multichannel systems

MEA Application Note: Cardiac Slices from Adult Mouse Ventricle

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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 real experiments with the MEA-System on the basis of 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 a complete protocol for the dissection of mouse heart and the preparation of ventricular slices for acute experiments.

Acknowledgement

Multi Channel Systems would like to thank all MEA users who shared their experience and knowledge with us. A major part of this document is based on the instructions provided by the Natural and Medical Sciences Institute in Reutlingen. We thank Dr. Udo Kraushaar for his help with this document.

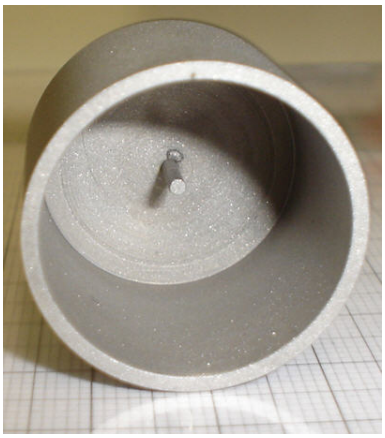
2 Material

2.1 Biological Materials

- 1 adult mouse, > 5 weeks old (1 adult mouse of your choice)

2.2 Technical Equipment

- MEA-System (with amplifier and data acquisition, see chapter “Suggested MEA-System”)
- Stimulus generator (internal or external)
- MEAs (microelectrode arrays)
- Ice
- Stereo microscope
- Inverted microscope. Necessary for aligning the electrode positions to the slice. If you prefer to use an upright microscope (mandatory in case of using 60EcoMEAs), you need, for example, a camera and a stereo microscope for documenting the electrode position. A picture of the slice on the electrode field can then be loaded into the MC_Rack program for aligning the data traces to the electrodes.
- “Heart Holder”, special device for blocking the ventricle in agar (see picture below)
- Oscillating microtome (for example, Leica, Integraslice, Campden) and blades
- Adjustable pipettes and pipette tips (20 µl and 1000 µl)
- Transfer pipettes (cut Pasteur pipettes with wide opening (approximately 0.5 cm)
- Large sharp scissors or guillotine
- Surgical instruments, for example a bone rongeur or scissors
- Narrow flat spatula
- Sharp forceps
- Curved straight forceps
- Small scissors
- Razor blade



Special device: Here called “Heart Holder”

2.3 Chemicals

Oxygen gas (O_2)

Carbogen gas (95 % O_2, 5 % CO_2)

NaCl

KCl

$CaCl_2$

KH_2PO_4

$NaHCO_3$

D-Glucose

$MgSO_4$

Cyanoacrylate glue (Pattex/Henkel, Düsseldorf, Germany)

100 % alcohol or acetone (for cleaning the MEA contact pads)

Low-melt agarose (Roth, Karlsruhe, Germany)

100.000 U/kg BW (Units per kg body weight) heparin sodium
(Hoffmann-La Roche, Grenzach-Wyhlen, Germany)

2.4 Media

2.4.1 Ca^{2+} -free Tyrodes solution

NaCl	155 mM
KCl	5.4 mM
NaH_2PO_4	0.33 mM
Glucose	10 mM
$MgCl_2$	1 mM
Hepes	10 mM
BDM (2,3-butanedione monoxime)	30 mM

Ca^{2+} -free Tyrodes solution: pH 7.4 adjusted with NaOH

Aerate the Tyrodes Solution with oxygen gas for 15 min.

2.4.2 Agarose

Dissolve low melting agarose in Ca^{2+}- free Tyrodes solution to obtain a 4 % Agarose concentration. Heat to boiling to dissolve, then cool to and store at 37 °C.

2.4.3 Tyrodes Solution

Add 0.9 mM $CaCl_2$ and aerate the Tyrodes Solution with oxygen gas for 15 min.

2.4.4 Dulbeccos Modified Eagles Medium

$CaCl_2$	1.8 mM
$MgSO_4$	0.8 mM
KCl	5.3 mM
$NaHCO_3$	44 mM
NaCl	110 mM
NaH_2PO_4	0.9 mM

1. Prepare the media as listed above.
2. Aerate the DMEM with carbogen gas for 15 min.
3. Refrigerate 200 ml Ca^{2+} -free Tyrodes solution until it is partially frozen.
4. Crush the frozen Ca^{2+} -free Tyrodes solution thoroughly, blend with hand blender and oxygenate on ice until use.

Note: To speed up the freezing and crushing of Ca^{2+} -free Tyrodes solution, you might consider to prepare Ca^{2+} -free Tyrodes solution ice cubes in advance, and store the ice cubes at –20 °C until use (30 – 45 min).

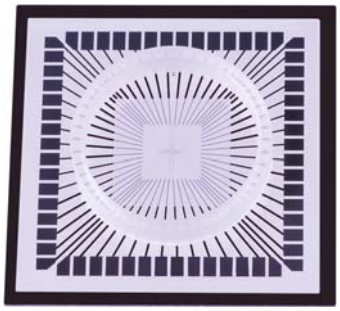
3 Methods

3.1 MEA Coating

Depending on the type of selected MEA, various coatings may be applied to the MEA surface to promote the adhesion of the slice. Suggestions for coating methods can be found in the MEA manual available in the “Download section” of the MCS web site.

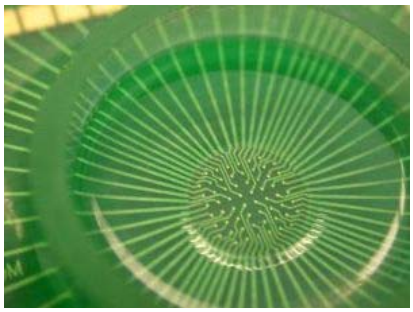
3.1.1 Standard MEAs

Standard MEAs (60MEA200/30iR-Ti, for example) should be coated either with cellulose nitrate or with polyethylenimine (PEI).



3.1.2 EcoMEAs

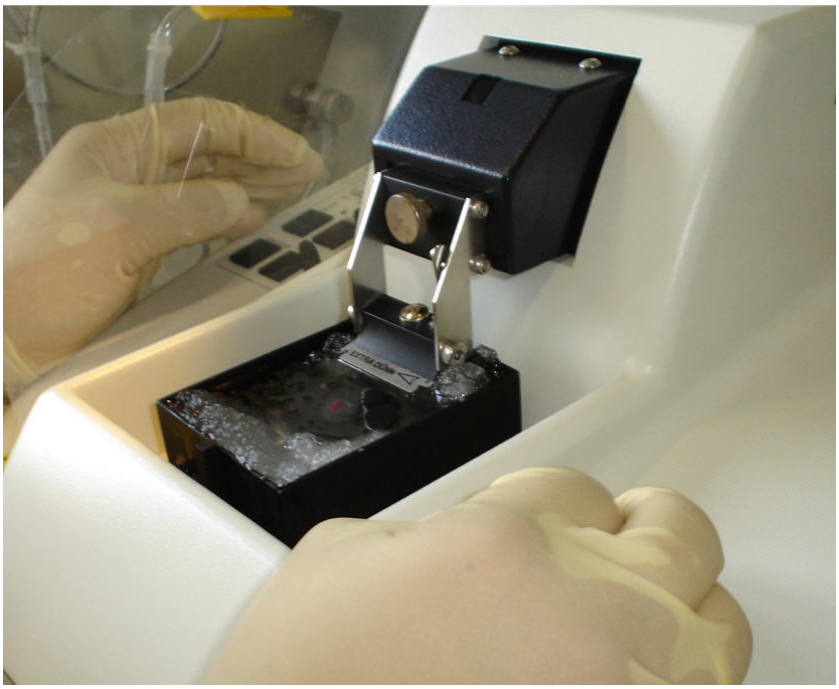
EcoMEAs (60EcoMEA or 60EcoMEA-Glass) should be coated either with cellulose nitrate or with polyethylenimine (PEI).



3.2 Setting Up the Vibratome

Note: As the design and handling of different vibratomes varies, please consult the manual of your vibratome for more details. Recommended: Leica VT1000s .

1. Fill outer vibratome chamber with ice.
2. Fill vibratome chamber with frozen Ca^{2+} -free Tyrodes solution. You might need to add some room temperature Ca^{2+} -free Tyrodes solution.



Vibratome overview: Heart mounted on agar, and base plate. Oxygenation for Ca^{2+} -free Tyrodes solution filled inner chamber. Slushed Ice filled outer chamber.

3.3 Sacrificing the Animal and Isolating the Heart

1. Sacrifice the animal by cervical dislocation or decapitation.

2. Mount animal on a preparation table (for example, icebox-lid covered with tissue).

3. Remove fur at the chest.

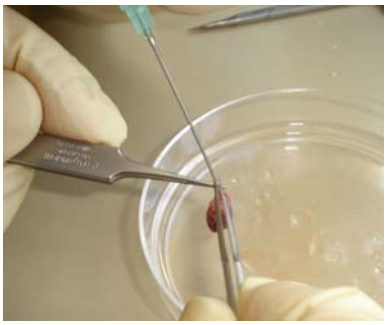
4. Open chest.

5. Remove pericardium.

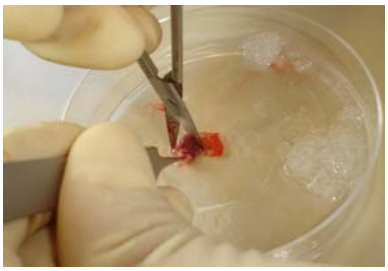
6. Isolate the heart and place it in a Petri dish filled with cold Ca^{2+}-free Tyrodes solution.

3.4 Preparations for Slicing

1. Perfuse the heart retrogradely with a syringe with Ca^{2+}-free Tyrodes solution with BDM.



2. Remove atria by cutting at the AV level.

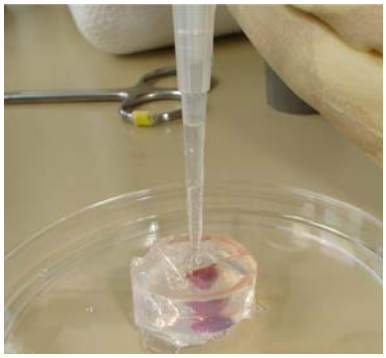


3. Mount the ventricles in the “Heart Holder”. Make sure to place the heart upside down with the pin of the holder into the ventricle cavity.

4. Fill “Heart Holder” with melted agarose.

5. Cool "Heart Holder” to speed up hardening of the agarose.

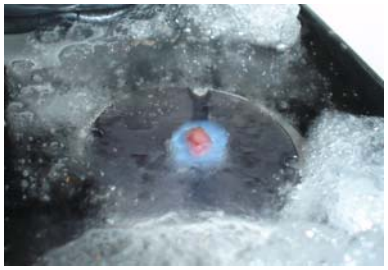
NOTE: Fill up the gap left by “Heart Holder” pole with agarose using a pipette tip. This will prevent the heart from detaching from the agarose block during slicing.



6. Trim agarose block.

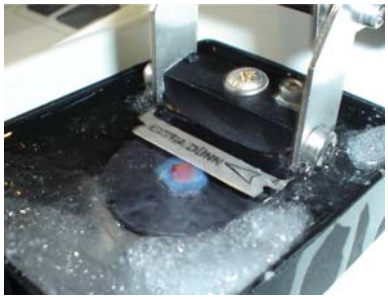
NOTE: Make sure the last cut at the heart cut area is directed as your intended slicing layer.

7. Mount heart jelly block in pre-refrigerated slicing platform with cyanoacrylate glue.
8. Place slicing platform in slicer.



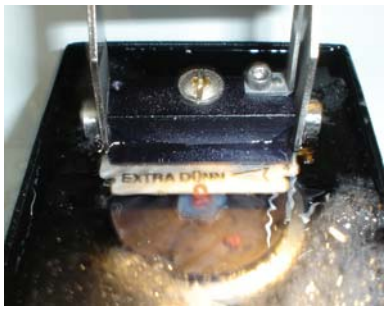
3.5 Slicing

1. Remove 2 - 3 slices at the heart tip and discard those.



2. Slice at 150 - 200 µm thickness.

   Note: It is very important to move the blade very slowly to minimize tissue damage.

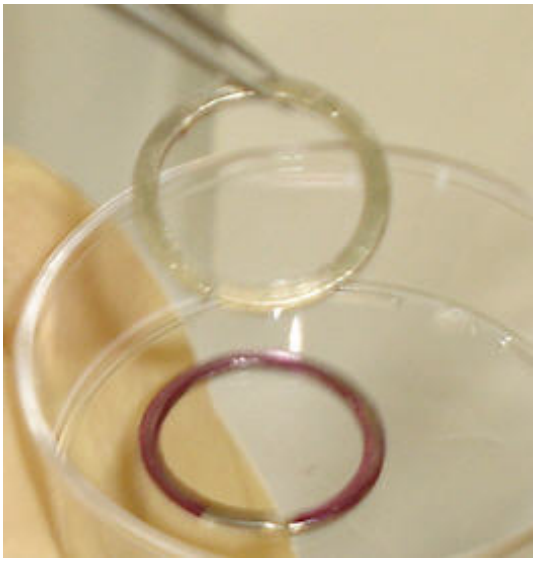


3. Transfer slice to oxygenated Tyrodes solution (with 0.9 mM $CaCl_2$).
4. Incubate for 30 min.
5. Transfer slice to MEA using a modified slice transfer glass Pasteur pipette.

3.6 Mounting the Slice onto the MEA

The recommended procedure described in the following instructions fixes the slice onto the coated MEA by **adhesion**.

An alternative method uses small **weights** (of a few hundred milligrams) to hold down the slice. This method is especially recommended if (uncoated) 3D-MEAs are used. However, this method might stress the slice and result in an altered potential distribution and propagation.

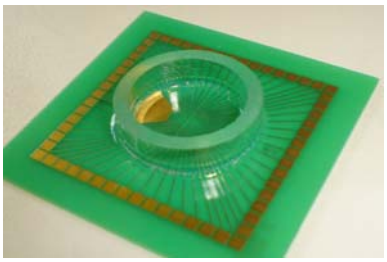


You can use either self-made wire grids or a shim with a nylon mesh in the middle to apply the weight. Some steel carriers with nylon meshes as slice hold-downs are also commercially available (for example from Warner Instruments, www.warneronline.com). Commonly used custom made weights are U-shaped flattened pieces of platinum wire (80 – 120 mg) glued onto a mesh, a sort of nylon stocking or wedding veil, for example.

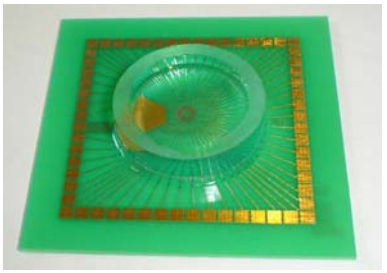
You can also glue another platinum wire from the other side of the mesh, symmetrically to the first piece of platinum. This kind of grid is less damaging because you can vary the pressure on the slice by changing the thickness of the second wire. The thickness of the wire that is placed onto the slice should match approximately the thickness of the slice. If you use 350 µm slices, the wire should be around 300 µm and not more than 350 µm; otherwise the grid will not hold the slice.

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 and a drop of Tyrodes solution with the transfer pipette onto the EcoMEA; center it roughly on the recording area.

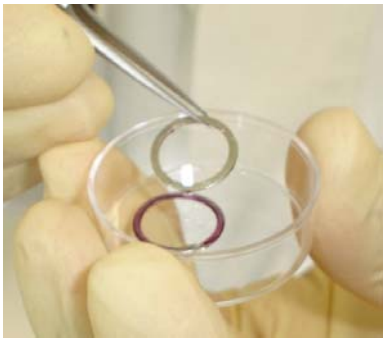


2. Position the slice by gently pushing it with a pipette tip from the sides into place. The region of interest should cover the recording area.
3. Remove the Tyrodes solution to fix the slice onto the EcoMEA: Hold the slice in position with a small pipette tip along the side and remove excess buffer with a pipette.

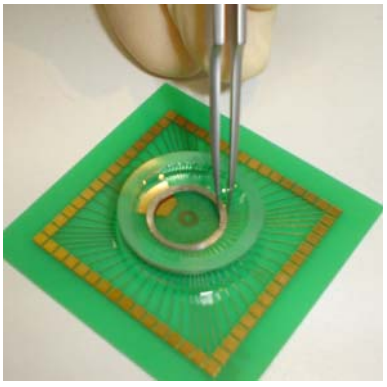


4. Cover the slice with a few drops of DMEM immediately. The buffer should be pipetted onto the slice carefully right from the top, rather than from the side, to avoid that the slice is floating up. Avoid falling drops that can damage the tissue.

   — OR —



5. If you prefer the alternative method of applying a grid: Position the slice in the desired position with approximately 200 µl Tyrodes solution left, then apply the grid and add oxygenated Tyrodes solution. Some MEA users prefer to nearly dry the slice on the array with filter paper wedges; others find it not necessary when using grids and prefer to change the position of the slice if the MEA culture chamber is filled with Tyrodes solution.
6. Store slices on MEAs in incubator till use.



4 Preparations for Recording

Note: We recommend the perfusion cannula with temperature control (PH01) for optimal environmental conditions. A two-channel temperature controller (TC02) allows to control both the MEA temperature (via the heating integrated into the amplifier) and the buffer temperature.

See the MC_Rack manual or online help for detailed application examples. For connecting and programming the stimulus generator (STG), please see the respective user manual. Please see the MEA manual for details on stimulation amplitudes and times that are supported by the MEA electrodes. Though TiN electrodes are very stable, an unsuitable stimulation pulse will irreversibly damage the electrodes.

We highly recommend the following preparations and tests before you start the experiment.

1. Test all connections.
2. Define your virtual rack specific to your application with the MC_Rack program and test it before use.
3. Define your stimulation file with the MC_Stimulus program and test it with the test model probe and with a MEA filled with recording buffer before use. It is recommended to test a range of stimulus amplitudes and locations prior to starting your actual experiment. If you are using an external stimulating electrode, the position of this electrode should be optimized in this step as well.
4. Set up the perfusion system, and test the perfusion with an old MEA. Adjust the grounding and shielding to avoid noise pickup and 50 Hz hum.
5. Set the temperature controller to 37 °C for heating the MEA culture chamber and to 32 °C for the buffer temperature.
6. Start carbogen aeration 15 min before mounting the slice.
7. Start the perfusion 15 min before mounting the slice at a low flow rate (0.5 exchanges/min) to maintain a stable oxygenation and pH.
8. Clean the MEA contacts with a soft tissue and pure alcohol or acetone.
9. Mount the MEA with the slice onto the amplifier as described in the MEA amplifier user manual.
10. Superfuse the slice with oxygenated Tyrodes solution prewarmed at 37 °C. The buffer volume should be exchanged 3 – 4 times per minute. The slice is mechanically stressed by activating the perfusion and should be perfused for about **half an hour** before recording. You can also control the parameter that you want to record, and start the recording as soon as you get a stable baseline, for example, as soon as the spike rate has stabilized.

    You are now ready for recording.

5 Suggested MEA-Systems

5.1 System Configurations

Depending on the throughput and the analysis requirements desired in your laboratory, different system configurations are recommended for the recording from cardiac slice preparations.

MEA2100-System-E: The MEA2100-System is the most advanced system MCS can offer today. It is as flexible as the USB-MEA60-System and can use the same MEAs, in addition to unique 120 electrode arrays. The **temperature controller** TC01/ TC02 regulates the temperature of the MEA and of the perfusion fluid via **the perfusion cannula** PH01. 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 and perfusion heating. The **filter band** of the DAQ can be changed by software. An additional box includes a unique freely programmable **DSP** for advanced closed loop experiments and many additional in and outputs for interface with other devices.

The MEA2100-System will fit equally well on upright and inverted microscopes. The system can be upgraded to operate up to four 60-channel or two 120-channel MEAs independently from one computer.

Approximately 20 electrode layouts with several additional options are available at the moment. The use of perforated MEAs is optional. It only make sense when the slice covers the perforated area. Cross sections through whole heart ventricles may not fulfill these requirement.

USB-MEA60-System-E: **60-channel** MEA recording system for inverted or upright microscopes. The **temperature controller** TC01/ TC02 regulates the temperature of the MEA and of the perfusion fluid via the **perfusion cannula** PH01. A **MEA1060 amplifier** allows recording up to 60 channels from one MEA. The three additional analog inputs can be used for feeding in data generated by other systems recording in parallel, for example, for patch clamp data. The additional digital inputs can be used for synchronizing the recording with the stimulation, or with external systems. This is the standard configuration for low-throughput academic research and high flexibility for a wide range of applications.

5.2 Microelectrode Arrays

Available MEAs differ in electrode material, diameter, and spacing. For an overview on available MEA types please see the Multi Channel Systems web site (www.multichannelsystems.com) or contact your local retailer.

The microfold structures formed by titanium nitride (TiN) result in a large surface area that allows the design of small electrodes with a low impedance and an excellent signal to noise ratio.

- **60MEA200/30iR-Ti**: Standard 8 x 8 layout, TiN electrodes for recording and stimulation, with substrate integrated reference electrode.
- **60EcoMEA:** EcoMEAs have a standard 8 x 8 layout, Gold electrodes and a substrate integrated reference electrode. They are opaque, that is why you need an upright microscope for controlling the slice.

5.3 Amplifier Specifications

In **MEA2100-Systems** the sampling rate, signal range and bandwidth can be adjusted via software control and is therefore suitable for a broad range of applications, from single unit spike recordings to field potentials from whole heart preparations.

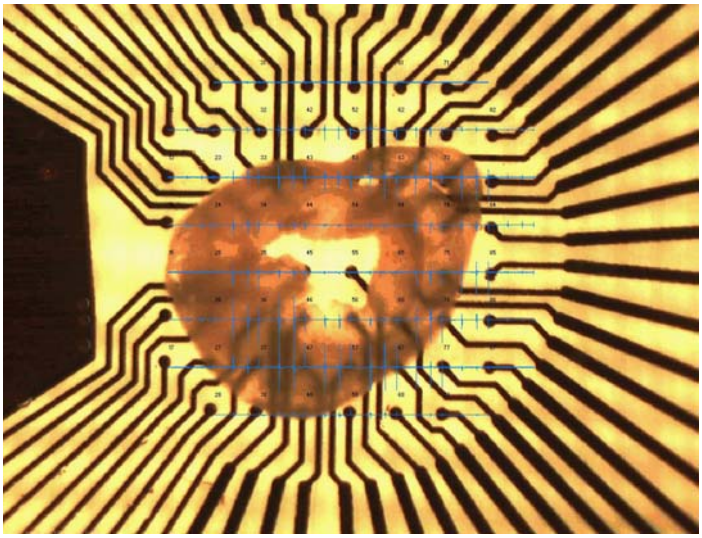
Though amplifiers with custom gain and bandwidth are available, Multi Channel Systems recommends the following settings for this application in **MEA1060** amplifiers.

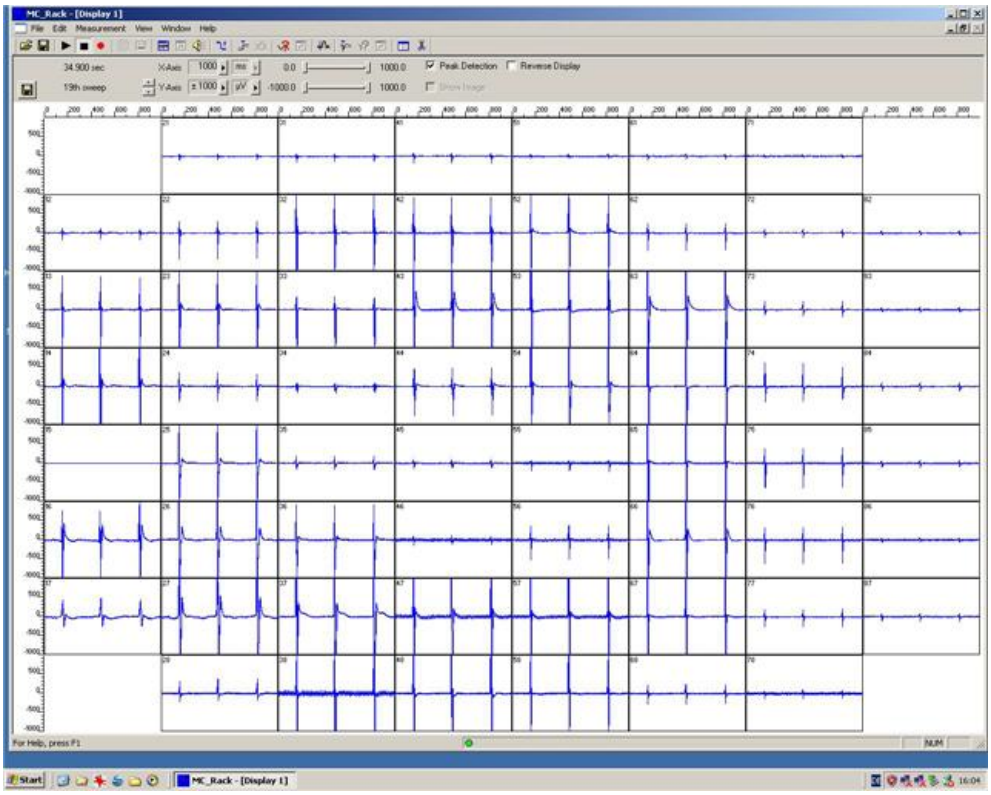
- Lower cutoff frequency: **1 Hz**
  With an even lower value, slow signal drifts will disturb the recordings and signal detection.
- Upper cutoff frequency: **3 kHz**
  Sufficient even for rapid depolarization waveforms. If only field potentials are of interest, a reduction of the upper cutoff frequency might be considered to reduce high frequency noise.
- Gain factor: **800 - 1000**

A note on **gain**: Traditional amplifiers for extracellular recordings often have 5000 x or even 10000 x gain switch options. A high gain, however, increases the high frequency noise level or requires a narrower filter band. Considering a 20 µV extracellular signal, we would receive 24 mV after a 1200 x amplification. An AD converter with an input range set from –400mV to 400 mV will resolve the signal in increments of 0.36 µV, which will definitely provide enough information, given the noise level of such systems in general. Therefore, there is no need for higher amplifications. Higher gains might result in clipping signals.

6 Results & Signals

Slices can be recorded using 60EcoMEAs or standard 60MEA200/30iR-Ti MEAs. The recorded cardiac field potential represents the extracellular reflection of the action potential (Halbach, Egert et al. 2003). Every single MEA electrode picks up the local field potential at that position in the slice. This allows to map propagation of cardiac action potentials in the slice and correlate field potential shape with the position in the slice.





7 References

Most work was done by the groups of Jürgen Hescheler in Cologne (Pillekamp, Reppel et al. 2005; Halbach, Pillekamp et al. 2006) and Ursula Ravens in Dresden.

Halbach, M., U. Egert, et al. (2003). "Estimation of action potential changes from field potential recordings in multicellular mouse cardiac myocyte cultures." Cell Physiol Biochem **13**(5): 271-84.

Halbach, M., F. Pillekamp, et al. (2006). "Ventricular slices of adult mouse hearts--a new multicellular in vitro model for electrophysiological studies." Cell Physiol Biochem **18**(1-3): 1-8.

Pillekamp, F., M. Reppel, et al. (2005). "Establishment and characterization of a mouse embryonic heart slice preparation." Cell Physiol Biochem **16**(1-3): 127-32.