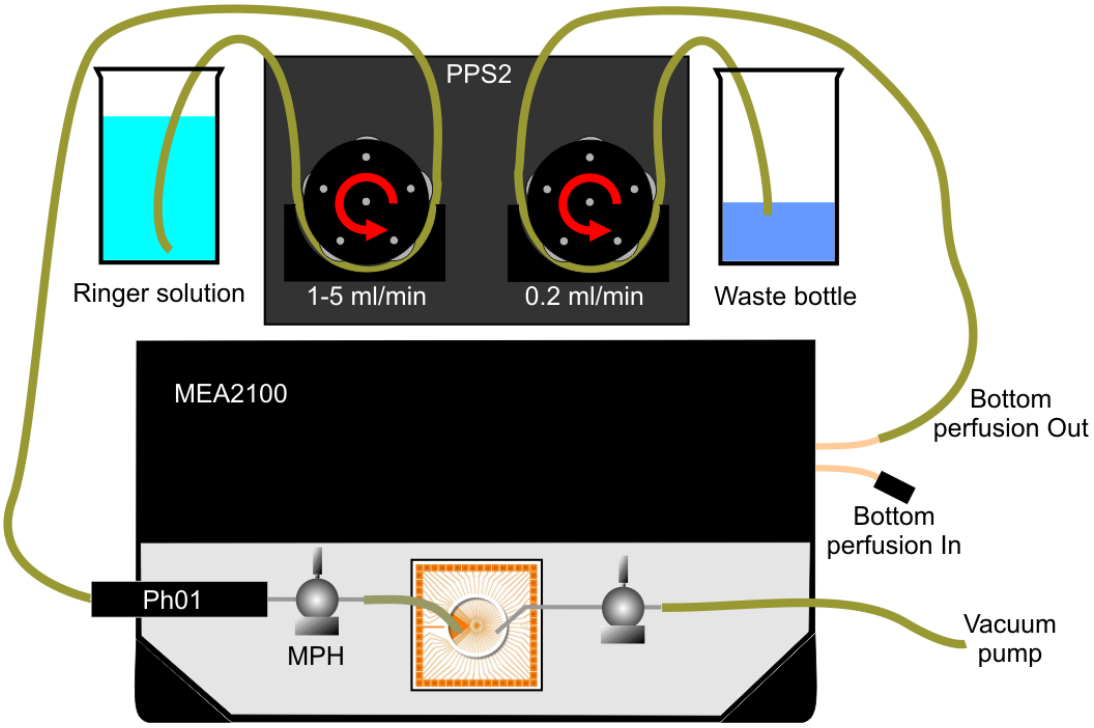
Close the Perfusion In port of the MEA-PGP/PE. Connect the Perfusion Out to a peristaltic pump. You can use the same pump that provides the Perfusion In from above, as long as the pump has at least two pump channels. Adjust the tubing diameter for both pump channels and the speed of the pump in such way that **2-5 ml/min** are pumped into the MEA chamber from above and **200 µl/min** are sucked trough the slice from below. For example, a combination of pump tubing with 3.17 mm and 0.44 mm inner diameter (Tygon black/white and yellow/green, www.liquid–scan.de) allows for a combination of 3 ml/min and 200 µl/min on the same peristaltic pump.

The rest of the solution is removed by the vacuum from the top. Different perfusion rates can be used, but the 200 µl/min trough the slice should not be exceeded. The 200 µl can be either recycled (see picture) or discarded.

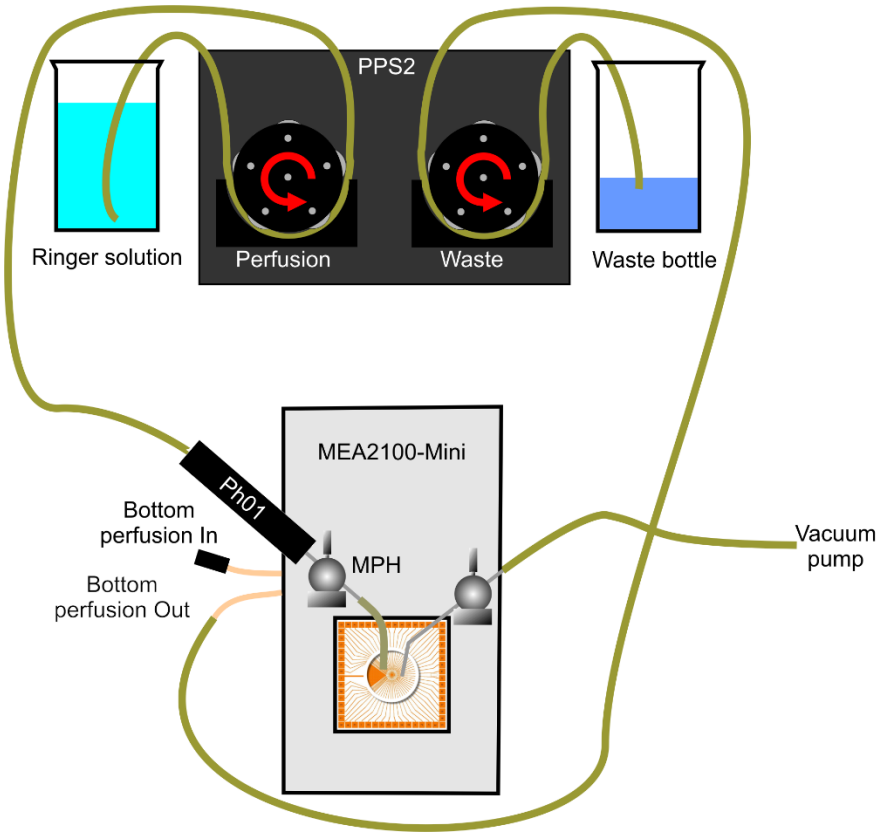


An easier and more flexible solution is to work with the **PPS2**, in combination with a vacuum pump. In that case, any flow rate can be adjusted independently for both perfusion channels without the need for specific tubing. The flow rate for the upper perfusion and the volume perfused through the slice can be varied just by setting the respective flow rates in the pump control.

MEA-2100 Setup



MEA-2100-Mini Setup

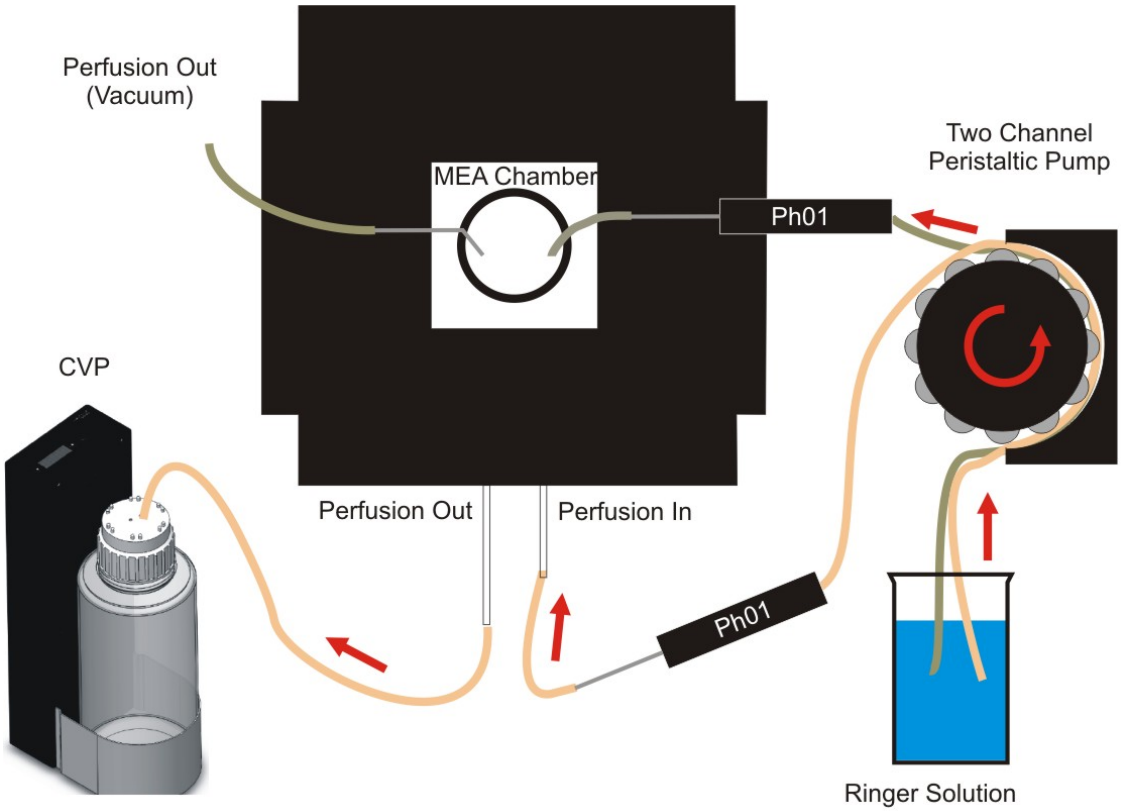


Both described options would work identically with a **MEA1060, MEA2100 or a MEA2100-Mini** amplifier.

4.3 Double Perfusion

In order to employ the full potential of the perforated MEAs, it is possible to use double perfusion of the slice, from the top and also trough the MEA-PGP/PE perfusion chamber from underneath. This double perfusion provides an optimal oxygen and nutrient supply throughout the slice. By using two PH01 perfusion cannulas, there is also a stable temperature control in this configuration.

Note: Be aware that the double perfusion is the most technically demanding option to work with pMEAs. It will most likely require some trial and error to work out the optimal parameters.



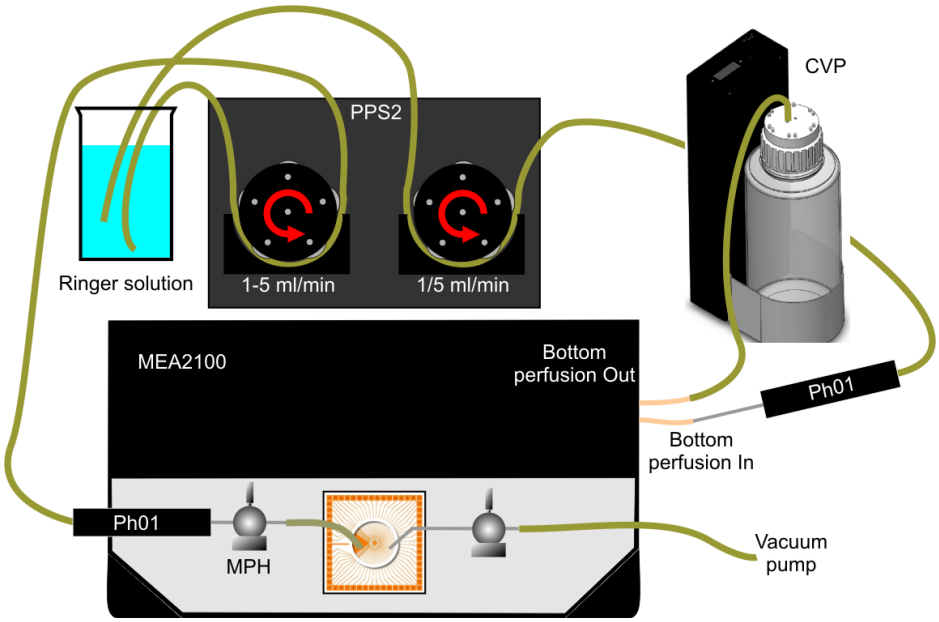
We recommend using an identical perfusion rate of 1-5 ml per minute for both perfusion cycles. Most likely, the temperature for both PH01 will have to be set a bit higher than the desired chamber temperature. Determine the **temperature offset** between PH01 and chamber with a thermometer and adjust the set value on the TC02 accordingly.

The controlled vacuum provided by the CVP should be set to 15-30 mbar (that means 15-30 mbar below atmospheric pressure). It is recommended to use a **valve** in the tubing to the CVP, as the CVP starts working immediately when switched on. For mounting of the slice

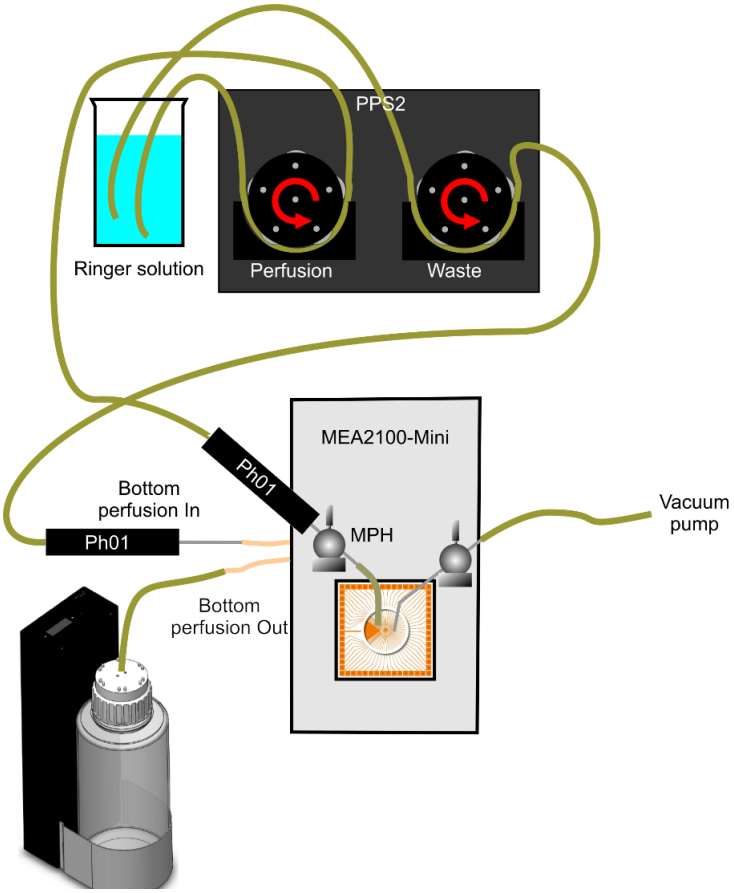
- close the valve
- position the slice
- set the CVP to 5 mbar
- open the valve
- increase the suction to 15-30 mbar
- start perfusion

For **MEA1060, MEA2100 or a MEA2100-Mini** amplifier, a PPS2 or any other peristaltic pump can be used. The CVP is mandatory for the double perfusion setup, though.

MEA-2100 Setup



MEA-2100-Mini Setup



The CVP works best if the tubing to the MEA-System is short, and completely filled with ACSF. Air bubbles in the tubing are compressible, and act as pressure buffers between the pressure sensor in the bottle, and the chamber underneath the pMEA. Ideally, place the CVP as close to the setup as possible, to keep the tubing short, and put it **on the same level or above** the MEA-System. This prevents ACSF from passively flowing to the waste bottle and creating air bubbles in the tubing.

5 Troubleshooting

Problem	Possible Solution
Slice is coming off	Increase suction; make sure slice sits at the bottom of the pMEA when suction is activated. Decrease perfusion flow rate from underneath.
Slice can't be removed after recording	Slice was sucked into the holes; decrease suction next time. Dissolve tissue with standard Terg-A-Zyme cleaning, as described on the MEA data sheets.
Edge of slice is flapping in the flow	Slice larger than perforated area; try to make slice as small as possible and center it on perforation.
Negative pressure dissipates fast	Not all holes of the perforation covered by tissue; center slice on perforated area.
Noise	Air bubbles in the MEA-PGP; fill chamber completely. Perfusion from underneath not grounded; usually, the MEA-PGP Perfusion In and –Out ports are grounded, induce additional grounding if necessary.

6 Suggested System Configurations

6.1 MEA2100-Mini-System

The MEA2100-Mini-System is the latest generation of MEA-Systems. Currently, headstages with 60 or 120 channels are available. Integrated current or voltage controlled stimulators can use any electrode as stimulation electrode. The system includes amplifier, data acquisition, and stimulators in one compact device, as well as floor heating. The filter band of the DAQ can be changed by software. The Interface Board includes a unique freely programmable DSP for advanced closed loop experiments and many additional in and outputs for interface with other devices. Approximately 20 electrode layouts with several additional options are available at the moment. The MEA2100-System will fit equally well on upright and inverted microscopes. Additionally, it can be upgraded with high flexibility from one to a maximum of eight headstages, operated by a single IFB.

Included accessories: Floor heating, internal stimulator
Recommended accessories: Temperature controller, Perfusion heating PH01, magnetic perfusion holders MPH, peristaltic perfusion system PPS2, Controlled Vacuum Pump CVP
Optional accessories: Video Table MEA-VMTC-1

Pro: Very compact, suitable for inverted and upright microscopes, selectable filter band, access to analog data, programmable DSP, easy to upgrade.
Contra: Electrodes not accessible for external stimulation, only two stimulation channels.

6.2 MEA2100-System

Headstage versions of the MEA2100 suitable for pMEAs are HS-60, HS2x60 and HS-120. The MEA2100 shares all advantages of the MEA2100-Mini. The system can be upgraded to operate up to four 60-channel or two 120-channel MEAs independently from one computer.

Included accessories: Temperature controller, floor heating, internal stimulator
Recommended accessories: Perfusion heating PH01, magnetic perfusion holders MPH, peristaltic perfusion system PPS2, Controlled Vacuum Pump CVP
Optional accessories: Video Table MEA-VMTC-1 or MEA-VMTC2

Pro: Very compact, suitable for inverted and upright microscopes, headstages for all MEA types available, selectable filter band, programmable DSP, three stimulation channels.
Contra: Electrodes not accessible for external stimulation devices, no direct access to analog raw data.

6.3 Suggested Perforated Microelectrode Arrays

Apart from perforated arrays, the MEA1060 and MEA2100-(2x)60 systems can be used with any type of 60 channel MEAs. The 120 channel MEA layouts can only be used with the MEA2100-120-System. The available pMEA layouts are specifically listed below. Perforated MEAs with 252 recording channels for the USB-MEA256 or the MEA2100-256 are unfortunately not possible due to technical restrictions.

6.3.1 For MEA2100-Mini-60, MEA2100-(2x)60-System or USB-MEA60-System

For the MEA2100-Mini-60, MEA2100-(2x)60 and the MEA1060, three perforated layouts are available. All have 30 µm diameter electrodes to provide optimal stimulation capabilities. The 60pMEA200/30iR-Ti has the classical 8x8 layout, and will cover a hippocampal slice well. The 60pMEA100/30iR-Ti has a 6x10 layout with 100 µm electrode spacing, and is more suited for smaller and/or more elongated preparations, as for example cortical slices. The 60Pedot-pMEA200/30iR-Ti-Au feature PEDOT coated electrodes with superior stimulation capabilities.

6.3.2 For MEA2100-Mini-120 or MEA2100-120-System

A 120 channel system is the best choice if a larger recording area should be combined with the advantages of perforated arrays. Two pMEA layouts are available, either with 100 µm or 200 µm spacing, both with a 12x12 electrode layout (120pMEA100/30iR-Ti and 120pMEA200/30iR-Ti).

6.3.3 Ring Options

The standard glass ring is best for running a perfusion and will also allow using the ALA MEA-Insert. A plastic ring should only be used if parallel patch clamp experiments are planned. See all ring options here.

7 Contact Information

Local retailer

Please see the list of official MCS distributors on the MCS web site.

User forum

The Multi Channel Systems MCS GmbH user forum has been closed, but can still be used as a knowledge base for FAQs.

Mailing list

If you have subscribed to the newsletter, you will be automatically informed about new software releases, upcoming events, and other news on the product line. You can subscribe to the newsletter on the contact form of the MCS web site.

www.multichannelsystems.com