MEA Application Note: Neuronal Cell Culture – Cultivation, Recording and Data Analysis
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 real experiments with the 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 a complete protocol for the preparation of dissociated neuronal cell cultures from different origins, either freshly prepared from embryonic rats or ready-to-use cryopreserved primary neurons. Suggestions for suitable MEA-Systems configurations and support files are also included.

It is assumed that the reader is familiar with the functions of the software Multi Channel Suite, MC_Rack and MC_Stimulus II, and the general terminology in the field.

1.2 Acknowledgement

Multi Channel Systems would like to thank all MEA users who shared their experience and knowledge with us, especially the following persons:

Daniel Wagenaar
Steve Potter
Wiebke Fleischer
Babben Tinner-Staines
Paolo Cesare
2 Primary Cortical or Hippocampal Neurons

A very useful video about the preparation, cultivation and recording of primary neurons can be found in the Journal of Visualized Results (JoVe), provided by Hales, Rolston and Potter.

An alternative protocol to the one described here is available from the Genes to Cognition Website.

2.1 Material

2.1.1 Biological Materials

Rat embryos E18 (Wistar Kyoto or Sprague Dawley)

2.1.2 Technical Equipment

- MEA-System (with amplifier and data acquisition, see chapter 0, Suggested System Configurations)
- Stimulus generator (optional)
- MEAs (microelectrode arrays)
- Sterile workbench
- Incubator set to 35 °C, 65 % relative humidity, 9 % O₂, 5 % CO₂
- Water bath at 37 °C
- Stereo microscope
- Inverted microscope
- Micropipettes and pipette tips (20 µl and 1000 µl)
- 15 ml BD Falcon tubes
- 40 µm nylon mesh cell strainer (BD Falcon)
- Sharp forceps
- Curved forceps
- Small scissors
- ALA-MEA-MEM Teflon membranes (ALA Scientific Instruments Inc.), see chapter 5 Long Term Culturing
2.1.3 Chemicals

- NaOH
- MgCl$_2$
- CaCl$_2$
- HEPES
- Phenol Red
- Na$_2$SO$_4$
- K$_2$SO$_4$
- Kynurenic acid
- DL-2-amino-5-phosphonovaleric acid (APV)
- Polyethylenimine (PEI)
- Laminin
- Sodium pyruvate
- Insulin
- Glutamate
- Phosphate buffered saline (PBS)
- Bovine serum albumin (BSA)
- Trypsin (Sigma)
- Papain suspension (Roche Applied Science, catalog No. 10108014001)
- DNase (Sigma)
- Horse serum (Donor Equine Serum from HyClone)
- Dulbecco’s Modified Eagle Medium (DMEM) (Gibco/Invitrogen)
- L-Alanyl-L-Glutamine (GlutaMAX™ from Gibco/Invitrogen)
- Hanks’ Balanced Salt Solution (HBSS) without Calcium / Magnesium (Gibco/Invitrogen)
2.1.4 Media

Culture Medium for Primary Cultures

- DMEM (may contain GlutaMAX™, depending on the supplier)
- 10 % horse serum
- 0.5 mM GlutaMAX™ (final concentration)
- 1 mM sodium pyruvate
- 2.5 µg/ml insulin

**Note:** Some work groups use trypsin, other papain or *Streptomyces griseus* protease for the enzymatic digestion of the tissue.

Enzyme Solution (Trypsin)

- HBSS
- 0.25 % trypsin
- 0.02 mg/ml DNAse
- Alternative Enzyme Solution (Papain)
- 1 ml Segal’s medium (see below)
- 200 µl papain suspension
- NaOH for adjusting the pH to 7.3

Alternative Enzyme Solution (Protease from *Streptomyces griseus*)

- Protease 2 mg/ml
- Earle’s balanced salt solution (EBSS)
- 1 mM MgCl₂
- 0.5 mM CaCl₂
Neuronal Cell Cultures

Segal’s medium (Banker & Goslin, p309ff.)

<table>
<thead>
<tr>
<th></th>
<th>Conc. (mM)</th>
<th>FW (g/mol)</th>
<th>mg (for 500 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MgCl₂·6 H₂O</td>
<td>5.8</td>
<td>203.31</td>
<td>590</td>
</tr>
<tr>
<td>CaCl₂·6 H₂O</td>
<td>0.25</td>
<td>147.02</td>
<td>18.4</td>
</tr>
<tr>
<td>HEPES</td>
<td>1.6</td>
<td>238.3</td>
<td>191</td>
</tr>
<tr>
<td>Phenol Red</td>
<td>0.001 %</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Na₂SO₄·10 H₂O</td>
<td>90</td>
<td>322.21</td>
<td>14500</td>
</tr>
<tr>
<td>K₂SO₄</td>
<td>30</td>
<td>174.26</td>
<td>2610</td>
</tr>
<tr>
<td>Kynurenic acid</td>
<td>1</td>
<td>189.2</td>
<td>95.6</td>
</tr>
<tr>
<td>APV</td>
<td>0.05</td>
<td>197.1</td>
<td>4.92</td>
</tr>
</tbody>
</table>

Note: Kynurenic acid takes a lot of stirring to dissolve. It is also recommended to add more NaOH while dissolving to keep the pH reasonable.

- Use 0.1 N NaOH (about 1 ml) for adjusting the pH to 7.3 before adding APV and Kynurenic acid.
- Again, use 0.1 N NaOH (about 1 ml) for adjusting the pH to 7.3 after having added APV and Kynurenic acid.
- Bring up to 500 ml after the final pH adjustment.
- Sterile filter, aliquot, and freeze in liquid nitrogen.

BSA / PBS

- Phosphate buffered saline (PBS)
- Bovine serum albumine (BSA)
2.2 Methods

2.2.1 Cleaning MEAs

- Leave MEA in 1 % Terg-A-Zyme solution overnight.
- Wash thoroughly with bdH₂O overnight or few hours.
- Let dry. Then place them in a plasma cleaner for 1-2 min (if available).

2.2.2 MEA Coating

Depending on the type of selected MEA, various coatings may be applied. Standard MEAs should be coated with Polyethylenimine or Laminin. Detailed suggestions for coating methods can be found in the MEA Manual available in the Download section of the MCS web site.

2.2.3 Dissection

1. Euthanize the animal with carbon dioxide.
2. Decapitate the animal with large sharp scissors or with a guillotine.
3. Open the skull carefully with a scissor and remove the brain.
4. Isolate the cortex or hippocampus with a scalpel.

2.2.4 Enzymatic Digestion

5. Chop the isolated sample into pieces of approximately 1 mm³ and transfer the fragments into a Falcon tube with 2–3 ml enzyme solution pre-warmed at 37 °C.
6. Digest the fragments with the enzyme solution at 37 °C for 15 min. Gently swirl the suspension every 5 min.
7. Gently wash the fragments in the culture medium twice: Gently swirl or invert the suspension of brain pieces in medium to wash away the protease, allow the pieces to settle, remove the supernatant with a pipette, and add 2–3 ml fresh medium. After the second wash, add 1 ml medium to the fragments.
8. Gently triturate the fragments by passing the preparation five times through the 0.78 mm wide opening of a 1000 µl pipette tip. The majority of cells should now be in suspension.
9. Transfer the supernatant containing the suspended cells into a fresh Falcon tube.
10. Add 1 ml medium to the remaining fragments, and triturate the remaining fragments once more.
11. Combine the supernatants from the two triturations in one tube, giving 2 ml cell suspension.
12. Remove the debris by gravity flow filtering the cell suspension through a 40 µm nylon mesh cell strainer (Falcon) into a 15 ml DB Falcon tube filled with BSA/PBS.
2.2.5 Plating and Culturing the Cells

13. Centrifuge the cell suspension at 160 g for 5 min.
14. Discard the supernatant and resuspend the cells in approximate 0.5 ml culture medium.
15. Count the cells under a microscope to determine the cell density by using a Neubauer chamber or an automated cell counter.
16. Plate the cells in a density of 1000–5000 cells per mm² (depending on your application) onto the recording field of the MEA.
17. Maintain the cells for 4–5 days in an incubator set to 35 °C, 65 % relative humidity, 9 % O₂, 5 % CO₂ before recording, depending on your application.
18. Check the pH of medium daily by eye, and change it as soon as the colour indicates that the medium is going acidic (shifting from pink to orange colour).

Note: The choice of plating density is up to the investigator. The higher the density, the sooner activity will be observed (as soon as 2-3 days in vitro), but the more often the culture will need to be fed. 5000 cells/mm² is very dense, and would require feeding about every 2 days, while 1000–3000 may only require feeding weekly or every 5 days. The best time for recording depends on the application: Many studies may be aimed at the development of the cultures, and therefore require recording as soon as possible. For other applications, it may be better to keep the cells longer in culture before starting the experiment. Cells are still developing, but the culture is more stable after about one month in culture. The culture can be used for several months or years.
3 Primary Neurons from Dorsal Root Ganglia (DRG)

This protocol was developed by Paolo Cesare from the NMI (paolo.cesare@nmi.de).

3.1 Material

3.1.1 Biological Materials

Young adult rats (4-6 weeks old Sprague Dawley).

3.1.2 Technical Equipment

- MEA-System (with amplifier and data acquisition, see chapter 0)
- MEAs (microelectrode arrays)
- Sterile workbench
- Incubator set to 37 °C, 5 % CO₂
- Water bath at 37 °C
- Stereo microscope and surgical instruments
- Inverted microscope
- Micropipettes and pipette tips (10, 100 and 1000 µl)
- 15 and 50 ml BD Falcon tubes
- 40 µm nylon mesh cell strainer (BD Falcon)

3.1.3 Cell Culture Reagents

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Amount</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagenase</td>
<td>500 mg</td>
<td>Sigma</td>
</tr>
<tr>
<td>Deoxyribonuclease I</td>
<td>11 mg</td>
<td>Cell Systems</td>
</tr>
<tr>
<td>Dispase II</td>
<td>1 g</td>
<td>Sigma</td>
</tr>
<tr>
<td>DMEM, High Glucose, GlutaMAX™</td>
<td>500 ml</td>
<td>Invitrogen</td>
</tr>
<tr>
<td>DPBS, Calcium, Magnesium</td>
<td>500 ml</td>
<td>Invitrogen</td>
</tr>
<tr>
<td>DPBS, no calcium, no magnesium</td>
<td>500 ml</td>
<td>Invitrogen</td>
</tr>
<tr>
<td>FBS, Certified, Heat Inactivated, USA</td>
<td>100 ml</td>
<td>Invitrogen</td>
</tr>
<tr>
<td>GlutaMAX™ supplement (100X)</td>
<td>100 ml</td>
<td>Invitrogen</td>
</tr>
<tr>
<td>Laminin</td>
<td>1 mgl</td>
<td>Sigma</td>
</tr>
<tr>
<td>N-2 Supplement (100X), Liquid</td>
<td>5 ml</td>
<td>Invitrogen</td>
</tr>
<tr>
<td>Neurobasal®-A Medium</td>
<td>500 ml</td>
<td>Invitrogen</td>
</tr>
<tr>
<td>NGF (2.5S)</td>
<td>100 µg</td>
<td>Promega</td>
</tr>
<tr>
<td>Penicillin-Streptomycin (100X)</td>
<td>20 ml</td>
<td>Invitrogen</td>
</tr>
<tr>
<td>Poly-D-lysine hydrobromide (70-150k)</td>
<td>5 mg</td>
<td>Sigma</td>
</tr>
</tbody>
</table>
3.1.4 Media Preparation

- DMEM (50 ml)
- 44.5 ml DMEM (High-glucose with GlutaMAX™)
- 5 ml Foetal Bovine Serum (FBS)
- 0.5 ml Penicillin/Streptomycin (PS)
- Neurobasal-A medium solution (50 ml)
- 49 ml Neurobasal-A-medium
- 500 µl Penicillin and Streptomycin
- 500 µl GlutaMAX™
- 1 Aliquot NGF (2.5 µg NGF; 10 µl); inal NGF is 100 ng/ml
- 1 Aliquot N2 supplement (250 µl; 1:100)
- Keep at ~4 °C

Digestion solutions

Solution 1:

- 5 ml DPBS+
- Collagenase: 7.5 mg
- Dispase: 15 mg
- Glucose: 9 mg

Solution 2:

- 5 ml DPBS+
- Dispase: 15 mg
- Glucose: 9 mg

3.1.5 Extracellular solution for MEA recording

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration [mM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>140</td>
</tr>
<tr>
<td>KCl</td>
<td>4</td>
</tr>
<tr>
<td>CaCl₂·2 H₂O</td>
<td>2</td>
</tr>
<tr>
<td>MgCl₂·6H₂O</td>
<td>1</td>
</tr>
<tr>
<td>HEPES</td>
<td>10</td>
</tr>
<tr>
<td>Glucose</td>
<td>4</td>
</tr>
</tbody>
</table>
3.2 Methods

3.2.1 Cleaning MEAs

- Leave MEA in 1 % Terg-A-Zyme solution overnight
- Wash thoroughly with bdH₂O overnight or few hours
- Let dry. Then place them in a plasma cleaner for 1-2 min

3.2.2 MEA Coating

- Prepare Poly-D-Lysine (PDL):
  - 100 µl PDL (1 mg/ml) into 10 ml sterile bdH₂O (1:100)
  - Add ~1 ml per MEA, under the hood, at RT, for ≥ 1 h
  - Remove PDL
  - Add sterile dH₂O and remove; repeat twice
- Prepare Laminine (Lm):
  - 20 µl Lm (1 mg/ml) into 250 µl DPBS+
  - Place 25 µl over the area covered by the microelectrodes. Leave at 37 °C until the end of the preparation (≥ 4 h)
  - Remove Lm
  - Add 30 µl Neurobasal medium and remove; repeat twice
  - Add 30 µl Neurobasal medium and keep at 37 °C until cells are ready for plating

3.2.3 DRG Isolation

- Prepare 4 small Petri dishes (35 mm) and 2 big (100 mm) with DPBS- and put at 4 °C
- Euthanize the animal with carbon dioxide
- Decapitate the animal with large sharp scissors or with a guillotine
- Remove the whole vertebral column and place it in chilled DPBS-
- Place the column with ventral part up. Trim well and remove tissues
- Very carefully cut the column in the midline
- Place half of it in DPBS- at 4°C
- Place the other half in DPBS- and start harvesting the ganglia (up to 30 DRG/rat) by carefully removing them with the help of a stereomicroscope
- Collect them in DPBS- and keep at 4 °C (in total you have 4 x 35 mm dishes with 7-8 ganglia each)
- Trim well, removing the nerves from the ganglia
3.2.4 **Enzymatic Digestion and Cell Dissociation**

- Put 5 ml filtered (0.2 µm) digestion Solution 1 in a small Petri dish
- Add all ganglia
- Incubate at 37 °C for 40 min
- Remove digestion Solution 1
- Add 5 ml filtered (0.2 µm) digestion Solution 2
- Incubate at 37 °C for 40 min. Stir gently every 15 min.
- Add 60 µl of DNAse I (80 Kunitz units/ml) to 6 ml of DMEM medium
- Remove digestion Solution 2
- Add DNAse solution
- Using a 1 ml Gilson pipette triturate well (x5) but carefully until ganglia are well dissociated
- Filter through a cell strainer (40 µm)
- Add DMEM medium up to 10 ml
- Centrifuge at 126 g (1000 tr/min) for 5 min at RT
- Carefully aspirate supernatant
- Resuspend the pellet into 200 µl of Neurobasal-A medium (with NGF and N2)

3.2.5 **Seeding DRG Neurons on the MEA**

- Use a hemocytometer to count the number of cells per µL. Expected yield: 100-300 cells/µl
- Dilute the cells to 100 cells/µl
- Take all the MEA out from the incubator
- Aspirate the medium (one MEA at a time) and add enough cell suspension to end up with 100000 cells/cm² inside the MEA dish
- Keep at 37 °C in the incubator, and after 1-2 hours gently add 800 µl of Neurobasal medium
- The following day exchange 50 % of the medium with fresh Neurobasal medium
4 Cryopreserved Primary Neurons

4.1 Material

4.1.1 Biological Materials

Ready to use cryopreserved primary neurons (from hippocampus or cortex) from QBM Cell Science.

4.1.2 Technical Equipment

- MEA-System (with amplifier and data acquisition, see chapter 0)
- Stimulus generator (optional)
- MEAs (microelectrode arrays)
- Sterile workbench
- Incubator set to 35 °C, 65 % relative humidity, 9 % O₂, 5 % CO₂
- Water bath at 37 °C
- Liquid nitrogen freezing tank
- Stereo microscope and Inverted microscope
- Micropipettes and pipette tips (1000 µl)
- 15 ml BD Falcon tubes
- ALA-MEA-MEM Teflon membranes (ALA Scientific Instruments)

4.1.3 Chemicals

- 70 % alcohol for disinfection
- Polyethylenimine (PEI)
- LamininGlutamine
- Neurobasal Medium with B-27 supplement (Gibco/Invitrogen)
- [Optional: penicillin/streptomycin]

4.1.4 Media

- Neurobasal medium
- B-27 supplement
- 0.5 mM Glutamine
- [optional: 50 U/ml penicillin/streptomycin]
- This medium is recommended by QBM Cell Science, the provider of the cryopreserved neurons.
4.2 Methods

4.2.1 Cleaning MEAs

- Leave MEA in 1 % Terg-A-Zyme solution overnight
- Wash thoroughly with bdH₂O overnight or few hours
- Let dry. Then place them in a plasma cleaner for 1-2 min

4.2.2 MEA Coating

Depending on the type of selected MEA, various coatings may be applied. Standard MEAs should be coated with Polyethylenimine or Laminin. Detailed suggestions for coating methods can be found in the MEA Manual available in the Download section of the MCS web site.

4.2.3 Thawing and Starting the Culture

**Important:** Do not centrifuge or vortex the cells. Keep the time between removing the vial from the liquid nitrogen tank and placing it into the water bath as short as possible.

1. Store the cells in liquid nitrogen until use.
2. Remove a vial with the cells from the liquid nitrogen and place it into a water bath pre-heated at 37 °C to thaw the cells.
3. After 2.5 minutes, remove the vial with the thawed cells and disinfect the outside of the vial with 70 % ethanol. Place it under a laminar flow hood. Proceed with the next step immediately after thawing.
4. Gently transfer 1 ml of the cells into a 15 ml centrifuge tube and immediately add 37 °C pre-warmed medium drop-wise onto the cells, while rotating the tube by hand. This procedure should take approx. 2 minutes. We recommend addition of 4 ml medium resulting in a final cell density of 800.000 per ml.

**Important:** Do not add the whole volume of the medium to the cells at once. This may result in an osmotic shock. If the same vial is to be used for several different experiments in parallel, mix the cells by pipetting slowly up and down once, then aliquot the cells into the appropriate vessels.

5. Mix the cell suspension by inverting the tube carefully, twice.
6. Transfer the cell suspension into the MEA culture chamber, about 0.6–1.6 ml (depending on the maximum volume of the MEA culture chamber).
7. Incubate the cells in an incubator for 4 hours.
8. Remove the medium from the cells leaving a small liquid layer to ensure that the cells do not dry out and add 1.6 ml fresh, 37 °C pre-warmed medium.
9. Incubate the cells at 37 °C with 95 % relative humidity and 5 % CO₂ until use.

**Important:** Cell death at the beginning of the cultivation should be considered normal. Generally, there are enough viable cells left for cultivation.

4.2.4 Maintenance

- Change the medium at day 5 after thawing.
- Replace 50 % of the medium with fresh, 37 °C pre-warmed medium every 3 to 4 days. Warm an appropriate amount of medium to 37 °C in a sterile tube. Remove 50 % of the medium from the cell culture. Replace with the warmed, fresh medium and return the cells to the incubator.
- Avoid repeated warming and cooling of the medium. Warm only the volume that is needed for a single procedure.
- Compensation for media loss due to evaporation should be taken into consideration. Add additional media whenever necessary.
- After 2–3 weeks of cultivation, the cells are ready for recording.
5 Longterm Culturing

In order to allow long term cultivation and recording, Multi Channel Systems recommends the use of Teflon membranes (fluorinated ethylene-propylene, 12.5 microns thick) developed by Potter and DeMarse (2001). The ALA-MEA-MEM membrane is produced in license by ALA Scientific Instruments Inc. and distributed via the worldwide network of MCS distributors.

The sealed MEA culture chamber with transparent semipermeable membrane is suitable for all MEAs with glass ring. A hydrophobic semipermeable membrane from Dupont that is selectively permeable to gases (O₂, CO₂), but not to fluid, keeps your culture clean and sterile, preventing contaminations by airborne pathogens. It also greatly reduces evaporation and thus prevents a dry-out of the culture.

The membrane covers come either with or without ports for continuous perfusion. Membrane covers are also available for 6-Well MEAs and 9-Well MEAs. Please see pictures below.
6 Recording

Activity from neuronal cell cultures on MEAs can be recorded starting a few days after plating, after the cells are firmly attached to the surface and a network has been formed. Activity usually begins at a relatively low frequency, with random spontaneous spikes detectable on several electrodes. Spike amplitudes can range from between 30 µV up to around 400 µV, if a cell with an ideal contact sits right on top of the electrode. Typical amplitudes are around 100 µV. The activity pattern changes over time as the culture matures. The spike frequency usually increases, and the pattern evolves in the direction of synchronized spike bursts in the whole network of cells. The higher the cell density, the sooner bursts will appear.

There are two options to record from these cultures, with or without a continuous perfusion. Perfusion is likely to get the culture contaminated in the long run, but allows long lasting recordings for hours with or without substance application. Without perfusion, either short, episodic recordings are possible without proper pH control or even longer lasting recordings, as long as the pH inside the culture is maintained.

6.1 Recording without Perfusion

To record from a cell culture for a few minutes only, it is enough to put a lid on the MEA dish and transfer the array in the amplifier for recording. The pH will be stable for 3-5 min. After the recording, the culture can be put back in the incubator. Even if the culture is not sterile any longer, repeated short recordings for a few days in a row are possible with this simple method.

To allow longer lasting recordings outside the incubator, the best way is to combine ALA-MEA-MEM covers with a CO₂ atmosphere. The heating plate of the MEA amplifier can keep the temperature stable, while the membrane covers prevent evaporation of the medium. A small confined chamber around the MEA connected to a 5 % CO₂ supply can provide a suitable atmosphere to keep the pH stable. Such a chamber is available for the MEA2100-System from Multi Channel Systems MCS GmbH. The MEA2100-CO₂-C will fit exactly on all available MEA2100 headstage variants.
Alternatively, a cell culture dish with a hole for tubing is usually sufficient. A ready-to-go climate chamber solution specifically designed for the MEA2100 headstage in combination with the ALA-MEA-MEM-PL is available from Okolab (oko-lab.com) under the part number H201-MEC-MEA.

To prevent evaporation of medium without using the ALA-MEA-MEM covers, it is possible to pipe the 5 % CO₂ gas through a tightly sealed bottle with water. This will result in a sufficiently humid atmosphere to limit evaporation.

In case of the USB-MEA-System, it is also possible to put the MEA1060 amplifier inside a dry incubator for extended recordings. As the usual 100 % humidity is missing, the use of ALA-MEA-MEM covers is mandatory to prevent evaporation of the medium.

Important: Only MEA1060 amplifiers are safe to be put in a dry incubator. The MEA2100-System produces more heat and might thereby compromise the temperature regulation of the incubator!

### 6.2 Recording with Perfusion

The most secure method to ensure stable conditions during a long lasting recording is to use a constant perfusion of the culture with culture medium. Usually very slow perfusion rates in the order of 100 µl per minute are enough. The easiest way is to use the ALA-MEA-MEM-PL membrane covers with perfusion ports, which will allow perfusion under stable conditions.

Important: The pH of medium can change while being pumped slowly through tubing, as not all tubing material is CO₂ impermeable! Short Teflon tubing is recommended.
6.3 MC_Rack Setup

6.3.1 Spontaneous Activity

To record spontaneous activity, usually a very simple rack is sufficient. A 10 Hz high pass filter to remove slow wave fluctuations is optional, but usually not necessary. A regular and/or a long term display is needed to display the ongoing activity. Additionally, a Spike Sorter in combination with an Analyzer can be used to monitor changes in spike rate over time. Please see the included rack file `Online_Recording_spontaneous.rck`. A sampling rate of 20 kHz is enough to capture the spike shape. In older MEA systems with MC_Card, adjust the input voltage range to the smallest value to achieve the optimal resolution.

![Rack Setup](image)

Please note that electrode raw data and spike cut outs are available as separate data streams in the recorder. It is recommended always to record the raw data. Any signal not recognized online by the spike sorter is lost when recording only spike cut outs.

6.3.2 Real-Time Feedback Stimulation

In all systems with USB data acquisition it is possible to detect signals in real-time and trigger an electrical feedback stimulation based on the detection of certain events. The following example shows a MEA2100-60-System with integrated stimulators, but similar experiments are also possible with a MEA1060-System with USB-ME64/128/256 data acquisition and an external stimulator STG4000.

The example shows a simple setup with feedback stimulation on only one stimulation channel on a single condition. More than one condition can be defined and all available stimulation channels can be started on independent conditions. The aim of the experiment is to try and synchronize different areas of a cell culture. It is assumed that the area in the upper left corner of the array shows strong synchronized activity, while in the lower right corner there is only weak, unsynchronized activity. Cell density in both areas should be comparable. Open the included rack file `Online_Recording_RTF.rck`. The real-time feedback tool filters the data with a 200 Hz filter and detects spikes with a threshold of four times the SD of the noise.

Important: The rack file will only open if a MEA2100-System is connected and running, as the real-time feedback function is dependent on operative hardware!
The condition set to trigger feedback stimulation is a combined spike rate of 100 Hz on four electrodes in the upper left corner of the array.

The Stimulator 1 will start on real-time feedback condition one (RF1). Every time this condition is fulfilled, the stimulator will deliver a short 100 Hz stimulation pulse train with 20 repeats to four electrodes in the lower right corner of the array.
Make sure to uncheck the function “Dedicated Stimulation Electrode”, or all four selected stimulation electrodes will be permanently connected to the stimulator and unavailable for recording.

A spike sorter in combination with an analyzer monitors and plots the spike rate. If the feedback stimulation synchronises the activity between both areas over time, this should be visible in the spike rate plot.

Note: When using the option Real-Time Feedback, it is recommended to record also the Digital Data Stream, as the condition of the digital bits shows the status of the stimulators and the real-time feedback conditions.
6.4 Multi Channel Experimenter Setup

Recording of spontaneous activity and also Real-Time Feedback are possible with the Experimenter software as well, the Real-Time Feedback works settings similar to MC_Rack. In addition, the Experimenter offers timer controlled recordings which are impossible in MC_Rack.

6.4.1 Spontaneous Activity

To record spontaneous activity, usually a simple experimental setup is sufficient. A high pass filter to remove slow wave fluctuations will improve spike detection. Filtered and/or unfiltered raw data can be monitored in the Data Acquisition or Filter tab. Additionally, a Spike Detector can be used to monitor the spike shapes online. Please see the included experiment (*.mse) file Online_Recording_Spontaneous.mse. A sampling rate of 25 kHz is enough to capture the spike shape. As all filtering and spike detection can be repeated offline in the Multi Channel Analyzer, only the unfiltered Electrode Raw Data needs to be recorded.
6.4.2 Timed Recordings

In contrast to MC_Rack, the Experimenter is able to go to standby in Recording mode, and wait for trigger inputs to re-start the recording without user intervention. In combination with the option to stop a recording file on a built-in timer in the Recorder, this offers several possible applications. Open the included *.mse file Online_recording_Timer.mse.

The Recording is set to Standby, the Trigger Generator creates triggers at pre-defined time points. Each trigger starts the recording of a file with a duration defined by the Timer in the Recorder. There are several possible applications:

**Continuous recording with multiple files:**
Set the Trigger Generator to periodic triggers with an interval of [File Duration+100 ms]. Set the file duration in the Recorder. In the example above, 5 minute (300000 ms) files will be created every 300100 ms. This would allow almost continuous recording with only 100 ms gaps without the problem of generating GB sized files.

**Automated episodic recordings:**
Set the Trigger Generator to periodic triggers with an interval of for example 2 hours, and set the required recording duration in the Recorder, for example 5 minutes. This would allow to automatically run repeated short recordings, possibly overnight, without user intervention. If the “List” option of the Trigger Generator is used, the recording times can also be set with more flexibility (for example 30 min, 1 h, 2 h, 5 h, 10 h).

**Automated recording with predefined gaps for substance application:**
It’s possible to record an experiment with several phases, for example different substance concentrations, with one file per phase and defined time gaps for substance application. Set the Trigger Generator to periodic triggers with an interval of for example 190 s, and set the recording duration in the Recorder to 3 minutes. This would generate 3 minute recordings with 10 s gaps in between, which would allow the application of a new concentration of a substance, and thereby exclude the related artefacts from the data. With one file per concentration, it’s also easier to extract analysis parameters in relation to concentration offline.
6.4.3 Real-Time Feedback Stimulation

In all systems with USB data acquisition it is possible to detect signals in real-time and trigger an electrical feedback stimulation based on the detection of certain events. The following example shows a MEA2100-60 System with integrated stimulators, but similar experiments are also possible with a MEA1060-System with USB-ME64/128/256 data acquisition and an external stimulator STG4000. The aim of the experiment is to try and synchronize different areas of a cell culture. It is assumed that the area in the upper left corner of the array shows strong synchronized activity, while in the lower right corner there is only weak, unsynchronized activity. Cell density in both areas should be comparable. Open the included rack file Online_Recording_RTF.mse. The real-time feedback tool filters the data with a 200 Hz filter and detects spikes with a threshold of five times the SD of the noise.

Important: The experiment file will only open if a MEA2100-System is connected and running, as the real-time feedback function is dependent on operative hardware!

The condition set to trigger feedback stimulation is a combined spike rate of 100 Hz (20 spikes in a 200 ms window) on four electrodes in the upper left corner of the array.

The Stimulator 1 will start on real-time feedback condition one (RF1). Every time this condition is fulfilled, the stimulator will deliver a short theta burst stimulation pulse train with 5 x 4 repeats to four electrodes in the lower right corner of the array. Repeated start condition during an ongoing stimulation will be ignored.

Make sure to uncheck the function “Dedicated Stimulation Electrode”, or all four selected stimulation electrodes will be permanently connected to the stimulator and unavailable for recording.

A Digital Event Detector monitors and stores the Stimulator activity as trigger events. The unfiltered raw data is recorded in the file, even though the spike detection for the Real-Time Feedback works on filtered data.
7 Data Analysis

7.1 Analysis with MC_Rack

When recording from neuronal cell cultures, the signals of interest are usually spikes. MC_Rack can detect spikes either by an individual threshold on each electrode, or by a combination of slope and amplitude of the signal. Spike detection works the same way online and offline. It is recommended to always record raw data and do the spike extraction afterwards. A basic spike sorting capability is also included. Once detected and possibly sorted, spikes can be analyzed with two instruments, the Analyzer and the Spike Analyzer. The Analyzer works in designated time bins, while the Spike Analyzer works event based, and creates one data point per inter spike interval. Please see the image below.

The following examples illustrate the use of both instruments.

7.1.1 Drug Application

Open the rack file Analysis_NMDA.rck and the data file Example_NMDA_Application.mcd. Data was recorded from a neuronal cell culture with striatum cells from rat (Wistar, E15). The file is merged from two 10 second recordings, the first 10 s were under control conditions, the other 10 s show the activity of the same cell culture after application of 5 µm N-methyl-D-aspartate (NMDA).

A long term display shows the complete 20 s of raw data. The MC_Rack Analyzer extracts the spike rate in 1 s bins. The Spike Analyzer plots the inter spike interval ISI and the detected events as time stamps in two different parameter displays. Under control conditions, the spontaneous spiking activity is organized in bursts. After NMDA application, the Analyzer shows a moderate increase in spike rate on most electrodes.
But the more striking difference is the change in spike pattern, which is obvious from the time stamp raster plot and the ISI. The spiking activity becomes more random, the ISI decreases. Instead of discrete bursts, there is now a more or less continuous and random spiking activity on all active electrodes.

(Data kindly provided by W. Fleischer, University of Düsseldorf)

7.1.2 Spike Sorting

Open the rack file Analysis_Sorting.rck and the data file Example_SpikeSorting.mcd. Data was recorded from a neuronal cell culture with hippocampal cells from rat (Wistar, E15), ten days after plating. The file contains one minute of spontaneous activity. A long term display shows 30 s of raw data. The Spike Sorter display shows detected events in an overlay plot. The colored spike markers are used to sort different spike populations roughly by shape on the most active electrodes.
The Spike Analyzer plots the spike rate and the detected events as time stamps in two different parameter displays, color coded for the sorted spike units.

The spike sorting capabilities of MC_Rack are limited, as the software is designed primarily as a data acquisition tool. To do more sophisticated spike sorting it is possible to export MC_Rack data to other software packages, like the Plexon Offline Sorter, or to Matlab with the Multi Channel Data Manager.

7.1.3 Spike Burst Analysis

Open the rack file Analysis_Bursts.rck and the data file Example_BurstAnalysis.mcd. Data was recorded from a neuronal cell culture with hippocampal cells from rat (Wistar, E15), 14 days after plating. The file contains two minutes of spontaneous activity from a single electrode only. Raw data is filtered with a 200 Hz high pass filter to remove slow wave components of spike bursts and thereby improve spike detection. Spikes are detected by threshold on the filtered data stream by the Spike Sorter tool. The Spike Sorter display shows the filtered raw data.
The Spike Analyzer plots the detected events as time stamps in a parameter display. Spike bursts are detected using the MaxInterval Method with user defined parameters. A colored line under the time stamps in the parameter display marks detected bursts. A second parameter display is used to plot the mean spike frequency inside bursts.

<table>
<thead>
<tr>
<th>Channel</th>
<th>Num. Of Spikes</th>
<th>Mean Spike Frequency [Hz]</th>
<th>Num. Bursts</th>
<th>Bursts per Second</th>
<th>Bursts per Minute</th>
<th>% of Spikes in Burst</th>
<th>Mean Burst Duration [ms]</th>
<th>Mean Spikes in Burst</th>
<th>Mean ISI in Burst [ms]</th>
<th>Mean Spike Frequency in Burst [Hz]</th>
<th>Mean Interburst Interval [ms]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2495.9</td>
<td>29.9</td>
<td>13.0</td>
<td>0.1</td>
<td>6.5</td>
<td>99.1</td>
<td>890.1</td>
<td>150.5</td>
<td>4.3</td>
<td>233.9</td>
<td>9592.6</td>
</tr>
</tbody>
</table>

The “Mean Spike Frequency in Bursts” and “Number of Spikes in Bursts” can be plotted online. Other burst parameters can only be calculated after the replayed file is finished or has been stopped. These additional parameters can be exported as ASCII data with the “Export Results” button (please see the screenshot above). The “Percentage of Spikes in Burst” is a good parameter to describe the bursting behavior of the cells.

### 7.2 Analysis with Multi Channel Analyzer

When recording from neuronal cell cultures, the signals of interest are usually spikes. The Analyzer can detect spikes offline, with the Spike Detector tool, or work with spike time stamps from then Spike Explorer. Spike detection works the same way online and offline. It is recommended to always record raw data and do the spike extraction afterwards. Spikes can be analyzed with two instruments, the Spike Analyzer and the Burst Analyzer.

The Spike Analyzer analyzes the inter spike intervals (ISI) between the time stamps and generates ISI, number and frequency plots, as well as ISI histograms. Spike frequencies are either generated event based (Instantaneous) or for discrete time bins. The Bin wise analysis creates one data point for each time bin. Number per time bin or frequency (Hz) is available as parameters. For 1 second time bins, number and frequency are identical. For other time bins, the frequency is the averaged frequency/s. In the example shown below, the results for 3 s bins would be:

\[(3+2+1)/3=2.0 \text{ and } (4+1+2)/3=2.3\]

Bin wise analysis allows averaging of spike activity over longer time periods.

<table>
<thead>
<tr>
<th>Binned results: 1 datapoint per time bin</th>
</tr>
</thead>
<tbody>
<tr>
<td>![Graph showing binned results]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Instantaneous results: 1 datapoint per spike interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>![Graph showing instantaneous results]</td>
</tr>
</tbody>
</table>

The Instantaneous (event based) analysis always works from one time stamp to the next, and creates one data point for each interval. Inter Spike Interval (ISI) and frequency are available as parameters.

The Burst Analyzer analyzes the inter spike intervals (ISI) between the time stamps and applies the Max. Interval algorithm to detect spike bursts. The Burst Analyzer uses the MaxInterval Method for detecting bursts:

- Scan the spike train until an inter spike interval is found that is less than or equal to Max. Interval.
- While the inter spike intervals are less than Max. End Interval, spikes are included in the burst.
- If the inter spike interval is more than Max. End Interval, the burst ends.
- Merge all the bursts that are less than Min. Interval between Bursts apart.
- Remove the bursts that have duration less than Min. Duration of Burst or have fewer spikes than Min. Number of Spikes.

Please see the [Multi Channel Analyzer manual](#) for more details. The following examples illustrate the use of both instruments.
7.2.1 Spike Frequency Analysis

Spike frequencies often vary a lot in between channels, and also on individual channels over time, one reason being that not every electrode records from the same number of cells simultaneously. Therefore, the instantaneous spike plots in the Spike Analyzer sometimes don’t show any obvious experiment-related changes at first glance. It is often more useful to average the spike counts in time bins to see changes.

The plots above show the same data in instantaneous mode (left) and averaged in 5 second bins (right). The same trend is obvious in both plots, but the binned data is more intuitive. A second way to illustrate also more subtle frequency changes is to use the ISI histograms.

The histograms may reveal a different distribution of spike frequencies on the same channel under different conditions, independently of the absolute number of spikes, which might remain more or less constant. The histogram data can be exported as ASCII data, and be used to compare different conditions on the same channel. This requires each condition to be recorded as an individual file (see also chapter 6.4.2, Timed Recordings). The graph below shows histograms for two conditions from the same channel, where the total number of detected spikes was similar, but the ISI/frequency distribution was quite different.
7.2.2 Spike Burst Analysis

Results of the Burst Analyzer heavily depend on the parameters selected for the MaxInterval algorithm. On electrodes with several continuously firing neurons, the algorithm might detect lots of long bursts, but by manual observation, one would not consider such activity as burst like. On the other hand, on electrodes with low general activity, no bursts will be detected, even if the activity is episodic and synchronized with other electrodes, as the number of detected spikes is too low to meet the Min. Duration of Burst and Min. Number of Spikes criteria.

Typical burst like behaviour are short periods of strong activity, with longer periods of no, or only sparse activity in between. During bursts, spikes are often superimposed by a slower field potential. Hence, the first step in burst analysis is to remove the field potential with a high pass filter. Subsequently, spikes can be detected with a Spike Detector, and the resulting time stamps can be used by the Burst Analyzer.

A reasonable approach is to optimize the parameters of the burst detection algorithm in a clearly bursting culture, till the detected bursts match what one would consider a burst by manual observation, and stick to those parameters afterwards for all cultures.

Some of the parameters the Burst Analyzer produces are displayed continuously, as they can be calculated for each individual burst: spike count, burst duration and mean spike frequency inside the burst. These parameters describe changes in bursting behaviour, like bursts getting longer or shorter. If the results are exported as ASCII data, all continuous parameters are sown for all channels underneath each other.
Some more parameters can only be calculated once the file has been **completely analyzed**. These so called **Numerical Results** are at the bottom of the ASCII file, below the continuous results.

The Interburst Interval indicates changes in burst frequency. Again, this requires that different experimental conditions, which need to be compared, were recorded in individual files. Bursting behaviour is well described by the parameter **% Spikes in Bursts**. A normal baseline value for a fairly active electrode in a culture with not synchronized, random activity is 10-20 %. In a bursting culture, this can go up to 90-100 %. As bursting is almost always accompanied by synchronization between the channels, the **% Spikes in Bursts** can also be used as an indicator for synchronization in a cell culture.

### 7.3 Analysis with 3rd Party Software

The Multi Channel **Data Manager** allows to convert single or multiple files to a number of other file formats, so the analysis can be carried out in 3rd party applications. **Input files** can be both from MC_Rack or the Multi Channel Suite.

**Output formats** include native file formats of Spike 2, Neuroexplorer and Plexon Offline Sorter. Additionally, export to generic formats HDF5, ASCII and European Data Format (*.edf) are available, which allows working in platforms as Matlab, Python or even Excel, Origin and IGOR Pro.
8 Suggested System Configurations

The following guidelines should give you an overview of the available systems. Please contact MCS or your local distributor to find the ideal system for your application.

8.1 MEA2100-System

The MEA2100-System is the most advanced system MCS can offer today. Available headstage versions are HS60, HS2x60, HS120 and HS256. It is as flexible as the USB-MEA60-System and can use the same MEAs, in addition to unique 120 or 256 electrode arrays. 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. 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. 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. The system can be upgraded to operate up to four 60-channel or two 120-or 256 channel MEAs independently from one computer.

Included accessories: Temperature controller, floor heating, internal stimulator.
Recommended accessories: Teflon membranes ALA-MEA-MEM.
Optional accessories: Perfusion heating PH01, magnetic perfusion holders MPH, peristaltic perfusion system PPS2.

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

8.2 USB-MEA60-Inv/Up-System

The USB-MEA60-System is a flexible system and uses 60 electrodes, each of which can be selected by software for stimulation and recording. The system includes amplifier and data acquisition in separate devices, floor and optional perfusion heating and can be supplemented with an external stimulator STG4000 for current or voltage stimulation. An optional blanking circuit removes stimulation artefacts. Approximate 20 electrode layouts with several additional options are available at the moment. Amplifiers are available in “Up” or “Inv” configuration, optimized for upright or inverted microscopes. The data acquisition allows real-time feedback stimulation by a specific digital signal processor for fast online signal analysis. The data acquisition and STG can be upgraded to operate up to four amplifiers independently from one computer. The USB-MEA60-BC-System with blanking circuit is recommended if electrical stimulation is planned on a regular basis.

Included accessories: Temperature controller, floor heating.
Recommended accessories: Teflon membranes ALA-MEA-MEM.
Optional accessories: Stimulator STG4000, perfusion heating PH01, magnetic perfusion holders MPH, peristaltic perfusion system PPS2.

Pro: Individual components (STG, DAQ) also usable for other experimental setups, very flexible applications, can be put in a dry incubator.
Contra: Many boxes and cables, relatively large footprint, especially in systems for parallel recordings from several MEAs.
8.3 USB-MEA256-System

The USB-MEA256-System is the legacy system of the MEA2100-256. It offers the highest number of recording channels available on a single MEA with metal electrodes. It is very compact and includes amplifier and data acquisition in one device, as well as floor heating and many additional in and outputs for interface with other devices. Five different MEA electrode layouts with additional options are available at the moment, including the 6-Well and 9-Well MEAs. While the USB-MEA256 allows also electrical stimulation with an external stimulator, the addressing of the stimulation electrodes has to be done manually and is far less comfortable than with the MEA2100- or MEA1060-BC-Systems. Therefore, it is not recommended if electrical stimulation is planned on a regular basis.

Included accessories: Temperature controller, floor heating.
Recommended accessories: Teflon membranes ALA-MEA-MEM.
Optional accessories: Stimulator STG4000, perfusion heating PH01, magnetic perfusion holders MPH, peristaltic perfusion system PPS2.

Pro: Highest channel number per MEA, high throughput experiments with 9-Well MEAs.
Contra: Can not be upgraded, not convenient for electrical stimulation, can not be put in a dry incubator.

8.4 Suggested Microelectrode Arrays

MEA2100-System
For the MEA2100, the same MEAs can be used as for the USB-MEA60-System, with the same advantages and disadvantages. In addition, 120-channel MEA layouts are available, which provide either a better resolution or a larger recording area.

USB-MEA60-System
For cell culture experiments, any MEA layout may be used, depending on the aim of the experiment. 30 µm electrodes are most suitable for electrical stimulation, because stronger stimulation pulses can be applied. The material (TiN or ITO) does not matter; only if sophisticated imaging on the arrays is planned ITO should be preferred. To avoid contamination of the cultures during recording sessions, MEAs with internal reference electrode should be used.

USB-MEA256-System
For the USB-MEA256, five different MEA electrode layouts are available at the moment, including the 9-Well MEAs. Thin MEAs are also available. Depending on the spacing of the electrodes, they either provide a very high resolution, or a large recording area.

Ring Options
The standard glass ring is best for running a perfusion and will also allow using the ALA MEA-MEM. A plastic ring should only be used if parallel patch clamp experiments are planned.
9 References

An up to date list of publications sorted by preparation or application can be found on the MCS web site http://www.multichannelsystems.com/publications.

10 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 provides the opportunity for you to exchange your experience or thoughts with other users worldwide.

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