



a division of **Harvard Bioscience, Inc.**

MEA Application Note: Neuronal Cell Culture Cultivation, Recording and Data Analysis



Imprint

Information in this document is subject to change without notice. No part of this document may be reproduced or transmitted without the express written permission of Multi Channel Systems MCS GmbH.

While every precaution has been taken in the preparation of this document, the publisher and the author assume no responsibility for errors or omissions, or for damages resulting from the use of information contained in this document or from the use of programs and source code that may accompany it.

In no event shall the publisher and the author be liable for any loss of profit or any other commercial damage caused or alleged to have been caused directly or indirectly by this document.

© 2020 Multi Channel Systems MCS GmbH. All rights reserved.

Printed: October 20

Multi Channel Systems MCS GmbH

Aspenhaustraße 21
72770 Reutlingen
Germany

Phone +49-7121-909 25 - 0
Fax +49-7121-909 25 -11

sales@multichannelsystems.com
www.multichannelsystems.com

Microsoft and Windows are registered trademarks of Microsoft Corporation. Products that are referred to in this document may be either trademarks and/or registered trademarks of their respective holders and should be noted as such. The publisher and the author make no claim to these trademarks.

Contents

1	Introduction	5
1.1	About this Application Note	5
1.2	Acknowledgement	5
2	Primary Cortical or Hippocampal Neurons	6
2.1	Material	6
2.1.1	Biological Materials	6
2.1.2	Technical Equipment	6
2.1.3	Chemicals	7
2.1.4	Media	8
2.2	Methods	10
2.2.1	Cleaning MEAs	10
2.2.2	MEA Coating	10
2.2.3	Dissection	10
2.2.4	Enzymatic Digestion	10
2.2.5	Plating and Culturing the Cells	11
3	Primary Neurons from Dorsal Root Ganglia (DRG)	12
3.1	Material	12
3.1.1	Biological Materials	12
3.1.2	Technical Equipment	12
3.1.3	Cell Culture Reagents	12
3.1.4	Media Preparation	13
3.1.5	Extracellular Solution for MEA Recording	13
3.2	Methods	14
3.2.1	Cleaning MEAs	14
3.2.2	MEA Coating	14
3.2.3	DRG Isolation	14
3.2.4	Enzymatic Digestion and Cell Dissociation	15
3.2.5	Seeding DRG Neurons on the MEA	15
4	Cryopreserved Primary Neurons	16
4.1	Material	16
4.1.1	Biological Materials	16
4.1.2	Technical Equipment	16
4.1.3	Chemicals	16
4.1.4	Media	16
4.2	Methods	17
4.2.1	Cleaning MEAs	17
4.2.2	MEA Coating	17
4.2.3	Thawing and Starting the Culture	17
4.2.4	Maintenance	17
5	Longterm Culturing	18

6	Recording	19
6.1	<i>Recording without Perfusion</i>	19
6.1.1	Recording outside an Incubator	19
6.1.2	Recording inside an Incubator	21
6.2	<i>Recording with Perfusion</i>	21
6.3	<i>MC_Rack Setup</i>	22
6.3.1	Spontaneous Activity	22
6.3.2	Real-Time Feedback Stimulation	22
6.4	<i>Multi Channel Experimenter Setup</i>	25
6.4.1	Spontaneous Activity	25
6.4.2	Timed Recordings	26
6.4.3	Real-Time Feedback Stimulation	28
7	Data Analysis	29
7.1	<i>Analysis with MC_Rack</i>	29
7.1.1	Drug Application	29
7.1.2	Spike Sorting	30
7.1.3	Spike Burst Analysis	31
7.2	<i>Analysis with Multi Channel Analyzer</i>	33
7.2.1	Spike Detection	33
7.2.2	Spike Frequency Analysis	35
7.2.3	Spike Burst Analysis	35
7.2.4	Network Burst Analysis	37
7.2.5	Analysis of Evoked Spikes	38
7.3	<i>Analysis with 3rd Party Software</i>	39
8	Suggested System Configurations	40
8.1	<i>MEA2100-System</i>	40
8.2	<i>MEA2100-Mini-System</i>	40
8.3	<i>Suggested Microelectrode Arrays</i>	41
9	References	42
10	Contact Information	42

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 MC_Rack and MC_Stimulus II or the Multi Channel Suite, 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 8, 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
- MEA-MEM Teflon membranes (ALA Scientific Instruments Inc.), see chapter 5 Long Term Culturing

2.1.3 Chemicals

- NaOH
- MgCl₂
- CaCl₂
- HEPES
- Phenol Red
- Na₂SO₄
- K₂SO₄
- 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

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

	Conc.(mM)	FW (g/mol)	mg (for 500 ml)
MgCl ₂ · 6 H ₂ O	5.8	203.31	590
CaCl ₂ · 6 H ₂ O	0.25	147.02	18.4
HEPES	1.6	238.3	191
Phenol Red	0.001 %		5
Na ₂ SO ₄ · 10 H ₂ O	90	322.21	14500
K ₂ SO ₄	30	174.26	2610
Kynurenic acid	1	189.2	95.6
APV	0.05	197.1	4.92

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 website.

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

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

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

Component	Concentration [mM]
NaCl	140
KCl	4
CaCl ₂ ·2 H ₂ O	2
MgCl ₂ ·6H ₂ O	1
HEPES	10
Glucose	4

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 μ m) 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 μ m) digestion Solution 2
- Incubate at 37 °C for 40 min. Stir gently every 15 min.
- Add 60 μ l 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 μ m)
- 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 μ l 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 μ L. Expected yield: 100-300 cells/ μ l
- Dilute the cells to 100 cells/ μ l
- 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 μ l 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 8)
- 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
- MEA-MEM Teflon membranes (MCS)

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 bdH_2O 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 website.

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 % CO_2 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

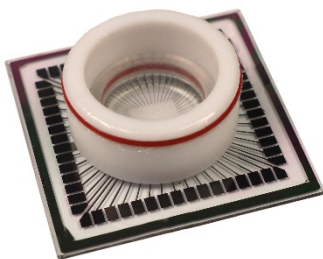
- Change the medium at day 5 after thawing.
- Replace 50 % of the medium with fresh, 37 °C pre-warmed medium every 3 to 4 days. Warm an appropriate amount of medium to 37 °C in a sterile tube. Remove 50 % of the medium from the cell culture. Replace with the warmed, fresh medium and return the cells 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 world-wide 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 (O_2 , CO_2), 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.

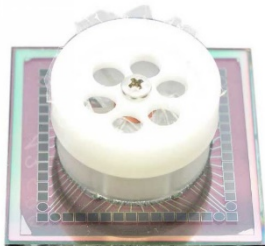
The [membrane covers](#) come either with or without ports for continuous perfusion. Membrane covers are also available for 6-Well MEAs and 9-Well MEAs. Please see pictures below.



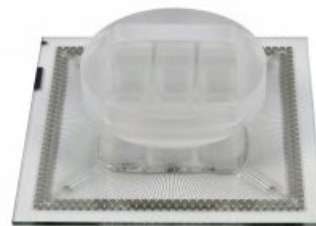
MEA-MEM



ALA-MEA-MEM-PL



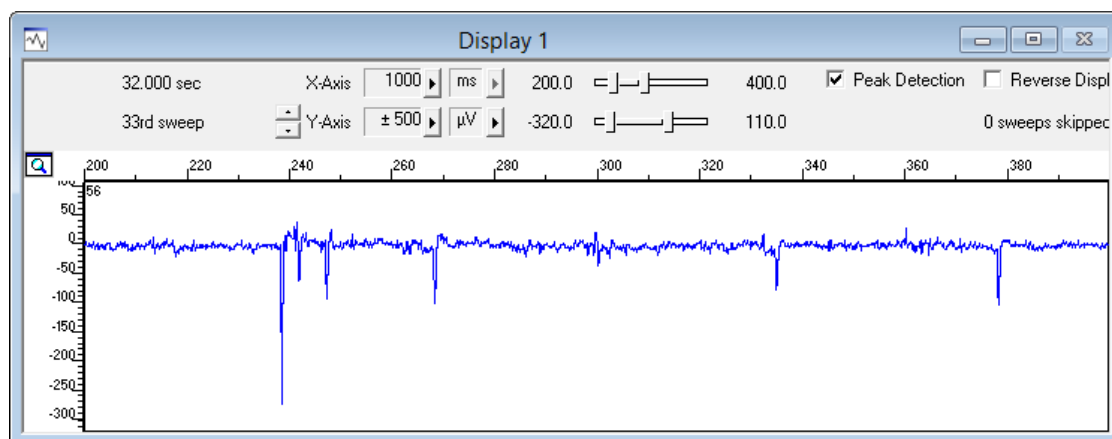
6well-CC



9well-CC

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.

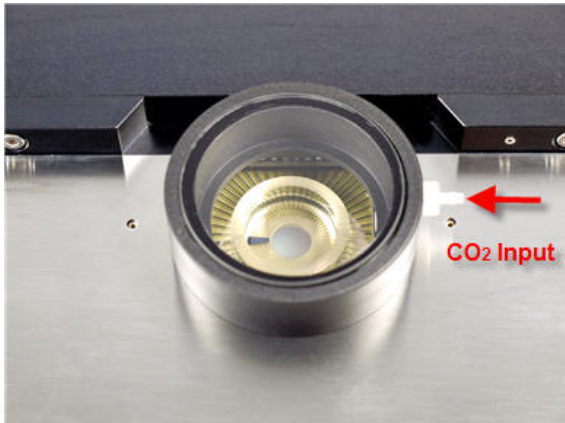
6.1 Recording without Perfusion

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.

6.1.1 Recording outside an Incubator

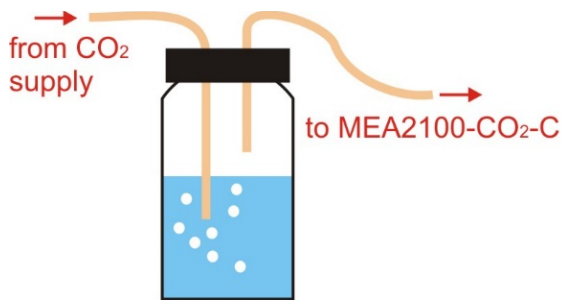
To allow longer lasting recordings outside the incubator, the best way is to combine MEA-MEM covers with a CO_2 atmosphere. The heating plate of the MEA amplifier can keep the temperature stable, while the **membrane covers** prevent evaporation of the medium. A small confined chamber around the MEA connected to a 5 % CO_2 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.

MEA2100-System with MEA2100-CO₂-C



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 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.



6.1.2 Recording inside an Incubator

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 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!

The new **MEA2100-Mini headstage** can also be used in a **humid incubator**, to allow extended recordings under stable conditions. Even though heat emission has been reduced as much as possible, there is still some excess heat from the active electronics. This will result in 2-3 °C above the set temperature of the incubator locally around the device. It is necessary to lower the set temperature of the incubator to around 34 °C. The exact settings depend on each individual incubator and the number of active headstages. Monitor the temperature inside the MEA dish to calibrate your setup.

Please also read about the Suspend Mode in chapter [6.4.2](#) to improve temperature management.

6.2 Recording with Perfusion

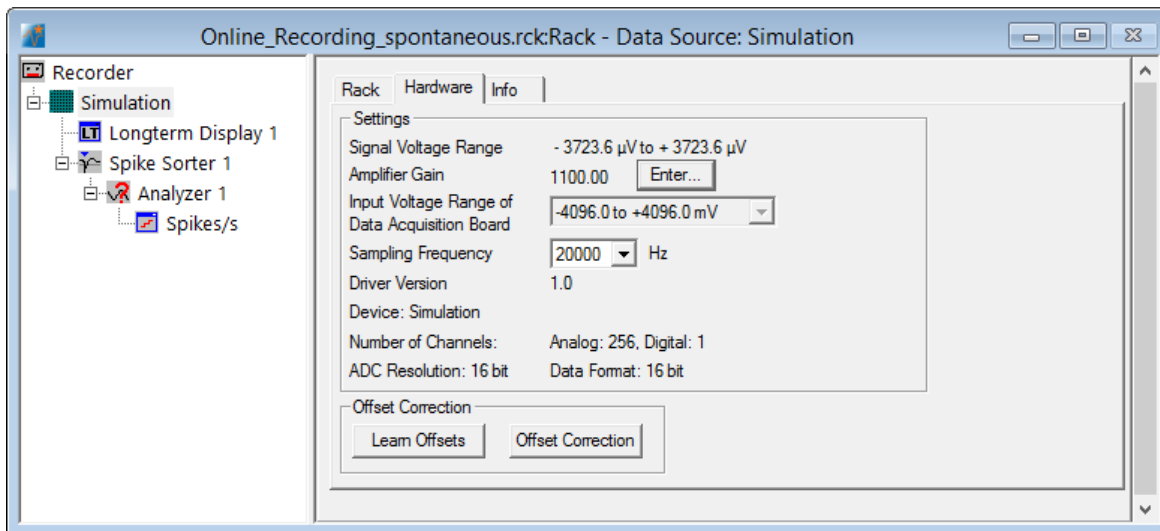
The most secure method to ensure stable conditions during a long lasting recording is to use a constant perfusion of the culture with culture medium. Usually very slow perfusion rates in the order of 100 µl per minute are enough. The easiest way is to use the **ALA-MEA-MEM-PL** membrane covers with perfusion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



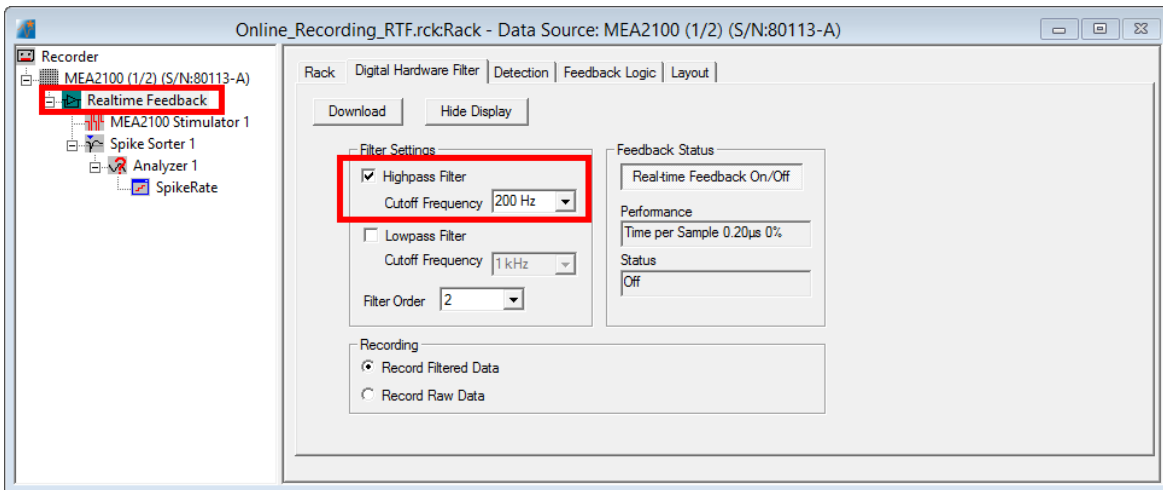
Please note that electrode raw data and spike cut outs are available as separate data streams in the recorder. It is recommended always to record the **raw data**. Any signal not recognized online by the spike sorter is lost when recording only spike cut outs.

6.3.2 Real-Time Feedback Stimulation

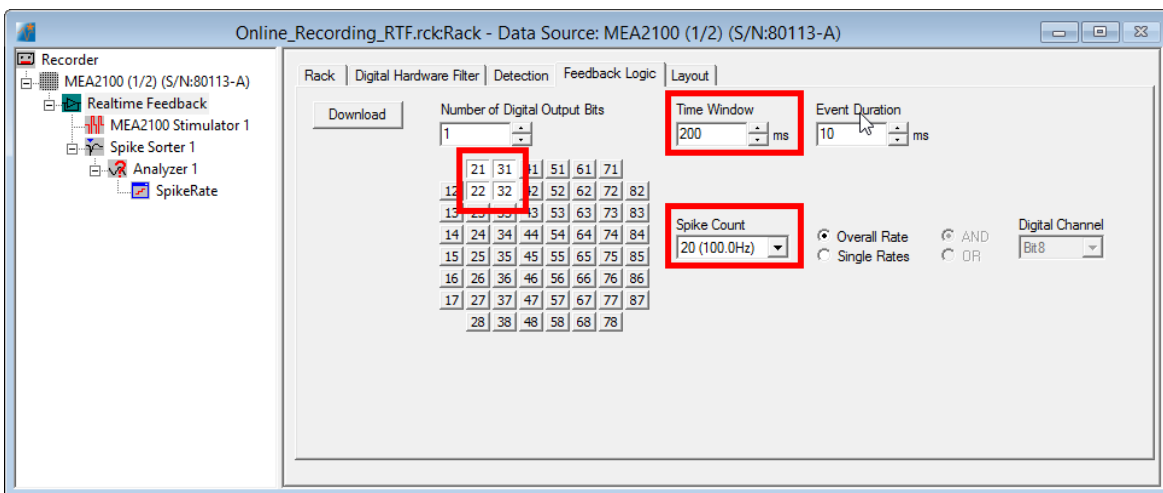
In all systems with USB data acquisition it is possible to detect signals in real-time and **trigger an electrical feedback stimulation** based on the detection of certain events. The following example shows a MEA2100-60-System with integrated stimulators, but similar experiments are also possible with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

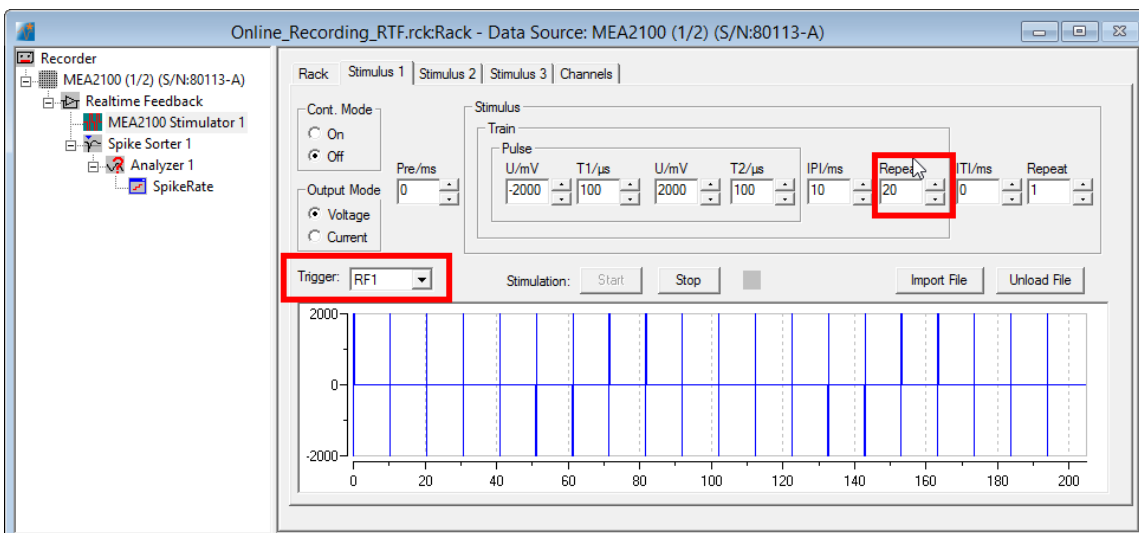
Important: The rack file will only open if a MEA2100-System is connected and running, as the Real-Time Feedback function is dependent on operative hardware!



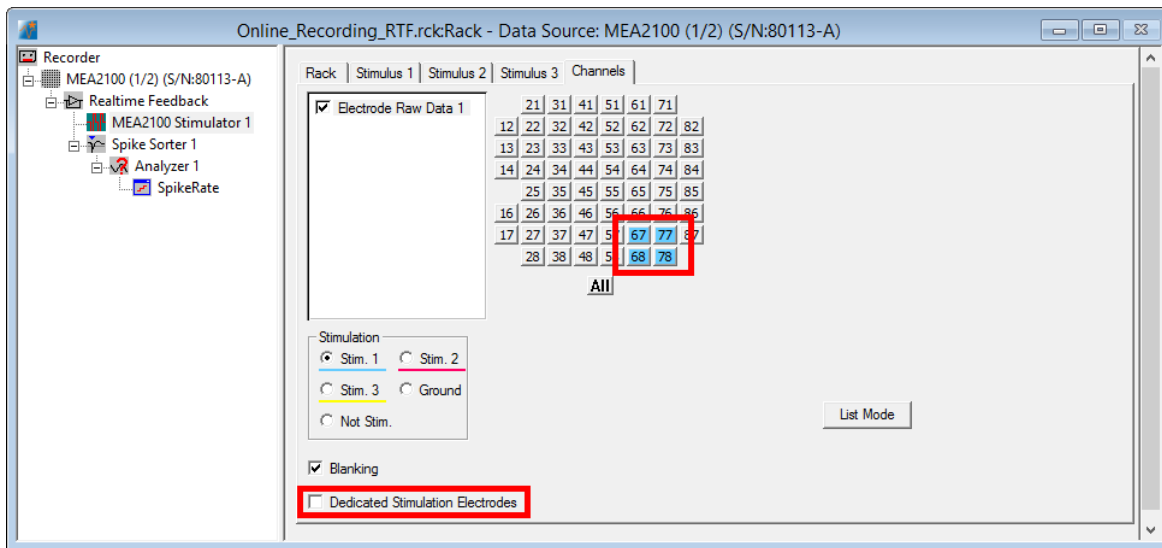
The condition set to trigger feedback stimulation is a combined spike rate of 100 Hz on four electrodes in the upper left corner of the array.



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.

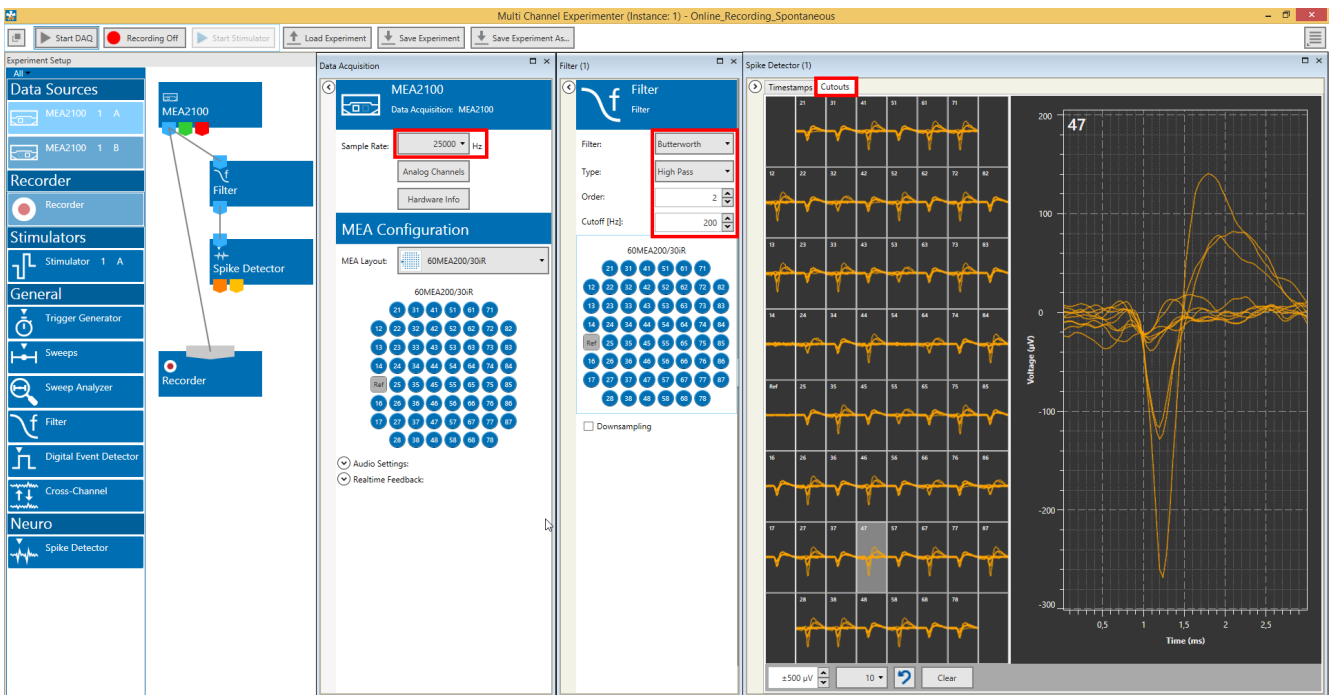
Note: When using the option Real-Time Feedback, it is recommended to record also the Digital Data Stream, as the condition of the digital bits shows the status of the stimulators and the Real-Time Feedback conditions.

6.4 Multi Channel Experimenter Setup

Recording of spontaneous activity and also Real-Time Feedback are possible with the Multi Channel Experimenter 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.

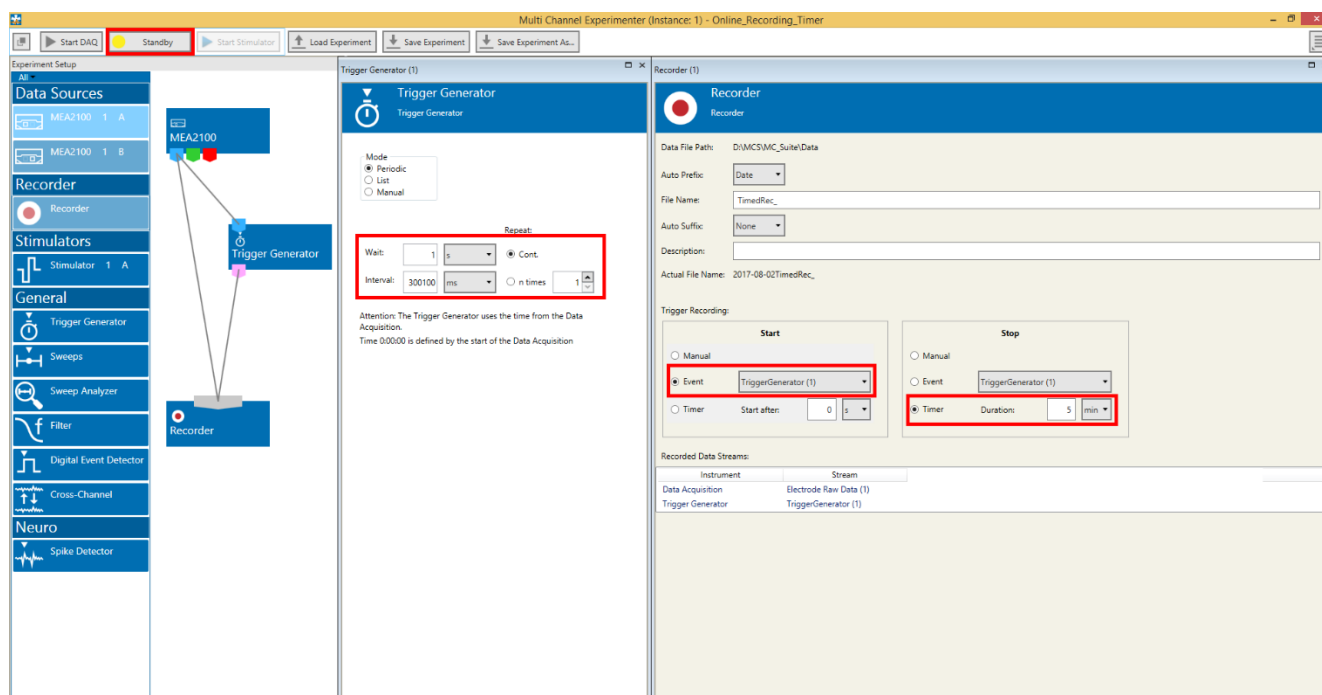
6.4.1 Spontaneous Activity

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 on line. 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 Multi Channel 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:

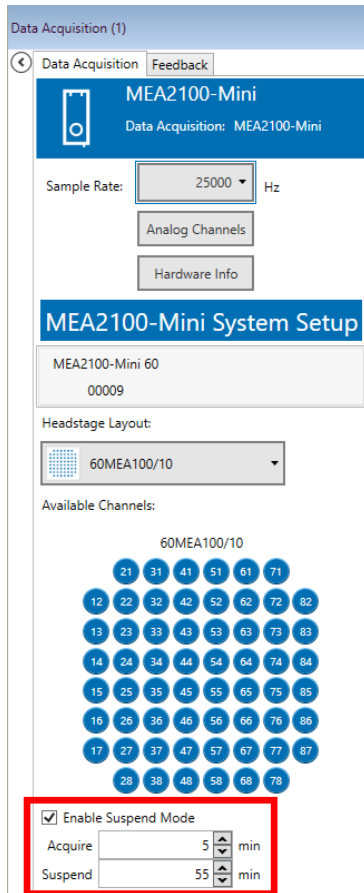
Set the Trigger Generator to periodic triggers with an interval of for example 2 hours, and set the required recording duration in the Recorder, for example 5 minutes. This would allow to automatically run repeated short recordings, possibly overnight, without user intervention. If the "List" option 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.

Suspend Mode in the MEA2100-Mini:

A similar functionality for automated episodic recordings, called Suspend Mode, is available for the MEA2100-Mini only. In addition to automatically recording episodes of data at predefined times, the headstages are actually switched off in between recordings, to reduce heat emission in case they are used in the incubator. The Suspend Mode is not as flexible as the automated recording with internally generated triggers. It is possible to set the recording time and the suspend time in between recordings. After starting the data acquisition, data will be recorded at the predefined intervals until manually stopped.

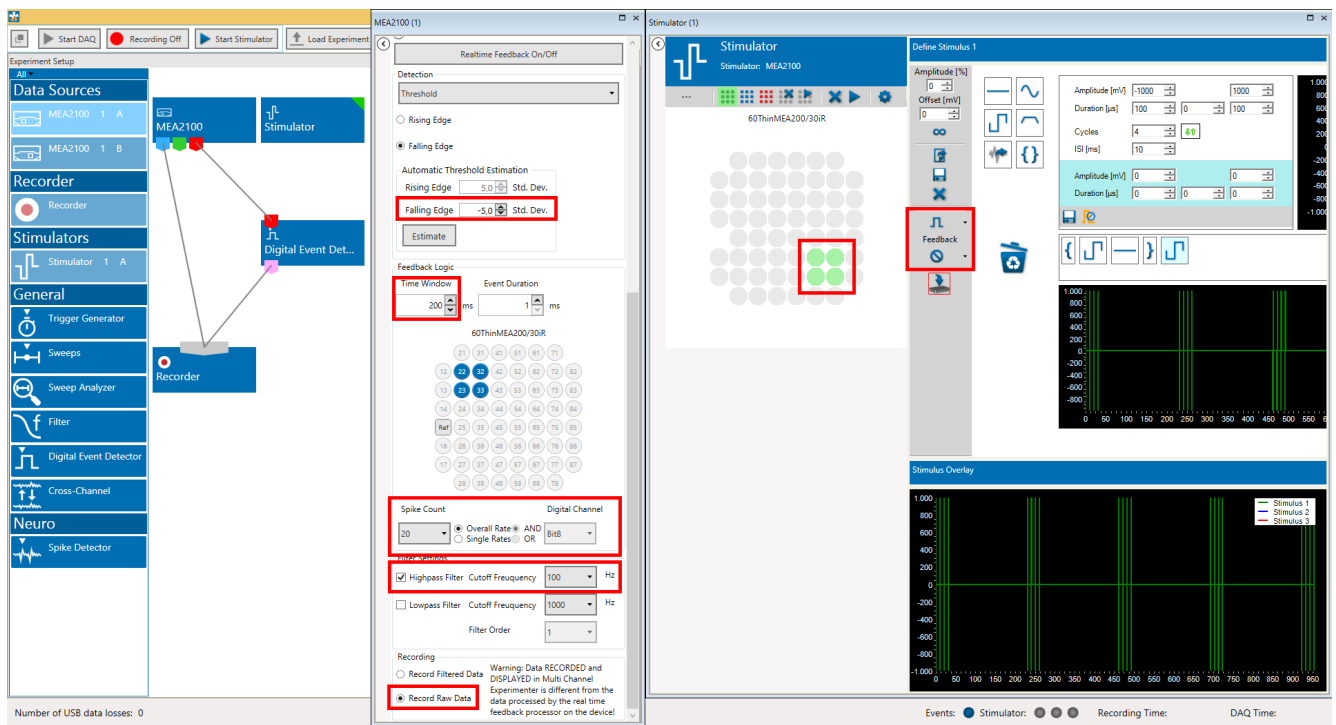


Important: The Suspend Mode function is not available for SCUs (Signal Collector Units) of the first generation. If you have a MEA2100-Mini System, but the function won't show up in the software, please get in touch with [MCS support](#) to figure out available upgrade options.

6.4.3 Real-Time Feedback Stimulation

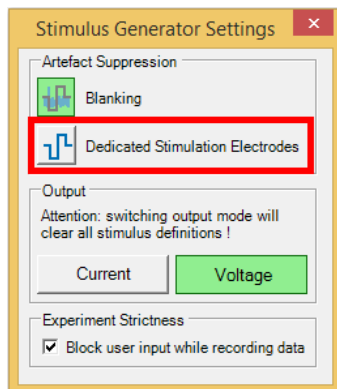
In all systems with **USB data acquisition** it is possible to detect signals in real-time and trigger an electrical feedback stimulation based on the detection of certain events. The following example shows a MEA2100-60-System with integrated stimulators, but similar experiments are also possible with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



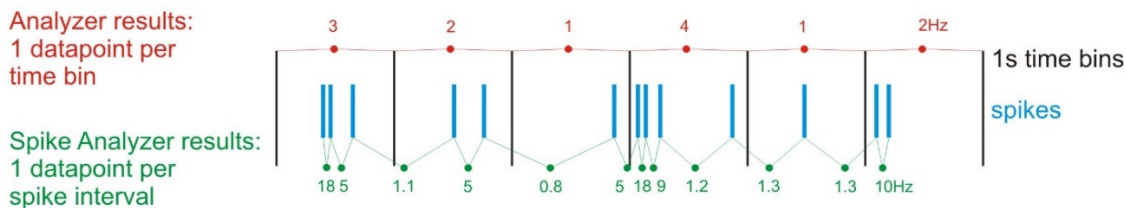
Make sure to uncheck the function “Dedicated Stimulation Electrode”, or all four selected stimulation electrodes will be permanently connected to the stimulator and unavailable for recording.

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

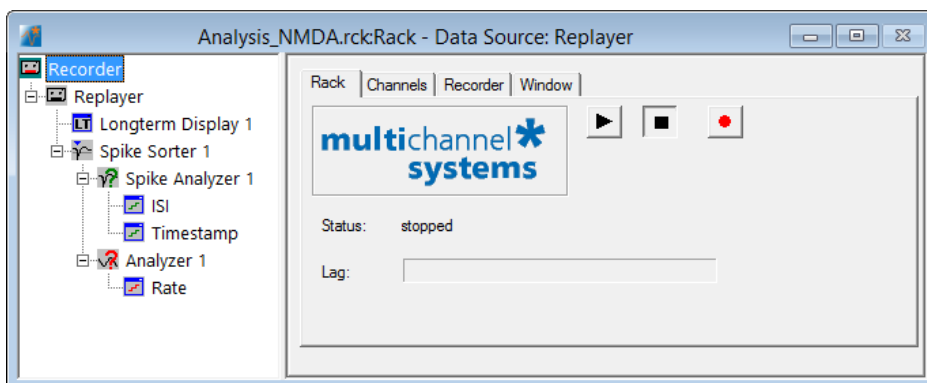
When recording from neuronal cell cultures, the signals of interest are usually spikes. MC_Rack can detect spikes either by an individual threshold on each electrode, or by a combination of slope and amplitude of the signal. Spike detection works the same way online and offline. It is recommended to 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.

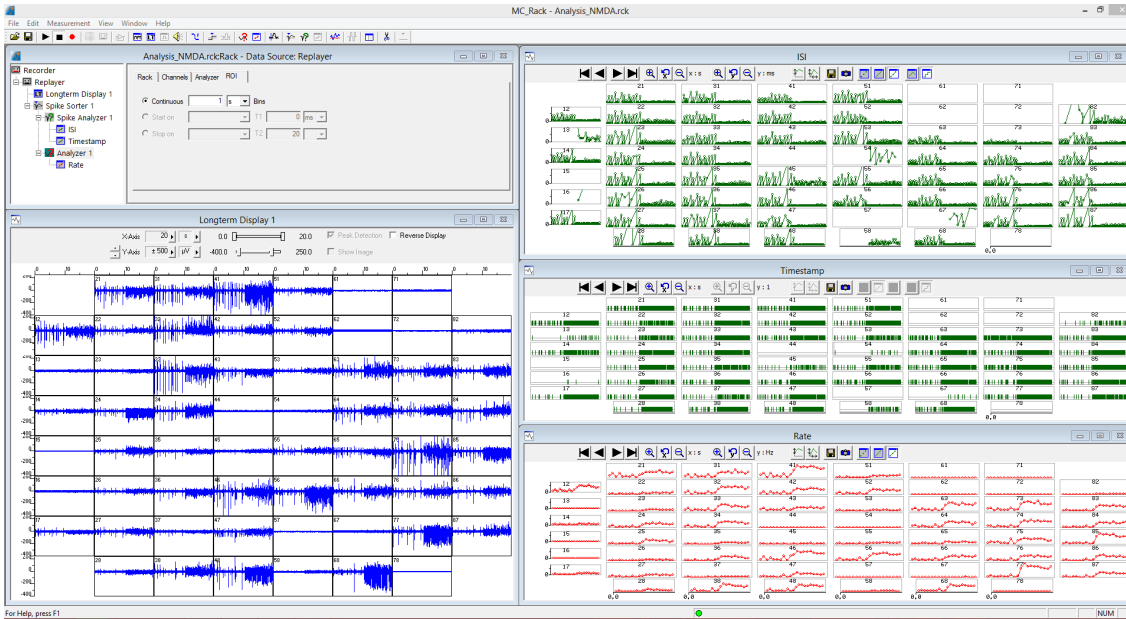
7.1.1 Drug Application

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 μ m 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.

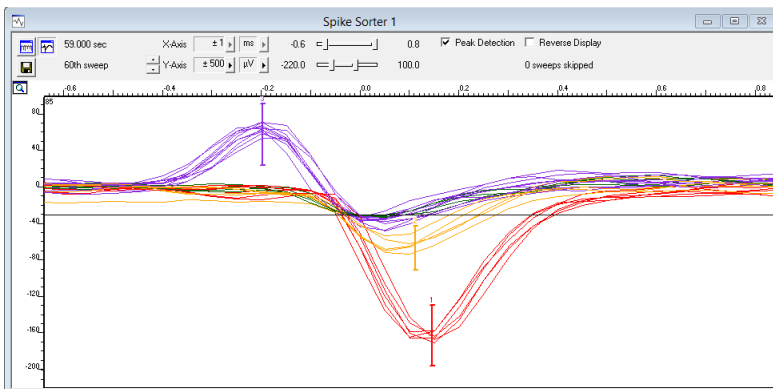
But the more striking difference is the change in spike pattern, which is obvious from the time stamp raster plot and the ISI. The spiking activity becomes more random, the ISI decreases. Instead of discrete bursts, there is now a more or less continuous and random spiking activity on all active electrodes.



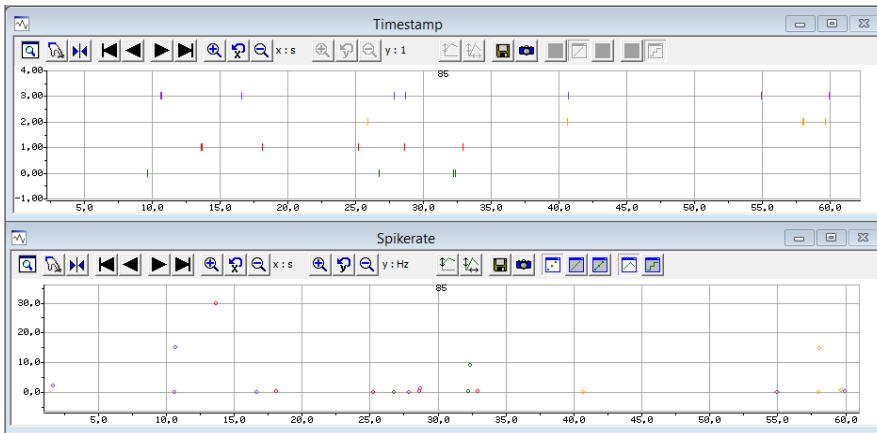
(Data kindly provided by W. Fleischer, University of Düsseldorf)

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.



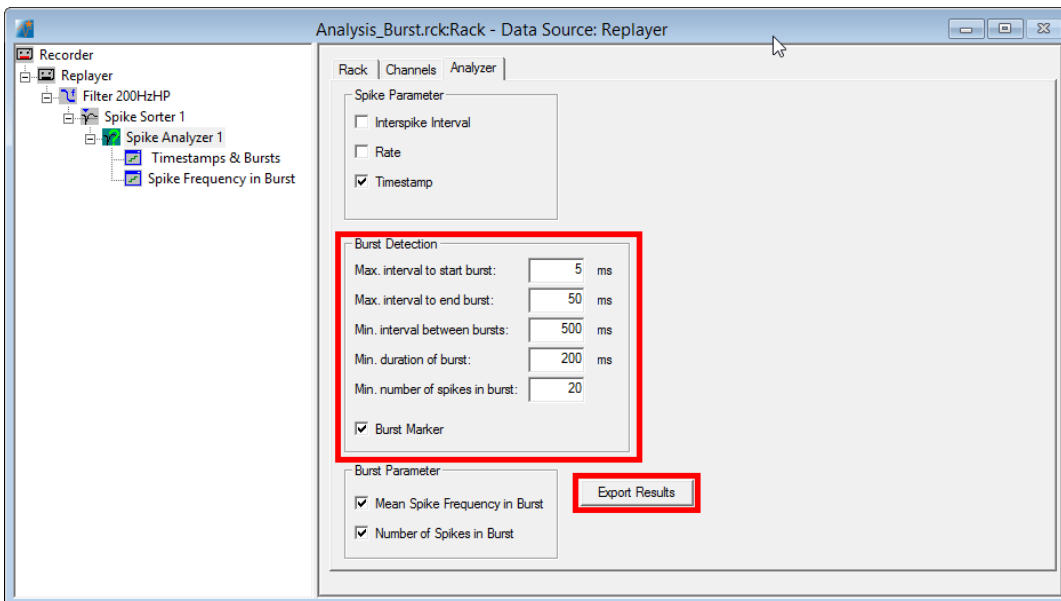
The Spike Analyzer plots the spike rate and the detected events as time stamps in two different parameter displays, color coded for the sorted spike units.



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.

	A	B	C	D	E	F	G	H	I	J	K	L
1	Numerical Results of Spike/Burst Analysis:											
2												
3	Channel/Unit	Num. Of Spikes	Mean Spike Frequency [Hz]	Num. Bursts	Bursts per Second	Bursts per Minute	% of Spikes in Burst	Mean Burst Duration [ms]	Mean Spikes in Burst	Mean ISI in Burst [ms]	Mean Spike Frequency in Burst [Hz]	Mean Interburst Interval [ms]
4	16_unit0	2499.0	20.9	13.0	0.1	6.5	99.1	810.1	190.5	4.3	233.9	9592.6

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.

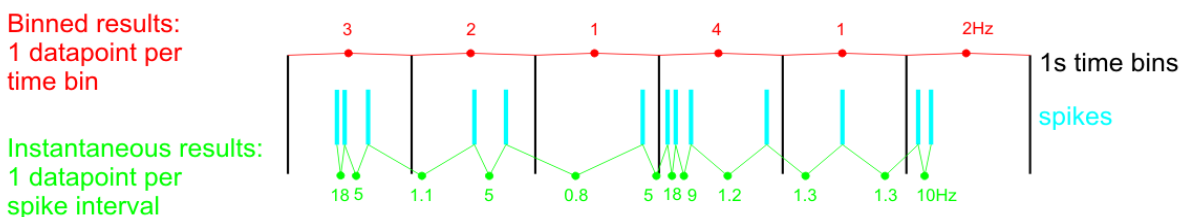
7.2 Analysis with Multi Channel Analyzer

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 three instruments, the Spike Analyzer, the Burst Analyzer and the PSTH tool.

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) are 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.



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*.

Finally, the **PSTH** tool can analyze spikes in relation to a trigger event, usually a stimulation of some sort. The tool need spikes and a trigger as input and can analyze spike parameters in a region of interest in relation to each trigger event, much like the Sweeps tool does for evoked EPSPs.

Please see the [Multi Channel Analyzer manual](#) for more details. The following examples illustrate the use of those instruments.

7.2.1 Spike Detection

Spike detection is the first step of the analysis. If not all spikes are detected, or if there are too many false positives, this compromises the quality of all further analysis steps. Usually a **high pass filter** is applied first, to remove slow wave potentials. After that, spike detection is done with the Spike Detector tool. Most often, the Threshold method is employed, where the detection level is adjusted to the noise level of each individual channel. The best way to proceed is to look for a quiet period **without activity** in the file and navigate there. Then click "Estimate" and "Explore", to send the segment of data without activity to the Spike Detector tool and thereby base the SD calculation on that data. Then click "Analyze", and the whole file will be analyzed without changing the thresholds. The reason to look for a quiet period is that activity increases the SD, and therefore the thresholds are further away from the noise than they should be.

The only option if there is too much activity all the time is to manually adjust the thresholds or try the other methods for spike detection (Manual Threshold or Slope, see the [Multi Channel Analyzer manual](#) for more details).

The screenshot displays the Multi Channel Analyzer software interface. The top menu bar includes 'Start Analysis', 'Explore', 'Load Experiment', 'Save Experiment', and 'Save Experiment as ...'. The 'Explore' button is highlighted with a red box and an arrow pointing to the 'Spike Detector' block in the 'Data Analysis Setup' workflow. A red text box with an arrow pointing to the 'Estimate' button in the 'Spike Detector' configuration panel contains the following text: "Click 'Estimate' and 'Explore'. The time stamp used for SD calculation will be shown. If satisfied with the thresholds, click 'Start Analysis'".

The 'Spike Detector (1)' configuration panel shows the following settings:

- Threshold: [Dropdown]
- Rising Edge
- Falling Edge
- Automatic Threshold Estimation:
 - Rising Edge: 5.0 Std. Dev.
 - Falling Edge: -5.0 Std. Dev.
- Estimate button (highlighted with a red box)
- Std. Dev. Calc. Begin: 00:00:06.977
- Dead Time: 3.0 ms
- Pre Trigger: 1.0 ms
- Post Trigger: 2.0 ms

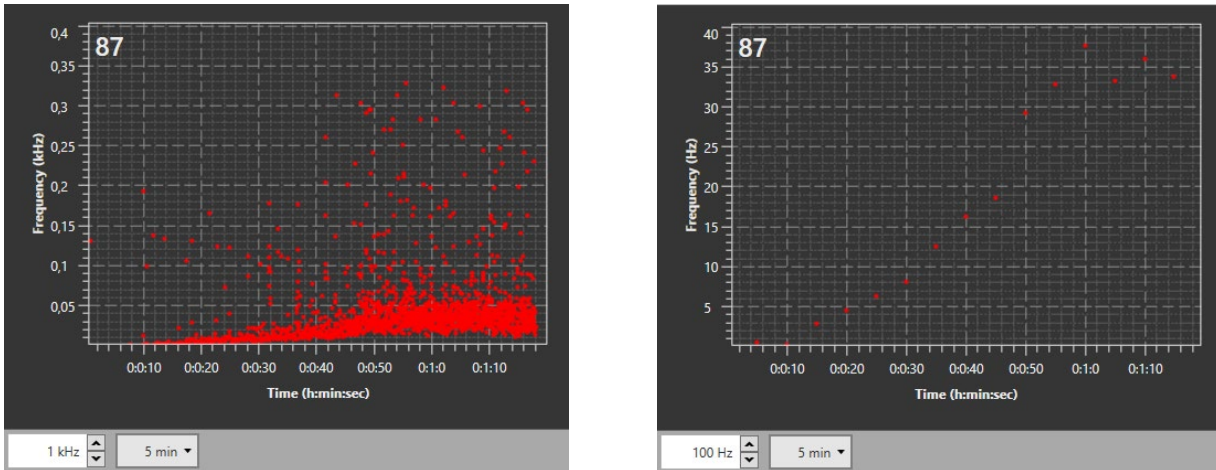
The 'Timestamps' table shows the following data:

Timestamp	Cutouts
21	31 41 51 61 71
22	32 42 52 62 72 82
23	33 43 53 63 73 83
24	34 44 54 64 74 84
Ref	35 45 55 65 75 85
26	36 46 56 66 76 86
27	37 47 57 67 77 87
28	38 48 58 68 78 88

The 'Voltage (µV)' plot shows a signal with a peak at 45 µV and a trough at -15.4 µV. A red text box with an arrow pointing to a flat segment of the signal contains the text: "If possible, select a segment of data with no activity on any channel".

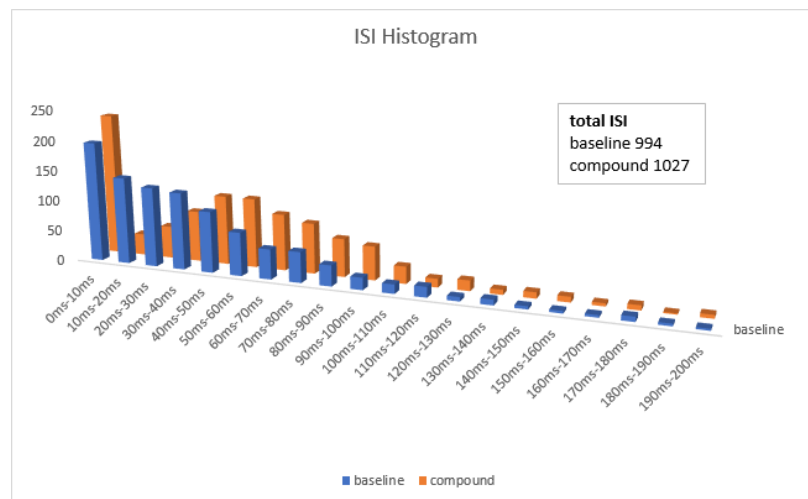
7.2.2 Spike Frequency Analysis

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's 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.

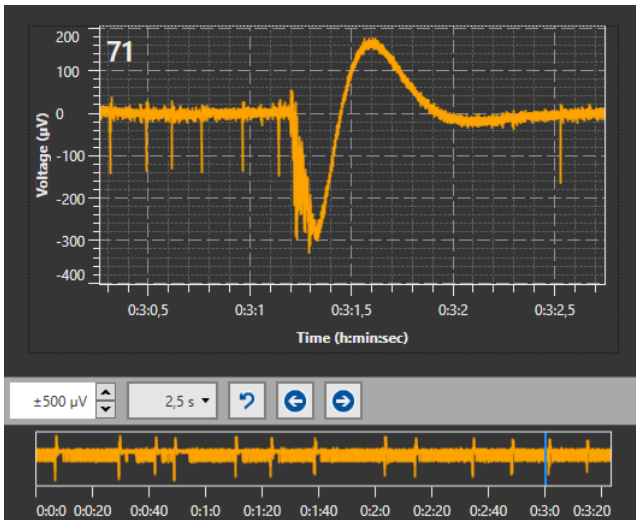


7.2.3 Spike Burst Analysis

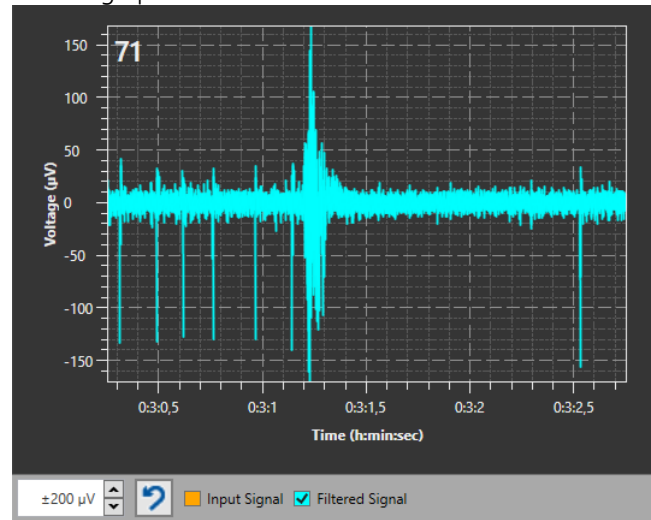
Results of the Burst Analyzer heavily depend on the parameters selected for the MaxInterval algorithm. On electrodes with several **continuously firing neurons**, the algorithm might detect lots of long bursts, but by manual observation, one would not consider such activity as burst like. On the other 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.

Raw data



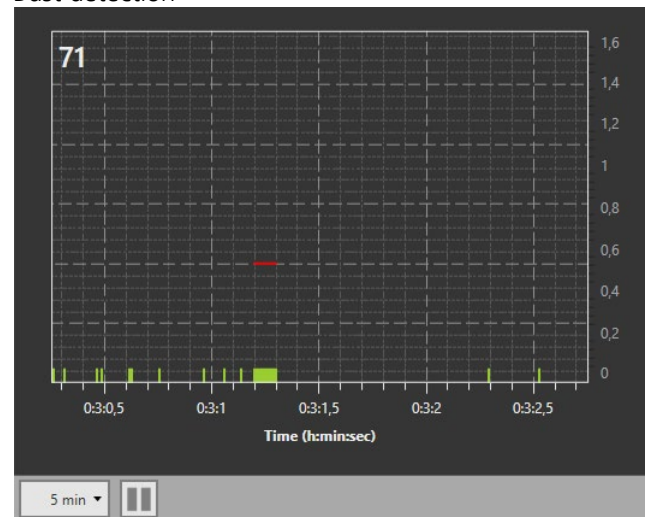
20Hz high pass filtered



Spike detection



Burst detection



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 shown for all channels underneath each other.

t	32	32	32
	Duration	Count	Frequency
[s]	[ms]	[-]	[Hz]
688,040	157,95		15
4,888,730	166,65		15
7,048,010	160,15		14
9,839,835	128,80		13
12,311,145	154,80		13
13,403,760	117,55		16
t	33	33	33
	Duration	Count	Frequency
[s]	[ms]	[-]	[Hz]
687,920	173,40		17
2,935,490	108,25		12
4,889,535	158,60		15
8,306,255	106,60		14
9,840,205	114,05		15
12,311,740	170,65		16
13,404,060	105,05		16
15,429,655	127,95		17

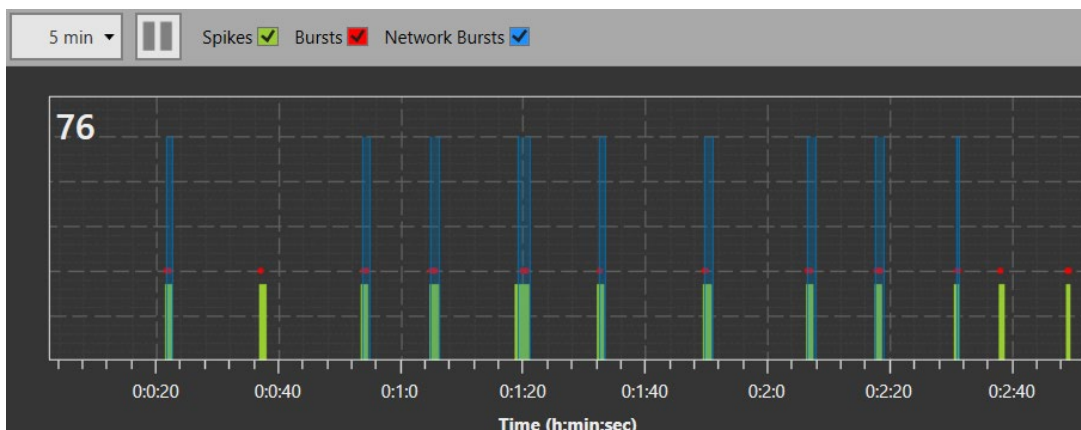
Some more parameters can only be calculated once the file has been **completely analyzed**. These so called **Numerical Results** are at the bottom of the ASCII file, below the continuous results.

Numerical Results:						
Channel	Number Of Spikes	Number Of Bursts	% Spikes in Bursts	Mean Burst Duration	Mean Spikes In Burst	Mean Interburst Interval
				[ms]		[ms]
12	2492	34	35,2	640,32	25,82	5401,15
13	276	10	55,1	178,02	15,20	20677,96
Ref	433	0	0,0	NaN	NaN	NaN
17	157	0	0,0	NaN	NaN	NaN
21	202	13	98,5	140,86	15,31	15500,88
22	175	1	9,7	151,50	17,00	NaN
23	524	13	72,7	242,86	29,31	15404,54
27	128	3	25,8	162,33	11,00	46702,55

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, please see next chapter.

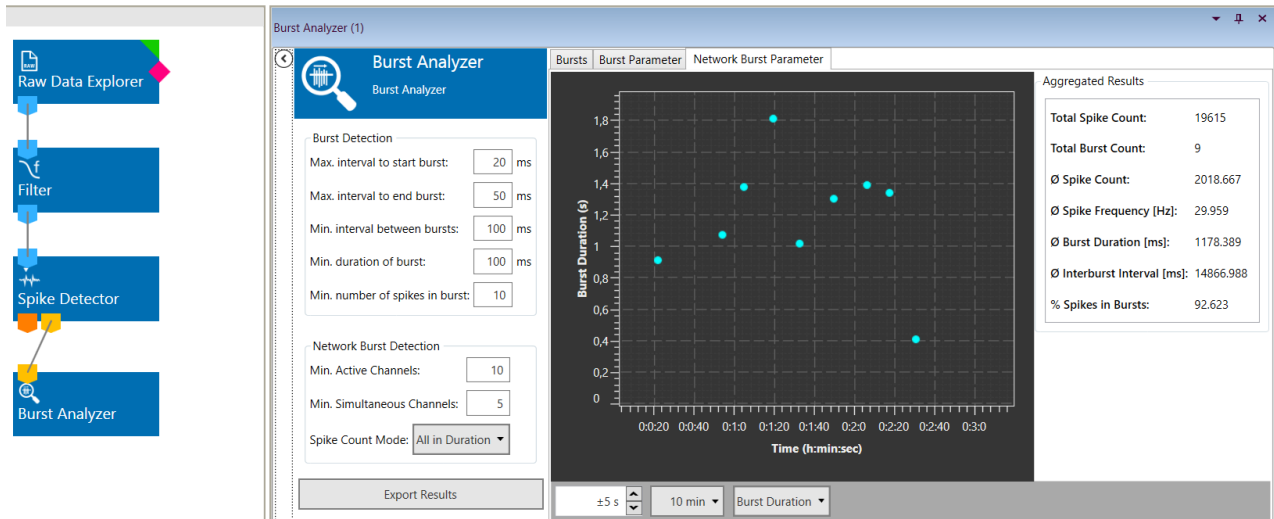
7.2.4 Network Burst Analysis

Bursting is very often **synchronized** throughout the neuronal network. So, after detecting bursts on individual channels, the next step is to look for synchronization between bursting channels. The user can select how many channels need to be active in total, and how many need to show synchronized (overlapping in time) bursts to define a network burst. A detected network burst will be labelled in blue.



Spikes occurring within the time of a network burst, but on electrodes not bursting themselves can be included (Spike Count Mode: **All in Duration**), or excluded (Spike Count Mode: **Only in Bursts**) for the subsequent calculation of network burst parameters.

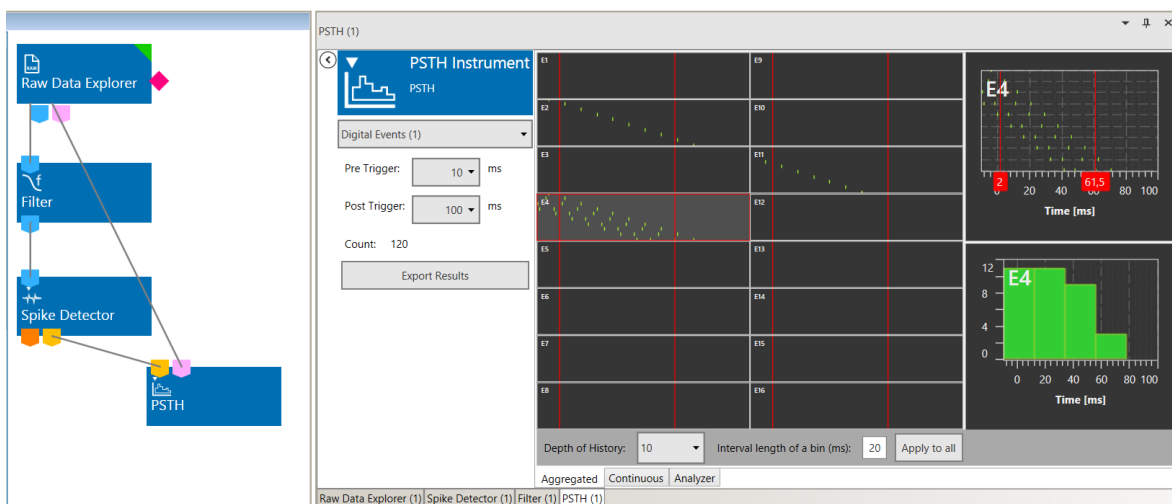
The **Network Burst Parameter** tab shows continuous and aggregated results for all network bursts. For each network burst, the Duration, Spike Count and average Spike Frequency is shown. The aggregated results include the average Spike Count, Frequency, Duration and Interburst Interval. The **Percentage of Spikes within Network** bursts is a very good and simple parameter to describe the synchronization of a network. Be aware the Spike Count Mode setting strongly influences this parameter (see above). All parameters can be exported as ASCII data.



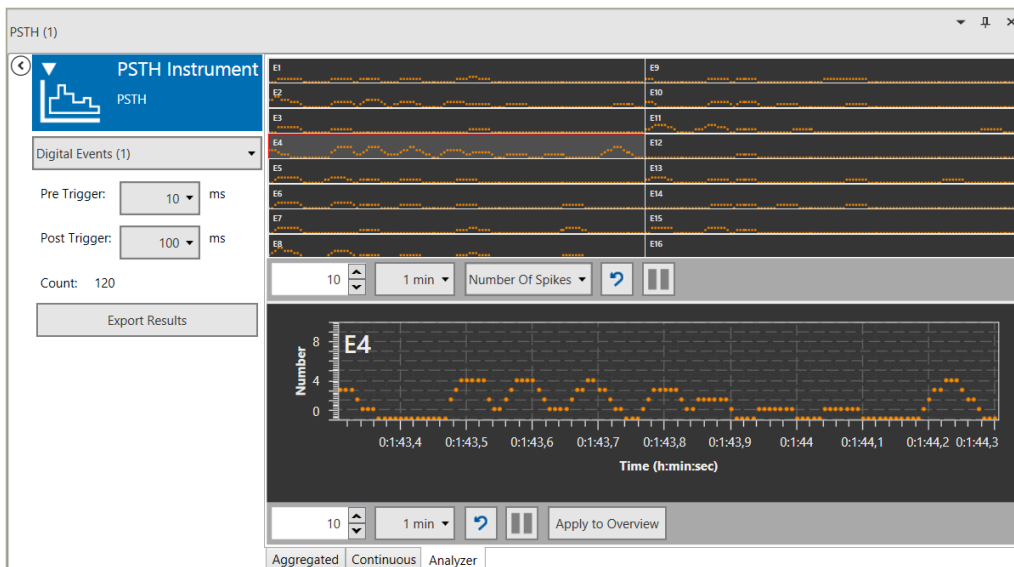
7.2.5 Analysis of Evoked Spikes

Spikes evoked by a light or electrical stimulation can be analysed using the PSTH (Peri Stimulus Time Histogram) tool. The PSTH tool needs Spike Time Stamps and the Triggers for the stimulation timepoints. Spikes can be directly loaded from a file or extracted offline from a raw data file. However, it is mandatory to **record the Triggers (pink port) during recording**. These can be the triggers generated by the internal stimulators, or triggers generated by the Digital Event Detector on an TTL from an external device. Triggers can not be generated offline.

Similar to the Sweeps Analyzer, the PSTH displays a time segment around each trigger. The duration of the segment can be selected (Pre Trigger and Post Trigger). Detected spikes are shown for several consecutive trigger events, and plotted in time bins as histogram. Within that time segment, a **Region Of Interest (ROI)** can be defined in relation to the trigger event.



Within that ROI, the Spike Count, average spike frequency (if there is more than one spike), and the time of the first detected spike in relation to the trigger is calculated, and plotted in the **Analyzer** tab.



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

8.1 MEA2100-System

Available headstage versions of the MEA2100 are HS-60, HS2x60, HS-120 and HS-256. 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 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, cannot be put in a dry incubator.

8.2 MEA2100-Mini-System

The MEA2100-Mini System is the latest generation of MEA-Systems. Currently, headstages with 60 or 120 channels are available. The MEA2100-Mini shares all advantages of the regular MEA2100. Additionally, it can be upgraded with high flexibility from one to a maximum of eight headstages, operated by a single IFB. The lower heat emission and sealed electronics make it possible to operate the headstages in a humid incubator. Up to 60 channels can be routed out as live analog data.

Included accessories: Floor heating, internal stimulator.

Recommended accessories: Temperature controller, Teflon membranes MEA-MEM.

Optional accessories: Perfusion heating PH01, magnetic perfusion holders MPH, peristaltic perfusion system PPS2.

Pro: Very compact, suitable for inverted and upright microscopes, Incubator friendly, selectable filter band, access to analog data, programmable DSP.

Contra: Electrodes not accessible for external stimulation devices, only two stimulation channels.

8.3 Suggested Microelectrode Arrays

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 PEDOT coated gold electrodes are particularly well suited for stimulation. 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.

MEA2100-System and MEA2100-Mini-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-MEA256-System and MEA2100-256-System

For the USB-MEA256 and MEA2100-256, 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 MEA-MEM. A plastic ring should only be used if parallel patch clamp or imaging experiments are planned, which require physical access from the top.

9 References

An up to date list of publications sorted by preparation or application can be found on the MCS website <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](#) 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