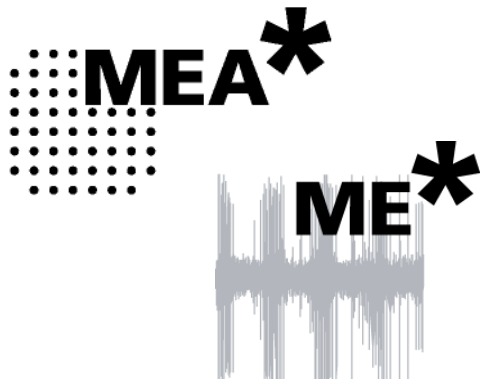


MC_Rack Tutorial: MEA Application Examples



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Multi Channel Systems

MCS GmbH

Aspenhaustraße 21

72770 Reutlingen

Germany

Fon +49-71 21-90 92 5 - 0

Fax +49-71 21-90 92 5 -11

info@multichannelsystems.com

www.multichannelsystems.com

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1 About the MEA Application Examples

MC_Rack is a very flexible recording and analysis program that can be customized for various applications. Different software configurations can be saved for later use in so-called **virtual rack** files, in analogy to a hardware rack configuration.

In this chapter, you will find a detailed description of **demo rack configurations** for various **typical applications**. You will find the demo racks together with demo data files in the **Tutorial** folder (**Offline** subfolder) on the installation volume. These racks are only suitable for replaying demo data files, not for new experiments, but if you follow the step-by-step instructions in the tutorial you will get an idea how to set up a rack for your particular application.

The different applications described in this tutorial are intended to be read separately, that is, important facts about MC_Rack are repeated in each section. On the other hand, not all MC_Rack features are explained in detail in each application section. In the chapters "Aim of Experiment", a short introduction into the application is given as well as a list of features that will be discussed in the section. For example, if you are interested in extracting spikes from brain slices, it might be interesting for you to study the "Retina: MicroERGs and Spikes" chapter to learn more about the spike detection methods in MC_Rack, even if you are not interested in the retina application itself. See list below.

Example racks that can be directly used for **experiments** are provided in the **Online** subfolder. You can load and modify these racks for your application instead of setting up a virtual rack from scratch. Some of the racks are very basic and provide a good introduction into basic MC_Rack features. Two of the basic racks are discussed in the Step by Step Tutorial. Others are more advanced and demonstrate the possibilities that MC_Rack offers. (If you have no data acquisition system (MC_Card or USB-MEA- or USB-ME-System) available, you can use the **Simulation** mode of the data source for working with the online sample racks, or load the offline sample racks into the **Replayer**).

You will find a list and a short description of all sample racks in the MC_Rack Sample Racks topic. This document may not always be up to date. For the latest documentation, please see the MC_Rack_SampleRacks_ReadMe.htm file in the **Tutorial** folder on the installation volume.

The following list provides an overview which application section focuses on which MC_Rack features.

<i>Application section</i>	<i>Discussed features</i>
Getting Started	Starting MC_Rack, recording data, replaying data files in general.
Long Term Potentiation (LTP)	Field potential (waveform) analysis, amplitude extraction, graphics and ASCII export .
Input/Output (I/O) Curve	Field potential (waveform) analysis, slope extraction.
Paired Pulse Facilitation (PPF) or Depression (PPD)	Field potential (waveform) analysis, amplitude extraction, triggering MC_Rack.
Spike Activity (Organotypic culture)	Digital filtering , spike detection (threshold mode) , spike rate extraction, two-dimensional false-color plots.
Retina: MicroERGs and Spikes	Digital filtering , spike detection (threshold and slope mode) .
Cardiac Signals	Excitation map , extraction of QT interval , averaging data.
Saving the Rack and Saving Data	Saving rack configuration files, recording data.

2 MC_Rack Sample Racks

The offline racks and the demo data are provided in the Tutorial folder on the installation volume. The demo data need to be copied to the correct file path. Please copy the **complete** MC_Rack **Tutorial** folder into the MC_Rack directory with the following path.

c:\Program Files\Multi Channel Systems\MC_Rack\

Otherwise the file paths of the data files are not valid and the program cannot find the files, resulting in an error message. This is no problem, however, but you will have to select the data files manually.

The intention of the **offline racks** is to show possible applications, and how to set up a rack. It is not possible to use offline racks for recording.

The **online racks** can be used as templates for your experiments instead of setting up new racks from scratch. You only have to adjust the parameters according to your needs.

3 Sample Racks - Overview

Neurophysiology	Retina	Cardiophysiology
<i>MEA / in vitro - Offline/Demo Racks</i>		
Hippocampus_IOCurve_Demo.rck		
Neuro_LTP_Demo.rck		
Neuro_PPF_Demo.rck		
Neuro_OTC_Spikes_Demo.rck		
Neuro_Cerebellum_Demo.rck		
Neurons_DrugApplications_Demo.rck		
	Retina_MicroERG_Demo.rck	
	Retina_Spikes_Demo.rck	
		Cardiomyocytes_ExcitationMap_Demo.rck
		Cardiomyocytes_QT-Analysis_Demo.rck
		hESC-CM_NoStim_Demo.rck
		hESC-CM_Stim_Demo.rck
		Cardiomyocytes_HL1_Demo.rck
		Murine-ESC-CM_EB_Iso.rck
		hESC-CM_EB_Iso.rck
		Cardio_WholeHeart_Chicken.rck
<i>ME / in vivo - Offline/Demo Racks</i>		
InVivo_Rat_Chronic.rck		
InVivo_RatBarrelCortex_FlexMEA.rck		

<i>Online/Template Racks</i>		
Display_Continuous.rck	Display_Continuous.rck	Display_Continuous.rck
Display_Triggered.rck	Display_Triggered.rck	Display_Triggered.rck
MEA1060-BC.rck		
MEA-120-System.rck	MEA-120-System.rck	MEA-120-System.rck
SpikeDetection.rck	SpikeDetection.rck	
SpikeDetectionModes.rck	SpikeDetectionModes.rck	
Neuro_Spikes_LED_Display.rck		
Neuro_LFP_SlopeAnalysis.rck		
Neuro_Spikes+FieldPotentials.rck	Neuro_Spikes+FieldPotentials.rck	
Bandpassfilter.rck	Bandpassfilter.rck	Bandpassfilter.rck
Neuro_LTP_analysis.rck		
Neuro_PPF.rck		
SpikeRate_5minBins.rck		
		Cardio_Averaging+ExcitationMap.rck
		Cardio_SignalRate+RRInterval.rck

4 MEA / in vitro - Offline/Demo Racks

The provided sample racks show two typical MEA applications and demonstrate the power and features of the data acquisition and analysis program MC_Rack. These racks are replaying recorded data files, that is, you can use these racks without a connected MEA-System.

For more information about the racks and how to set them up, please refer to the Step by Step Tutorial in the MC_Rack Help.

4.1 Neurobiology

4.1.1 Acute Hippocampus Slice: fEPSP Analysis, Recording of an I/O Curve

Consider you have recorded from an acute hippocampal slice on a Microelectrode Array (MEA) with 64 channels and you like to analyze the input-output relationship of an electrical stimulation.

The biological sample was 300 μm thick (see picture) and stemmed from a 22 days old rat. The tissue has been stimulated with one of the substrate integrated electrodes (#65).

A monopolar electrical stimulation with biphasic voltage pulses (duration of 100 μs per phase) with amplitudes from 500 to 3000 mV, in 250 mV steps, was applied. Individual voltage steps were repeated 5 times with a delay of 5 s, that is, 5 x 500 mV, then 5 x 750 mV, then 5 x 1000 mV, and so on. The Sync Out signal of the STG (stimulus generator) was used to trigger the recording sweeps, with a pre-trigger of 10 ms and a time window of 70 ms.

This example shows a recording with the new MEA amplifier with blanking circuit, featuring a superior stimulus artifact suppression. The Sync Out signal of the STG also triggered the MEA amplifier. The electrodes were transiently (for 500 μs : Sync Out pulse 300 μs long, Wait settings 200 μs) separated from the amplifier stage during stimulation. Thus, stimulus artifacts could be efficiently prevented on all recording channels.

(Data kindly provided by Dr. Frank Hofmann, University of Heidelberg, Germany)

- Rack file: Hippocampus_IOCurve_Demo.rck
- Data file: I-Ocurve_Demo.mcd
- Image file: Hippocampus_IOCurve.jpg

4.1.2 LTP-Demo rack: Theta burst induced Long Term Potentiation (LTP)

This example file was recorded from an acute hippocampal slice on a Microelectrode Array (MEA) with 64 channels with the aim to analyze long-term potentiation (LTP) following electrical stimulation.

The 300 μm thick acute hippocampus slice was prepared from a 21 days old rat. The slice was stimulated every 60 s with a single biphasic pulse (1.5 V amplitude, 100 μs per phase) by one of the embedded MEA-electrodes (No. 75).

LTP was induced by an LTP train with 100 pulses of same amplitude and duration as the test stimulus, and a frequency of 100 Hz. The Sync Out signal of the STG (stimulus generator) has been used to trigger the recording, with a pre-trigger of 30 ms and a time window of 100 ms. Data was recorded before and after the LTP induction; the LTP induction itself was not recorded.

As the data was acquired with a MEA1060-BC amplifier with blanking circuit, all MEA electrodes were disconnected from the amplifier shortly during stimulation, in this case for 540 μs (300 μs Sync Out pulse of the STG, plus intrinsic Wait of 40 μs , plus user defined Wait of 200 μs). This efficiently prevented stimulus artifacts.

(Data kindly provided by Dr. Frank Hofmann, University of Heidelberg, Germany)

- Rack file: Neuro_LTP_Demo.rck
- Data file: LTP-Demo.mcd
- Image file: LTP_slice.jpg

4.1.3 Paired pulse facilitation (PPF) and Depression (PPD)

In Paired Pulse Facilitation (PPF) or Depression (PPD) experiments, the stimulus is followed by a second stimulus, typically 20-60 ms later. The amplitude of the sum potential of the neuronal response to the second stimulus is compared to that of the first one. It is higher in case of a paired pulse facilitation, or lower in case of a paired pulse depression. Some electrodes recorded PPF, and others PPD in this demo data file. For example, you can see a PPF on electrode 53, and a PPD on electrode 66.

In MC_Rack, two (or more) regions of interest (ROI) can be defined, one triggered by the first stimulus, the other by the second stimulus, for example. The peak-to-peak amplitudes for both ROIs can be extracted automatically and online.

The example data file was recorded from a 300 μm thick acute hippocampus slice that was prepared from a 32 days old rat. The slice was stimulated with a single biphasic pulse (2 V amplitude, 100 μs per phase) by one of the embedded MEA-electrodes (No. 64). The second stimulus pulse was applied 20 ms following the first one. Recording was triggered by applying the Sync Out signal of the stimulus generator to one of the analog input channels (A1). Recorded sweeps are 200 ms, with a pre-trigger time of 30 ms, and a post-trigger time of 170 ms.

As the data was acquired with a MEA1060-BC amplifier with blanking circuit, all MEA electrodes were disconnected from the amplifier shortly during stimulation, in this case for 540 μs (300 μs Sync Out pulse of the STG, plus intrinsic Wait of 40 μs , plus user defined Wait of 200 μs). This efficiently prevented stimulus artifacts.

(Data kindly provided by Dr. Frank Hofmann, University of Heidelberg, Germany)

- Rack file: Neuro_PPF_Demo.rck
- Data file: PPF_Data.mcd
- Image file: PPF_Image.jpg

4.1.4 OTC Spikes Demo rack: Organotypic co-culture with spontaneous spike activity

Consider an organotypic co-culture of the dentate gyrus (DG) and the entorhinal cortex (EC) taken from a PND 6 rat, cultured for seven days. Both subregions show an independent spontaneous spike activity. Total recording time has been 60 seconds.

(Data kindly provided by Dr. Frank Hofmann, University of Heidelberg, Germany)

- Rack file: Neuro_OTC_Spikes_Demo.rck
- Data file: Neuro_OTC_Spikes_Demo.mcd
- Image file: OTC Spikes Image.jpg

4.1.5 Acute slices from cerebellum

You can also study acute slices from the cerebellum with the MEA-System, for example for drug screening. The demo data shows typical signals from the cerebellum. Four selected electrodes of interest are marked with a white circle in the slice picture. Any standard MEA, for example with a medium spatial resolution (200/30), can be used for recordings from acute slices.

The demo rack shows the raw data from all 60 electrodes, and extracts the spike rate from four selected electrodes of interest.

(Data kindly provided by Dr. Ulrich Egert, University of Freiburg, Germany)

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- Rack file: Neuro_Cerebellum_Demo.rck
- Data file: Cerebellum_Demo.mcd
- Image file: Cerebellum.jpg

4.1.6 Neuronal cell culture, drug testing

Data was recorded from a neuronal cell culture with rat striatum cells. The example shows the application of NMDA. Two demo data files are provided: neurons_NMDA_control.mcd before drug application, neurons_NMDA_5 μ M after drug application. The Analyzer extracts the spike number in 100 ms bins: Under control conditions, over 60 % of these bins are empty, that is, without spike activity. This demonstrates the organization of the spontaneous spiking activity in bursts. After NMDA application, the spiking activity is more random, demonstrated by the fact that only 8 % of the bins are empty. The overall spiking activity measured in 2 s bins and displayed in spike numbers is increased, too, but the main difference is how the activity is organized.

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

- Rack file: Neurons_DrugApplication_Demo.rck
- Data files: neurons_NMDA_control.mcd (before compound application), neurons_NMDA_5 μ M.mcd (after compound application)

4.2 Retina

4.2.1 Field potential electroretinogram

The electroretinogram (ERG) is an extracellular field potential generated by the cells in the retina. So-called microERGs can be recorded with the MEA-System from retina explants, for example, from rat or chicken.

(Data kindly provided by NMI Reutlingen, Germany)

- Rack file: Retina_MicroERG_Demo.rck
- Data file: Retina_ERG.mcd

4.2.2 Spiking activity

Spiking activity is visible in the ERG traces as well and can be easily extracted from the underlying field potential with the Spike Sorter in Slope mode.

Alternatively, the field potentials can be removed by a 300 Hz High Pass filter, and spikes can be detected by setting a threshold. This sample rack demonstrates both methods, and the spike sorting feature.

The raster plot highlights the regular spike pattern.

(Data kindly provided by NMI Reutlingen, Germany)

- Rack file: Retina_Spikes_Demo.rck
- Data file: Retina.mcd

4.3 Cardiophysiology

4.3.1 Excitation map (chicken embryonic cardiomyocytes)

The two-dimensional MEA layout is ideally suited for showing the waveform propagation and measuring the conduction velocity in a cardiomyocyte culture. The demo data shown here was recorded from chicken embryonic cardiomyocytes at a sampling rate of 10 kHz with a gain of 1200, and a bandwidth of 1 Hz to 3 kHz.

- Rack file: Cardiomyocytes_ExcitationMap_Demo.rck
- Data file: Cardiomyocytes_60Ch.mcd

4.3.2 Excitation map (HL1 cell line)

The demo data used here was recorded from a cardiomyocyte cell line from mouse that retains a differentiated cardiac myocyte phenotype: HL-1 from Dr. W. Claycomb. The demo data file Cardiomyocytes_HL1.mcd was recorded from HL-1 cells at a sampling rate of 10 kHz with a gain of 1000, and a bandwidth of 1 Hz to 3 kHz.

- Rack file: Cardiomyocytes_ExcitationMap_HL1.rck
- Data file: Cardiomyocytes_HL1.mcd

4.3.3 QT prolongation

The QT interval is an important parameter for drug testing. It can be extracted from the cardiac field potential recorded with the MEA-System. The demo data shown here was recorded from chicken embryonic cardiomyocytes at a sampling rate of 10 kHz with a gain of 1200, and a bandwidth of 1 Hz to 3 kHz. The data was filtered with a digital 2 Hz high pass filter in MC_Rack.

- Rack file: Cardiomyocytes_QT-Analysis_Demo.rck
- Data file: Cardiomyocytes_QT.mcd

4.3.4 Recording from human embryonic stem cell derived cardiomyocytes (hESC-CM)

Cardiomyocytes from human embryonic stem cells develop a spontaneous beating pattern and can be used for basic research, for example, in the field of implantation medicine, or for drug screening, for example, for arrhythmogenic effects. hESC-CM present the most advanced model in cardiac *in vitro* physiology. Recorded extracellular field potentials resemble remarkably electrocardiogram signals. MEA recordings can be combined with other methods, for example, calcium imaging, optical recordings, patch clamp. This example data file shows the spontaneous activity of hESC-CM. (cell density: 10-15,000 cells per square mm, bandwidth: 1 Hz to 3.5 kHz, amplifier gain: 1200, voltage input range: -2000 mV to +2000 mV, sampling frequency 10 KHz, electrode diameter: 30 micrometer, electrode spacing: 200 micrometer)

(Data kindly provided by Ofer Binah, Rappaport Institute, Haifa, Israel)

- Rack file: hESC-CM_NoStim_Demo.rck
- Data file: hESC-CM_NoStim.mcd

4.3.5 Pacing hESC-CM with electrical stimulation

hESC-CM are usually paced at the lowest rate and voltage as possible, for example, a culture beating with 1 Hz would be paced at 950 ms rate with a typical biphasic stimulus pulse of +/-2 mV and 2 ms for each phase. For this experiment, MEA 200/30 with additional 4 pairs of large stimulating electrodes were used. Following the stimulus artifact, you can see the fast activation spike and the slow repolarization waveform. (cell density: 10-15,000 cells per square mm, bandwidth: 1 Hz to 3.5 kHz, amplifier gain: 1200, voltage input range: -2000 mV to +2000 mV, sampling frequency 10 KHz, electrode diameter: 30 micrometer, electrode spacing: 200 micrometer) (Data kindly provided by Ofer Binah, Rappaport Institute, Haifa, Israel)

- Rack file: hESC-CM_Stim_Demo.rck
- Data file: hESC-CM_Stim.mcd

4.3.6 Recording from HL-1 cell line

The demo data used here was recorded from a cardiomyocyte cell line from mouse that retains a differentiated cardiac myocyte phenotype: HL-1 from Dr. W. Claycomb. The demo data file Cardiomyocytes_HL1.mcd was recorded from HL-1 cells at a sampling rate of 10 kHz with a gain of 1000, and a bandwidth of 1 Hz to 3 kHz.

The signal rate and RR interval are graphed.

- Rack file: Cardiomyocytes_HL1_Demo.rck
- Data file: Cardiomyocytes_HL1.mcd

4.3.7 Effect of Isoproterenol and E-4031 on mouse embryoid bodies

This demo data file shows a complete drug testing experiment on a cardiac body derived from human embryonic stem cells. The cardiac body was developed on an MEA. Under control conditions, the signal rate was stable at about 0.75 Hz.

During wash in of 10 μ M Isoproterenol (applied at 64 s after recording start), the signal rate increased to 1.2 Hz. The effect was completely reversible by wash out (not recorded). As a result of the reduced RR interval, the QT interval was also decreased by 14 %.

E-4031 had then been added (at 188 s) in a concentration of 100 nM (wash in not recorded). The QT interval was increased by 70 %.

The demo data was recorded with an MEA1060 amplifier with a gain of 1200, and a bandwidth of 1 Hz to 3 kHz and an MEA-60-System, with a sampling rate of 10 kHz. Data was recorded in separate files and then merged to a single data file with MC_DataTool. The signal rate and the interspike interval (ISI) are graphed based on the automated signal detection in MC_Rack.

- Rack file: Murine-ESC-CM_EB_Iso.rck
- Data file: Murine-ESC-CM_EB_Iso.mcd

4.3.8 Effect of Isoproterenol on human embryoid bodies

The effect of Isoproterenol was tested on a cardiac body derived from human embryonic stem cells. The compound almost doubled the signal rate. The cardiac body was developed on an MEA. See also the MEA Application Note "Human Embryonic Stem Cell Derived Cardiac Myocytes (hESC-CM)". Under control conditions, the signal rate was stable at about 0.75 Hz.

During wash in of 1 μ M Isoproterenol (applied at 64 s after recording start), the signal rate increased to 1.2 Hz. The effect was completely reversible by wash out (not recorded). As a result of the reduced RR interval, the QT interval was also decreased by 14 %.

E-4031 had then been added (at 188 s) in a concentration of 100 nM (wash in not recorded). The QT interval was increased by 70 %.

The demo data was recorded with an MEA1060 amplifier with a gain of 1200, and a bandwidth of 1 Hz to 3 kHz and an MEA-60-System, with a sampling rate of 10 kHz. Data was recorded in separate files and then merged to a single data file with MC_DataTool. The signal rate and the interspike interval (ISI) are graphed based on the automated signal detection in MC_Rack.

(Data kindly provided by Dr. William Sun, ES Cell International, Singapore)

- Rack file: hESC-CM_EB_Iso.rck
- Data file: hESC-CM_EB_Iso.mcd

4.3.9 Recording from chicken embryonic whole-heart

The demo data shown here was recorded from an E13 chicken embryonic whole-heart preparation at a sampling rate of 10 kHz with a gain of 1200, and a bandwidth of 1 Hz to 3 kHz. The signal rate and RR interval are graphed.

- Rack file: Cardio_WholeHeart_Chicken.rck
- Data file: Cardio_WholeHeart_Chicken.mcd

4.4 ME / in vivo - Offline/Demo Racks

4.4.1 Chronic motor cortex recordings from rat

Spike recordings were obtained from vibrissae motor cortex in a chronically implanted awake behaving rat. A custom-made platinum/tungsten (95/5%) electrode in glass with an impedance of 1 MOhm, an MPA8I, an FA64I (bandwidth: 300 Hz - 5 kHz, for spike recordings), and an ME64-System were used. One of the electrodes in the array was used as a reference electrode for the differential MPA8I amplifier. Total gain was 2000; the sampling rate was 20 kHz. (Data kindly provided by Dr. Cornelius Schwarz, University Tübingen, Germany)

- Rack file: InVivo_Rat_Chronic.rck
- Data file: InVivo_Rat_Chronic.mcd

4.4.2 MicroEEG recordings with FlexMEA from rat barrel cortex

Field potential recordings were obtained from the surface of the cortex, just above S1bf (Primary Somatosensory Cortex, barrel field), which is the primary somatosensory representation of the vibrissae of the rat. The acute experiment was done in an anaesthetized rat. The FlexMEA was kept in the same position during the experiment, but the mechanically stimulated vibrissa was varied. The resulting field potentials were analyzed after filtering the data with a digital low pass filter. The analysis of typical responses allows to locate the FlexMEA position relative to the positions of the barrels. This demo file shows the response to stimulation of one of the vibrissae.

The FlexMEA was connected directly to an MPA32I with an FM-ADPT-32 adapter. An FA32I (bandwidth: 1 Hz - 5 kHz) was used as a filter amplifier. Recordings were made in MC_Rack with an ME-32 System. Reference electrodes in the array were used for the differential MPA32I amplifier. Total gain was 5000; the sampling rate was 20 kHz. The time stamps of the stimulus were recorded on the analog (not amplified) input channel A2. The Averager in MC_Rack was used to increase the S/N ratio. (Data kindly provided by Dr. Jean-Francois Léger, Ecole Normale Supérieure, Paris, France)

- Rack file: InVivo_RatBarrelCortex_FlexMEA.rck
- Data file: InVivo_RatBarrelCortex_FlexMEA.mcd

4.5 Online

4.5.1 Continuous monitoring and recording: Display_Continuous.rck

This is a very basic sample rack that contains only a **Data Display**. You can use it for a first test of your MEA amplifier and noise level with the provided model test probe, for example. You will need a fully set up MEA-System for using this rack.

4.5.2 Triggered recording: Display_Triggered.rck

This is a very basic sample rack for recording triggered sweeps, for example after stimulation. The selected trigger input is bit 0 of the digital MC_Card input.

Parameters:

- Recorder: Start time (pretrigger) *-100 ms*, window extent (sweep time) *300 ms*
- Trigger Detector: Dead time *3 ms*, digital input *bit 0*

4.5.3 Basic rack for MEA1060-BC amplifier: MEA1060-BC.rck

Basic rack for recording triggered sweeps with gain settings (1100) for a standard MEA1060-BC amplifier.

4.5.4 Basic rack for double MEA layout: MEA-120-System.rck

A basic rack with two raw data displays, one for each MEA.

4.5.5 Basic spike detection: SpikeDetection.rck

Demonstrates basic spike detection in Threshold mode. One display shows the raw data and the spike detection; the spike detection level can be adjusted for each channel separately.

Both analyzers available in MC_Rack are used in this rack for extracting the spike rate: The standard Analyzer operates based on discrete time intervals (bins or region of interest). Extracted parameters are graphed in the Parameter Display. The event-based Spike Analyzer was specifically designed for extracting statistical parameters such as the interspike interval (ISI) or the rate from Spike data streams generated by the Spike Sorter. Extracted parameters are graphed in the Spike Analyzer Display.

Parameters:

- Recorder: Spike cutouts and spike rate selected for recording
- Spike Sorter: Spike detection level for each channel

4.5.6 Spike detection - threshold and slope mode: SpikeDetectionModes.rck

This is a more advanced rack demonstrating both methods of spike detection in comparison:
(a) Raw data is filtered by a high pass filter; spikes are detected by defining a threshold.
(b) Spikes are detected by waveform (Slope mode) in unfiltered data.

Parameters:

- Spike Sorter (Threshold mode): Spike detection level for each channel
- Spike Sorter (Slope mode): Slope and amplitude for all channels

4.5.7 Visualizing activity with software LEDs: `Neuro_Spikes_LED_Display.rck`

Similar rack as SpikeDetection, but with an LED display. Analyzer extracts spike number in 10 ms bins. Spiking activity is indicated by lighting up of the corresponding electrode in the False Color "LED" display. Another display shows the spike cutouts.

Parameters:

- Spike Sorter (Threshold mode): Spike detection level for each channel

4.5.8 Slope analysis of local field potentials: `Neuro_LFP_SlopeAnalysis.rck`

This rack is based on the Triggered_Display rack. The slope of the sum potential is extracted from the region of interest (ROI), and then plotted vs. time. For example, for LTP experiments.

Parameters:

- Recorder: Start time (pretrigger) *-100 ms*, window extent (sweep time) *300 ms*
- Trigger Detector: Dead time *3 ms*, digital input *bit 0*
- Analyzer: ROI (after stimulus) *2-5 ms*

4.5.9 Recording spikes and field potentials in parallel: `Neuro_Spikes+FieldPotentials.rck`

Rack for recording spikes and field potentials in parallel. A 300 Hz high pass filter removes separates field potentials from spikes, and vice versa. Spikes and spike rates are extracted from the high-pass filtered data; field potentials and peak-to-peak amplitudes are extracted from the low pass filtered data. An additional display shows the unfiltered raw data.

Parameters:

- Spike Sorter (Threshold mode): Spike detection level for each channel

4.5.10 Digital filtering: `Bandpassfilter.rck`

A combination of two digital filters acts as a bandpass filter: The first filter is a 300 Hz high pass filter, the following is a 3 kHz low pass filter, resulting in a total bandwidth of 300 Hz to 3 kHz. The display monitors the filtered and the unprocessed raw data.

Parameters:

- Filters: Adjustment of the cutoff frequencies

4.5.11 LTP experiments: `Neuro_LTP_analysis.rck`

For recording and analyzing LTP experiments. The Sync Out signal of the STG (stimulus generator) will be used for triggering the recording, with a pre-trigger of 30 ms and a time window of 200 ms. Connect the Sync Out to the digital input bit 0 of the data acquisition computer.

The Raw Data Display monitors the raw data. One Analyzer extracts the minimum and maximum of the response in region of interest I (shortly after the stimulus pulse), the other Analyzer extracts the slope of ROI II (the linear part of the falling edge of the field potential). As an example, the parameters are extracted only from channels 41 and 42. Please select the channels where you expect the largest response. Minimum and maximum are plotted versus time in a common display; the slope is plotted vs. time in a separate display.

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Parameters:

- Recorder: Start time (pretrigger) *-30 ms*, window extent (sweep time) *200 ms*
- Recorder: Channels of interest, for recording to hard disk
- Analyzers and Parameter Displays: Channels of interest (*41, 42*) for extracting parameters, regions of interest

4.5.12 Paired pulse facilitation (PPF): **Neuro_PPF.rck**

In PPF experiments, the stimulus is followed by a second stimulus, typically 50-60 ms later. The amplitude of the sum potential of the neuronal response to the second stimulus is higher than to the first one. The Sync Out signal of the STG (stimulus generator) will be used for triggering the recording, with a pre-trigger of 30 ms and a time window of 200 ms. Connect the Sync Out to the digital input bit 0 of the data acquisition computer.

In MC_Rack, two regions of interest (ROI) can be defined, one triggered by the first stimulus, the other by the second stimulus. The peak-to-peak amplitudes for both ROIs are extracted automatically and online. For triggering the two Analyzers, you could either use one of the additional Sync Out channels of the STG 2000 series, or you can use the standard recording trigger as well (as in this example). In this case, the dead time of the Trigger Detector should be set to a higher value (for example, 1000 ms) to make sure that only the first trigger input (correlating to the first stimulus pulse) is used for triggering, and the second is skipped.

Parameters:

- Recorder: Start time (pretrigger) *-100 ms*, window extent (sweep time) *200 ms*
- Recorder: Channels of interest, for recording to hard disk
- Analyzers and Parameter Displays: Channels of interest for extracting parameters, regions of interest

4.5.13 Longterm experiments: **SpikeRate_5minBins.rck**

The MEA-System and the flexible data stream management of MC_Rack is ideally suited for longterm experiments, for example 24 h monitoring of the spike rate, for days or even weeks.

As demonstrated with this sample rack, the spike rate can be extracted in, for example, bins of 5 minutes, that is, the spike rate is continuously extracted in 5 min intervals.

In this example, the spike cutouts, and the extracted spike rate is stored onto hard disk. To limit the file size, a new data file is generated each 4 hours.

Parameters:

- Recorder: Channels of interest, for recording to hard disk
- Analyzer: Width of the time bin: *5 min*, parameter of interest: *Rate*

4.5.14 Cardio recording: **Cardio_Averaging+ExcitationMap.rck**

The MEA-System can be used for in vitro studies of the electrical properties of cardiac myocytes and, for example, effects of drug candidates on field potential waveforms and kinetics. The two-dimensional MEA layout is ideally suited for showing the waveform propagation and measuring the conduction velocity in a cardiomyocyte culture. The field potential duration corresponds to the action potential duration, that is, the QT interval in an electrocardiogram. It is measured from minimum of the Na⁺ peak to the maximum / minimum of the IKr current peak.

If you study spontaneously active cultures, you can trigger the analysis based on the easily detectable sodium peak of any one channel, in this example, channel 51. Please select a channel that shows a very clear and regular waveform.

Based on this trigger, the Averager sums up the waveforms. The signal-to-noise ratio and signal quality improves significantly with each window that is summed up. For example, the IKr current peak (T wave) should be very clearly visible after 50 windows.

The Analyzer extracts the maximum corresponding to the IKr current peak (T wave) following the sodium peak.

Parameters:

- Recorder: Channels of interest, for recording to hard disk
- Analyzer and Parameter Display: Channels of interest for extracting parameters, region of interest
- Averager: Number of summed up windows (50)

4.5.15 Cardio recording: Cardio_SignalRate+RRInterval.rck

This template rack shows how cardiac signals can be automatically detected with the Spike Sorter. The signal rate and the RR interval as parameters that characterize the beating rhythm are monitored online during the experiment, and are saved together with the raw data. The export features of the event-based parameter displays allow a direct export of the extracted parameters in universal ASCII format, or as a graphics file.

In this configuration, the Spike Sorter operates in Threshold mode, that is, a signal is detected when the threshold is reached. The signal detection level can be adjusted for each recording electrode, according to the signal to noise ratio. The StdDev feature allows to automatically determine an appropriate threshold value for each channel in parallel, based on the standard deviation.

The Spike Analyzer extracts the signal rate and the interspike interval. Both parameters are plotted over time in the Spike Analyzer Display.

Parameters:

- Recorder: Channels of interest, for recording to hard disk
- Spike Analyzer: Channels of interest for extracting parameters, appropriate detection level for signal detection
- Spike Parameter Display: Channels of interest for extracting parameters, x and y axes ranges

5 Getting Started

5.1 First Steps in MC_Rack

In this chapter, you will find a description of basic tasks and tools that are needed for **all** demo racks presented in the tutorial. Go through this chapter before you start building an application specific rack.

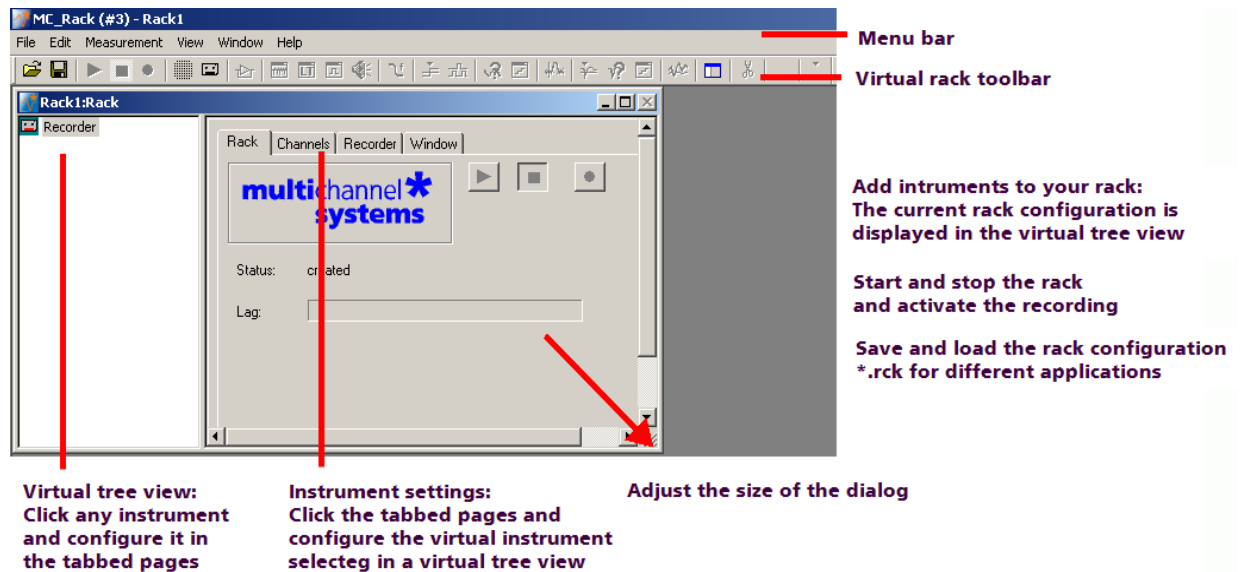
You will learn in this chapter

- How to start MC_Rack.
- How to create a data file (**Recorder**).
- How to replay previously recorded data (**Replayer**).
- How to save a rack for future use.

5.2 Starting MC_Rack

- Double-click the **MC_Rack** icon or select **MC_Rack** from the **Start** menu. The program starts. One window opens automatically. This is your **virtual rack** configuration, which is blank after program start. Therefore, most commands and buttons are unavailable. You can choose from various software features, so-called virtual instruments, and assemble the virtual rack according to your specific application. You will learn in this tutorial how to set up racks for some typical applications.

MC_Rack main window (empty)



The virtual rack

The title bar shows the file name of the **virtual rack** (the default name before saving the rack under a custom name is **Rack1**). The white pane on the left of the virtual rack window holds the **virtual rack tree view pane**, where all virtual instruments that are part of this rack are represented by icons and (customizable) individual instrument names.

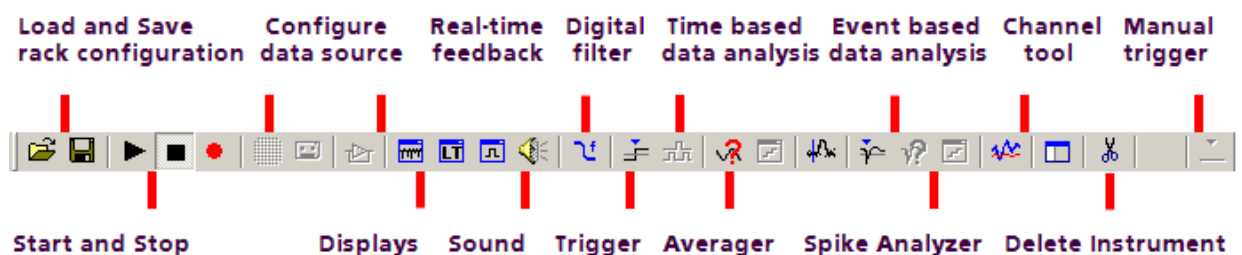
After program start, the virtual rack is almost empty, it holds only a single virtual instrument the **Recorder**, represented by a small cassette player icon. The context-sensitive grey pane on the right shows the **virtual instrument settings** (organized in tab pages) of the virtual instrument that is selected (highlighted in blue) in the virtual rack tree view pane on the left. If you have added more instruments to the rack, you were able to click through the instruments in the tree view pane, and click the tabbed pages to review or change any settings.

At this point, only the **Recorder** settings are available. The first **Rack** tabbed page is always the same for all virtual instruments here, you can **start** and **stop** MC_Rack (that is, the **data acquisition** or the **Replayer**), and activate the **recording** (that is, writing data to **hard disk**).

The **Lag** status bar gives you information on the computer performance; the lower the **Lag**, the better the performance. When the **Lag** exceeds the maximum, MC_Rack will be stopped automatically, and you will be informed about a performance limit of the computer by an error message. The complexity limit of the virtual rack depends directly on the computer performance. To avoid data loss during over night recordings, for example, test the rack configuration thoroughly under realistic conditions (that is, the signal rate should be as expected in the real experiment) before starting the experiment. If you have trouble with the computer performance, please see the chapter Error Messages in the Troubleshooting section for more information on how to optimize the rack configuration.

In the **Recorder** settings, you select the **data streams** and **channels** that you want to save to the hard disk. You define the **path** and the **file** in which the acquired data will be saved, and you define other parameters like the recording mode (**continuous** or **triggered**), and the **maximum file size**.

The **Recorder** shows you the currently available **disk space** on the target hard disk. Please check the disk space and estimate how long you still can write data to the hard disk always before starting an experiment. Otherwise, data loss will occur when the disk is full. For example, if you record 60 electrode channels at a sampling frequency of 25 kHz, the data rate is 3 MB/s, that is 10.8 GB/h.



MC_Rack toolbar. For a more detailed information on the toolbar buttons, please see "Toolbar" under General User Interface.

The MC_Rack toolbar shows all main functions available in MC_Rack. You can click on a **virtual instrument** button to insert a virtual instrument into your **rack configuration**. Please note that virtual instruments that need an **input data stream** that is generated by another virtual instrument (for example, the **Spike Analyzer**, which needs a **Spike** data stream generated by the **Spike Sorter**) can only be placed **in series** with the required virtual instrument and are otherwise not available (indicated by a gray button color). In an empty rack, only the data source, that is, the **data acquisition** or the **replayer**, are available to start with. After the data source was inserted into the rack, other virtual instruments will be available.

5.3 Creating a Data File

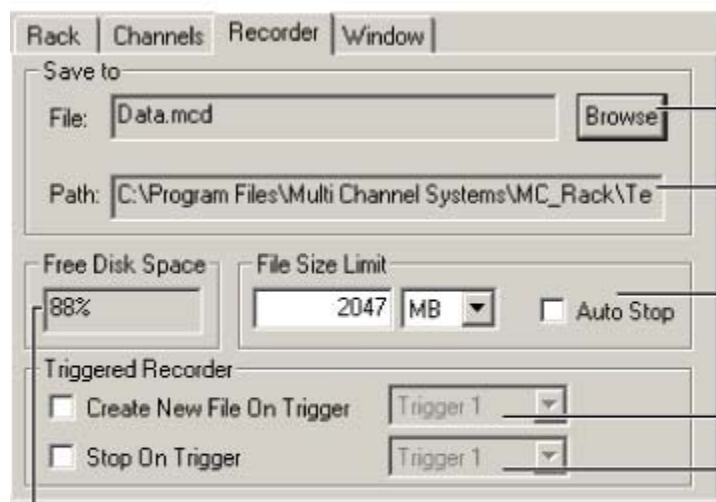
If you want to write the parameters that you will extract in an offline analysis to the hard disk, you have to specify the file name and path in the **Recorder**. (It is not possible to change or overwrite existing data files in MC_Rack, for example, adding the spike rate to an existing data file, but you can record the raw data together with the spike rate stream to the same file.)

Choosing the file name and path

1. Click the **Recorder** tab.
2. **Browse** your folders and select a path.
3. Type a file name into the text box.
4. Confirm by clicking **Save**.

The data file is then generated automatically when you start MC_Rack in **recording** mode. The file extension for the data files is ***.mcd**.

Recorder settings



Current **file name**. Click **Browse** to change the file name and path.

Current **file path**.

Enter the desired **file size limit**. The file is closed when the limit has been reached. Select **Auto Stop** if you want to stop the recording. Otherwise, a **new file** is generated.

Generate **new files** on a **trigger** event.

Stop recording on a **trigger** event.

Displays available disk space on the currently selected hard disk.

File size limit

When the maximum file size specified by the user has been reached, a new file is generated automatically. The file name is extended by four digits, counting up, for example LTP-Parameters0001.mcd, LTP-Parameters0002.mcd, and so on.

If you rather prefer that the recording is completely stopped when a file has reached the maximum size, please select the option **Auto Stop**.

For information on more options, please see "Generating Data Files" in the MC_Rack Features section.

Selecting data streams and channels

As has been said before, the fate of each single channel is **independent** from other channels. You can pick exactly the channels you like to save from all generated data streams. For example, you can decide to save only **one** channel of **raw data**, but the **peak-to-peak amplitude** results of **all**, or of a **specific selection** of channels.

→ Click the **Channels** tab.


As long as the rack is still empty, you see an empty box. There are no channels available at this point, because you have not chosen a data source yet (**MC_Card** for online data acquisition or **Replayer** for replaying data files). Without a data source, there are no data streams available for recording. Remember later, when you have completed the rack, to assign the channels that you like to save to the data file to the **Recorder**.

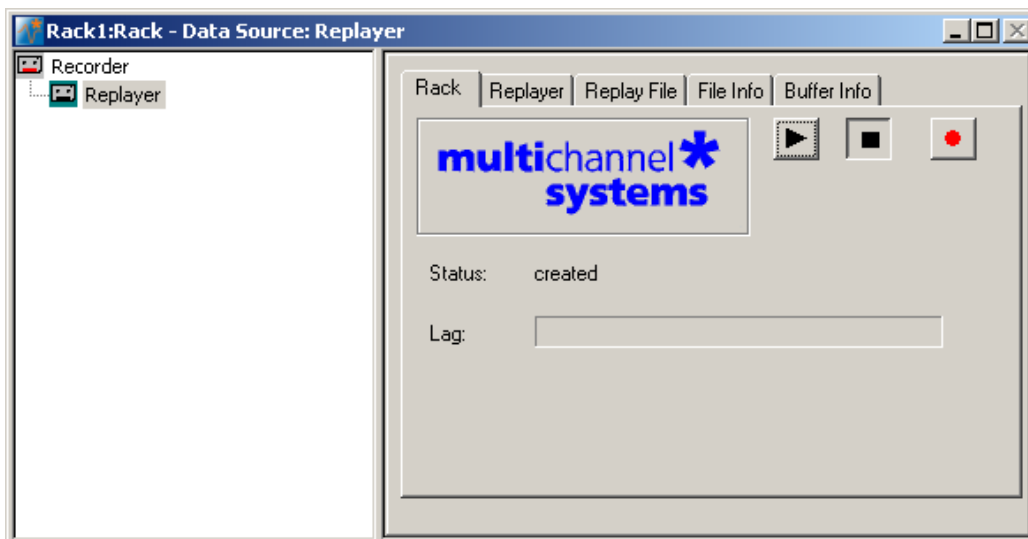
After you added a **data source**, you will see the electrode raw data streams provided by the data source (for example, electrode raw data, analog data, and digital data from the **MC_Card**, or the data streams included in the data file loaded into the **Replayer**). If the rack file contains virtual instruments that **generate** data streams such as Spikes from a **Spike Sorter** or Parameter streams from an **Analyzer**, these data streams will be available for recording as well.

5.4 Replaying Data

In MC_Rack, the virtual **rack** configuration you use to record and analyze data online and offline, and the **data files** are in principle **independent** from each other. You can reuse a rack for several experiments and generate separate data files. You can then load the generated data file with another rack later for further offline analysis. We will be doing this now for demonstration purposes in this tutorial. (The general design of a rack is basically the same for online and for offline analysis.)

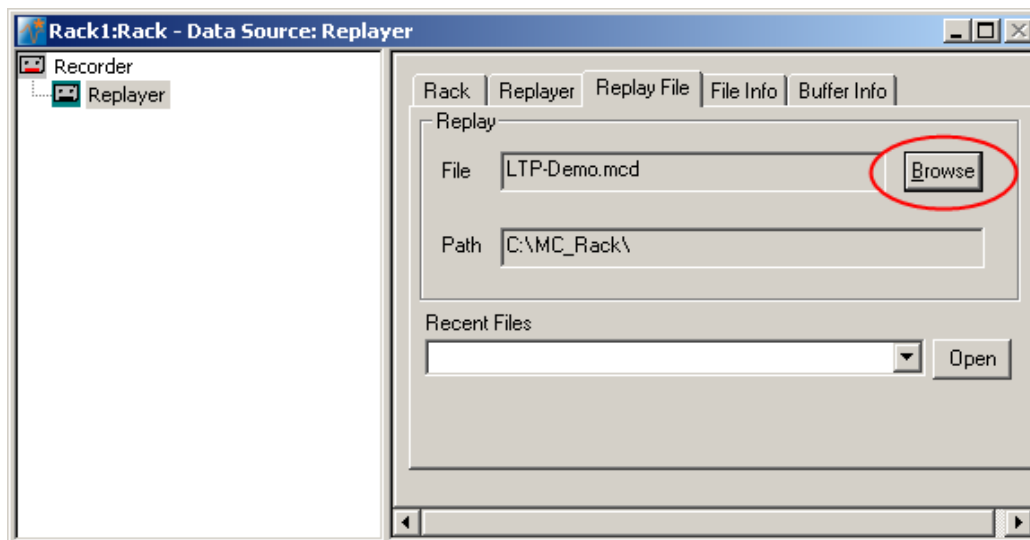
Firstly, you define the input data (the data source). Because you will be doing an offline analysis with data saved previously, you need the **Replayer**. The **Replayer** works like a tape deck; you load a data file and you can then start and stop replaying the data streams by mouse-click. You can record the data streams you extracted in an offline analysis (for example, the spike rate, field potential amplitude, or digitally filtered data) by pressing the red **Record** button and then replaying the data file.

1. On the toolbar, click  to add a **Replayer** to your rack. The **Replayer** property sheet opens.



2. Click the **Replay File** tab. Here, you load the file you would like to review or to analyze offline.

- Click **Browse** and browse your folders. Choose the desired file, for example, **LTP-Demo.mcd** or **OTC Spikes Demo.mcd** from the **MC_Rack Sample Racks** folder on the installation volume, and click **Open** or double-click the file name to open it.

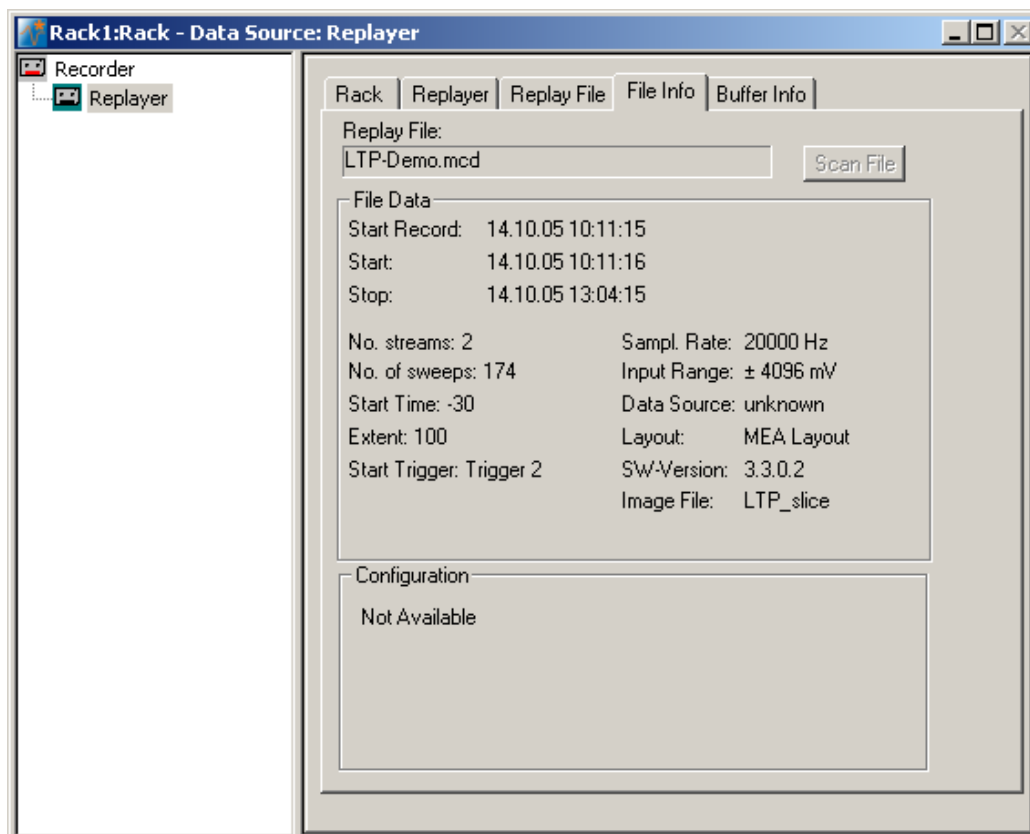


Later on, you can choose recently used files from the **Recent Files** drop-down list. Load a file by clicking **Open**.


5.5 File Information

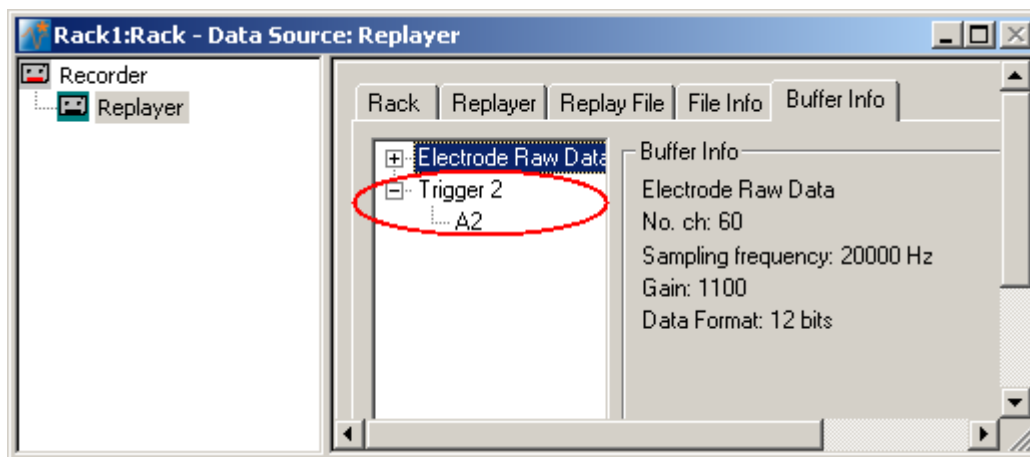
Let us have a look at the file specifications of the data file loaded into the **Replayer** now.


- Click **File Info**.

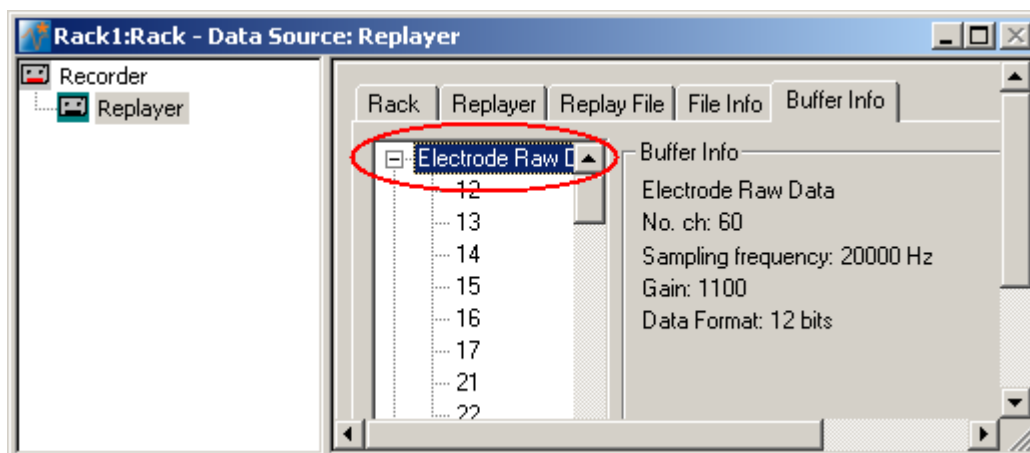


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- You see the **date** and **time** when the data has been recorded and the **sampling rate**. The example screen shot shows that the LTP demo data was recorded **triggered**, that is, the data streams are not continuous, but the data has been cut out and recorded around a trigger event (in this case, the stimulus). The **time window of each sweep** has been set to 100 ms, and the recording has been **started** 30 ms before the trigger event. The total **number of sweeps** is 174.) The **sampling rate** was 20 kHz, and the **input range** of the MC_Card was set to +/-4096 mV. The MC_Rack **software version** for recording was 3.3.0.2 (this information might be required for support issues), and the name of the **image file** that was linked to the data file is **LTP_slice**.
- Click **Buffer Info**.
On the left pane, you see the **data streams** that are present in the data file. (In the example screen shot: **Electrode Raw Data**, and **Trigger 2**). Please see the chapter "About Data Streams" for more information.
- Click  to expand the data streams, for example **Trigger 2**.
The example screen shot shows that the analog (not amplified, that is, gain = 1) channel A2 has been used as a trigger. In this case, the recording has been synchronized to the stimulus generator (STG) via the Sync Out of the STG. As long as the Trigger 2 data stream is selected (highlighted), you can see the parameters of the trigger, for example, the trigger detection level and the dead time of the trigger.






- Click **Electrode Raw Data**.
You see that there are 60 channels present.
- Click  to expand the tree. You see the **channel numbers** according to the MEA layout. The **gain** for the electrode channels was set to 1100, the standard gain of the MEA1060-BC amplifier.




5.6 Starting Replaying and Recording

When you have finished the virtual rack or if you have opened a preconfigured virtual rack from the Tutorial folder, you are ready to start the replaying and recording.

- Click **Start**  (either on the **Measurement** menu, the toolbar, or the **Rack** tabbed page) to start the data acquisition. Each virtual instrument in your rack starts to process the channels and data streams that were assigned to it.
- Click first **Record**  and then **Start**  to write data to the hard disk. The data from the electrodes selected in the **Recorder** is saved to the file and location specified in the **Recorder**.



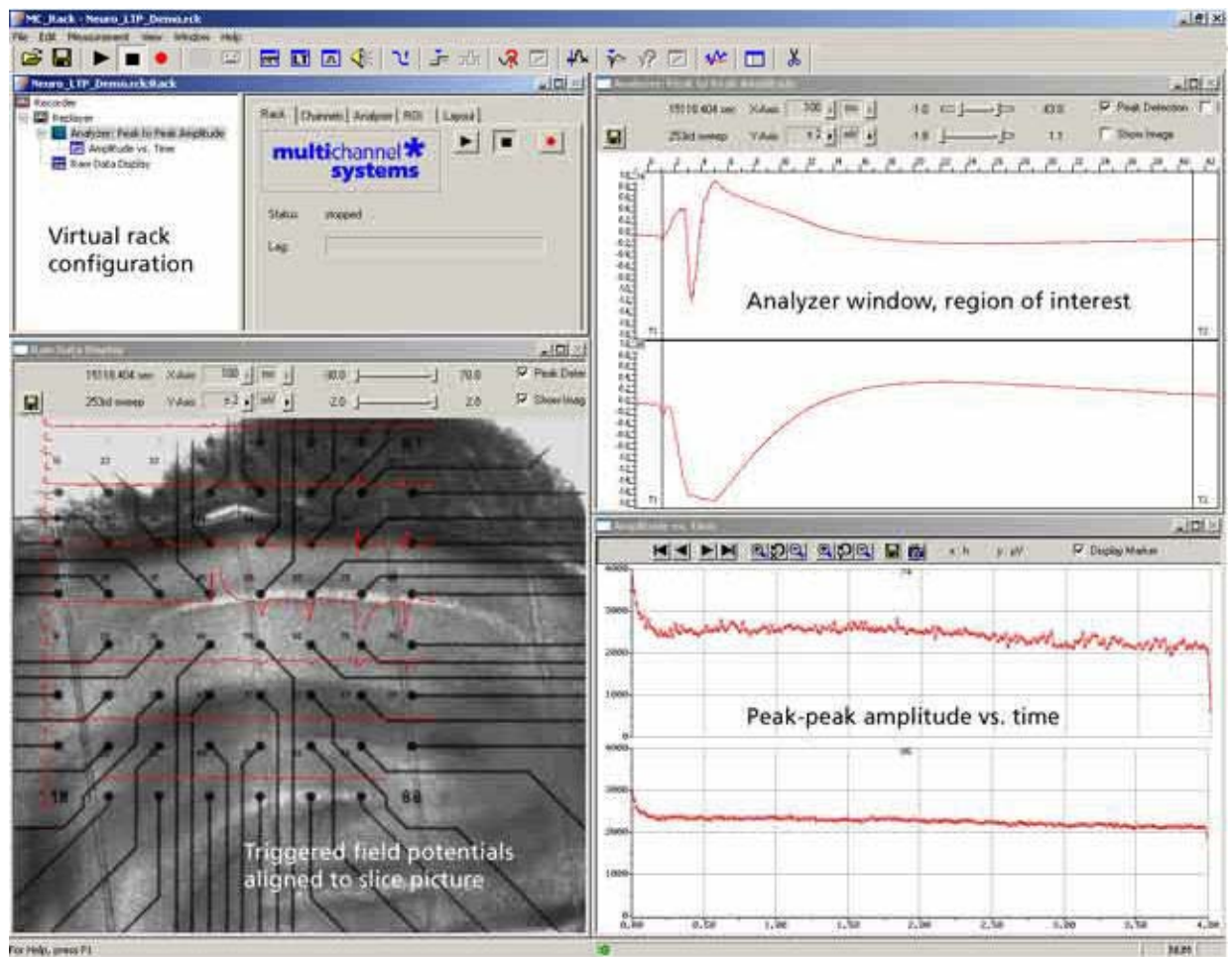
- Click **Stop**  to stop the replaying of data.



Warning: **Only** data of the channels and data streams that were **selected** in the **Recorder** are saved in the data file when you start a recording. Data is **only** saved to the hard disk when the red **Record** button is pressed **in**.

6 Long Term Potentiation (LTP)

6.1 Aim of Experiment



This experiment was performed on an **acute hippocampal slice** on a Microelectrode Array (MEA) with 64 channels with the aim to analyze **long-term potentiation (LTP)** following electrical stimulation.

The 300 μm thick acute hippocampus slice was prepared from a 21 days old rat. The slice was stimulated every 60 s with the **test stimulus**, a single biphasic pulse with 1.5 V amplitude and 100 μs duration per phase, by using one of the embedded MEA electrodes (No. 75).

MC_Rack Tutorial: MEA Application Examples

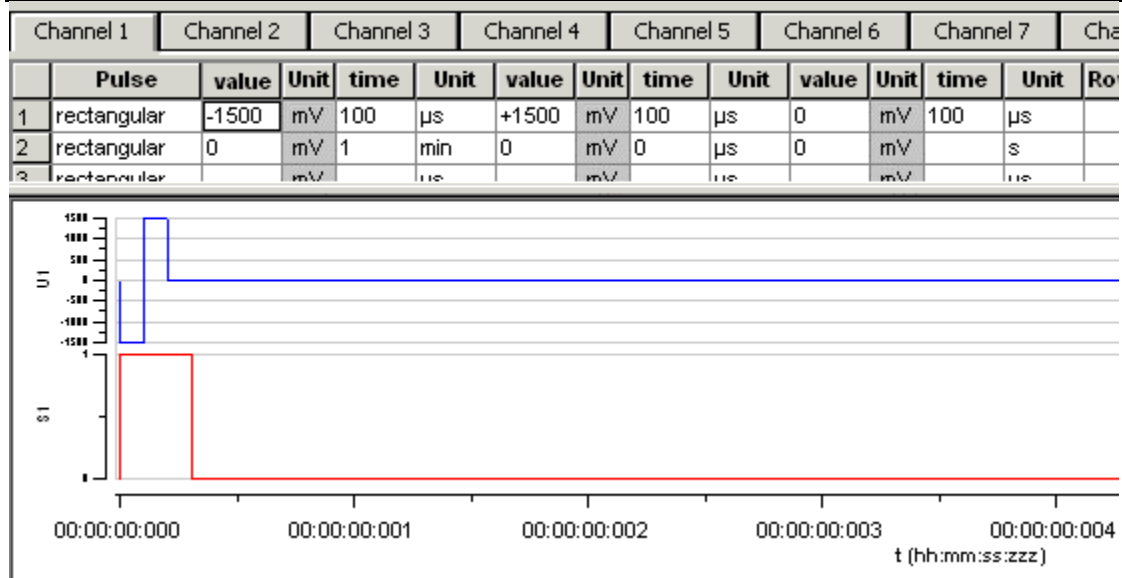


Fig. 1 Test stimulus for LTP experiment

The screen shot from the MC_Stimulus program shows the biphasic test pulse (amplitude: 1.5 V, duration: 100 µs per phase, inter-stimulus-interval: 60 s). The red trace is the Sync Out (TTL) pulse that was used for triggering the recording.

LTP was induced by an **LTP train** with **100 pulses** of same amplitude and duration as the test stimulus, and a frequency of 100 Hz. The Sync Out signal of the STG (stimulus generator) has been used to trigger the recording, with a pre-trigger of 30 ms and a time window of 100 ms. Data was recorded before and after the LTP induction; the LTP induction itself was not recorded.

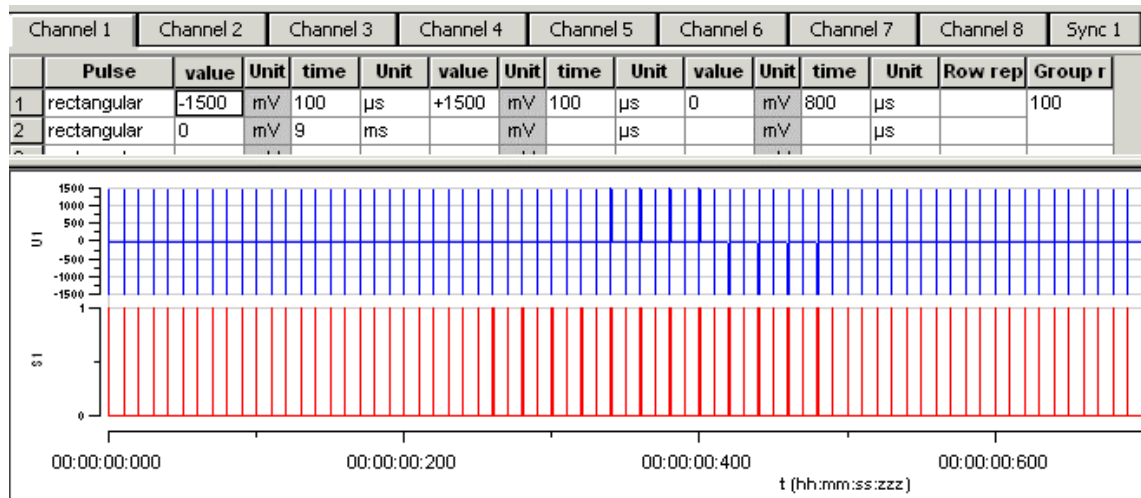


Fig. 2 Pulse train of 100 biphasic pulses for LTP induction

Amplitude: 1.5 V, duration: 100 µs per phase, inter-stimulus-interval/frequency: 10 ms / 100 Hz)

As the data was acquired with a MEA1060-BC amplifier with **blinking circuit**, all MEA electrodes were disconnected from the amplifier shortly during stimulation, in this case for **540 µs** (300 µs Sync Out pulse of the STG, plus intrinsic **Wait** of 40 µs, plus user-defined **Wait** of **200 µs**). This efficiently prevented stimulus artifacts.

The demo data used in this part of the tutorial was kindly provided by Dr. Frank Hofmann, University of Heidelberg, Germany.

Task

Show the impact of an LTP train on the LTP peak-to-peak amplitude by an offline analysis; graph, print and save the results.

Note: In this chapter, you will set up an offline analysis rack (for the demo data file) step by step. You can set up a virtual rack for online recordings and analysis likewise, simply add the **MC_Card** as the data source to the rack instead of the **Replayer**.

- Rack file: **Neuro_LTP_Demo.rck**
- Data file: **LTP-Demo.mcd** (recorded **after** LTP induction) , **preLTP-Demo.mcd** (recorded **before** LTP induction)
- Image file: **LTP_slice.jpg**


Please see also the MEA Application Notes for more information on the preparation techniques and experiments.

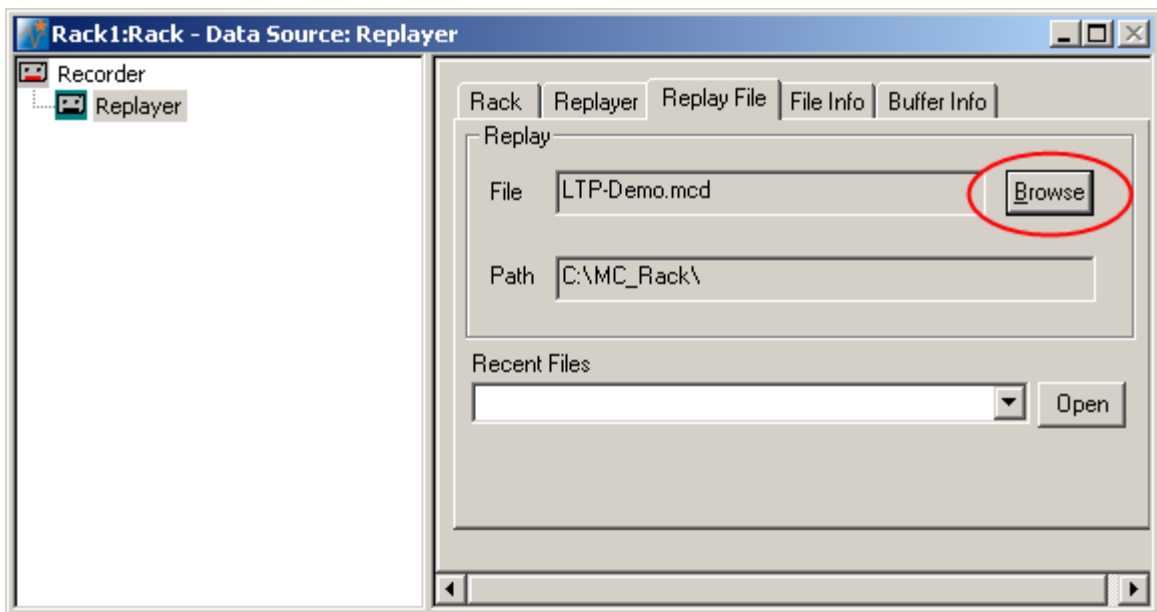
You will learn in this chapter ...

- How to define **basic functions** in your **rack configuration**:
 - Record data (**Recorder**)
 - Analyze field potentials, for example, extract peak to peak amplitudes (**Analyzer**)
 - Plot the amplitude vs. time (**Parameter Display**)
- How to **customize** the rack:
 - Modify the display layout (all display types)
 - Rename virtual instruments for a better overview (all virtual instruments)
- More **details**:
 - Align the data traces to a slice picture (**Analyzer**, **Data**, or **Averager** display)
 - Customize the colors of the traces and axes in the display (all display types)

6.2 Preparations

We recommend that you take some time for rebuilding the virtual rack for this application step by step in this tutorial, but if you prefer having a look at the completed rack or if you get stuck during the tutorial, you can also open the rack file "Neuro_LTP_Demo.rck". Click **Open** on the **File** menu to open the rack file.


1. Copy the **complete** MC_Rack **Tutorial** folder from the installation volume into the MC_Rack program directory with the following path "c:\Program Files\Multi Channel Systems\MC_Rack\"
2. Start MC_Rack or click **New** on the **File** menu to generate a new virtual rack file configuration.
3. Click  on the toolbar to add a **Replayer** to your virtual rack.
4. In the tree view pane of the virtual rack, select the **Replayer** and click the **Replay File** tab. Click the **Browse** button and browse to the **Offline** subfolder of the **Tutorial** folder, and load the data file **LTP-Demo.mcd** into the **Replayer**.



6.3 Analyzing Field Potentials

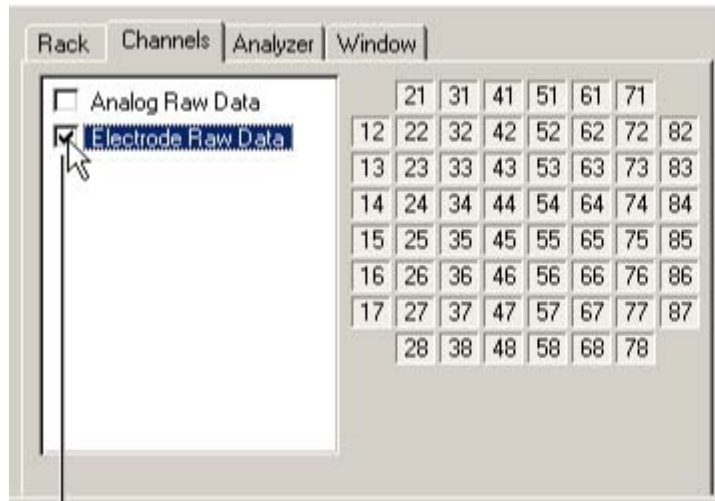
You can use a virtual **Analyzer** for **extracting parameters** of interest, for example, the **minimum** or **maximum**, the **amplitude**, or the **slope** from a **region of interest (ROI)** of the field potentials.

As the data was recorded on a **trigger** (the Sync Out pulse of the stimulator) and the region of interest needs to be specified relative to a trigger event, the **Analyzer** will also operate based on this trigger. In an online experiment, you would now have to define the trigger with a **Trigger Detector**. In the data file, the trigger was already recorded together with the raw data, and you can use the existing trigger for triggering the **Analyzer**. (In this experiment, more than one trigger were used, but trigger 2 was used for recording the data, trigger 1 was not saved. Therefore, there is only trigger 2 available in the data file.) You can see this information on the **File Info** page of the **Replayer**.

1. On the toolbar, click  to add an **Analyzer** to the virtual rack configuration.
2. Select now the channels you are interested in. Start with all channels, you can then later decide to deselect channels with no LFP responses (for optimizing the computer performance). For example, you can deselect the stimulating electrode No. 75, or electrodes that are not covered by the slice.

3. In the tree view pane of the virtual rack, select the **Analyzer** and click the **Channels** tab of the **Analyzer**.
4. Click the data stream **Electrode Raw Data**. On the right half of the **Channels** page, the channels appear in the standard 8 x 8 MEA grid.
5. Select the check box next to the stream name to select **all** channels or click the buttons with the electrode numbers to pick **single** channels. The check box next to the stream name appears shaded if some, but not all channels have been selected.

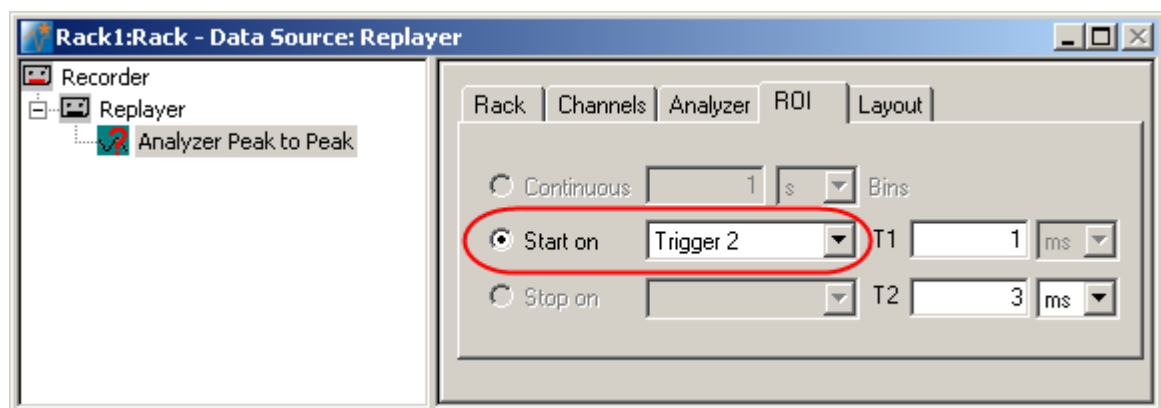
Selecting all channels of a data stream



The channels of the selected data stream appear as toggle buttons in the designated channel layout. You can select single channels by clicking the corresponding buttons. Selected channels appear pressed in.

Click the check box next to a stream name to select **all** channels.

6. Click the **ROI** tabbed page, which stands for **region of interest**. **Start on Trigger 2** is preselected, because the recording of this data file was triggered on trigger 2. The option **Continuous** is not available, because the sample data is not a continuously recorded file.



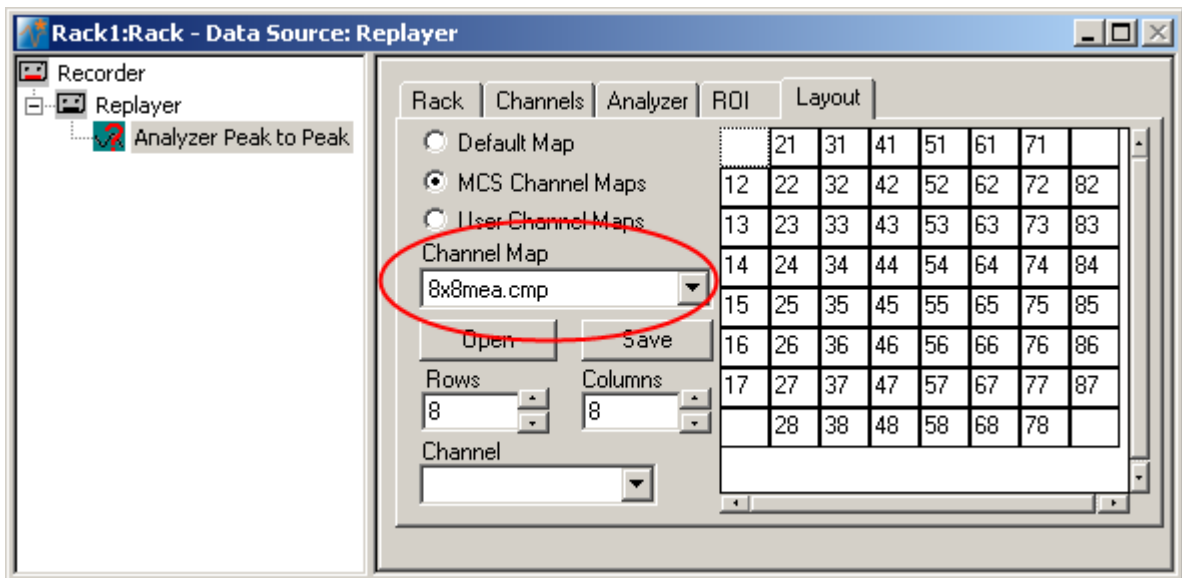
The **Analyzer** displays the electrode channels in the layout of the **Channel Map** you have created or loaded.

6.4 Customizing the Display Layout

The following information applies not only to the **Analyzer** display, but also to other displays, such as the **Raw Data** display or the **Parameter** display.

Hint: Channel maps are provided for all MEA types available from Multi Channel Systems. You can download channel maps for all MEAs available from **Ayanda Biosystems** from the Ayanda web site (<http://www.ayanda-biosys.com/download.html>). You can also set up custom channel maps with a preselected number of electrodes and save them for later use.

1. In the tree view pane of the virtual rack, select the **Analyzer** and click **Layout**.
2. To load a **Channel Map** in the MEA layout, click **Open**.
3. Browse your folders and open the MC_Rack program folder.
4. Select the appropriate MEA layout, for example, the **8x8mea.cmp** file for standard 8 x 8 MEAs, and click **Open**. The MEA layout appears on the right side of the dialog box, and the corresponding display is updated accordingly.

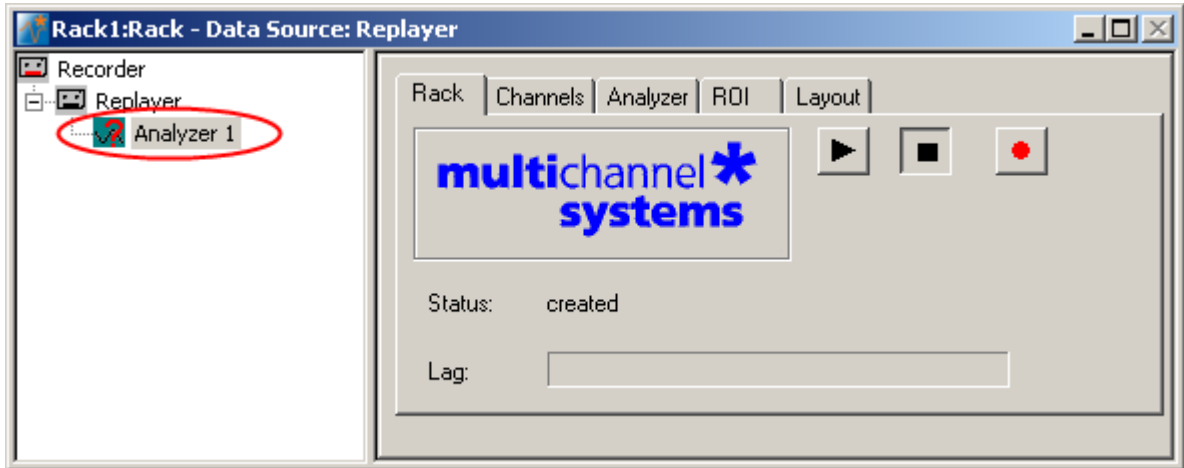


5. Switch to the **Analyzer** display. You can choose the appropriate range of the y-axis from the drop-down list. The x-range and the display refresh rate are defined by the sweep time. You can see on the **File Info** page of the **Replayer** that the data is triggered and that one sweep is 100 ms long. Therefore, the maximum range of the x-axis is 100 ms. You can fine-tune the ranges with the sliders.

6.5 Renaming Virtual Instruments

You can rename instruments for a better overview. This is especially useful if you use the same type of virtual instrument several times, for different purposes, in the same rack.

1. In the tree view pane of the virtual rack, select the **Analyzer 1**, and then click its name (do not double-click it). An insertion point appears.



2. Type in the new name, for example **Peak-Peak Amplitude**.

6.6 Zooming into a Single Channel

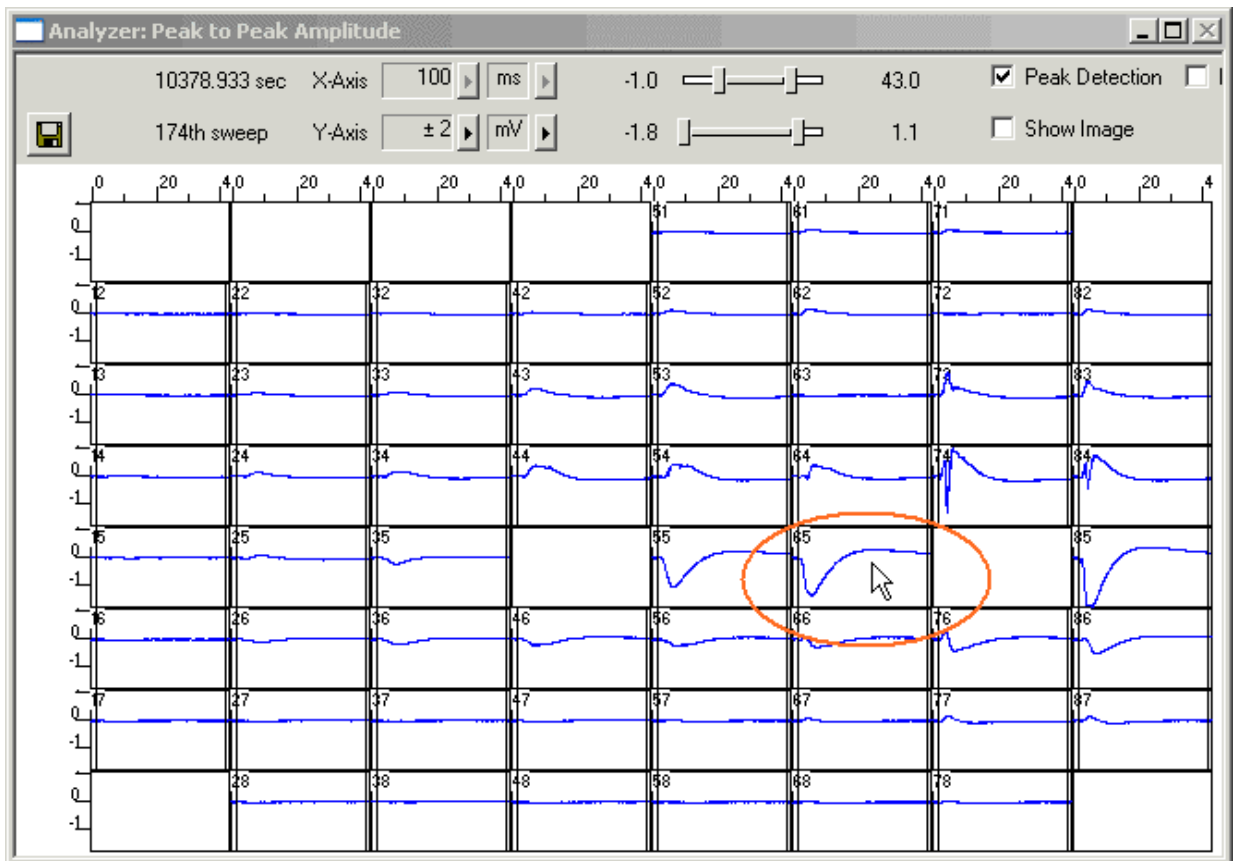
In all displays, you can switch to a single channel by double-clicking the channel in the display.

1. Click **Start**  (either on the **Measurement** menu, the toolbar, or the **Rack** tabbed page) to start the replaying of the data.

Each virtual instrument in the rack configuration starts to process the channels and data streams that were assigned to it. For example, data is graphed in the displays, and so on. You can adjust the replaying **speed** on the **Replayer** tabbed page of the **Replayer**.

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2. Double-click channel No. 65 in the **Analyzer** display. As the pathway that is going to be analyzed in this experiment is directed from the right to the left in the slice, the electrodes of interest are left of the stimulating electrode.

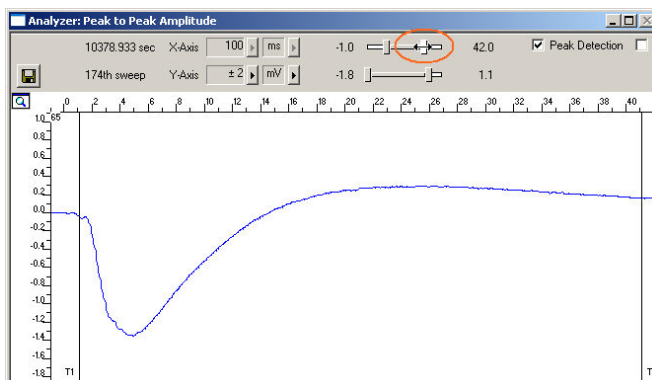


The magnifying-glass icon  on the top left indicates that you have zoomed to a single channel.

3. **Zoom in** the y-axis by moving the two sliders **to each other**.



4. **Move the range** by clicking in the **middle** between the two sliders and dragging with your mouse or pressing the LEFT ARROW and the RIGHT ARROW keys. For larger steps, you can also use the PAGE UP / PAGE DOWN keys.
5. **Zoom the x-axis** as well until you have centered on the response following the stimulus (at time point 0).



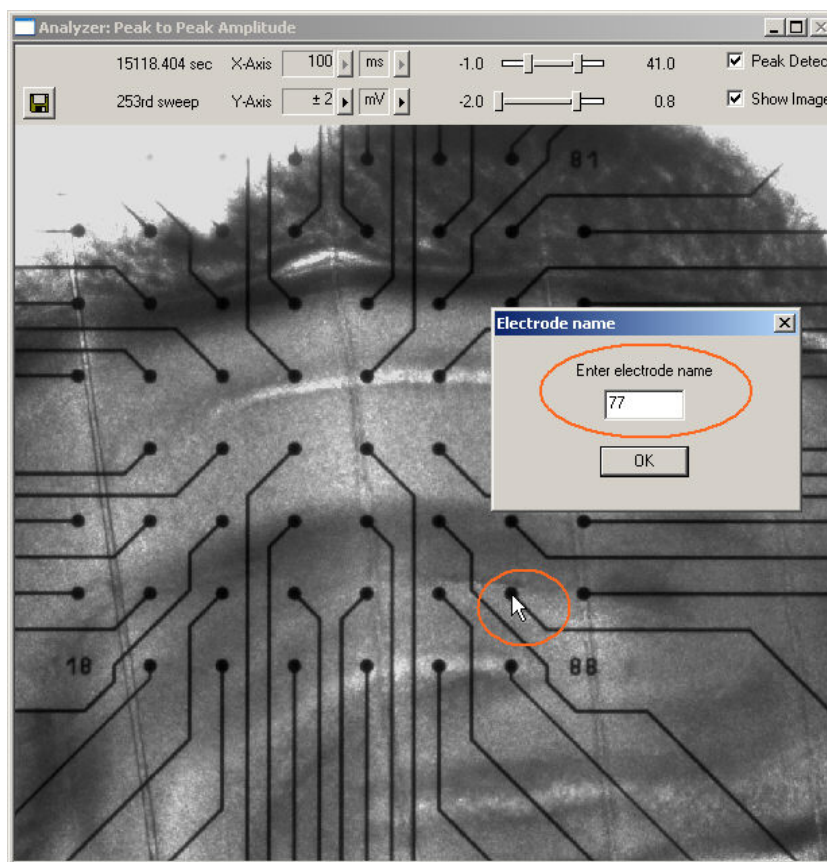
6. Double-click the display again to switch back to the full channel view. The settings have been applied to all channels.

6.7 Aligning the Data Traces to a Slice Picture

You can load a digital photograph of the acute slice as a background picture and align the signals to the electrodes and the regions of the slice.

Hint: Crop the picture in a graphics program so that only the electrode array is visible. This helps saving (unused) space on the screen.

1. On the **File** menu, click **Load Image**.
2. Browse your folders and open the **Tutorial** folder on the installation volume.
3. Select the **LTP_slice.jpg** file and click **Open**.
4. On the **Analyzer** or **Data Display**, click **Show Image**.
You can now see the slice picture.
5. Now, you map the channels to the electrodes in the picture. Hold down the **SHIFT key**, point to any electrode in the picture, for example No. 77, and **double-click**.

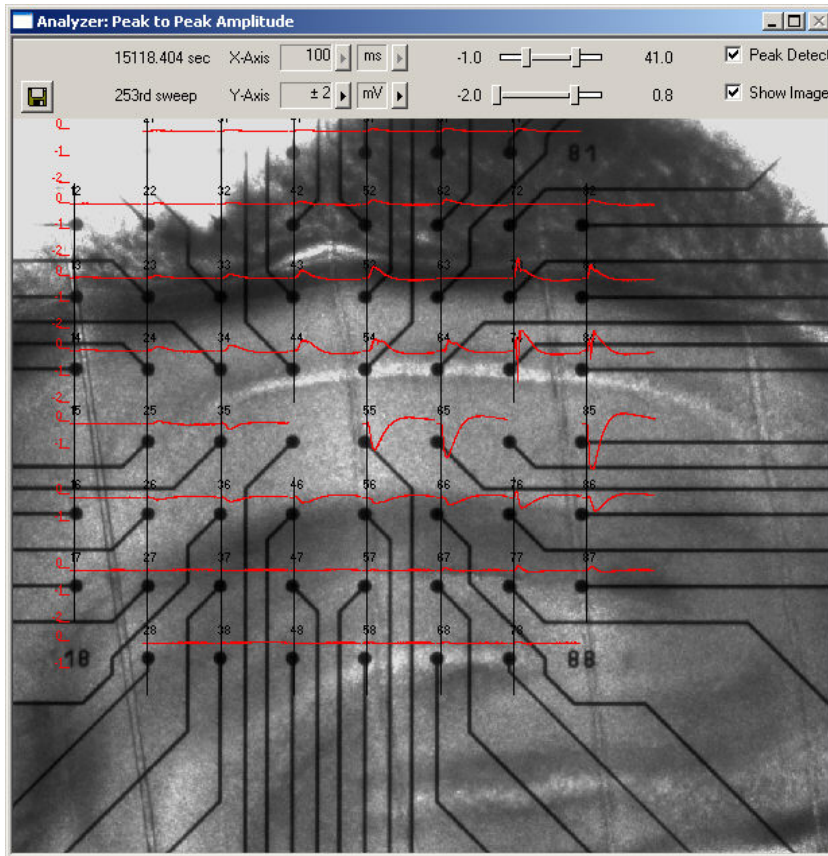


A text box opens.

6. Type in the number of the electrode according to the MEA layout, for example 77.
7. Hold down the **CTRL key**, point to any electrode in **another** row and column, for example No. 44, and **double-click**.
8. Type in the number of the electrode according to the MEA layout, for example 44. The data traces appear on top of the picture.

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9. You can resize the window by dragging the window frame with the mouse. The graphic will be scaled accordingly.



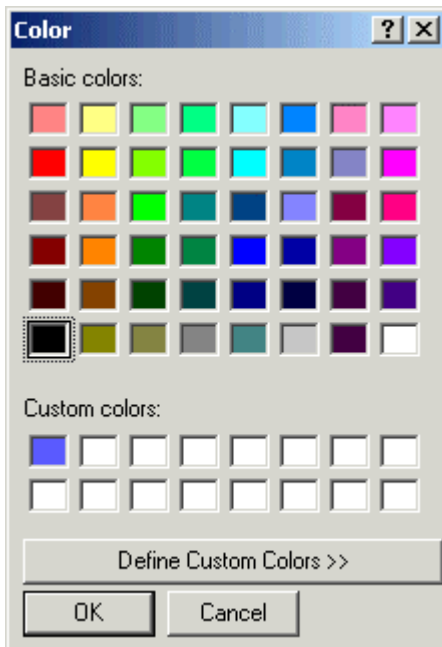
Note: If you have made a mistake, simply repeat the assignment from step 58.

Hint: If you **record** data with a background picture in your rack, the file path of the picture is saved together with the data, that is, it is opened automatically when you replay the file in a rack that has a **Display** with the **Show Image** option selected. (File paths are absolute, that means MC_Rack cannot find picture files if they have been moved to another folder or directory. You will be informed by an error message.)

6.8 Customizing the Display Colors

It may be difficult to see the traces or the axes because the contrast of the trace color to the background color is too low. You can define the colors of the traces and the axes to improve the view.

1. Point to any trace or to an axis and right-click. The **Color** dialog box opens.

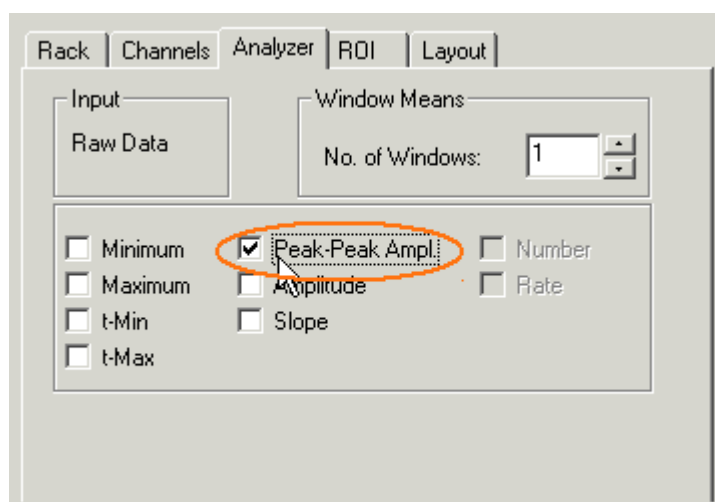


2. Choose a color from the palette or set up a custom color. The color of all traces or of the axes is changed accordingly.

6.9 Extracting Parameters

In triggered mode, the **Analyzer** extracts the parameters of interest from a distinct **time window** relative to the **trigger event**, the region of interest (**ROI**). It is very important to define this time frame appropriately for **all** channels of interest, because otherwise you will not obtain any proper results from the **Analyzer**.

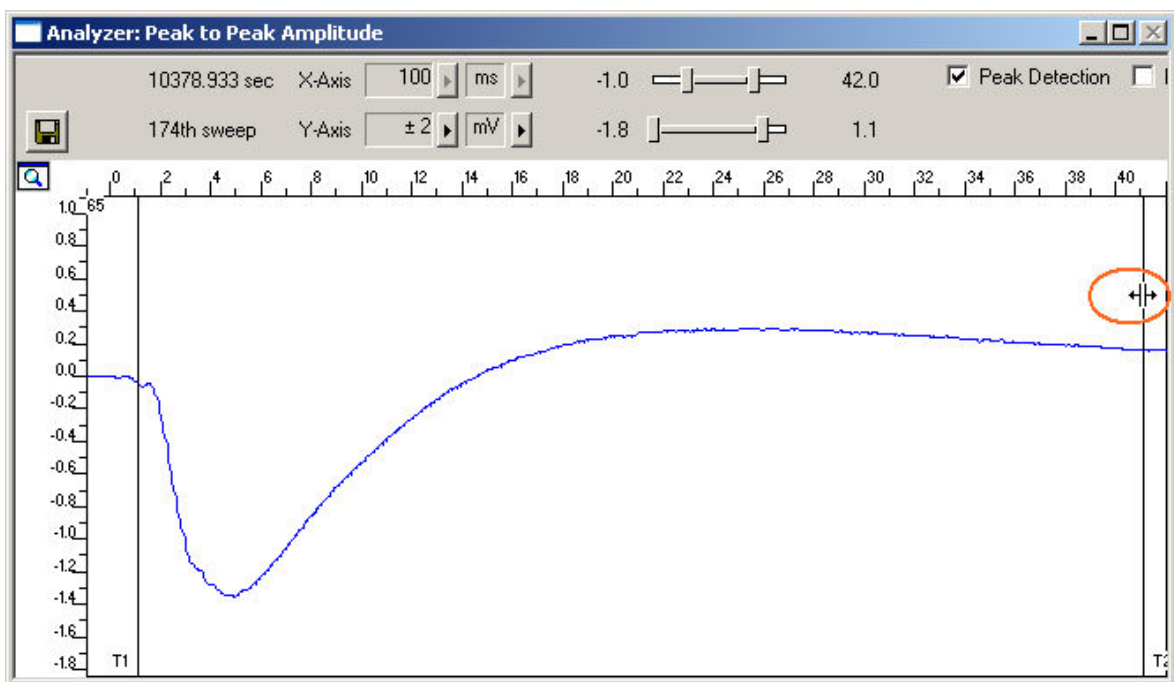
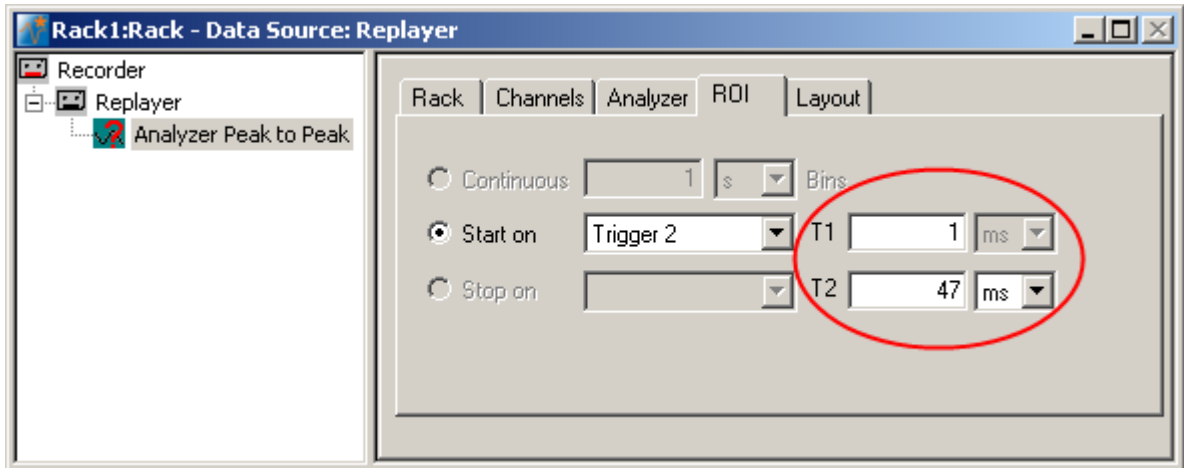
1. In the tree view pane of the virtual rack, select the **Analyzer**, and click the **Analyzer** tab. Select the parameters you like to extract, **Peak-Peak Ampl.** in this case.



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- Now, you have to define the **ROI**. Look at the Raw Data traces again in the **Analyzer** display and select a range that includes the biological responses on all channels. Shortly after the stimulus / trigger event (time = 0) will be fine. You can define the region of interest with by dragging the two vertical bars in the display, or by editing the **T1** and **T2** boxes on the **ROI** tabbed page of the **Analyzer**. For example, the start time (= **T1**) can be set to 1 ms (after the trigger event) and the stop time (= **T2**) 41 ms.

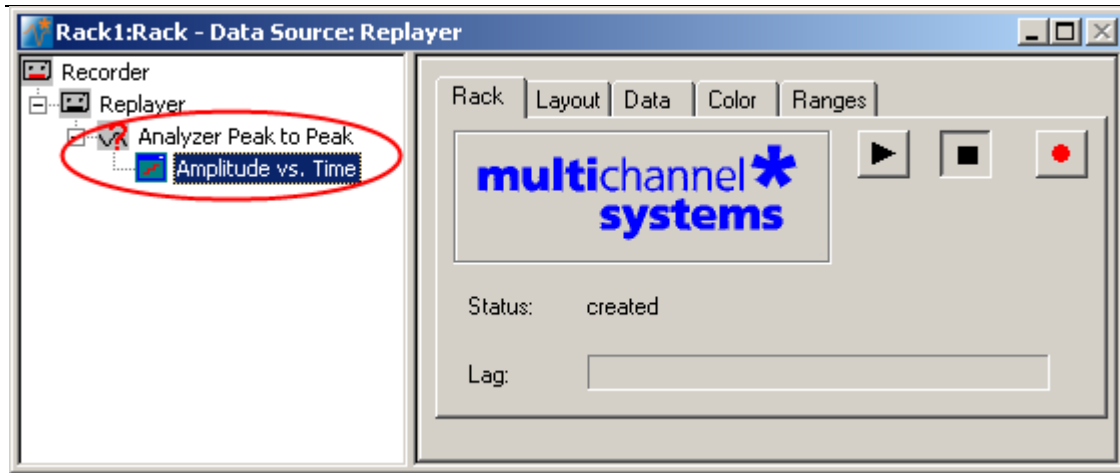
The selected parameters of interest will then be extracted from the region of interest, and can then be recorded to a data file, and / or plotted using a **Parameter** display.



6.10 Plotting Amplitude versus Time

Add a display to graph the peak-to-peak parameter. As a parameter data stream is very different from raw data, MC_Rack features a specialized display type, the **Parameter Display**, which is optimized for parameter streams.

- In the tree view pane of the virtual rack, select the **Analyzer** and click  on the toolbar to add a **Parameter Display** in **series** with the **Analyzer** to the virtual rack. (It would not be possible to add the **Parameter Display** in parallel to the **Analyzer**, as the replayed data file does not contain any parameter streams as input streams for the display, and virtual instruments can only use the output streams of other virtual instruments that are upstream in the virtual rack tree.) Rename the display to **Amplitude vs. Time**.

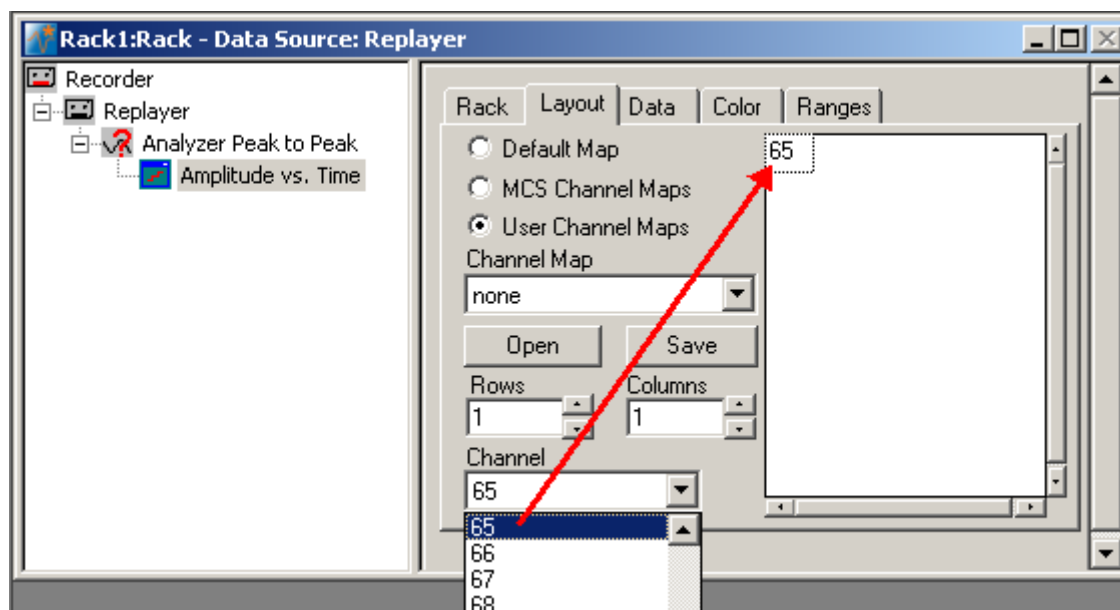


Customizing the display layout

You can define the layout for each display separately. If you have selected all channels as an input for the analyzer, you may want to see the plot for all channels as well, for example. On the other hand, you may be interested in **monitoring** only one or two representative channels online (for saving space on the screen or for saving computer performance). (You can still **save** the parameters of all channels in the **data file** to review it later or to analyze it further in your custom evaluation software.) Set up a custom layout with only **one** electrode of interest now.

[Hint: Try typing the channel numbers instead of selecting them from the drop-down list with the mouse.](#)

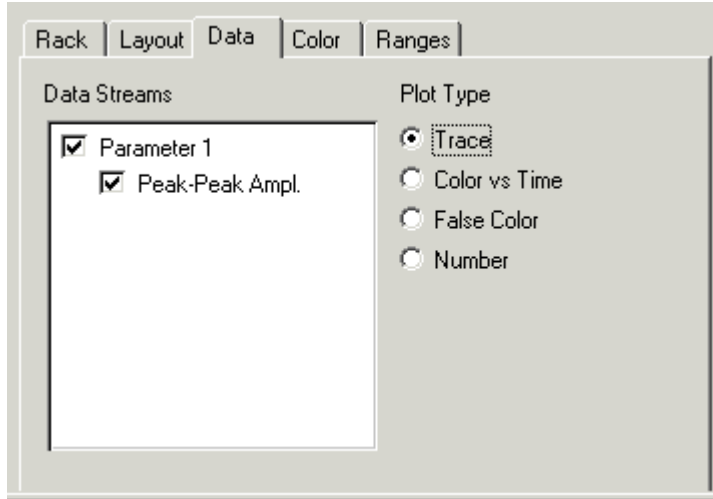
1. Click the **Layout** tab.
The default layout is loaded. In the **Rows** and **Columns** boxes, you can now modify the layout.
2. Type or select the number of **Rows**, for example 1, and the number of **Columns**, for example 1.
On the right, a custom layout with a single channel appears.
3. Assign the appropriate channel numbers to the empty slots. Click the empty slot to select it.
The active slot is highlighted by a dotted line.
4. Select 65 for the active slot from the **Channel** list, or type **65**.
The **Parameter Display** is updated accordingly. Likewise, you can set up any custom configuration for all display types.



Selecting data streams

Now, select the data that you like to be displayed.

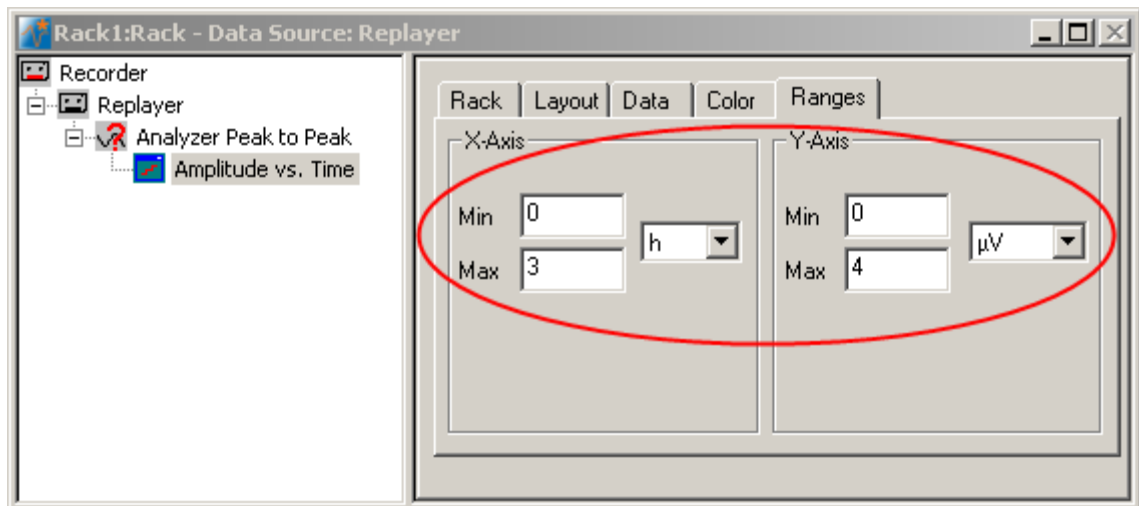
1. Click the **Data** tab.
The **Peak-to-Peak Amplitude** is already preselected. The plot type is set to **Trace**, meaning that a curve is plotted. These settings are ok and do not have to be modified in the moment.




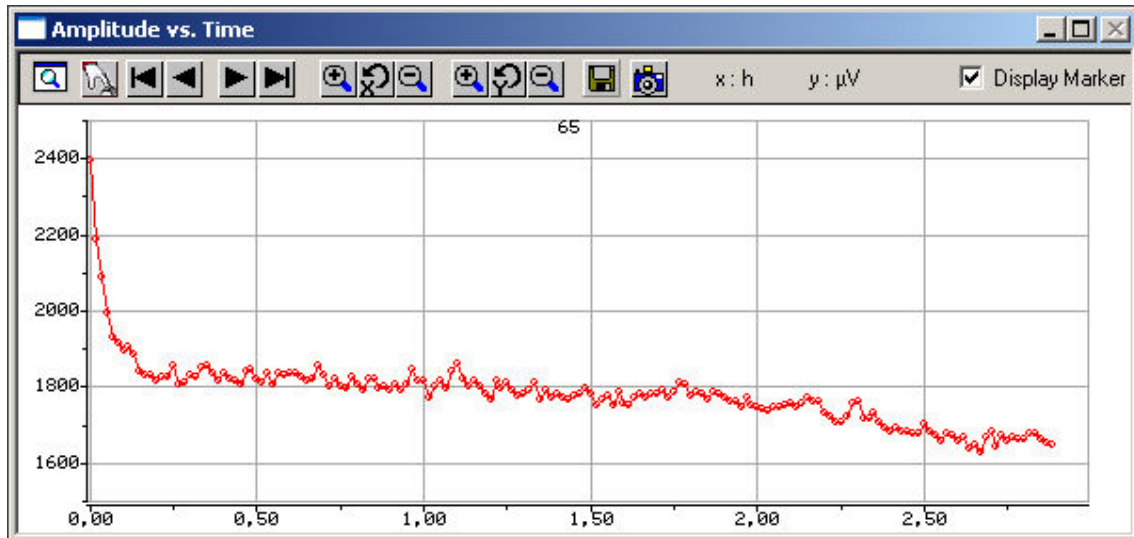
Setting the window extent

As you can see on the **File Info** page of the **Replayer**, the total recording time of the example file is about 3 hours. Adjust the ranges of the y-axis as well.

1. Click the **Ranges** tab.
2. Enter 3 h for the x-axis range, and 4 mV for the y-axis.




1. Click **Start**  to graph the plots. The baseline and the LTP induction were not recorded. The high amplitudes at the beginning demonstrate the effect of short term potentiation. LTP starts after about 20 min following the LTP induction. The amplitude slowly decreases over time.



6.11 Zooming and Scrolling

Zooming to a single channel





To provide an overall view of the ongoing activity, normally **all channels** (depending on the layout) are shown in the parameter display.

- Simply double-click a channel to have a closer look at it. The magnifying glass icon  in the upper left corner indicates the zoom mode.

Double-click the zoomed channel again to switch back to the **overall view**.





Scrolling back and forward


You can scroll the data forward and backward along the time axis. You are able to look for a region of the plot you are interested in and snapshot the displayed region.

- On the tool bar, click  to scroll **backward** and  to scroll **forward**.
- Click  if you like to jump to the **beginning** of the plot.
- Click  if you like to jump to the **end** of the plot.

Scaling the x- and y-axis

You can zoom the x- and y-axis in and out separately. The left button array on the toolbar corresponds to the x-axis and the right button array to the y-axis.

- Decide which axis you like to scale and click the corresponding  button to zoom the axis in and  to zoom it out. The display will be updated accordingly.
- To reset the scaling of the x-axis to default, click .
- Likewise, click  to reset the scaling of the y-axis.


- If you have zoomed to a single channel, you can click the Apply to All button  to apply the settings to all channels. If you do not use this button and switch back to the overall view, any changes to the settings are lost.

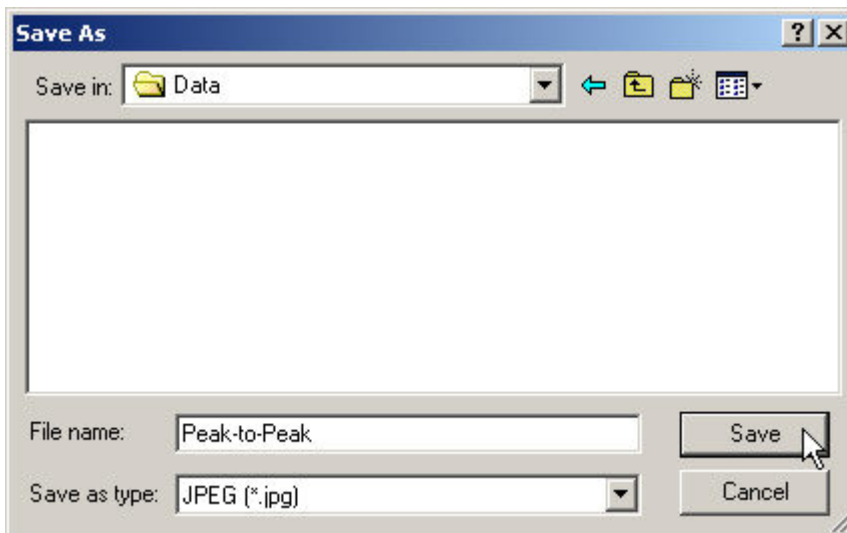
6.12 Saving a Picture

You can take a screen shot of the **Parameter Display**. This feature is especially useful for quickly setting up illustrations and presentations. Each visible channel on your display is saved as a separate image file. Each file has the chosen file name extended by channel number. If, for example, the entered file name is *Peak-to-Peak*, the file name of channel 41 will be *Peak-to-Peak_41.jpg*.

Note: Only the channels that are currently shown on the display are captured. Therefore, if you want to capture all channels, click the Snapshot button when all channels are visible. If you want to capture only a single channel, zoom this channel and then click the Snapshot button.

Hint: If you need more features for data screen shots, Multi Channel Systems recommends to use a commercially available screen shot program.

1. On the **Parameter Display** toolbar, click the **Snapshot** button  to take screen shots of the **displayed** channel(s). The **Save As** dialog box opens.
2. Select an image file format from the drop-down list:
(*.emf), Enhanced Meta File format (vector graphics)
(*.bmp), bitmap
(*.jpg), compressed bitmap in JPEG format
3. Enter a file name.



The **file name** will automatically be **extended** by the **channel number**.

4. Confirm by clicking **Save**.


6.13 ASCII Export

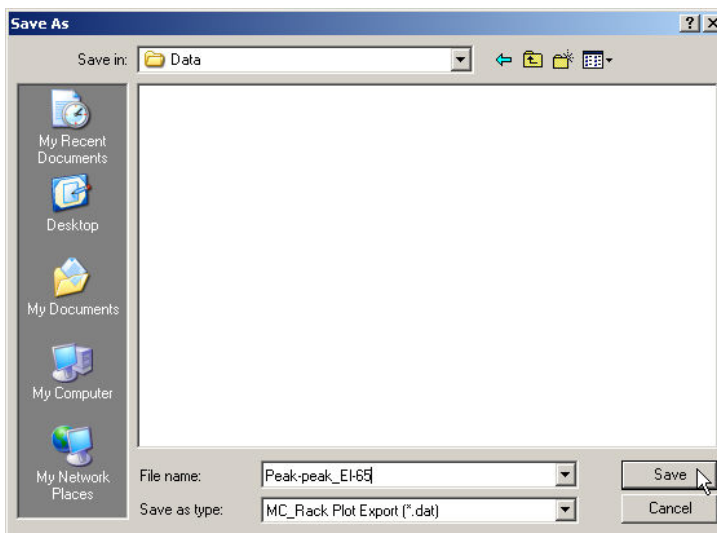
You can save the displayed data as an **ASCII file** (text without formatting). You may then send this data to an editor or to your custom analyzing software, for example. This feature is also very convenient if you want to generate custom graphics, for example, with Microsoft Excel or Micrococcal Origin.

Saving data is only possible when you have stopped the recording. The **Save** button will reappear on the toolbar.

Note: Only the channels that are currently shown on the display are saved. In the example given below, only the Peak-to-Peak amplitude of the channel 65 will be saved. Therefore, if you want to save all channels **in a single file**, click the **Save** button when all channels are visible. If you want to save the parameter values of a single channel only, zoom this channel and then click the **Save** button.

Hint: If you need more features, for example, export of raw data, use the add-on program MC_DataTool that comes together with ME- / MEA-Systems.

1. Click the **Save** button  on the **Parameter Display** toolbar to open the **Save As** dialog. The **Save As** dialog box will be opened.
2. Type in a **file name** and **path** and click **Save**.



You can now open and process the file with any text editor, spreadsheet, or any other custom evaluation software. The following screen shot shows how it looks when the file is opened with a standard spreadsheet. The first row contains the axes headers. The first column contains the x-values (in hours), the second column the y-values of channel 65 (in microvolts).

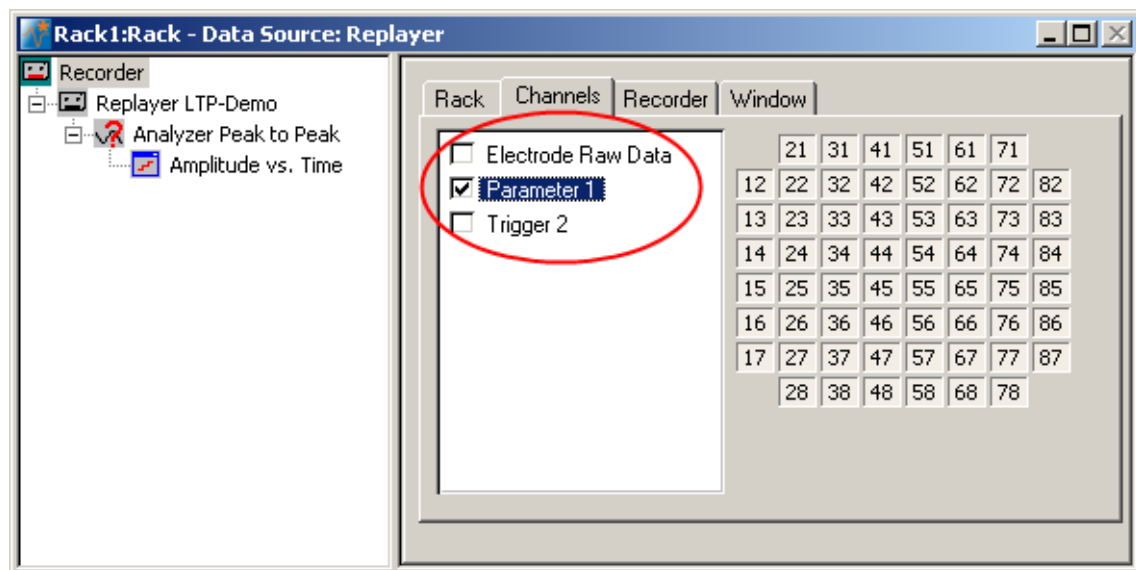
	A	B
1	T[h]	65-Height[μ V]
2	0.000	2.396.364
3	0.017	2.189.091
4	0.033	2.090.909
5	0.050	1.994.545
6	0.067	1.930.909
7	0.083	1.918.182
8	0.100	1.898.182
9	0.117	1.905.455
10	0.133	1.887.273
11	0.150	1.842.698

6.14 Recording Analyzed Data

Now, the virtual rack for offline analysis of LTP experiments has been completed, and you can select the data that should be recorded, that is, saved in the generated data file. It is important to know that MC_Rack **never** modifies or overwrites an **existing** data file. That means, in an offline analysis, the extracted parameter streams are stored in a **new** data file. Therefore, you need to specify a file name and path in the **Recorder** for both online and offline analysis.

Hint: If you want to store raw data and extracted parameters in the same data file, you can select the already existing raw data traces in a replayed file for rerecording them together with the extracted parameter streams.


1. Select the **Recorder** and click the **Channels** tab.
You see all data streams on the left. You can now select any channels you like, independently from the channels you view on screen with MC_Rack. To select the **Electrode Raw Data** stream does not make much sense in this case, because the data file already exists and is only replayed in this tutorial. But if you performed an online analysis, you would probably want to save the raw data (cutout sweeps triggered on the stimulus) of the relevant channels.
2. In this case, we are interested in the **Peak to Peak** parameters. Select the **Parameter 1** data stream. All available channels appear in a button array on the right side. (Remember: Available are those channels that have been assigned to the **Analyzer** and thus have been processed by the **Analyzer**. If you change the **Analyzer** input, you will see that the recordable output will be changed accordingly.)
3. Select all channels by selecting the check box, or pick single channels. All channels selected on this page will be saved in the specified file when you start the recording. (If you have not already specified the file and path on the **Recorder** tabbed page, do so now.)



Click the **Record** button and then **Start** again.

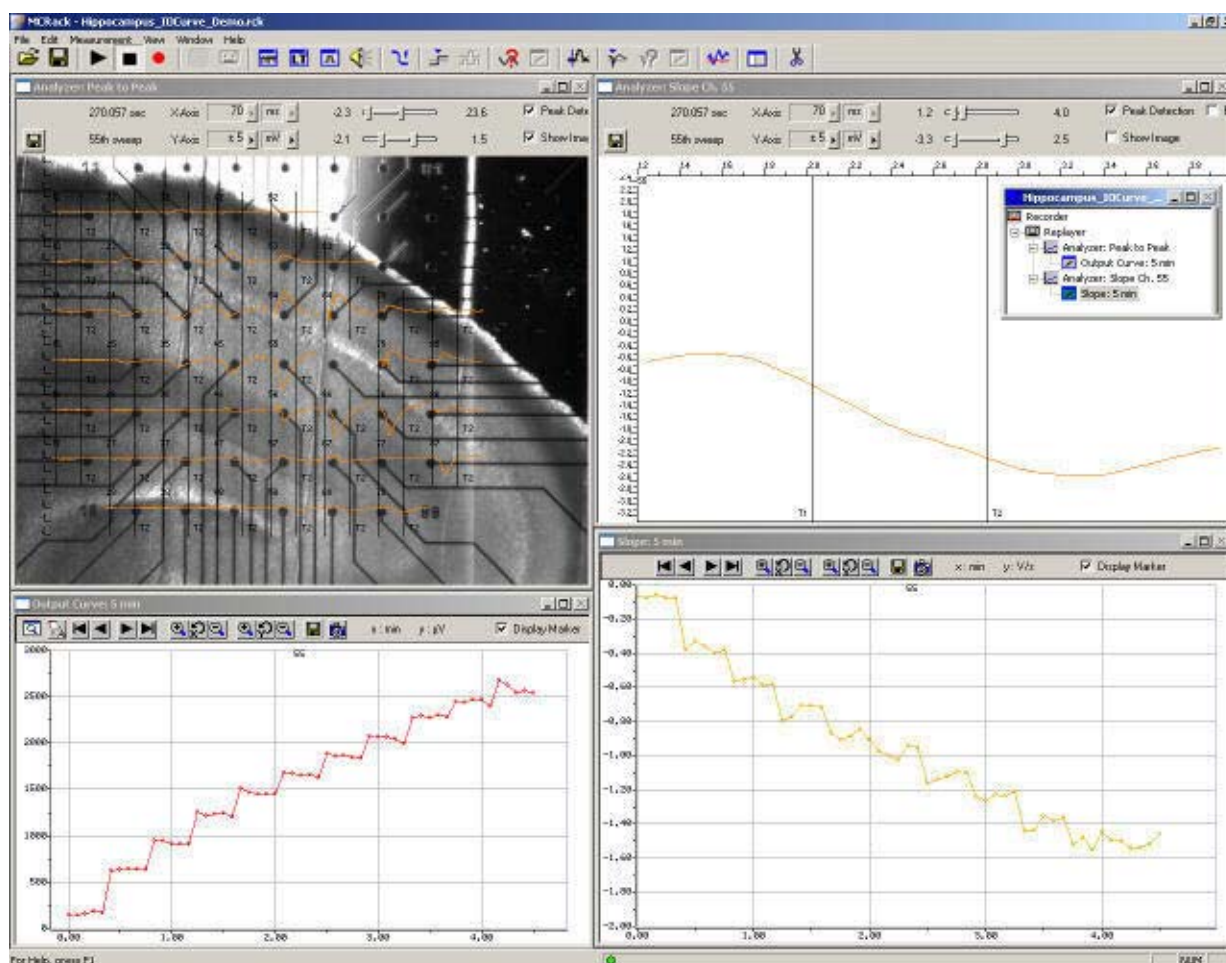


4. The data will now be replayed and the selected data streams will now be saved to the specified location. You can then replay the newly generated file with the **Replayer** and a **Parameter Display**. You can also export the data to your custom graphics or analysis software.

Note: Do not forget to enable the recording by clicking the **Record** button  !
Otherwise, no data will be stored.

7 Input/Output (I/O) Curve

7.1 Aim of Experiment



This example file was recorded from an **acute hippocampal slice** on a Microelectrode Array (MEA) with 64 channels with the aim to analyze the **input-output (I/O) relationship** of the biological response to the electrical stimulation. The **magnitude** of the **synaptic response** is relevant in this experiment. It can be assessed based on the **minimum** or **peak-to-peak amplitude** of the field excitatory postsynaptic potential (fEPSP) or on the **slope** of the initial response decay. The latter has the advantage that it is a more linear measure of the EPSP strength when population spikes mask the fEPSP. It is, however, more sensitive to noise and more difficult to extract with propagating waves of activity.

The biological sample was 300 μm thick and stemmed from a 22 days old rat. The tissue was stimulated with one of the substrate integrated electrodes (# 65). A monopolar electrical stimulation with biphasic voltage pulses (duration of 100 μs per phase) with amplitudes from 500 to 3000 mV, in 250 mV steps, was applied. Individual voltage steps were repeated 5 times with a delay of 5 s, that is, 5 x 500 mV, then 5 x 750 mV, then 5 x 1000 mV, and so on. The Sync Out signal of the STG (stimulus generator) was used to trigger the recording sweeps, with a pre-trigger of 10 ms and a time window of 70 ms.

This example shows a recording with the new MEA amplifier with blanking circuit (MEA1060-BC), featuring a superior stimulus artifact suppression. The Sync Out signal of the STG also triggered the MEA amplifier. The electrodes were transiently, in this case for **540 μs** (300 μs Sync Out pulse of the STG, plus intrinsic **Wait** of 40 μs , plus user-defined **Wait** of **200 μs**) separated from the amplifier stage during stimulation. Stimulus artifacts could be efficiently prevented on all recording channels.

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The demo data used in this part of the tutorial was kindly provided by Dr. Frank Hofmann, University of Heidelberg, Germany.

Task

Analyze the **input-output (I/O) relationship** of the biological response to the electrical stimulation by an offline analysis; graph, print and save the results.

Note: In this chapter, you will set up an offline analysis rack (for the demo data file) step by step. You can set up a virtual rack for online recordings and analysis likewise, simply add the **MC_Card** as the data source to the rack instead of the **Replayer**.

- Rack file: Hippocampus_IOCurve_Demo.rck
- Data file: I-Ocurve_Demo.mcd
- Image file: Hippocampus_IOCurve.jpg


Please see also the MEA Application Notes for more information on the preparation techniques and experiments.

You will learn in this chapter ...


- How to define **basic functions** in your **rack configuration**:
 - Select electrodes of interest / deselect unused electrodes.
 - Analyze field potentials, for example, extract peak-to-peak amplitude and the slope in parallel (**Analyzer**)
 - Plot the extracted parameters vs. time (**Parameter Display**)
- How to **customize** the rack:
 - Modify the display layout (all display types)
- More **details**:
 - Align the data traces to a slice picture (**Analyzer**, **Data**, or **Averager** display)

7.2 Preparations

We recommend that you take some time for rebuilding the virtual rack for this application step by step in this tutorial, but if you prefer having a look at the completed rack or if you get stuck during the tutorial, you can also open the rack file "Hippocampus_IOCurve_Demo.rck". Click **Open** on the **File** menu to open the rack file.

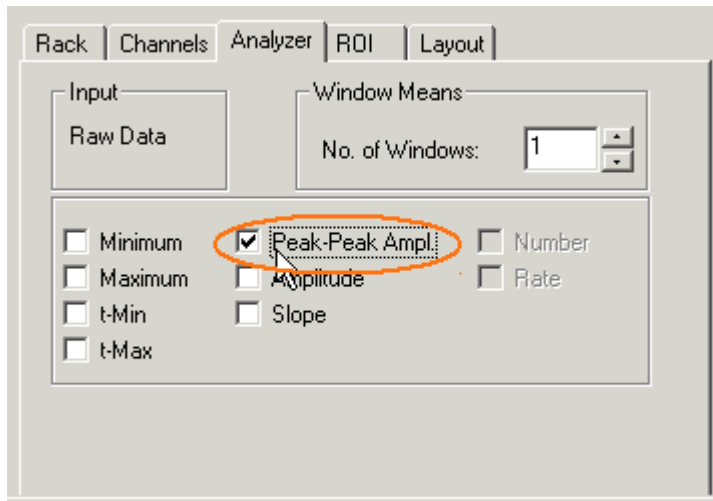
1. Copy the **complete** MC_Rack **Tutorial** folder into the MC_Rack directory with the following path "c:\Program Files\Multi Channel Systems\MC_Rack\"
2. Start MC_Rack or click **New** on the **File** menu to generate a new virtual rack file configuration.
3. Click  on the toolbar to add a **Replayer** to your virtual rack.
4. In the tree view pane of the virtual rack, select the **Replayer** and click the **Replay File** tab. Click the **Browse** button and browse to the **Offline** subfolder of the **Tutorial** folder, and load the data file **I-Ocurve_Demo.mcd** into the **Replayer**.

7.3 Extracting the Peak to Peak Amplitude

1. For extracting the peak-to-peak amplitude and the slope, we will need an **Analyzer**.
Click  on the toolbar to add an **Analyzer** to the virtual rack.

The **triggered Analyzer** display with the **region of interest** appears because the data was recorded as triggered sweeps.

2. Rename the **Analyzer** to **Peak to Peak** to distinguish it from the slope analyzer that we will set up later.
3. Select the **Analyzer** in the tree view pane. Select the parameters you like to extract, **Peak-Peak Ampl.** in this case.



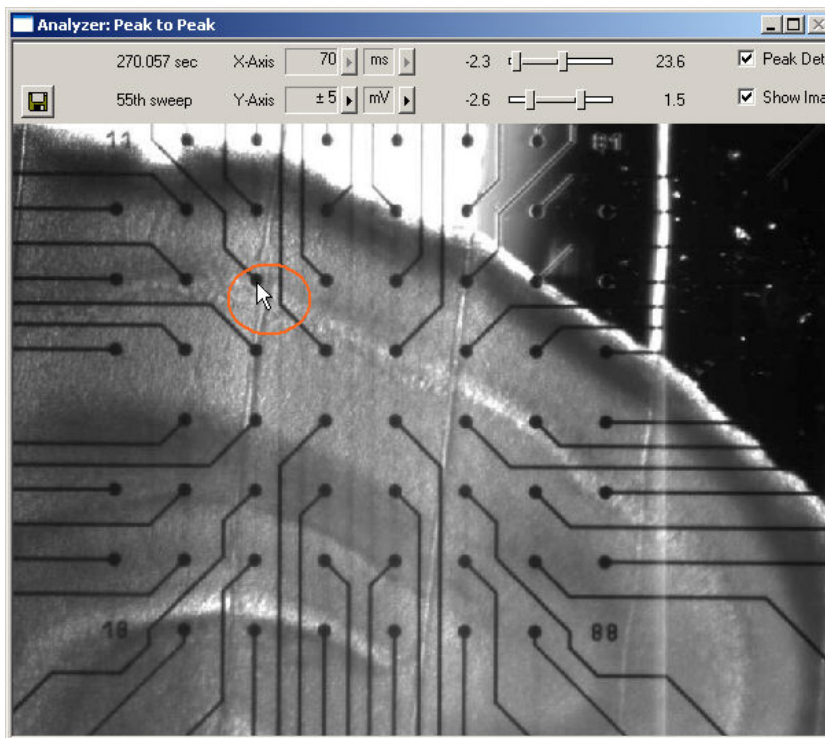
7.4 Aligning the Electrode Pattern to the Slice

You can load a picture (from a digital camera, for example) of the acute slice as a background picture of the display and align the traces to the electrode positions.

1. On the **File** menu, click **Load Image**.
2. Browse your folders and open the **Tutorial** folder on the installation volume.
3. Select the **Hippocampus_IOCurve.jpg** file and click **Open**.
4. On the **Analyzer** window, click **Show Image**.
You can now see the slice picture.

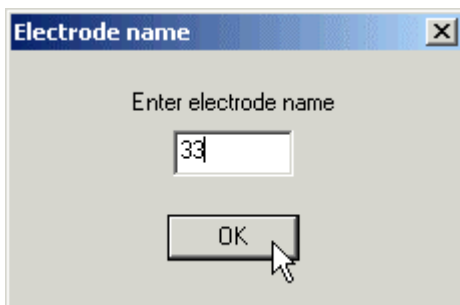
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- Now, you map the channels to the electrodes in the picture. Hold down the **SHIFT** key, point to any electrode in the picture, for example, No. 33, and **double-click**.



A text box opens.

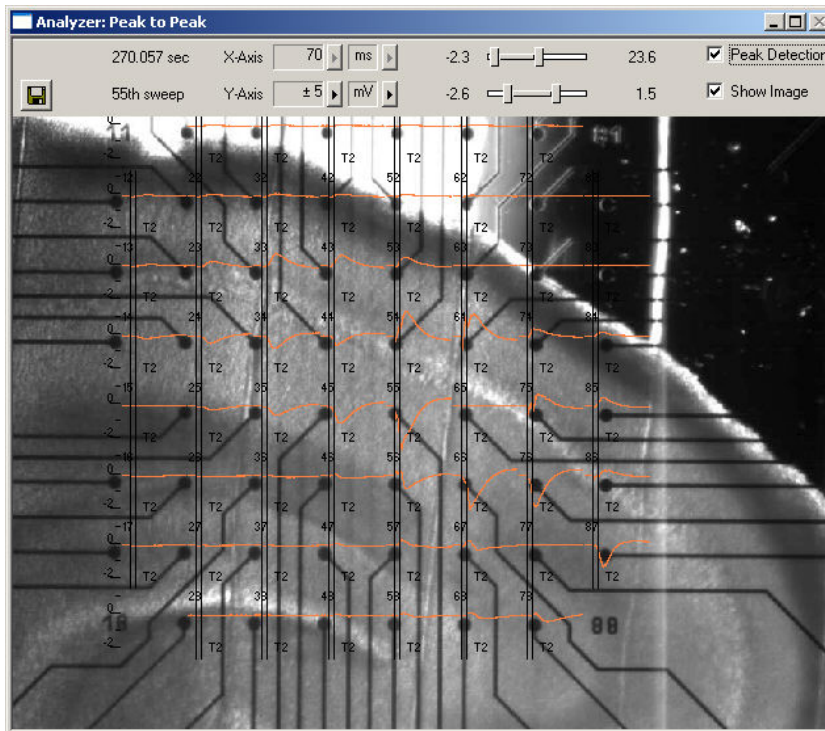
- Type in the number of the electrode according to the MEA layout, for example 33.



- Hold down the **CTRL** key, point to any electrode in **another** row, for example the bottom right, and **double-click**.
- Type in the number of the electrode according to the MEA layout, for example 78. The electrode numbers appear on top of the picture in the typical 8x8 grid.
- Click **Start**  (either on the **Measurement** menu, the toolbar, or the **Rack** tabbed page) to start the replaying of the data.

Each virtual instrument in the rack configuration starts to process the channels and data streams that were assigned to it. For example, data is graphed in the displays, and so on. You can adjust the replaying **speed** on the **Replayer** tabbed page of the **Replayer**.

The data traces appear on top of the picture.



Note: If you have made a mistake, simply repeat the assignment from step 58.

Hint: If you record data with a background picture in your rack, the file path of the picture is saved together with the data, that is, it is opened automatically when you replay the file in a rack that has a display with the **Show Image** option selected. (File paths are absolute, that means MC_Rack cannot find picture files if they have been moved to another folder or directory. You will be informed by an error message.)

7.5 Deselecting Unused Electrodes

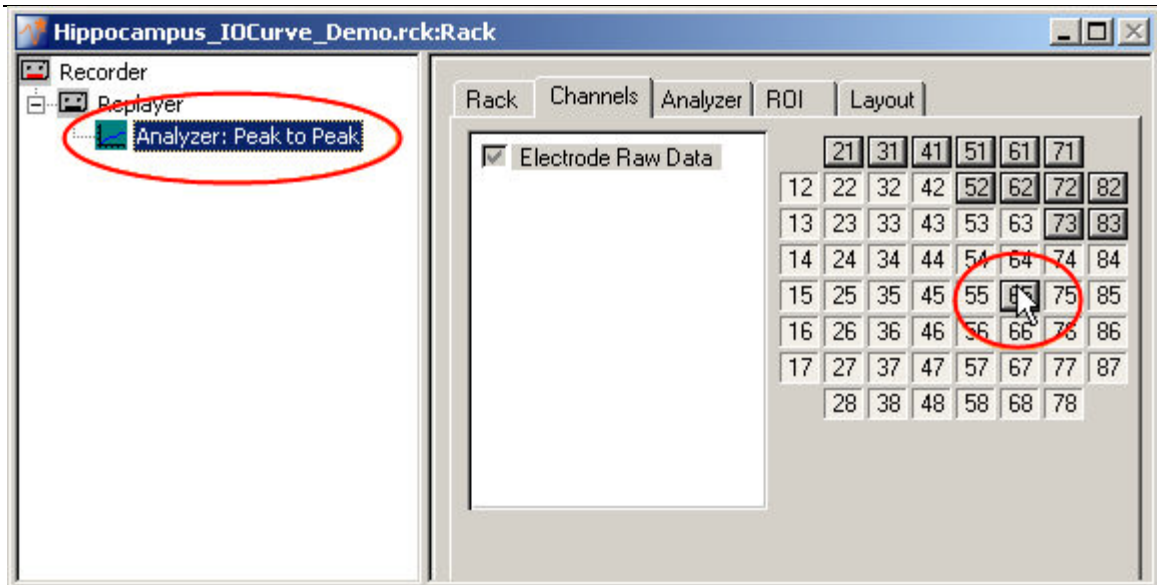
One of the main advantages of MC_Rack is the flexible data management. You can now **deselect** any **unused electrodes**, for example, the electrodes that are not covered by the slice or the stimulating electrode, in the **Analyzer**, so that the peak to peak amplitude is extracted only from the selected electrodes.

Hint: With a **MEA Switch**, you could use unused channels on the data acquisition board for recording from an additional amplifier.

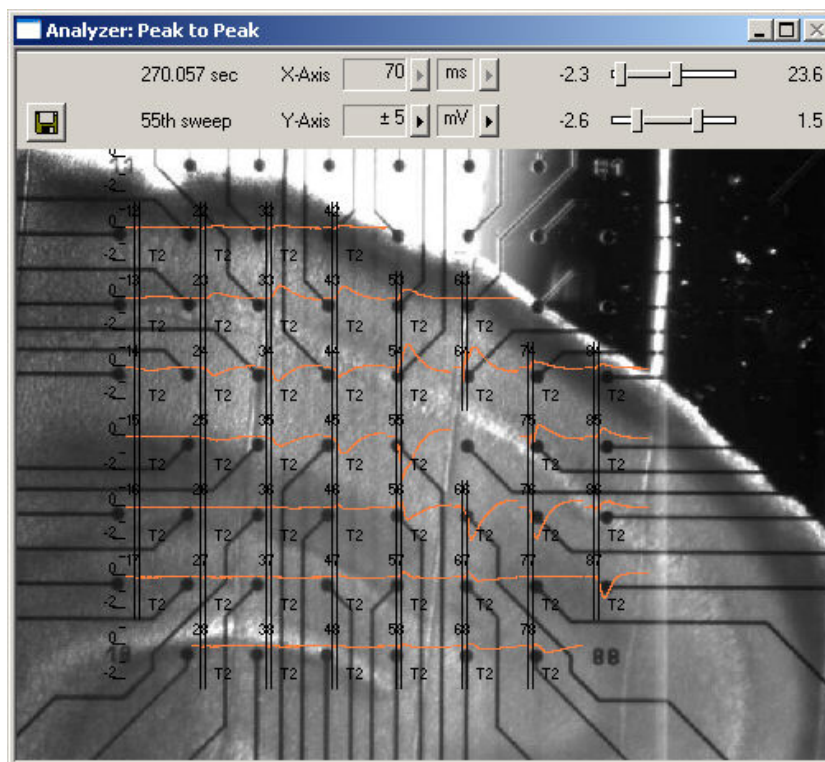
Note: The **Recorder** that writes data to the hard disk is an independent virtual instrument, that is, you have to select the channel numbers that you want to save in the generated data file, for each data stream separately, on the **Channels** tabbed page of the **Recorder**.

1. In the tree view pane of the virtual rack, select the **Analyzer**, and click the **Channels** tab.
2. Click the data stream **Electrode Raw Data**.
On the right half of the **Channels** page, the channels appear in the MEA-layout. You can select the check box next to the stream name to select all channels, or click single buttons to pick single channels. The check box next to the stream name appears shaded if some, but not all channels have been selected.
3. Deselect the stimulating electrode 65, and all unused electrodes such as 21, 31, 32, 33, 34, 35, 36, 37, 52, 62, 72, 82, 73, and 83, by clicking the corresponding channel buttons. **Selected** channel buttons appear **pressed in**.

MC_Rack Tutorial: MEA Application Examples



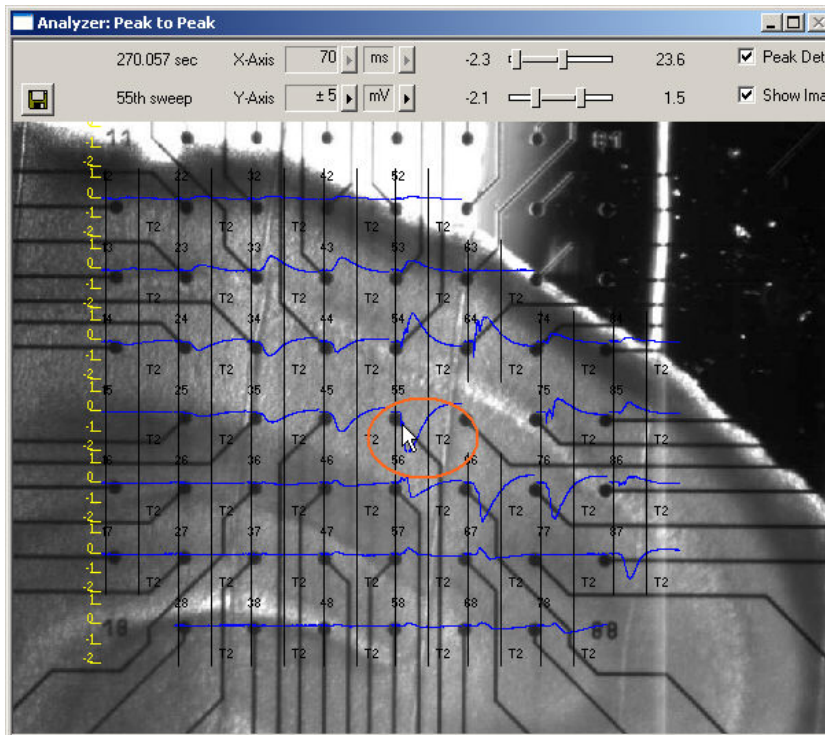
If you start the rack again, only the traces from selected electrode are displayed (and only the peak to peak amplitudes from selected electrode channels are extracted).



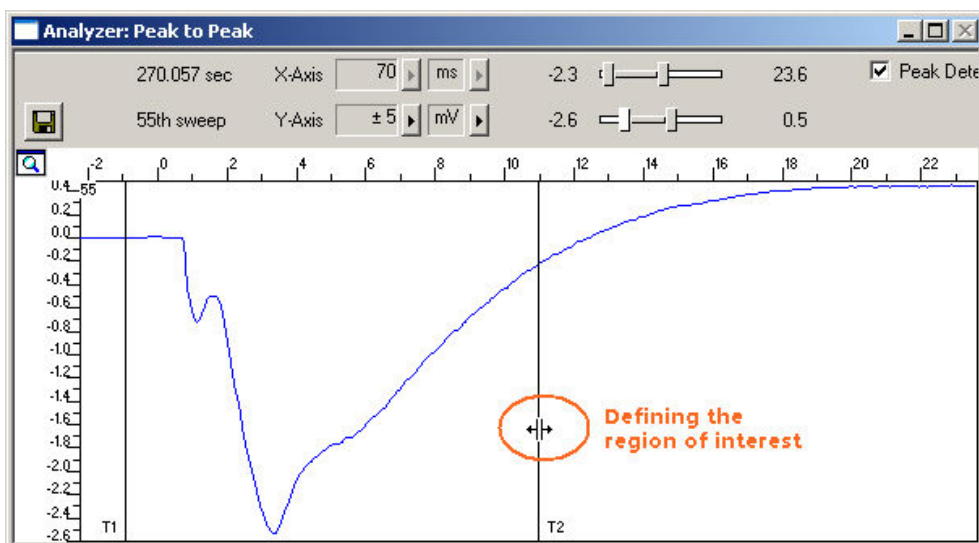
7.6 Defining the Region of Interest

You have to define a time range relative to the trigger event as the region of interest (**ROI**). The parameter of interest, the peak-to-peak amplitude in this case, is then extracted from the data trace in the region of interest. You can define the region of interest with the two vertical bars in the **Analyzer** display or by editing the **T1** and **T2** boxes on the **ROI** tabbed page of the **Analyzer**.

1. Double-click on any electrode with a large response to zoom into the data recorded from this electrode.




2. Position the bars so that the field potential is clearly inside the region of interest. As the demo data was recorded with a MEA1060-BC amplifier with blanking circuit, there is no stimulus artifact that you would need to exclude from the analysis.



7.7 Plotting the Intensity of the Biological Response

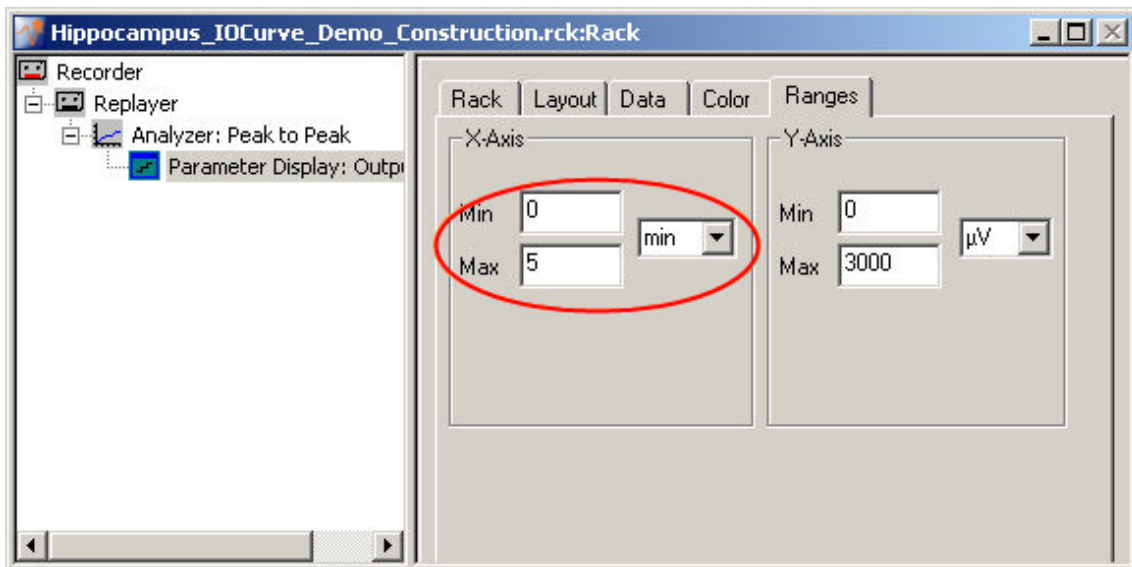
As a measurement for the response to the stimulus, we will plot the **peak-peak amplitude** and the **slope** versus time. We will start with the peak-peak-amplitude. For graphing the extracted data, we will need a **Parameter Display**.


- In the tree view pane of the virtual rack, select the **Analyzer** and click  on the toolbar to add a **Parameter Display** in **series** with the **Analyzer** to the virtual rack. (It would not be possible to add the **Parameter Display** in parallel to the **Analyzer**, as the replayed data file does not contain any parameter streams as input streams for the display, and virtual instruments can only use the output streams of other virtual instruments that are upstream in the virtual rack tree). You can rename it so that you can identify it later in the virtual rack.

Setting the window extent

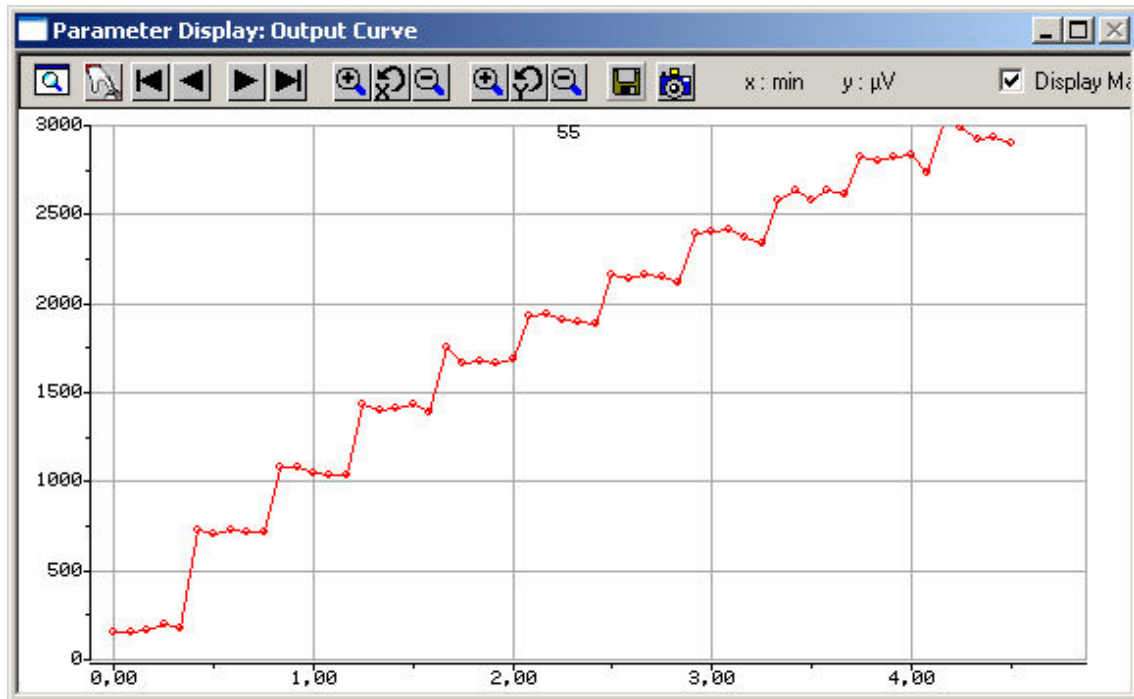
As this demo data file has about 4 min recording time (see **File Info** page of the **Replayer**), 5 min will be fine for the x-axis range.

1. In the tree view pane of the virtual rack, select the **Parameter Display**, and click the **Ranges** tab.
2. Enter 5 min for the x-axis range, and adjust the y-axis to the maximum response, for example, 3000 μV .




1. Click **Start**  to generate the plots. You can select the option **Display Marker** to mark each extracted data point in the plot.

The output is nicely increasing with the increasing stimulus amplitude.



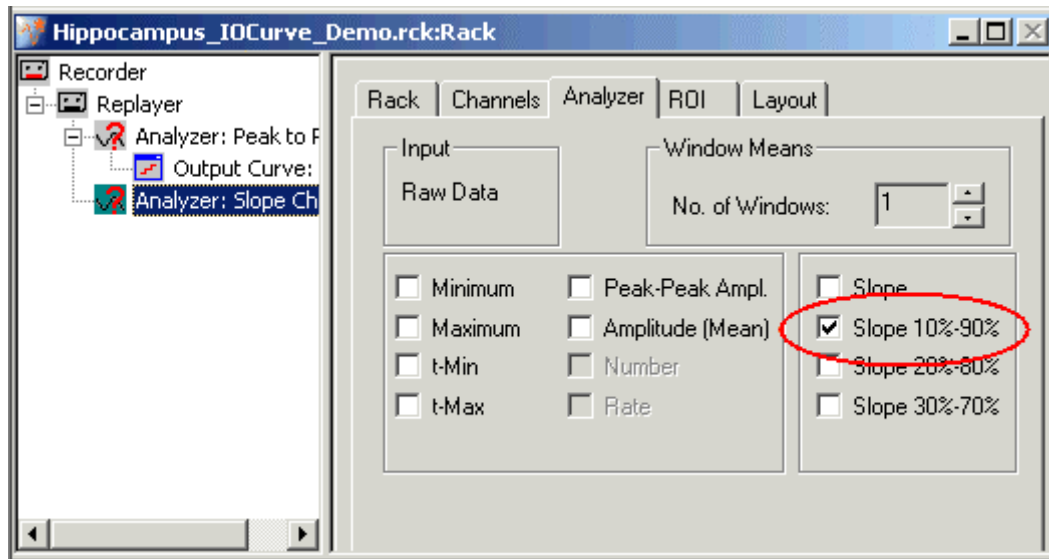
7.8 Extracting the Slope

In triggered mode, the **Analyzer** extracts the parameters of interest from a distinct **time window** relative to the **trigger event**, the region of interest (**ROI**). You could use the same **Analyzer** for extracting both the peak-to-peak amplitude and the slope, but as two distinct regions of interest are required for extracting the two parameters, we will need to set up a second **Analyzer**.

1. Click  on the toolbar to add a second **Analyzer** to the virtual rack.
2. Rename the **Analyzer** to **Slope** to distinguish it from the first analyzer.
3. In the tree view pane of the virtual rack, select the **Analyzer**, and click the **Channels** tab. Select the electrode channels that you would like analyze, for example, channel 55.
4. Click the **Analyzer** tab and select the parameters you like to extract, slope in this case. You have the choice between four options: **Slope** is the slope of a straight line fitted through all data points in the ROI. When choosing **Slope 10% / 90%**, only the data points in the range of 10% / 90% of the peak-peak amplitude (stretching from minimum to maximum) in the region of interest is considered for fitting the line. This is generally more useful as the exact timing of the regions of interest may differ slightly from channel to channel and it might be difficult to position the ROI to fit all channels of interest.

MC_Rack Tutorial: MEA Application Examples

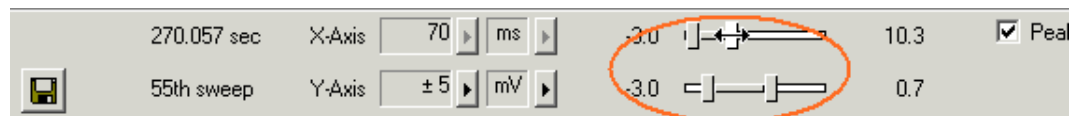
Select checkbox slope 10% / 90%.



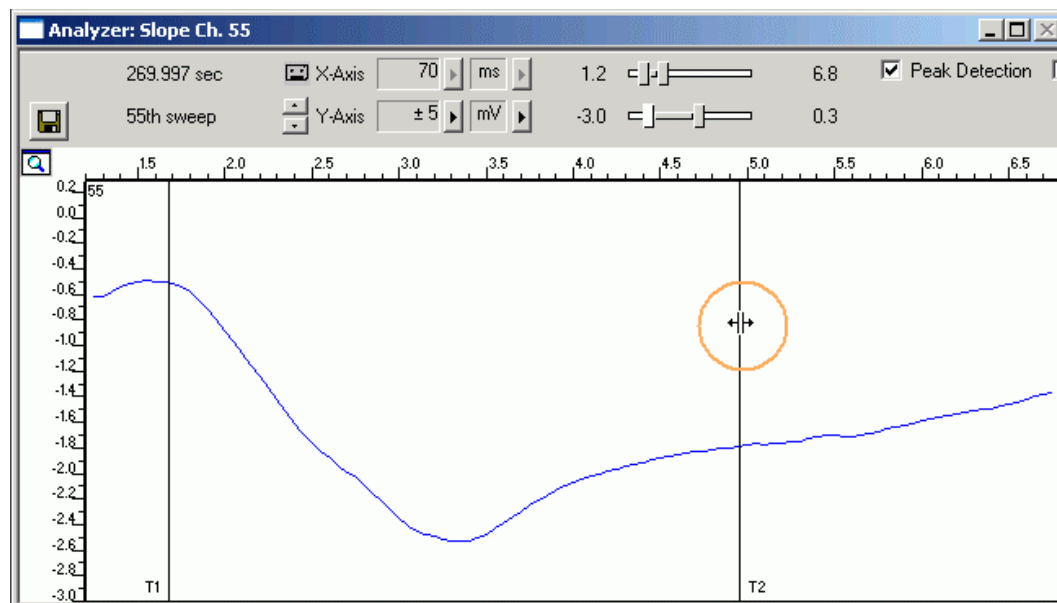
Defining the ROI

For extracting the slope, you need to frame ideally only the linear part of the response decay with the ROI cursors.

1. Zoom into a single channel and use the sliders to adjust the ranges so that you can clearly see the linear part of the response decay.



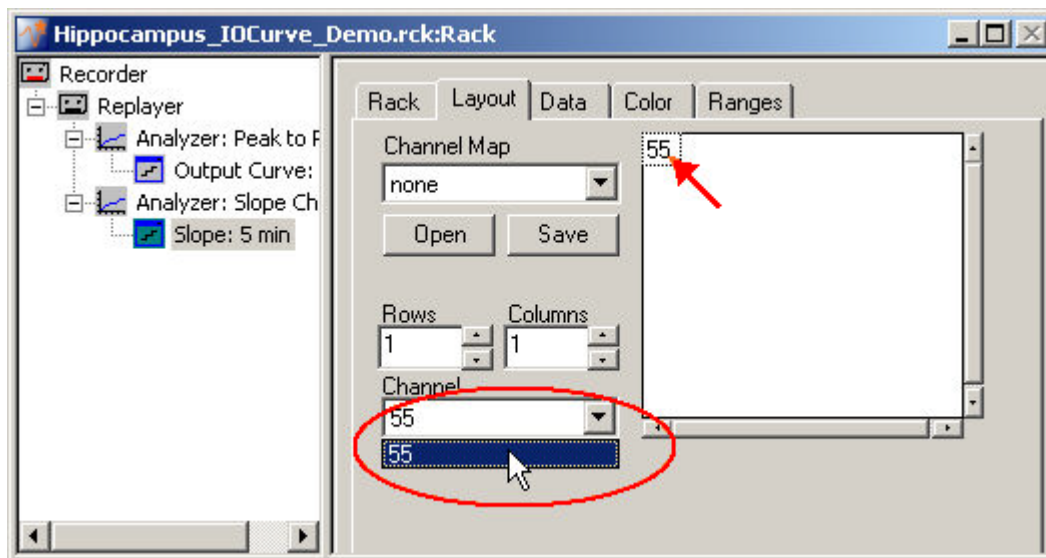
2. Define the region of interest with the vertical bars **T1** and **T2**. When choosing the **Slope 10%90%** option, it is not required to position T1 and T2 exactly, as 10 percent, that is, the non-linear part of the response, is not regarded. The slope is then computed from a linear fit regression line (Least Square Algorithm) in the ROI.




7.9 Customizing Displays

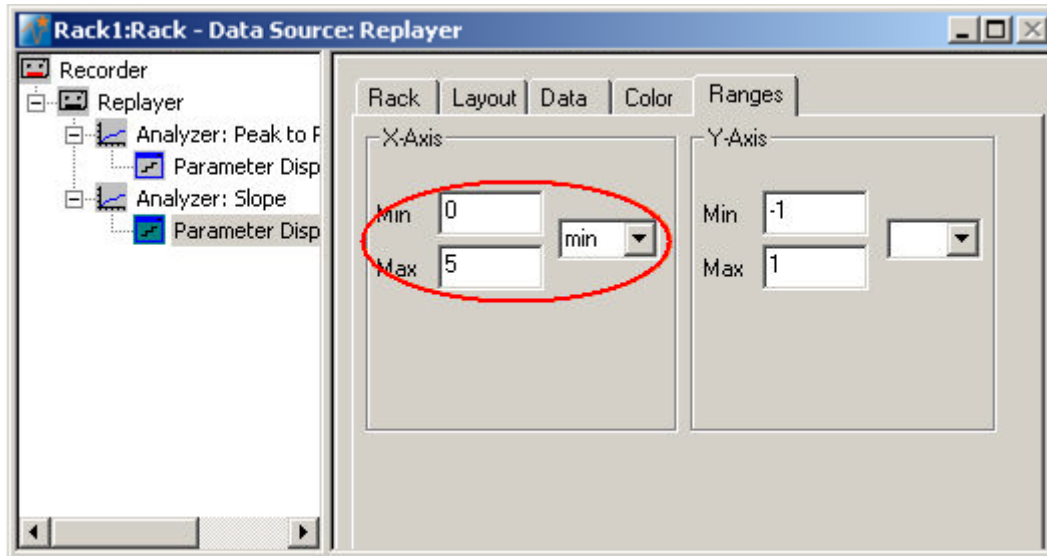
As it may not be useful or possible to extract the slope from all channels, you can set up your custom layout for the **Analyzer** or the **Parameter** display (this is true for all displays), to save space on the screen. You may be interested only in one or two representative channels. (Remember: You can save the parameters of all channels in the **Recorder** to review it later or to analyze it further in your custom evaluation software.)

1. Click the **Layout** tab.
The default layout is loaded. In the **Rows** and **Columns** boxes, you can now modify the layout.
2. Type or select the number of **Rows**, for example 1, and the number of **Columns**, for example 1.
On the right, a custom layout with a single channel appears.
3. Assign the appropriate channel numbers to the empty slots. Click the empty slot to select it.
The active slot is highlighted by a dotted line.
4. Select the channel of interest, for example, 55, for the active slot from the **Channel** list, or type **55**. The **Parameter Display** is updated accordingly. Likewise, you can set up any custom configuration for all display types.



7.10 Plotting a Slope Curve

1. On the toolbar, click  to add a second **Parameter Display** following the second **Analyzer** to the virtual rack configuration. (If it follows the first **Analyzer** in the tree view, it will not be possible to assign the output data stream of the second **Analyzer**, that is, the slope, to this **Parameter Display**.) You can rename it so that you will identify it later in the virtual rack.
2. Adjust the x-axis range to the total recording time of about 5 minutes.



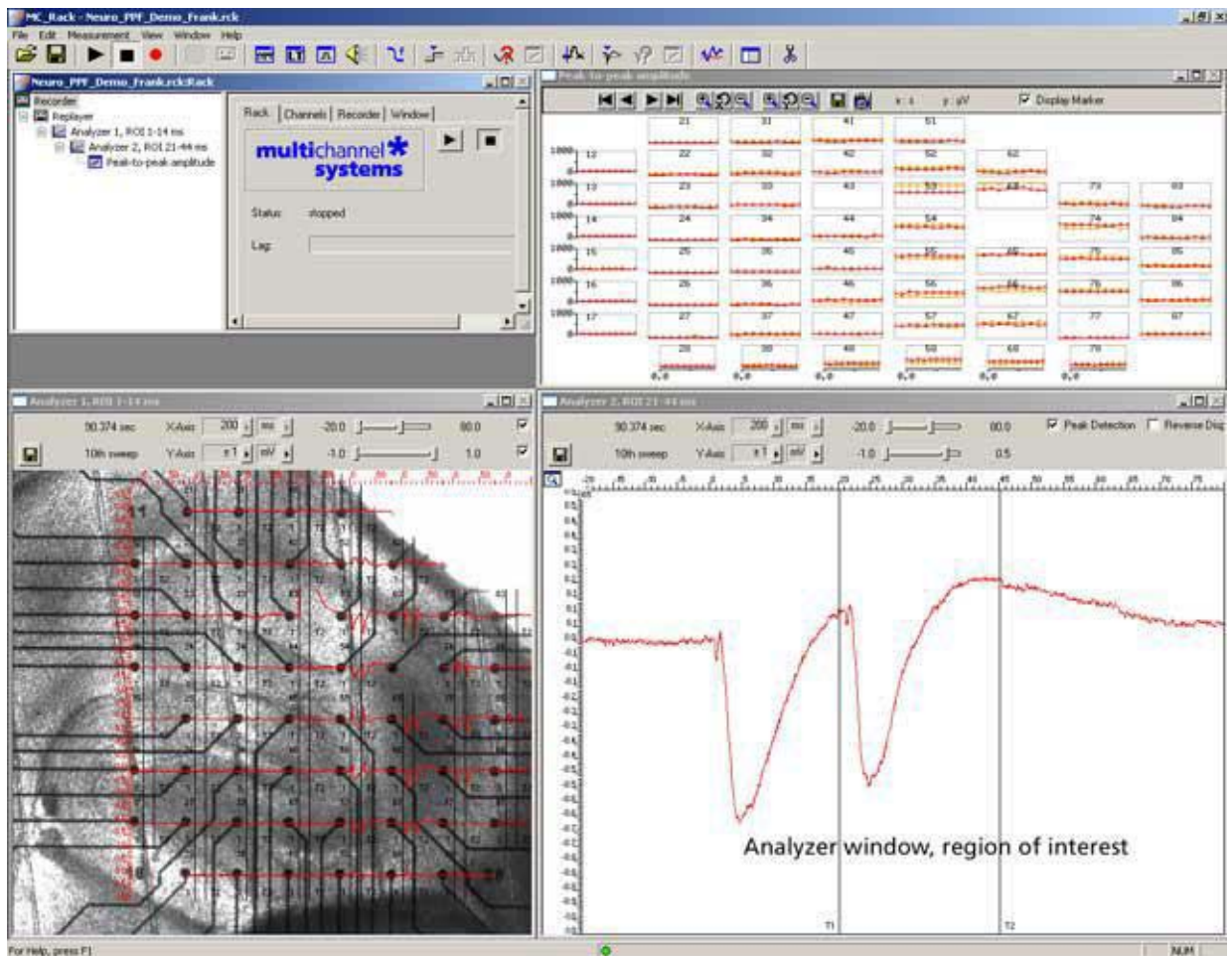
3. Start the rack to generate the plot.

The slope becomes more negative, that is, the decay time decreases, with an increasing stimulus amplitude.



8 Paired Pulse Facilitation (PPF) or Depression (PPD)

8.1 Aim of Experiment



In **Paired Pulse Facilitation (PPF)** or **Depression (PPD)** experiments, the stimulus is followed by a second stimulus, typically 2080 ms later. The magnitude of the population response to the second stimulus is compared to that of the first one. It is higher in case of a PPF, or lower in case of a PPD.

In MC_Rack, two (or more) regions of interest (ROI) can be defined, one triggered by the first stimulus, the other by the second stimulus, for example. The peak-to-peak amplitudes for both ROIs can be extracted online.

The example data file was recorded from a 300 μm thick **acute hippocampus slice** that was prepared from a 32 days old rat. The slice was stimulated with a single biphasic pulse (2 V amplitude, 100 μs per phase) by one of the embedded MEA-electrodes (No. 64). The second stimulus pulse was applied 20 ms following the first one. Recording was triggered by applying the Sync Out signal of the stimulus generator to one of the analog input channels (A1). Recorded sweeps are 200 ms, with a pre-trigger time of 30 ms, and a post-trigger time of 170 ms.

As the data was acquired with a MEA1060-BC amplifier with blanking circuit, all MEA electrodes were disconnected from the amplifier shortly during stimulation, in this case for **540 μs** (300 μs Sync Out pulse of the STG, plus intrinsic **Wait** of 40 μs , plus user-defined **Wait** of 200 μs). This efficiently prevented stimulus artifacts.

Some electrodes recorded PPF and others PPD in this demo data file.

The demo data used in this part of the tutorial was kindly provided by Dr. Frank Hofmann, University of Heidelberg, Germany.

MC_Rack Tutorial: MEA Application Examples

Task

Extract the field potential amplitude from two distinct regions of interest by an offline analysis; graph, print and save the results.

Note: In this chapter, you will set up an offline analysis rack (for the demo data file) step by step. You can set up a virtual rack for online recordings and analysis likewise, simply add the **MC_Card** as the data source to the rack instead of the **Replayer**.

- Rack file: Neuro_PPF_Demo.rck
- Data file: PPF_Data.mcd
- Image file: PPF_Image.jpg


Please see also the MEA Application Notes for more information on the preparation techniques and experiments.

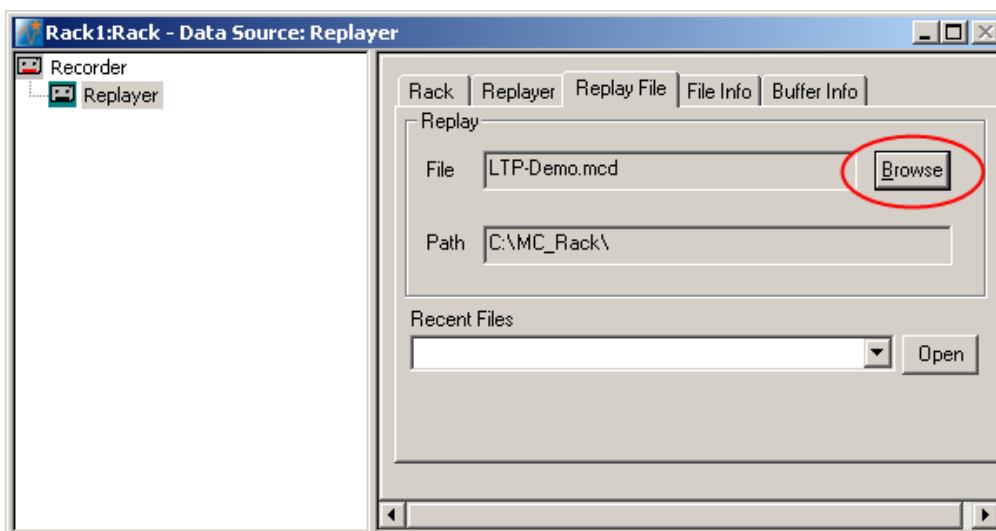
You will learn in this chapter ...

- How to define **basic functions** in your **rack configuration**:
 - Trigger MC_Rack on a TTL pulse (**Trigger Detector**)
 - Extract the peak-to-peak amplitude from two distinct regions of interest (**Analyzer**)
 - Plot the amplitude vs. time (**Parameter Display**)

8.2 Preparations

We recommend that you take some time for rebuilding the virtual rack for this application step by step in this tutorial, but if you prefer having a look at the completed rack or if you get stuck during the tutorial, you can also open the rack file "Neuro_PPF_Demo.rck". Click **Open** on the **File** menu to open the rack file.


1. Copy the **complete** MC_Rack **Tutorial** folder from the installation volume into the MC_Rack program directory with the following path "c:\Program Files\Multi Channel Systems\MC_Rack\"
2. Start MC_Rack or click **New** on the **File** menu to generate a new virtual rack file configuration.
3. Click  on the toolbar to add a **Replayer** to your virtual rack.
4. In the tree view pane of the virtual rack, select the **Replayer** and click the **Replay File** tab. Click the **Browse** button and browse to the **Offline** subfolder of the **Tutorial** folder, and load the data file **PPF_Data.mcd** into the **Replayer**.

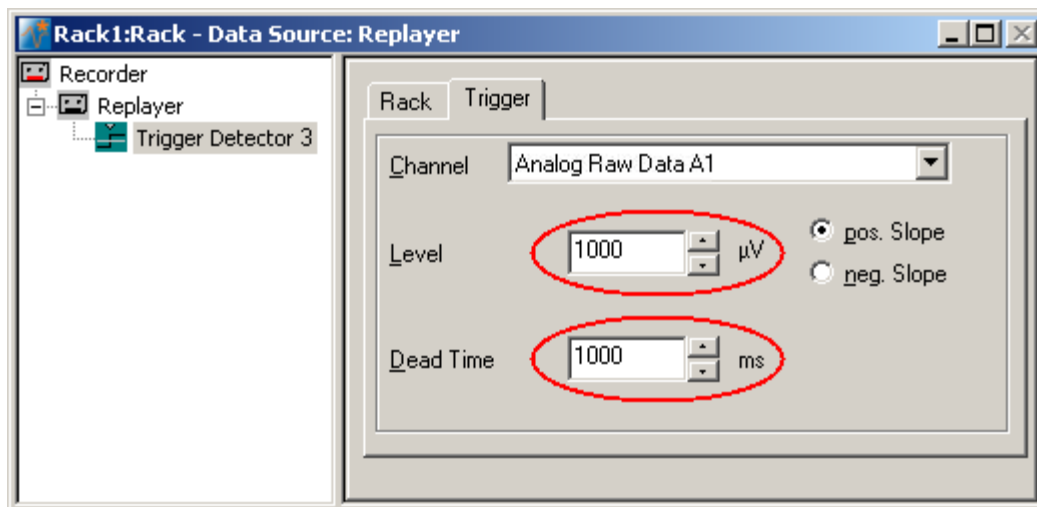


8.3 Triggering MC_Rack on the Stimulus

The analog input channel A1 was used to feed in the TTL signal from the Sync Out channel of the stimulus generator. You can use this TTL pulse to trigger the **Recorder** or any other virtual instruments. (As the trigger data stream was saved together with the data file, you can of course also use this trigger for replaying the data as well, but we recommend to set up a **Trigger Detector** on your own in this tutorial to learn how to do it.)


Note: It is recommended to use the digital input port for feeding in TTL signals. The analog inputs are intended for analog signals, like patch clamp data, for example.

1. Click  on the toolbar to add a **Trigger Detector** to the virtual rack. The **Trigger Detector** is automatically named **Trigger Detector 3**, as the replayed data file already contains 3 trigger data streams, so the data stream generated by this new **Trigger Detector** will be **Trigger 4**. You can rename it if you wish.
2. In the tree view pane of the virtual rack, select the **Trigger Detector**, and click the **Trigger** page.
3. From the **Channel** list, select the **Analog Raw Data A1** channel, to which the Sync Out channel of the stimulus generator was connected.
4. Enter a threshold. As the TTL signal has a 5 V amplitude, and the input voltage range of the MC_Card was +/- 2048 mV, 1 V should be fine.
5. Enter a dead time that is long enough so that the second stimulus of the PPF protocol does not generate a second trigger event, that is, longer than 20 ms. To be on the safe side, enter 1000 ms, for example.



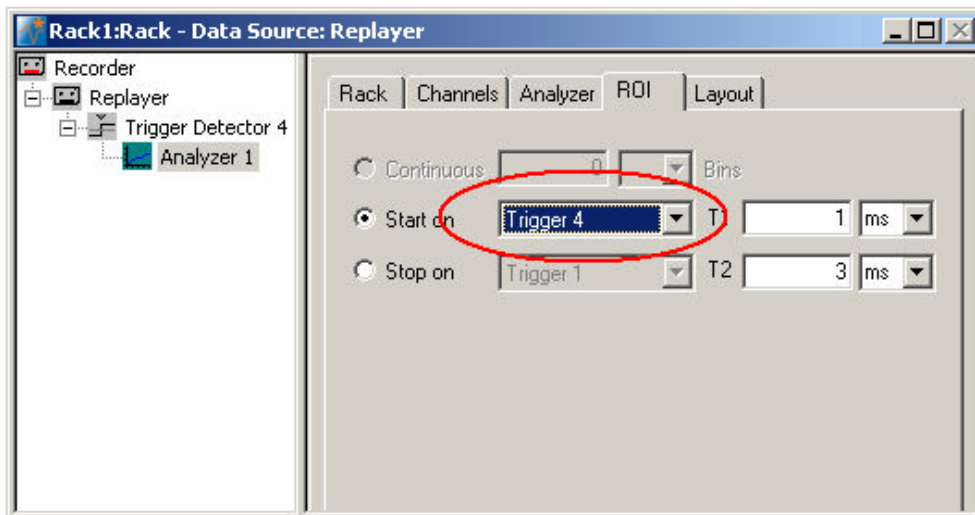
8.4 Extracting the Peak to Peak Amplitude

For extracting the peak-to-peak amplitude, you will need an **Analyzer**.

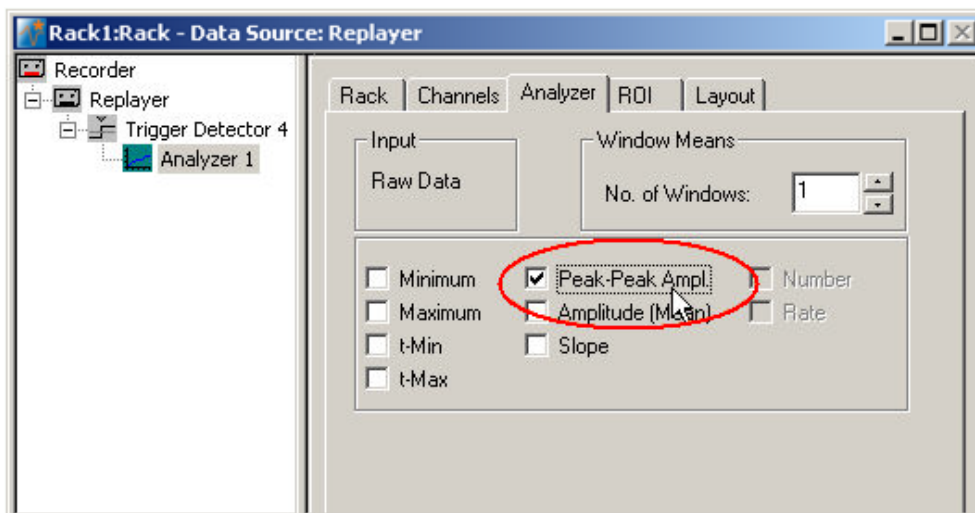
1. In the tree view pane of the virtual rack, select the **Trigger Detector** and click  on the toolbar to add an **Analyzer** in **series** with the **Trigger Detector** to the virtual rack. (If you would put both instruments in parallel, you would not be able to use the trigger stream generated by the **Trigger Detector** for triggering the **Analyzer**, because virtual instruments can only use the output streams of other virtual instruments that are upstream in the virtual rack tree.) You can rename it so that you can identify it later in the virtual rack.

The **triggered Analyzer** display with the **region of interest (ROI)** appears because the data was recorded as triggered sweeps.

2. Rename the **Analyzer** to **ROI 1** to distinguish it from the second analyzer that we will set up later.
3. In the tree view pane of the virtual rack, select the **Analyzer**, and click the **ROI** tabbed page. The recording trigger **Trigger 1** is already preselected, but you can also select the **Trigger 4** that is generated by the **Trigger Detector 4**.



4. Click the **Channels** tab, and select the **electrodes of interest** (default is all).
5. In the tree view pane of the virtual rack, select the **Analyzer**. Select the **parameters of interest**, **Peak-Peak Ampl.** in this case.



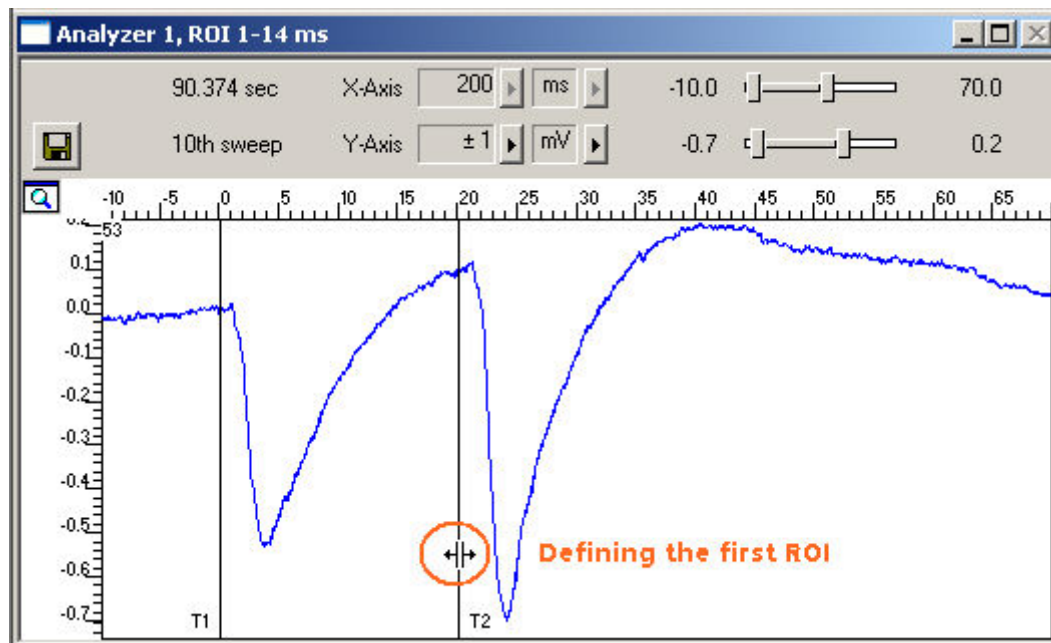
8.5 Defining the Region of Interest

In triggered mode, the **Analyzer** extracts the parameters of interest from a distinct **time window** relative to the **trigger event**, the region of interest (**ROI**). The parameter of interest, the peak-to-peak amplitude in this case, is then extracted from the data trace in the region of interest. You can define the region of interest with the two vertical bars in the **Analyzer** display or by editing the **T1** and **T2** boxes on the **ROI** tabbed page of the **Analyzer**.

1. Click **Start**  (either on the **Measurement** menu, the toolbar, or the **Rack** tabbed page) to start the replaying of the data.


Each virtual instrument in the rack configuration starts to process the channels and data streams that were assigned to it. For example, data is graphed in the displays, and so on. You can adjust the replaying **speed** on the **Replayer** tabbed page of the **Replayer**.

2. Double-click on any electrode channel with a large response in the **Analyzer** display to **zoom** into the data recorded from this electrode. Use the sliders to adjust the ranges so that you can clearly see the response.
3. Position the bars **T1** and **T2** so that the field potential following the first stimulus is clearly inside the ROI.



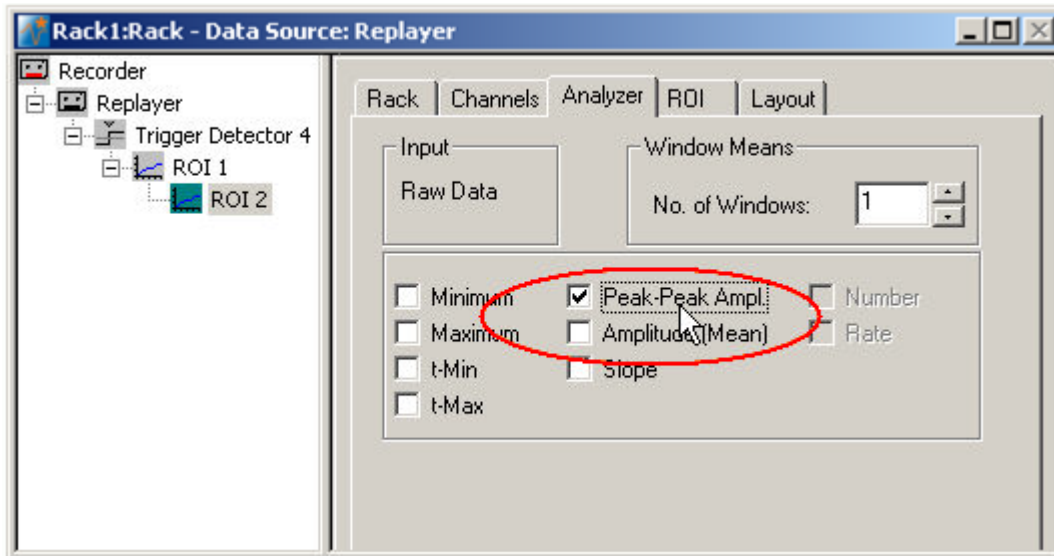
8.6 Defining the Second Region of Interest

For defining the second region of interest in order to extract the amplitude of the response to the **second** stimulus, you will need to set up a second **Analyzer**. You need to configure the second **Analyzer** similar to the first one, except the distinct ROI.

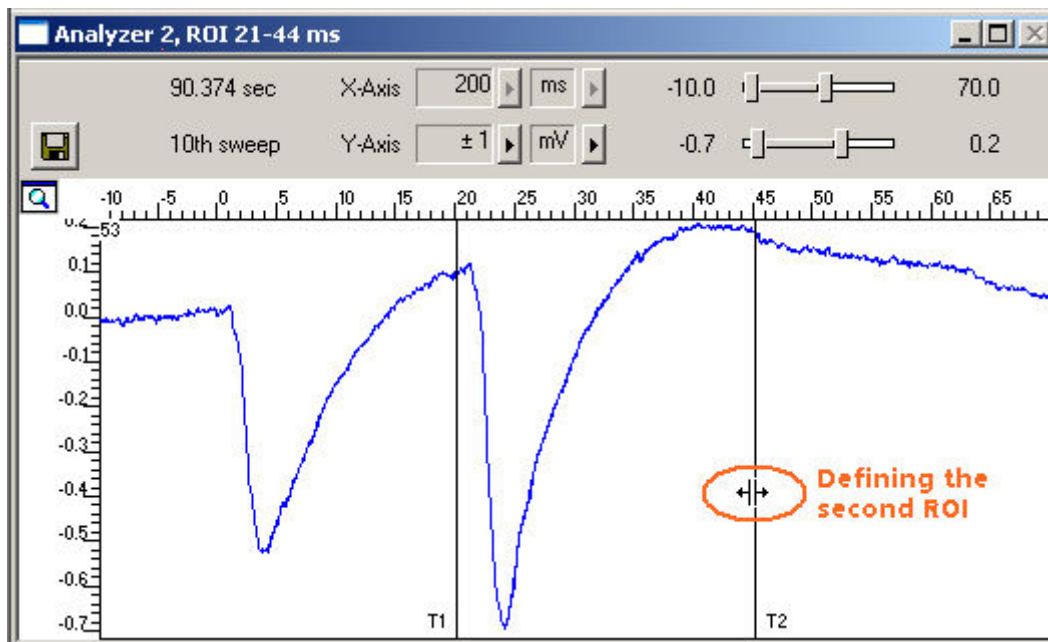
1. Click  on the toolbar to add a second **Analyzer** in **serial** to the first **Analyzer** to the virtual rack. (You will then be able to overlay the parameters extracted from both ROIs in the same **Parameter Display**. If you put the instruments in parallel, you would need two separate **Parameter Displays** for graphing the traces.)
2. Rename the **Analyzer** to **ROI 2** to distinguish it from the first analyzer.
3. In the tree view pane of the virtual rack, select the **Analyzer**, and click the **Channels** tab. Select the same electrode channels that you have selected for the first **Analyzer**.

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- Click the **Analyzer** tab and select the parameters you like to extract, again **Peak-to-peak Ampl.** in this case.




- Click the **ROI** tabbed page. The recording trigger **Trigger 1** is already preselected, but you can also select the **Trigger 4** that is generated by the **Trigger Detector 4**.
- In the **Analyzer** window, double-click on any electrode with a large response to zoom into the data recorded from this electrode. Use the sliders to adjust the ranges so that you can clearly see the response.
- Position the bars **T1** and **T2** so that the field potential following the second stimulus is clearly inside the region of interest, or enter the appropriate values (T1 and T2 of the first Analyzer + 20 ms) directly into the **T1** and **T2** text boxes on the **ROI** page.



8.7 Plotting Amplitude versus Time

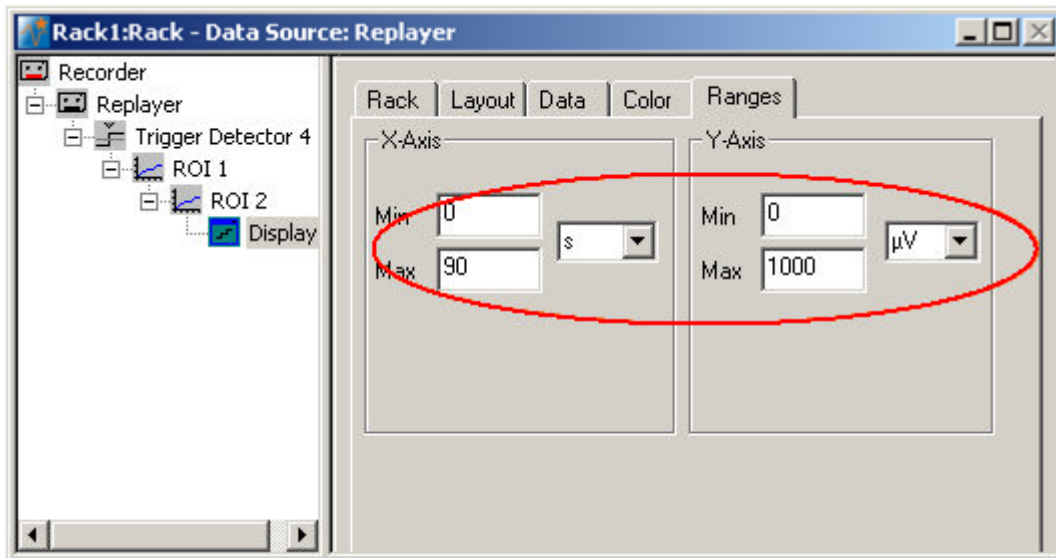
Add a display to graph the Peak-to-Peak plot. As a parameter data stream is very different from raw data, MC_Rack features a specialized display type, the **Parameter Display**, which is optimized for parameter streams.

- In the tree view pane of the virtual rack, select the second (bottom) **Analyzer** and click  on the toolbar to add a **Parameter Display** in **series** with both **Analyzers** to the virtual rack. (It would not be possible to add the **Parameter Display** in parallel to the **Analyzers**, as the replayed data file does not contain any parameter streams as input streams for the display, and virtual instruments can only use the output streams of other virtual instruments that are upstream in the virtual rack tree.)

Setting the window extent

The total recording time of the example file is 90 s, as you can see on the **File Info** page of the **Replayer**. Adjust the ranges of the y-axis as well.

1. Click the **Ranges** tab.
2. Enter 90 s for the x-axis range, and 1000 μV for the y-axis.



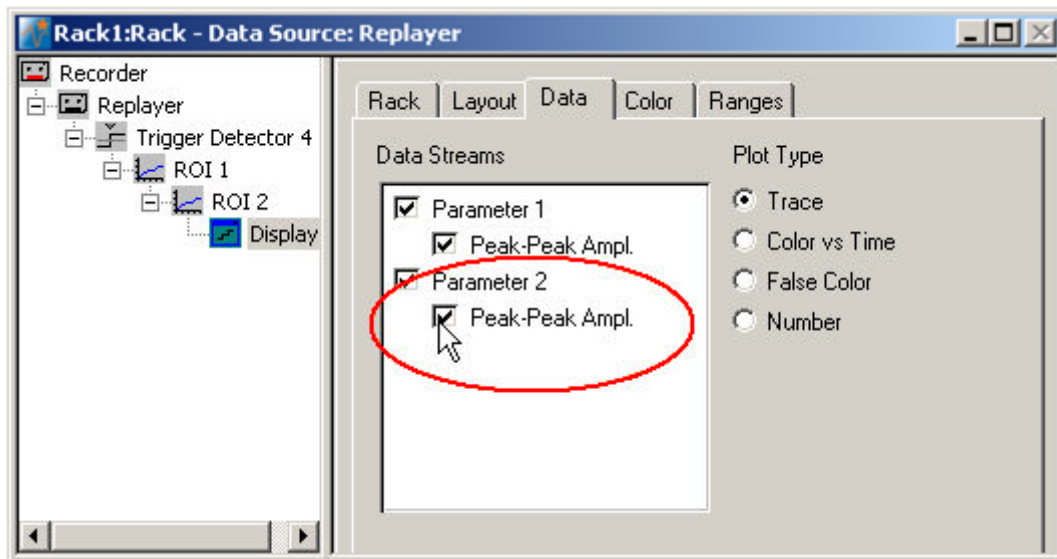
Selecting data streams


Now, select the data that you like to be displayed.

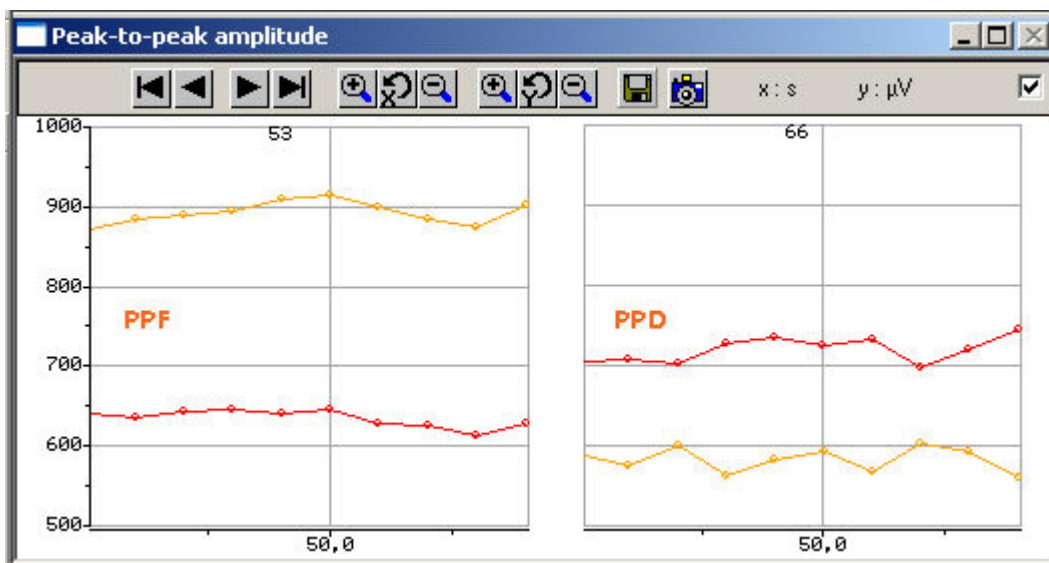
Note: You can only plot the data streams in the parameter plot that are generated by virtual instruments that are higher in the hierarchy of the rack tree and directly connected with the **Parameter Display**. In this rack configuration, both **Analyzers (ROI 1 and ROI 2)** are **upstream** the **Parameter Display** in the hierarchy (see screen shot below); therefore, both **Parameter 1** and **Parameter 2** streams can be used by the display, that is, are visible on the **Data** page. If the two **Analyzers** were set up in two separate tree branches (in parallel), you would need two separate **Parameter Displays**, each downstream of each of the **Analyzers**.

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1. In the tree view pane of the virtual rack, select the **Display 1**, and select the data streams that you want to plot. You can overlay the extracted amplitudes from both regions of interest, or you can set up two individual **Parameter Displays** for plotting the responses separately.

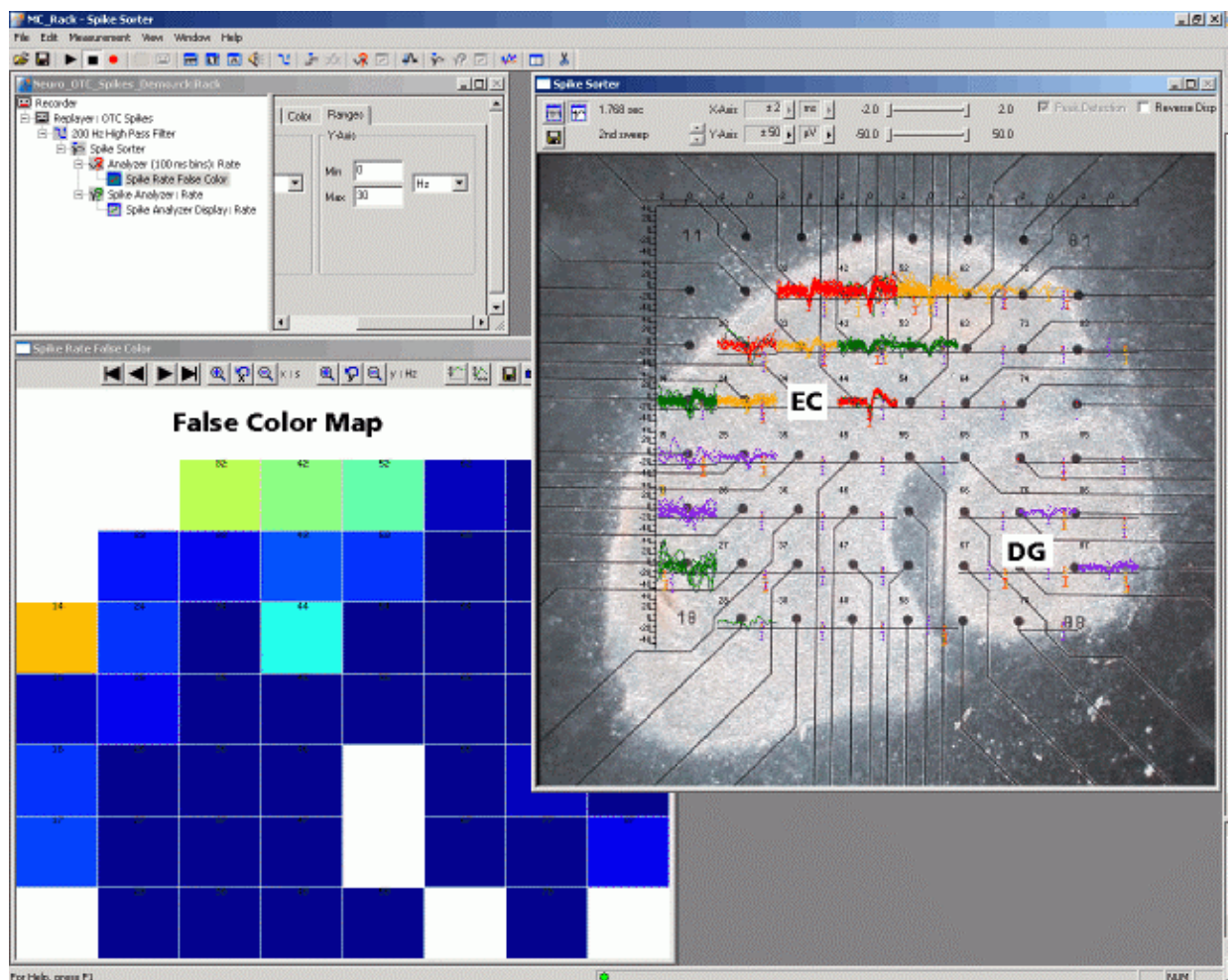


2. Click **Start**  to plot the extracted parameters. The two different data streams are plotted in different colors (red from the first pulse, yellow from the second pulse). You can see a PPF on electrode 53, and a PPD on electrode 66, for example.



9 Spike Activity (Organotypic culture)

9.1 Aim of Experiment



Organotypic slices of the **dendate gyrus (DG)** and the **entorhinal cortex (EC)** taken from a PND 6 rat were **co-cultured** on MEAs for seven days. Both subregions showed an independent spontaneous spike activity.

In an acute experiment, the slice (generally from adult animals) is used directly after preparation and discarded after the experiment. Slices from neonatal animals can also be kept in prolonged culture, over several weeks or even months, as so called **organotypic cultures (OTC)**. Even though the synaptic organization is not exactly the same as in native tissue, the main characteristics and functions are preserved. OTCs have the advantage that they allow to observe the electrophysiological activity over a longer period of time on the same slice. Also, organotypic slices tend to thin out on the MEA, which is advantageous for optical imaging. OTC experiments are especially useful for **longterm experiments**, for example, for monitoring the **development** of the neuronal networks and its electrophysiological activity, the behavior of co-cultured slices from different regions and the **regeneration** of tissue (like in this experiment), or longterm **drug effects**.

The demo data used in this part of the tutorial was kindly provided by Dr. Frank Hofmann, University of Heidelberg, Germany.

Task

Extract spikes and visualize the two-dimensional distribution of activity by an offline analysis; graph, print and save the results.

MC_Rack Tutorial: MEA Application Examples

Note: In this chapter, you will set up an offline analysis rack (for the demo data file) step by step. You can set up a virtual rack for online recordings and analysis likewise, simply add the **MC_Card** as the data source to the rack instead of the **Replayer**.

- Rack file: Neuro_OTC_Spikes_Demo.rck
- Data file: Neuro_OTC_Spikes_Demo.mcd
- Image file: OTC Spikes Image.jpg


Please see also the MEA Application Notes for more information on the preparation techniques and experiments.

You will learn in this chapter ...

- How to define **basic functions** in your **rack configuration**:
 - Remove field potentials with a filter for a following spike extraction
 - Detect spikes
 - Analyze the spike rate
 - Visualize the spatial distribution of spike activity



9.2 Preparations

We recommend that you take some time for rebuilding the virtual rack for this application step by step in this tutorial, but if you prefer having a look at the completed rack or if you get stuck during the tutorial, you can also open the rack file "Neuro_OTC_Spikes_Demo.rck". Click **Open** on the **File** menu to open the rack file.

1. Copy the **complete MC_Rack Tutorial** folder from the installation volume into the MC_Rack program directory with the following path "c:\Program Files\Multi Channel Systems\MC_Rack\"
2. Start MC_Rack or click **New** on the **File** menu to generate a new virtual rack file configuration.
3. Click  on the toolbar to add a **Replayer** to your virtual rack.
4. In the tree view pane of the virtual rack, select the **Replayer** and click the **Replay File** tab. Click the **Browse** button and browse to the **Offline** subfolder of the **Tutorial** folder, and load the data file **Neuro_OTC_Spikes_Demo.mcd** on the installation volume.

9.3 Monitoring Raw Data

You are ready to start to replay the data. We start with a simple **Data Display** for monitoring the raw data traces first.

1. On the toolbar, click  to add a **Data Display** to the virtual rack and assign the **Electrode Raw Data** stream to it. Make sure that an 8x8 MEA Channel Map is loaded on the **Layout** page of the display.
2. Click **Start**  (either on the **Measurement** menu, the toolbar, or the **Rack** tabbed page) to start the replaying of the data.

Each virtual instrument in the rack configuration starts to process the channels and data streams that were assigned to it. For example, data is graphed in the displays, and so on. You can adjust the replaying **speed** on the **Replayer** tabbed page of the **Replayer**. The recorded data traces are displayed in the (continuous) **Data Display**.

3. Zoom in the ranges so that you can see the signals clearly.

Peak Detection

With **Peak Detection** switched off, you do not see any spike bursts, the data looks atypical for spikes.

Peak Detection is a very important display option. The massive amount of data points retrieved in the range of the time axis, 1000 ms in this case, that means 2500 data points at a sampling rate of 25 kHz, are reduced to a few pixels on the display, about 90 pixels. That means, about 28 data points are reduced to one. Without **Peak Detection**, only every 28th data point is actually plotted. The other 27 are skipped. As you can imagine, faster signals like spikes cannot be identified in the data at all.

With **Peak Detection**, the highest and the lowest data point of the five pixels in this example are taken and connected to a straight vertical line. This results in a much more realistic representation of the data. On the other hand, this feature needs a higher computer performance for the internal data handling. You may want to deselect this option when you have a limited computer performance, indicated by a **Performance Limit** error message., or if you do not need the feature, because you are recording slow field potentials only.

1. Select **Peak Detection** on the display toolbar for a more realistic data representation, and start the **Replayer** again.

On the following screen shots, you can see that the spikes are only visible if **Peak Detection** has been selected. This option affects only the **graphical representation** of data, **not** the recorded or exported data.

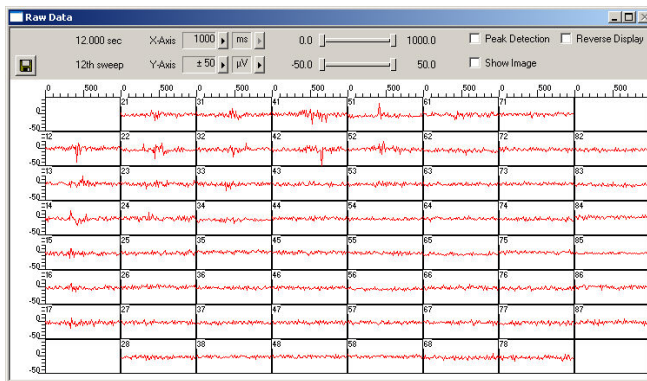


Fig. 3 Without Peak Detection

Due to the graphical representation of the data, no fast signals like spikes are visible in the display.

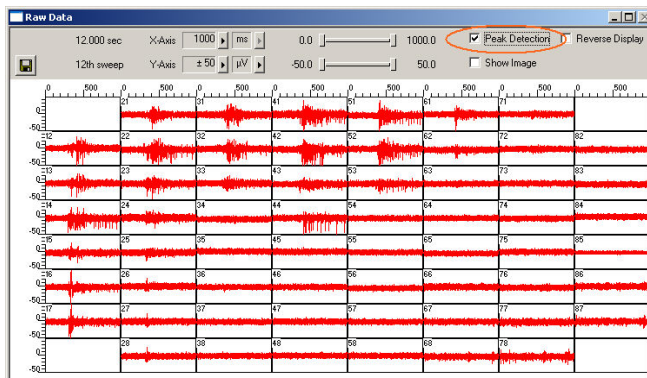
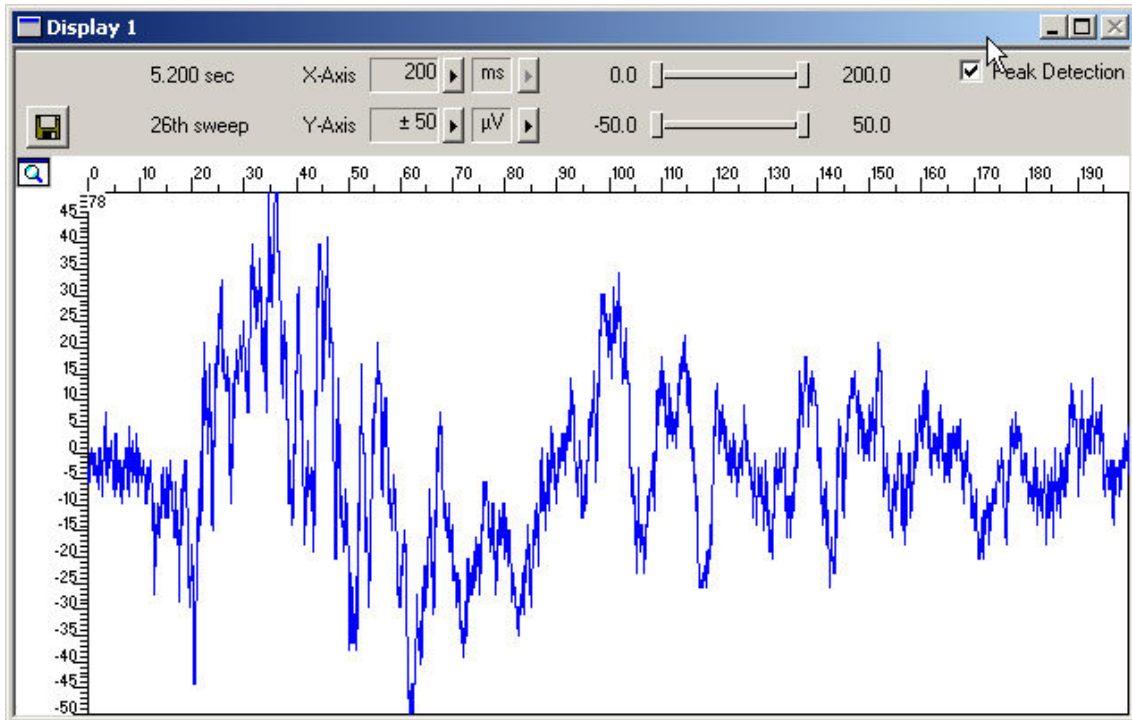


Fig. 4 With Peak Detection



This is the same data as and display ranges as in the preceding screen shot. With **Peak Detection**, you now see bursting activity on the upper left half of the MEA.

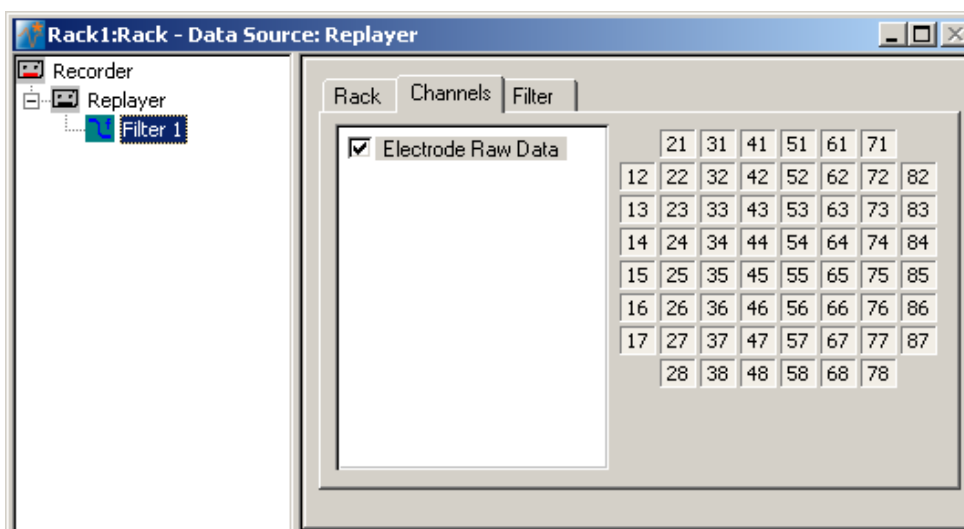
9.4 Removing Field Potentials with a Filter

1. Zoom in the signals a bit more. As you can see, **spike signals** are superimposed on **local field potentials**, which are built up by signals of cells firing simultaneously.

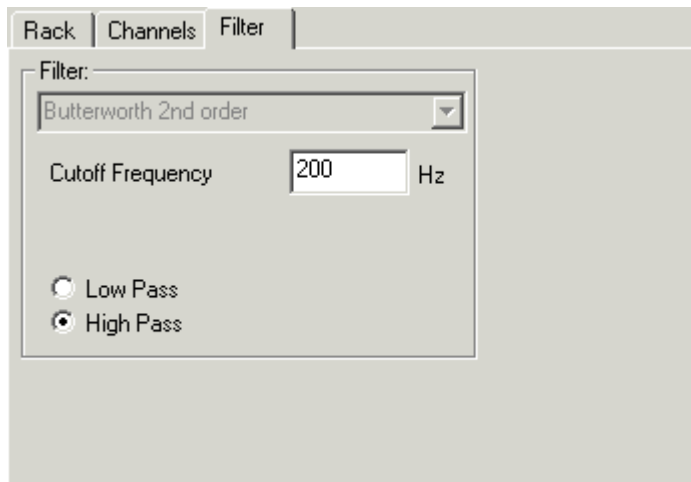



If you would try to detect peaks by a **threshold**, you would miss most of the peaks. To circumvent this problem, you can use a digital filter that removes slow signals, that is, field potentials. In the current version of MC_Rack, a second order Butterworth filter is available. Please be aware that all filters deform signals. It has always to be made a compromise between good filtering and low signal deformation. (The other option would be to use the **Slope** mode of the **Spike Sorter**. In this mode, the **Spike Sorter** detects spikes on the basis of the waveform instead of a threshold, and thus is able to extract spike regardless of the baseline or underlying field potentials.)

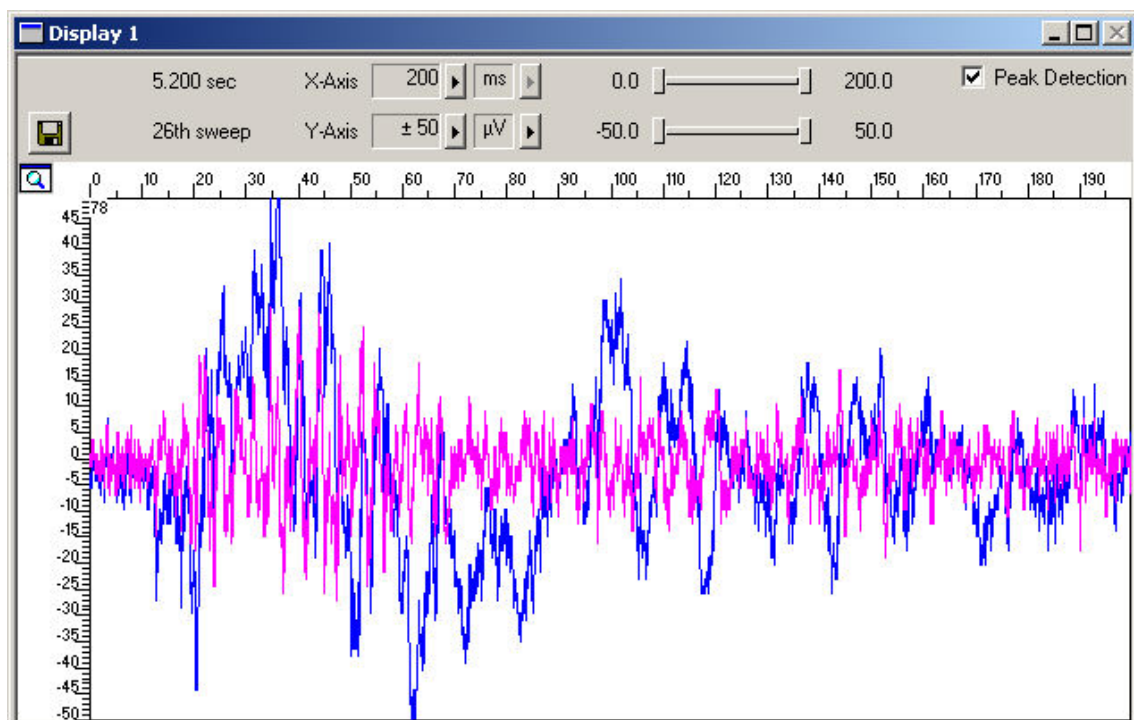
2. Click the **Delete Instrument**  button to remove the **Data Display** again to save computer performance.
3. On the toolbar, click  to add a **Filter** to your rack.
4. Click the **Channels** tab and select all channels.



- Click the **Filter** tab.



- Select **High Pass**.
You can choose between two filter types: **Low Pass** and **High Pass**. As the name suggests, a low pass filter passes the lower frequency components but attenuates the higher frequency components of signals. To remove slow components (with a low frequency) and to let pass high frequency signals, you need a high-pass filter.
- Type in **200 Hz** as the **Cutoff Frequency**. The high-pass filter now cuts off frequencies below the specified 200 Hz. For spike activity, you usually use a **Cutoff Frequency** of 200 Hz or above, depending on the frequency of the noise and the slope / waveform of the spikes. The cutoff frequency of the filter must be compatible with the frequencies present in the signal of interest.
- Add a **Data Display** to your rack. Load the 8x8 MEA **Channel Map**. On the **Data** tabbed page, select the **Electrode Raw Data** and the **Filtered Data** streams.
- Click **Start**  to start the **Replayer**, and zoom the traces.
The filtered data traces are displayed superimposed (magenta) on the unfiltered data (blue). You see that the field potentials have been more or less removed. You can now remove the display again from the rack to save computer performance.



9.5 Detecting Spikes


The **Spike Sorter** detects spikes and features an online (or offline) spike sorting into up to three units. The spike cutouts form a new data stream that can be analyzed further (or recorded to the data file).

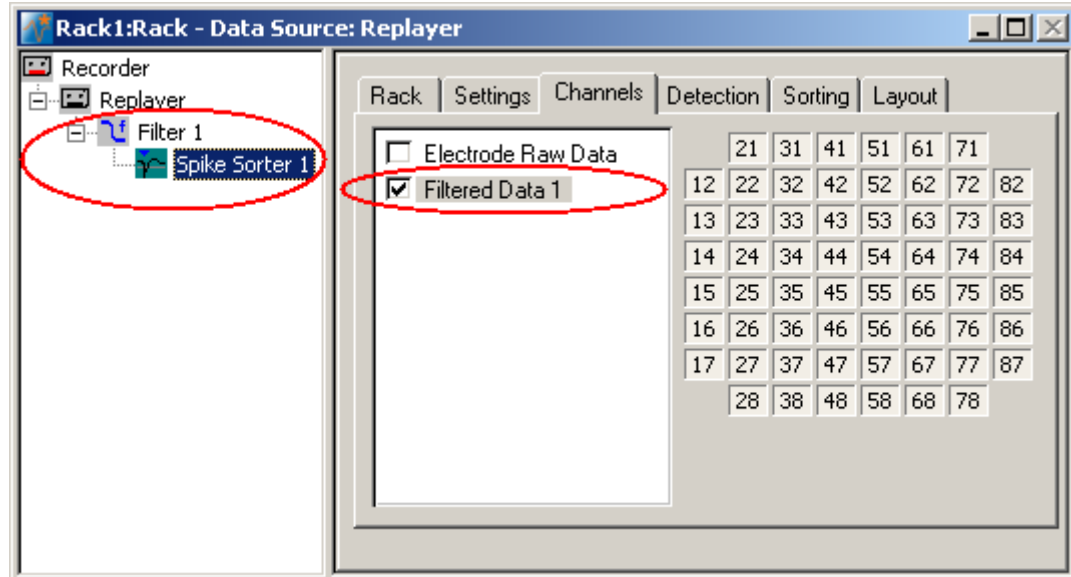
There are two modes for detecting spikes with MC_Rack. One method uses a **threshold**, the other recognizes the **amplitude** and **slope** of the waveform of interest. Both methods regard either the **rise** or the **fall** of the input signal, depending on your settings.

The **threshold** method is especially useful if the overall baseline is stable, and spikes appear approximately on the same height. If local field potentials are underlying the spikes, or if you have an unstable baseline, spikes will be missed, or noise signals may be detected as spikes with the threshold method. In this case, the **Slope** method is more appropriate.

In this tutorial, we have used a digital high pass **Filter** to remove field potentials and to prepare the data for using the **Threshold** method.

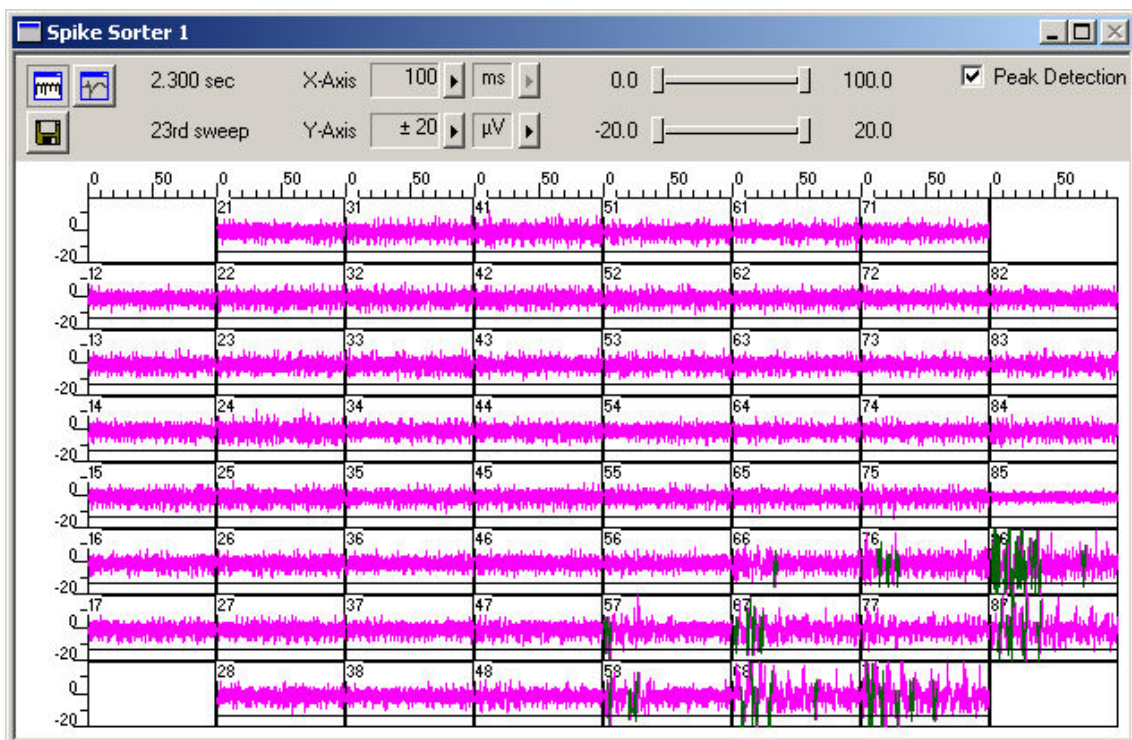
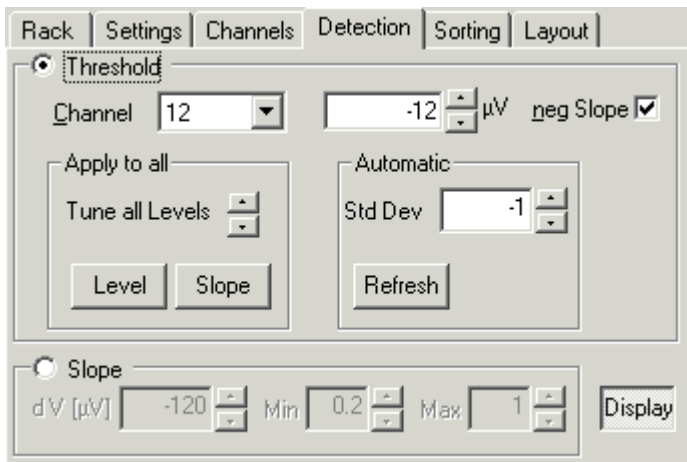
The **Spike Sorter** has a special display where you can either set the threshold, or sort spikes in an interactive kind of way. You can set the level for each channel separately (manually or automatically), or you can choose the same level for all channels.

1. In the tree view pane of the virtual rack, select the **Filter**. On the toolbar, click  to add a **Spike Sorter** in **series** with the **Filter** to the virtual rack. (If you put the **Spike Sorter** in parallel to the **Filter**, it would not be possible to use the filtered data stream as an input stream for the **Spike Sorter**, because virtual instruments can only use the output streams of other virtual instruments that are upstream in the tree view as input streams.) The **Spike Sorter** display appears (in detection mode).
2. Click the **Channels** tab and assign the **Filtered Data** stream to the **Spike Sorter**.




3. Select the **Peak Detection** option of the display. Click the **Start** button and zoom the display until you can see the signals clearly. You see that the baseline is on a similar level for all channels and that the spikes are quite small (approximately 10 to 15 μV).
4. Click the **Detection** tab. Make sure the **Threshold** method is selected. You can now set the detection threshold. Start with a value of 15 μV .
5. Type in 15 in the text box. This value is now applied only to the **selected** channel. Under **Apply to All**, click **Level** to apply the new detection level to all channels. Spike cutouts appear in a different color in the display. The detection level is visible as a horizontal straight line. You see that the level could be set a bit higher though to catch more spikes.

- Use the option **Tune all levels** to modify the level of all channels until the detection level seems to be optimized (approximately to $-12\ \mu\text{V}$). You can also click and drag the detection level (black line) of each channel in the **Spike Sorter** display with your mouse.

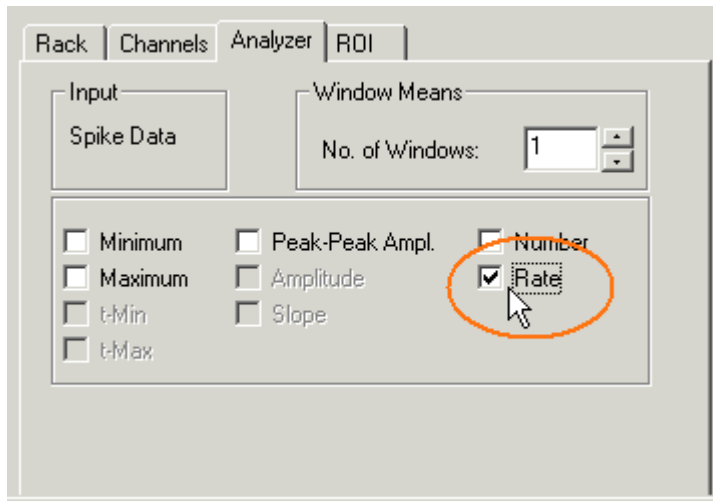


9.6 Analyzing the Spike Rate

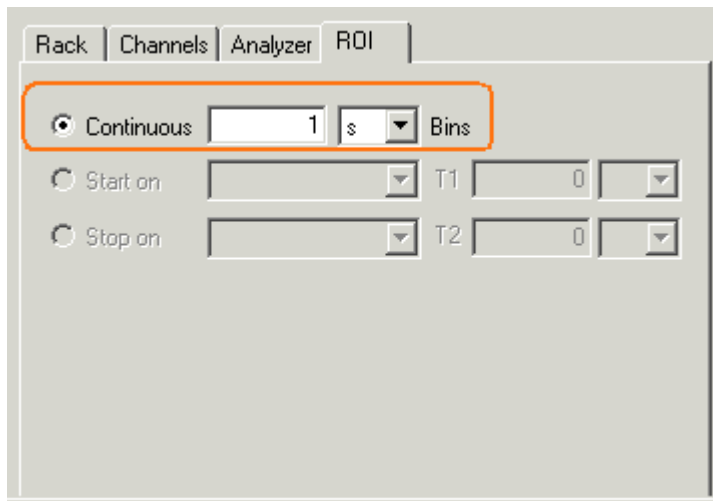
Hint: Instead of using the time-interval based **Analyzer**, you can also use the event-based **Spike Analyzer** for extracting the spike rate and the interspike-interval. Please see [MC_Rack Features > Analyzing Data > Analyzing Data](#) for details.

- On the toolbar, click  to add an **Analyzer** in **series** with the **Spike Sorter** to the virtual rack. (You cannot put the **Analyzer** in parallel to the **Spike Sorter**, because virtual instruments can only use the output streams of other virtual instruments that are upstream in the tree view as input streams.)
- In the tree view pane of the virtual rack, select the **Analyzer**. Click the **Channels** tab.
- Select all channels of the **Spikes** data stream.
- Click the **Analyzer** tab.

5. Select **Rate**.




The data stream is continuous, therefore the **Analyzer** should process the data continuously, too. Internally, the **Analyzer** operates on distinct pieces of data, so called bins. You can enter the bin size. Standard is 1 s, which is ok if the overall spike activity is going to be monitored.

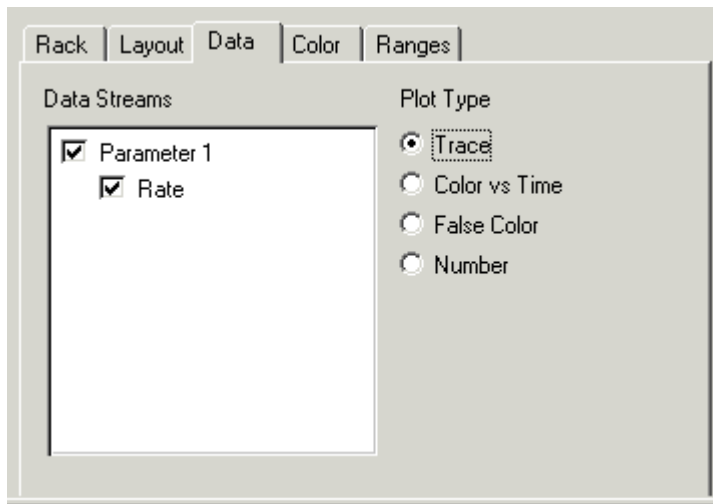


9.7 Plotting the Spike Rate

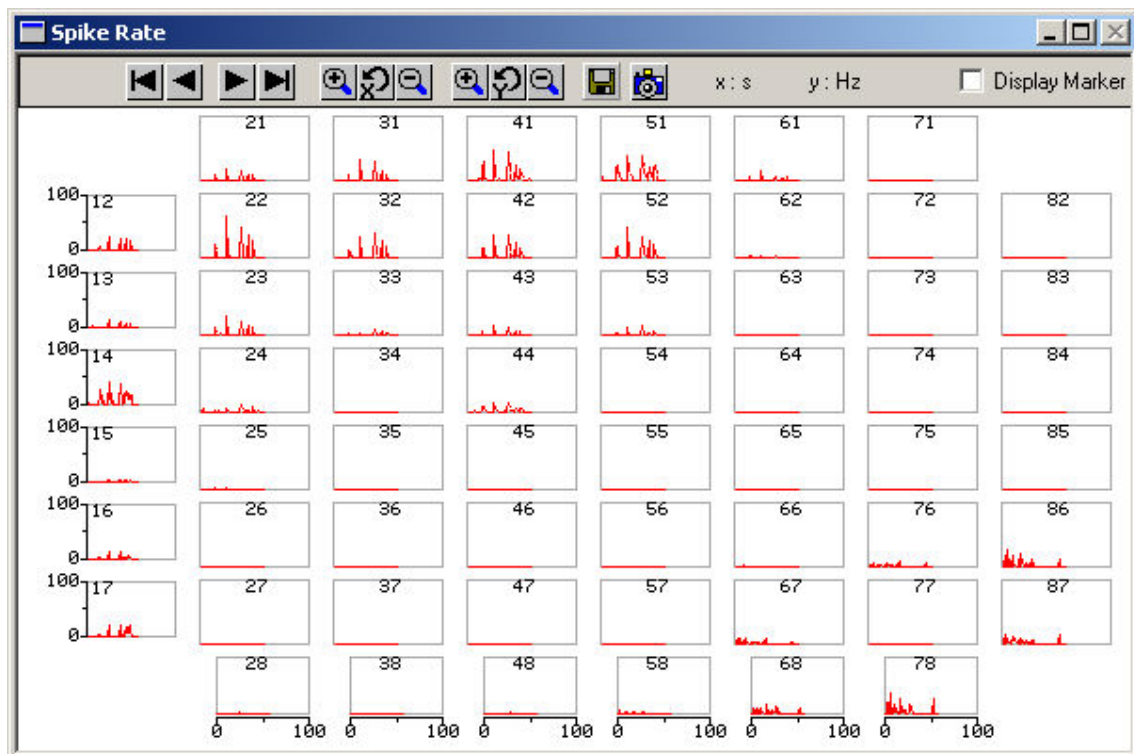
For plotting extracted parameters, we will need a **Parameter Display**, which features are optimized for graphing **Parameter** streams.

1. In the tree view pane of the virtual rack, select the **Analyzer** and click  on the toolbar to add a **Parameter Display** in **serial** with the **Analyzer** to the virtual rack. (It would not be possible to add the **Parameter Display** in parallel to the **Analyzer**, as the replayed data file does not contain any parameter streams as input streams for the display, and virtual instruments can only use the output streams of other virtual instruments that are upstream in the virtual rack tree.)
2. In the tree view pane of the virtual rack, select the **Parameter Display** and rename it to **Spike Rate**. Click the **Layout** tab to load the MEA Channel Map.

- Click the **Data** tab and select the **Parameter 1 Rate** stream.



- Start the **Replayer**.
The spike rate is plotted over time. You clearly see the two active tissues and the non-active area in the middle. The highest rate is about 60 Hz.



9.8 Visualizing the Spatial Distribution

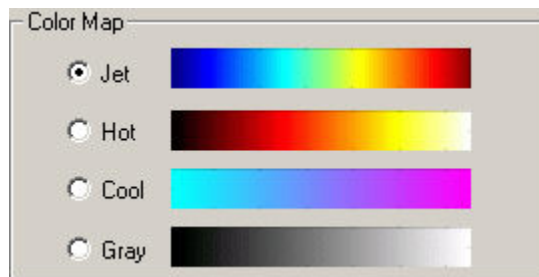
The **Trace** plot is good for monitoring the spike rate over the complete recording time. A **False Color** plot is the better choice for getting an idea of the spatial distribution and whether two regions fire simultaneously or independently. In a **False Color** plot, a specific color map is assigned to the parameter values, in this case, the spike rate.

- Select the **Parameter Display** and click the **Color** tab.
You see four color palettes. The selected **Color Map** defines the colors used for the plot. Each **Color Map** consists of **64 different colors** arranged in a particular order.

The **minimum and maximum range of the y-axis** (defined in the **Range** page) is assigned to the selected **Color Map**, that is, the **most left color corresponds to the minimum y-value** selected, and the **most right color corresponds to the maximum y-value**. The interval between min and max is straight-proportional to the colors in-between. The color of the actual value is picked from the **Color Map** accordingly. **Parameter values outside the defined min/max range** are plotted in **Black**.

Example: If the range was set from min = 0 Hz to max = 64 Hz, each color of the map would correspond to 1 Hz steps. Spike rates lower than 1 Hz would then be plotted as the first (most left) color of the color map, spike rates between 1 Hz and 2 Hz would be plotted as the second color of the color map, and so on. A value above 64 Hz would be plotted in black.

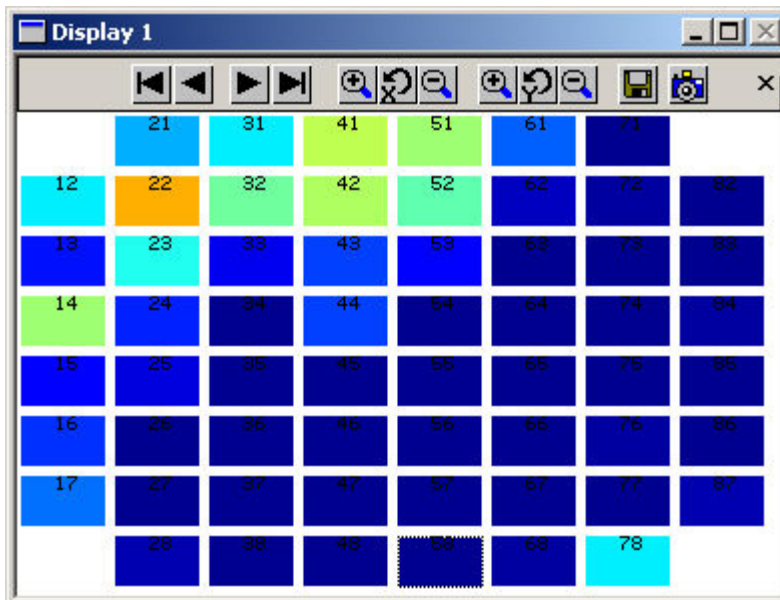
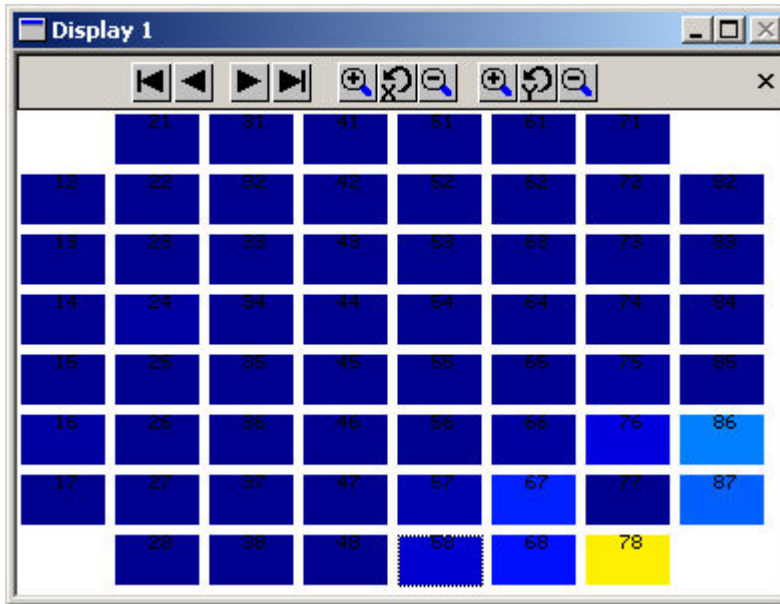
That means, for example, for the **Gray Color Map**, the lighter the color becomes, the higher is the spike rate.



1. Click the **Data** tab and select the **False Color** plot type.
2. Click the **Ranges** tab and set the y-axis to the maximum rate, that is, 100 Hz.
3. Start the **Replayer**.

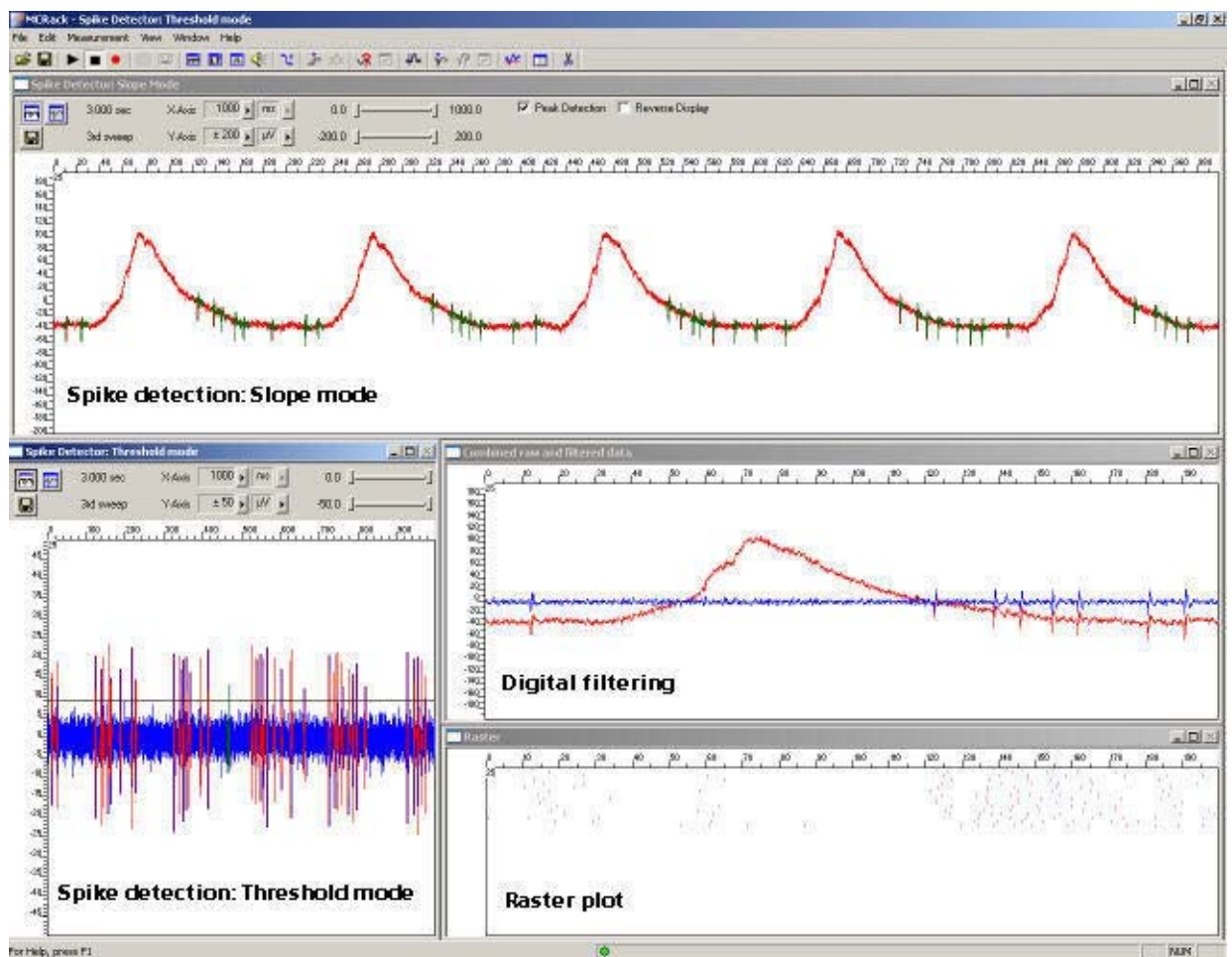
Spike Activity (Organotypic culture)

The first screen shot shows the right region firing, the second shows the left slice firing. You see now clearly that both brain slices fire independently. The **Jet** Color Map has been used here.



10 Retina: MicroERGs and Spikes

10.1 Aim of Experiment



The **retina** is a peripheral, easily accessible part of the central nervous system. **Stimulation** with **light pulses** results in a complex signaling by neurons within the layers of the retina. The retinal ganglion cells transmit retinal information to higher visual centers in the brain via their axons that form the optic nerve. Retinal function can be affected by acute injuries, intoxications, or retinal diseases, either inherited or acquired, resulting in visual impairment or even blindness.

The **electroretinogram (ERG)** is an **extracellular field potential** generated by the cells in the retina. So-called **microERGs** can be recorded with the MEA-System from **retina explants**, for example, from rat or chicken. For the recording of light-evoked activity, a retinal segment with the pigment epithelium, is placed ganglion cell site down on an MEA. Local ERGs (microERGs) with the typical components and **ganglion cell spikes** can be recorded with appropriate filter settings with the MEA-System. The prominent components of the microERG can be pharmacologically identified.

This *in vitro* **retina sensor** can be used for assessing effects of pharmacological compounds and putative therapeutics, drug side effects, and consequences of degeneration-related processes on retinal signalling.

(Reference: A. Stett, U. Egert, E. Guenther, F. Hofmann, T. Meyer, W. Nisch, H. Haemmerle; Biological application of microelectrode arrays in drug discovery and basic research. Analytical and Bioanalytical Chemistry, 2003. 377, 486-495.)

Task

Extract the **spiking activity superimposed** on **microERG field potentials** by an offline analysis; graph, print and save the results.

Note: In this chapter, you will set up an offline analysis rack (for the demo data file) step by step. You can set up a virtual rack for online recordings and analysis likewise, simply add the **MC_Card** as the data source to the rack instead of the **Replayer**.

10.1.1 Field potential electroretinogram

The example data shows a typical microERG recording. The demo data was kindly provided by the NMI Reutlingen, Germany.

- Rack file: **Retina_MicroERG_Demo.rck**
- Data file: **Retina_ERG.mcd**

10.1.2 Spiking activity

Spiking activity is visible in the ERG traces as well and can be easily extracted from the underlying field potential with the **Spike Sorter** in **Slope** mode.

Alternatively, the field potentials can be removed by a digital 300 Hz High Pass filter, and spikes can be detected by setting a threshold. This sample rack demonstrates both methods, and the spike sorting feature.

The raster plot highlights the regular spike pattern.

The demo data used in this part of the tutorial was kindly provided by the NMI Reutlingen, Germany.

- Rack file: **Retina_Spikes_Demo.rck**
- Data file: **Retina.mcd**


Please see also the MEA Application Notes for more information on the preparation techniques and experiments.

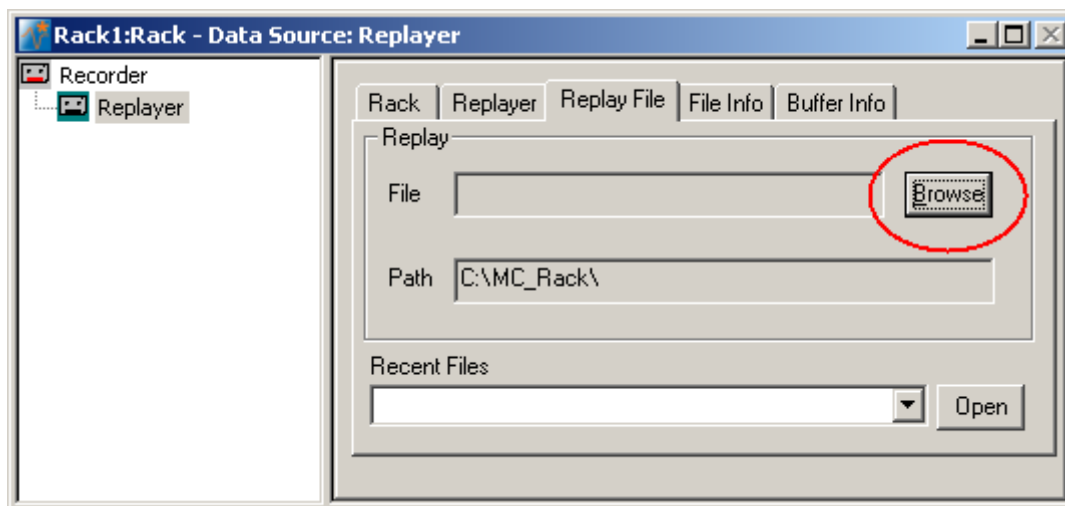
You will learn in this chapter ...

- How to define **basic functions** in your **rack configuration**:
 - Remove field potentials for extracting spike signals by digital filtering (**Filter**).
 - Detect spikes by defining the spike detection level (**Spike Sorter, Threshold** mode).
 - Detect spikes with an underlying field potential based on the waveform (**Slope** mode).
- How to **customize** the rack:
 - Modify the display layout (all display types).

10.2 Preparations



We recommend that you take some time for rebuilding the virtual rack for this application step by step in this tutorial, but if you prefer having a look at the completed rack or if you get stuck during the tutorial, you can also open the rack file **Retina_Spikes_Demo.rck**. Click **Open** on the **File** menu to open the rack file.

1. Copy the **complete** MC_Rack **Tutorial** folder from the installation volume into the MC_Rack program directory with the following path "c:\Program Files\Multi Channel Systems\MC_Rack\"
2. Start MC_Rack or click **New** on the **File** menu to generate a new virtual rack file configuration.
3. Click  on the toolbar to add a **Replayer** to your virtual rack.
4. In the tree view pane of the virtual rack, select the **Replayer** and click the **Replay File** tab. Click the **Browse** button and browse to the **Offline** subfolder of the **Tutorial** folder, and load the data file **Retina.mcd** into the **Replayer**.



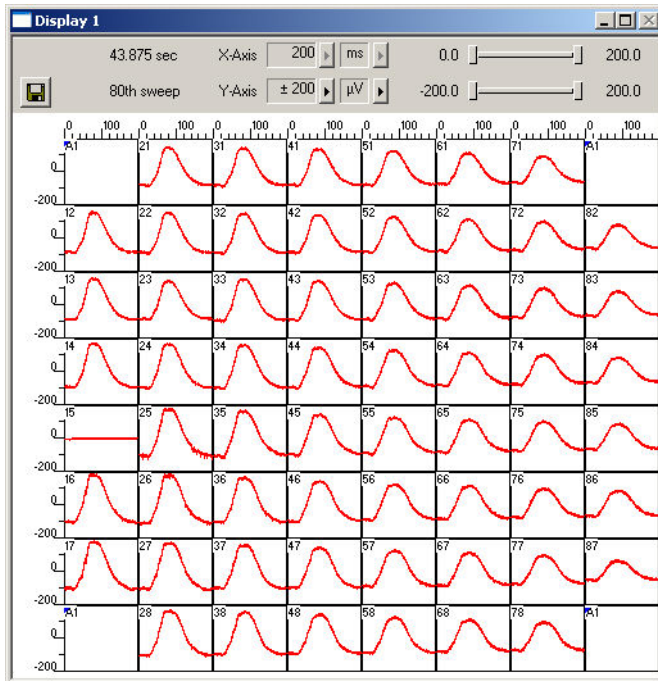
10.3 Monitoring Field Potentials and Spikes

With the MEA-System, you can record slow signals like field potentials and fast signals like spikes in parallel, depending on the amplifier settings.

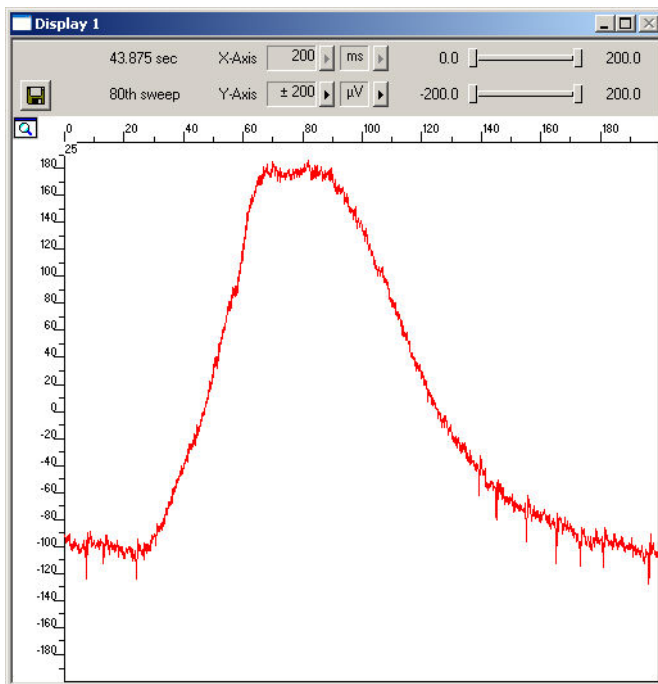
1. Click  on the toolbar to add a **Data Display** to the virtual rack.
2. Click **Start**  (either on the **Measurement** menu, the toolbar, or the **Rack** tabbed page) to start the replaying of the data.
Each virtual instrument in the rack configuration starts to process the channels and data streams that were assigned to it. For example, data is graphed in the displays, and so on. You can adjust the replaying **speed** on the **Replayer** tabbed page of the **Replayer**.

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- Zoom the axes to see the signals clearly. You see large sum potentials on all electrodes. Spikes are not detectable on all channels, but you can see spikes on electrode 25.




- Zoom into the data recorded from electrode 25 by double-clicking display 25.




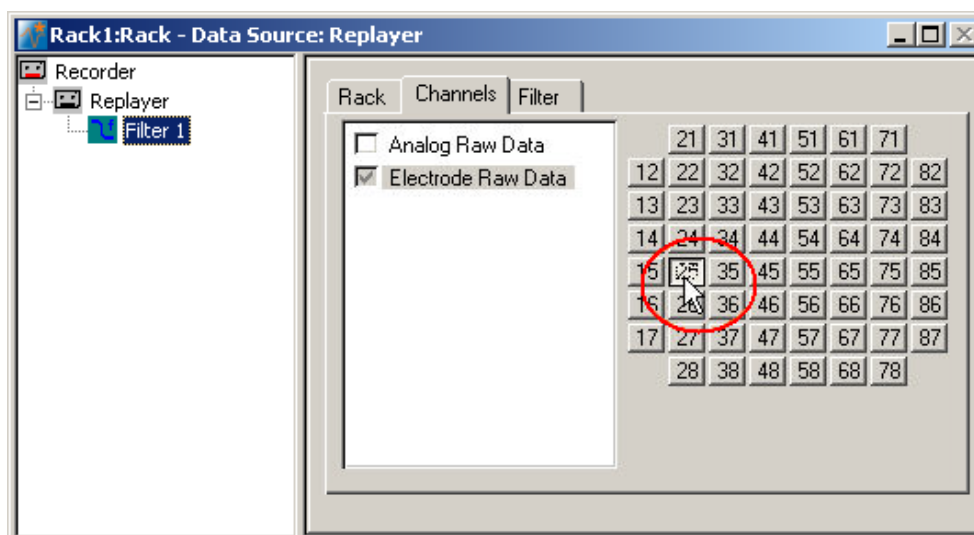
You see spike signals superimposed on the large field potential. It is impossible to detect all the spikes by defining a threshold. To circumvent this problem, you can use a filter that removes slow signals, that is, field potentials. In the current version of MC_Rack, a second order Butterworth filter is available. Please be aware that all filters deform signals. It is always a compromise between good filtering and low signal deformation.

Another option would be to detect spikes by their waveform, that is, use the **Slope** mode of the **Spike Sorter**. We will look into this alternative later.

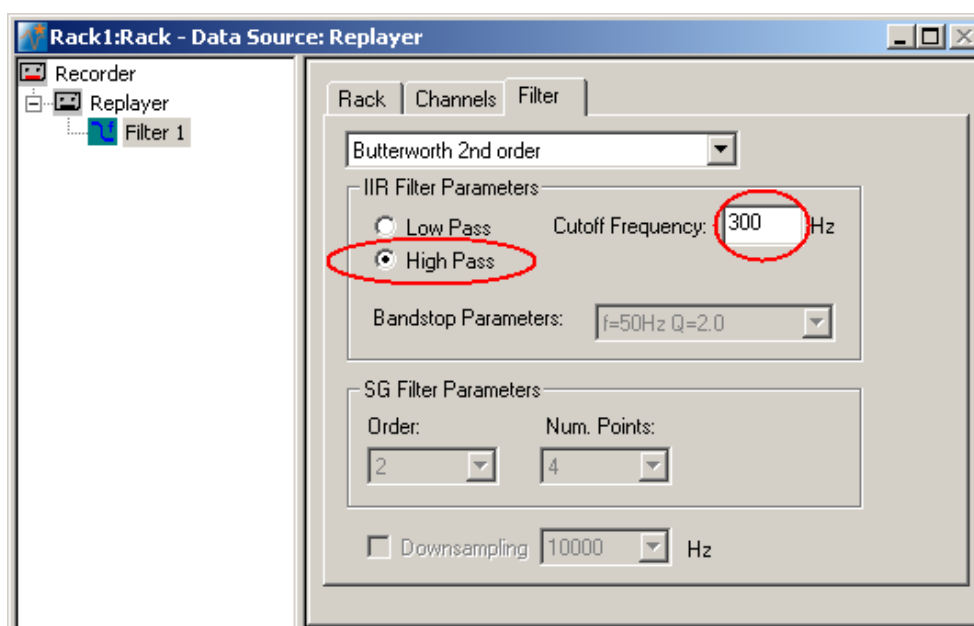
- Click the **Delete Instrument**  button to remove the **Data Display** again to save computer performance.

10.4 Removing Field Potentials with a Digital Filter


1. On the toolbar, click  to add a **Filter** to the virtual rack.
2. In the tree view pane of the virtual rack, select the **Filter 1**. Click the **Channels** tab and select the channel of interest, number 25 in this case. (Digital filtering is quite demanding regarding the computer performance and should be limited to the channels of interest.)

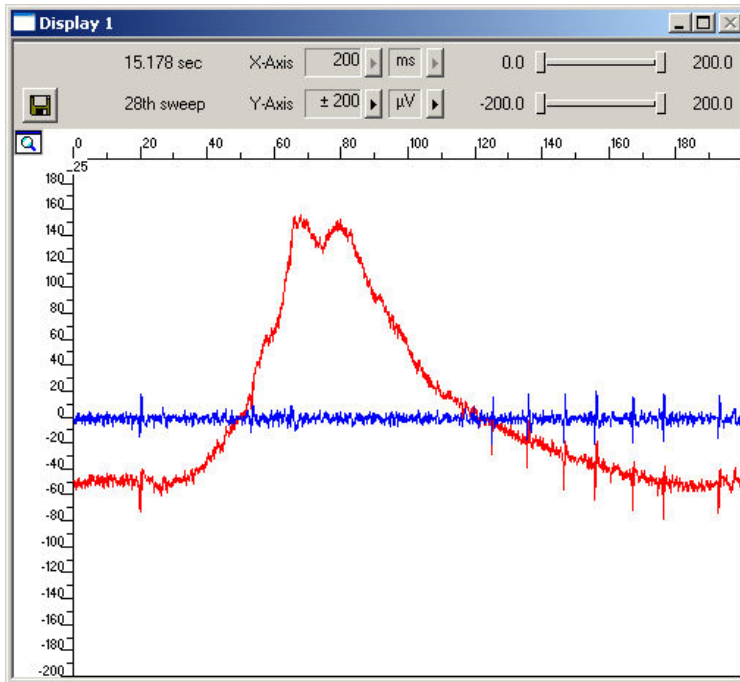


3. Click the **Filter** tab.
4. Select **High Pass**.
You can choose between two filter types: **Low Pass** and **High Pass**. As the name suggests, a low pass filter passes the lower frequency components but attenuates the higher frequency components of signals. To remove slow components (with a low frequency) and to let pass high frequency signals, you need a high pass filter.
5. Type in **300 Hz** as the **Cutoff Frequency**. The high-pass filter now cuts off frequencies below the specified 300 Hz. For spike activity, you usually use a **Cutoff Frequency** of 200 Hz or above, depending on the frequency of the noise and the slope/waveform of the spikes. The cutoff frequency of the filter must be compatible with the frequencies present in the signal of interest.

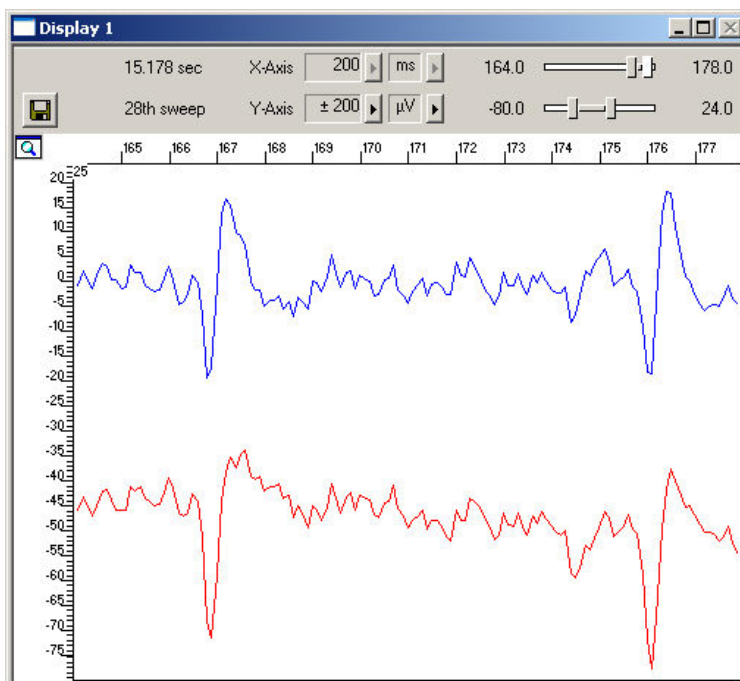


MC_Rack Tutorial: MEA Application Examples

6. Add a **Data Display** to your rack. Load the 8x8 MEA **Channel Map**. On the **Data** tabbed page, select the **Electro Raw Data** and the **Filtered Data** streams.
7. Click **Start**  to start the **Replayer**, and zoom the traces. The filtered data traces are displayed superimposed (blue) on the unfiltered data (red). You see that the field potentials have been removed.



8. If you zoom into single spikes, you can also observe slight differences in the waveforms that are always obtained when filtering data. The negative component is slightly smaller after filtering, for example. This does not matter for analyzing timing and patterns, like extracting the spike rate, but you should keep this in mind when analyzing and comparing spike waveforms, for example, when you sort spikes.




10.5 Detecting Spikes (Threshold Mode)

The **Spike Sorter** detects spikes, and features an online (or offline) spike sorting into up to three units. For example, you can use the **Spike Sorter** for sorting units from ON and OFF cells. The spike cutouts form a new data stream that can be analyzed further (or recorded to the data file). The unit information is saved in the data file and can be used for the analysis with external programs. The **Spike Sorter** has a special display where you can either set the spike detection level, or sort spikes in an interactive kind of way.

There are two modes for **detecting spikes** with MC_Rack. One method uses a **threshold**, the other recognizes the **amplitude** and **slope** of a waveform. Both methods regard either the **rise** or the **fall** of a signal, depending on the settings.

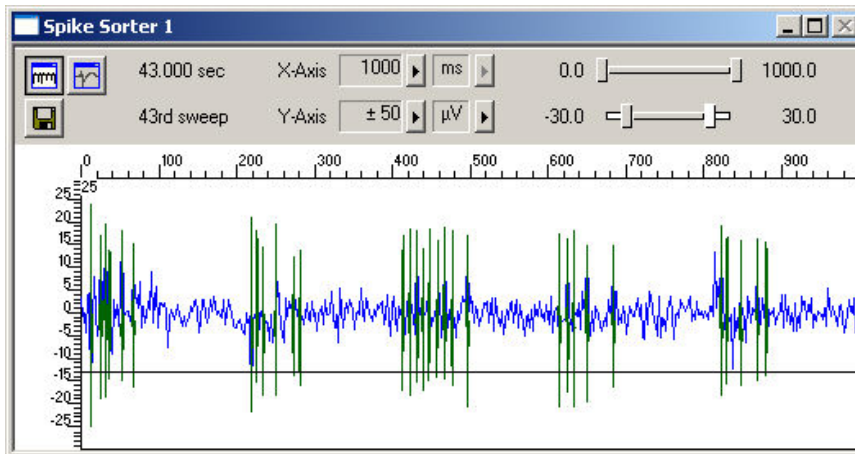
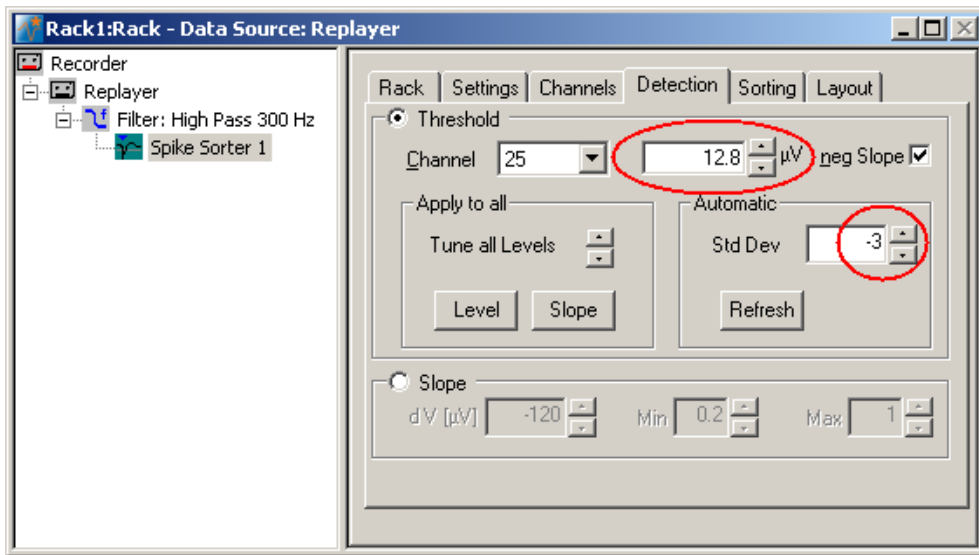
The **Threshold** method is especially useful if the overall baseline is stable, and spikes appear approximately on the same height. You can set the spike detection level for each channel separately (manually or automatically), or you can choose the same level for all channels. If local field potentials are underlying the spikes, or if you have an unstable baseline, spikes will be missed, or noise signals may be detected as spikes with the threshold method. In this case, the **Slope** method is more appropriate.



In this tutorial, we have used a digital high pass **Filter** to remove field potentials and to prepare the data for using the **threshold** method. We will also demonstrate later how to extract spikes using the **Slope** method from unfiltered data.

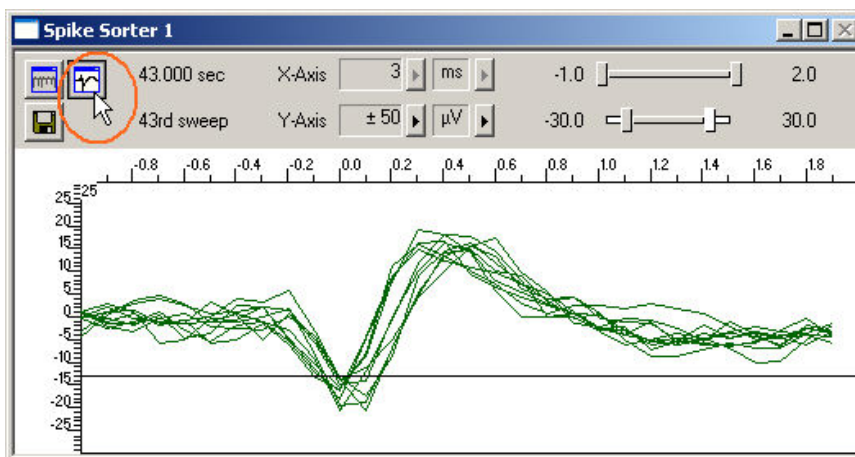
1. On the toolbar, click  to add a **Spike Sorter** in **series** with the **Filter** to the virtual rack. (If you put the **Spike Sorter** in parallel to the **Filter**, it would not be possible to use the filtered data stream as an input stream for the **Spike Sorter**, because virtual instruments can only use the output streams of other virtual instruments that are upstream in the tree view as input streams.) The **Spike Sorter** display appears (in detection mode).
2. Select the option **Peak Detection** in the **Spike Sorter** display.
3. Click the **Channels** tab and assign the **Filtered Data** stream to the **Spike Sorter**. As the Filtered Data stream includes only channel 25, only this channel will appear in the **Spike Sorter** window. All other window slots appear empty. You may want to customize the display layout at this point.
4. Click the **Start** button and zoom the display until you can see the signals clearly. You see that the baseline is on a similar level for all channels and that the spikes are quite small (approximately 10 to 20 μV).
5. Click the **Detection** tab. Make sure the **Threshold** method is selected. You can now set the detection threshold. Start with a value of 15 μV , for example.
6. Type in 15 in the text box. This value does apply now to the selected channel. Under **Apply to All**, click **Level** to apply this detection level to all channels. You can also use the **Automatic** feature to automatically assign an **individual** detection level to **each** channel, which is very convenient if you have multiple channels for extracting spikes. The standard deviation of each data trace is used to estimate its spike threshold. A time interval of 500 ms is used to calculate the standard deviation. You set the factor, by which the standard deviation is multiplied. The sign of the factor determines whether the spike detection level is positive or negative. You generally will use a negative factor. A value between -1 and -4 is appropriate for most applications. For example, enter a standard deviation of 2, and click **Refresh** to calculate the standard deviation and apply the result. Spikes appear in a different color on the display. The detection level of 8.5 determined for this channel by the **Automatic** feature is visible as a horizontal straight line.

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- The level may still be a bit too near the noise level. You can then fine tune the detection level by dragging the horizontal line of each channel in the **Spike Sorter** display with your mouse, or use a higher standard deviation. If you have multiple channels, you can use the option **Tune all levels** to modify the level of all channels at once.



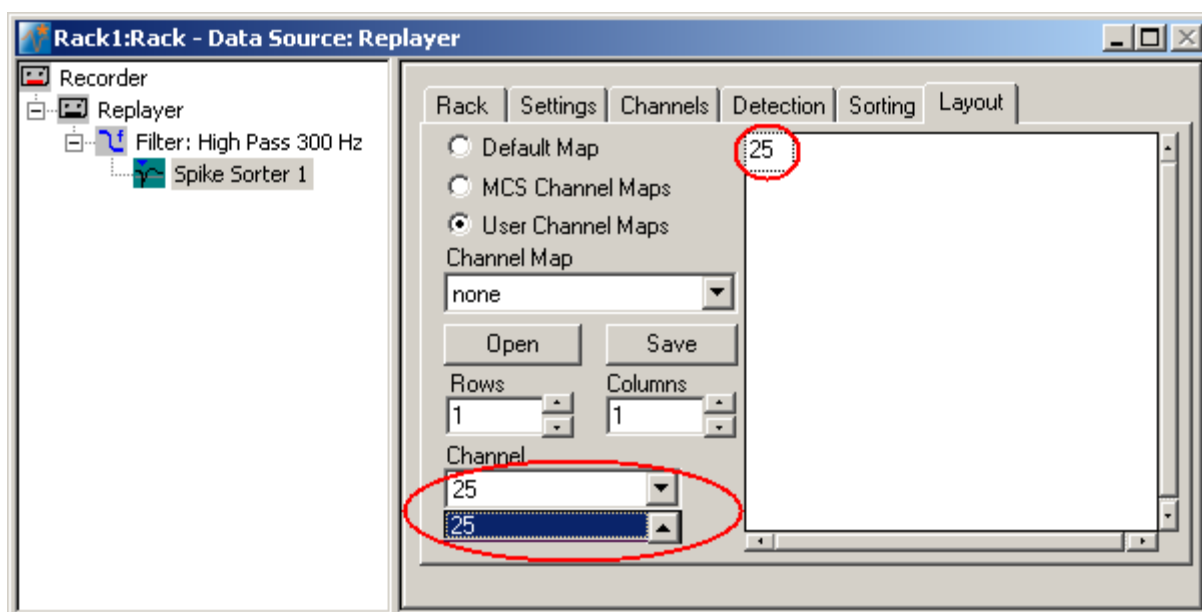
- You can then switch to the spike **overlay** mode of the display by clicking the  button on the display toolbar, and switch back again to the other mode with the  button. Depending on the application, you could then use the spike sorting feature for discriminating signals from ON and OFF ganglion cells, for example.



10.6 Customizing Displays

As it may not be useful to extract spikes from all channels, you can set up your custom layout for the **Spike Sorter** (this is true for all displays), to save space on the screen. You may be interested only in one or two channels. (Remember: You can save the raw data of all channels in the **Recorder**.)

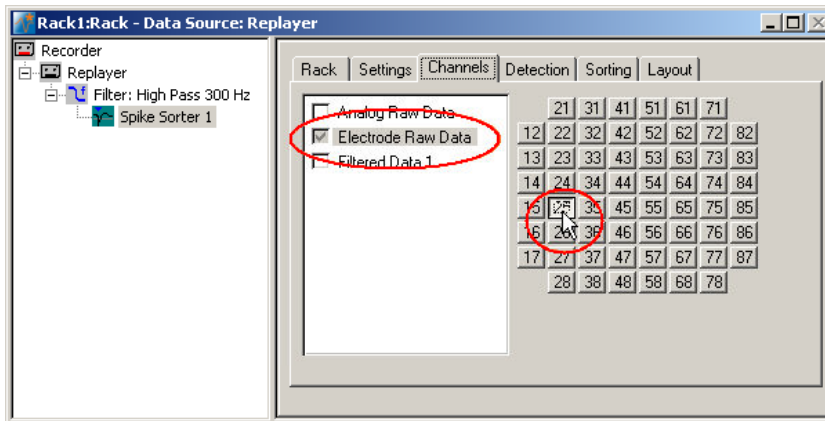
1. In the tree view pane of the virtual rack, select the **Spike Sorter** (or the display that you want to customize) and click the **Layout** tab. The default layout is loaded. In the **Rows** and **Columns** boxes, you can now modify the layout.
2. Type or select the number of **Rows**, for example 1, and the number of **Columns**, for example 1. On the right, a linear layout with two channels appears.
3. Assign the channel numbers to the empty slots, for example channel 25: Click to select the empty slot in the white layout pane, then pick 25 from the **Channel** list. The active slot is highlighted by a dotted line. The **Spike Sorter Display** is updated accordingly.



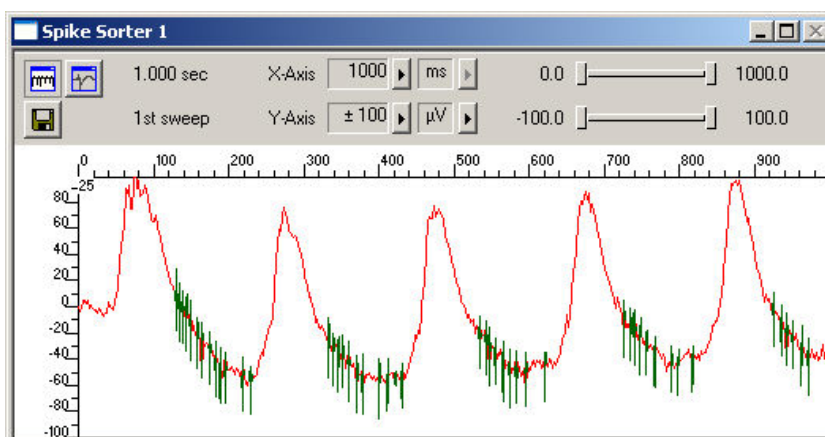
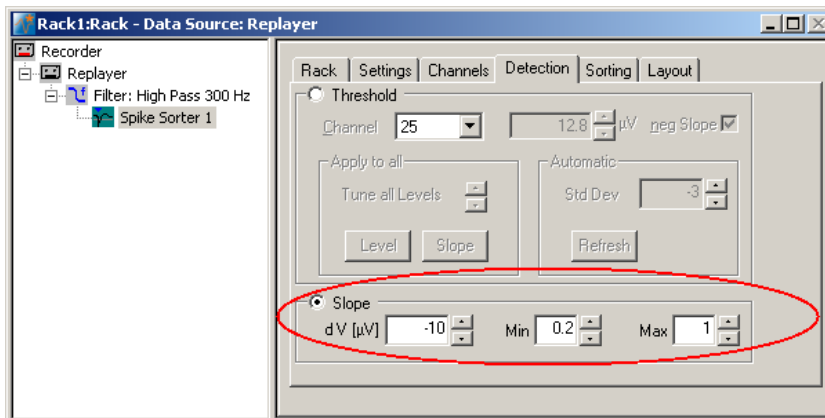
10.7 Detecting Spikes (Slope Mode)

In **Slope** mode, spike detection is performed on the waveform rather than the spike level. Therefore, it is possible to extract spikes even if field potentials are underlying the spikes, as in this example. Therefore, you can extract the spikes from the unfiltered data with this method.

1. In the tree view pane of the virtual rack, select the **Spike Sorter**, and click the **Channels** tab.
2. Click the **Electrode Raw Data** stream, and then select channel 25.



3. Click the **Detection** tab, and select the **Slope** method.
4. You now have to play around with the amplitude (**dV**), and the **minimum** and **maximum** slope until you achieve satisfying results. For more information on the **Slope** mode, please see "MC_Rack Features", "Spike Sorter", "Detecting Spikes by Waveform". For example, you know that the minimum amplitude should be around 10 mV for this data, so you can try 10 for dV, and then play around a bit with the parameters.

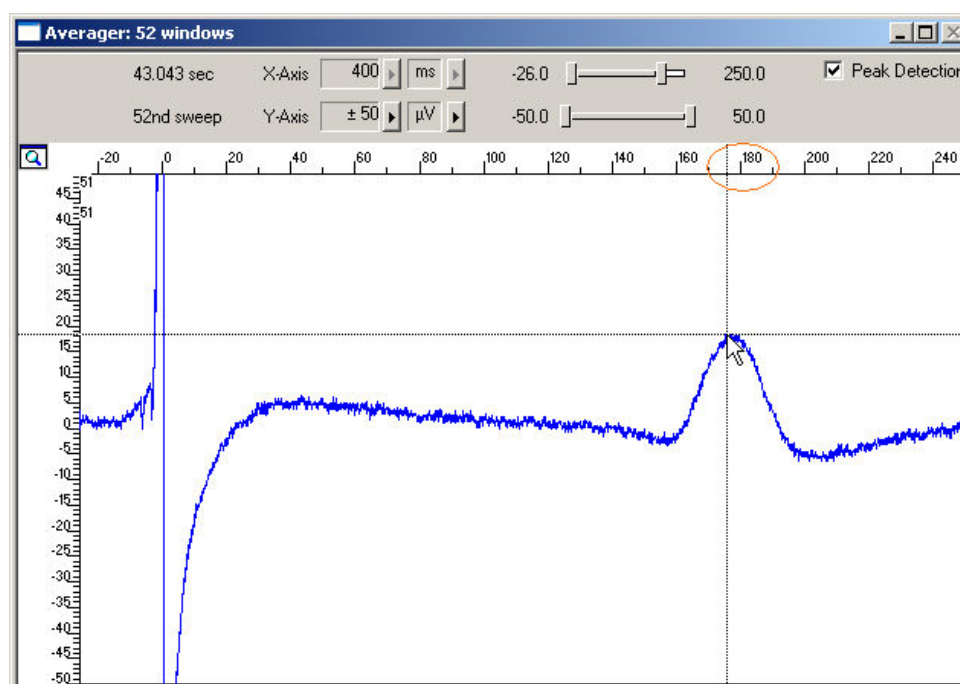


11 Cardiac Signals

11.1 Manual Measurement of the QT Interval

If you need the averaged data as the basis for the analysis, you can measure the parameter with the crosshair tool. As the Na^+ peak corresponds to the trigger event, the time point of the T wave is the relevant parameter.

1. Right-click on the display and select the option **Display Crosshair**. A fine dotted line at the position of the mouse pointer.
2. Move the mouse pointer on the T wave peak. You can now read the time point and amplitude of the peak on the scale. In this example, the QT interval is about 180 ms long.



11.2 Monitoring the QT Interval

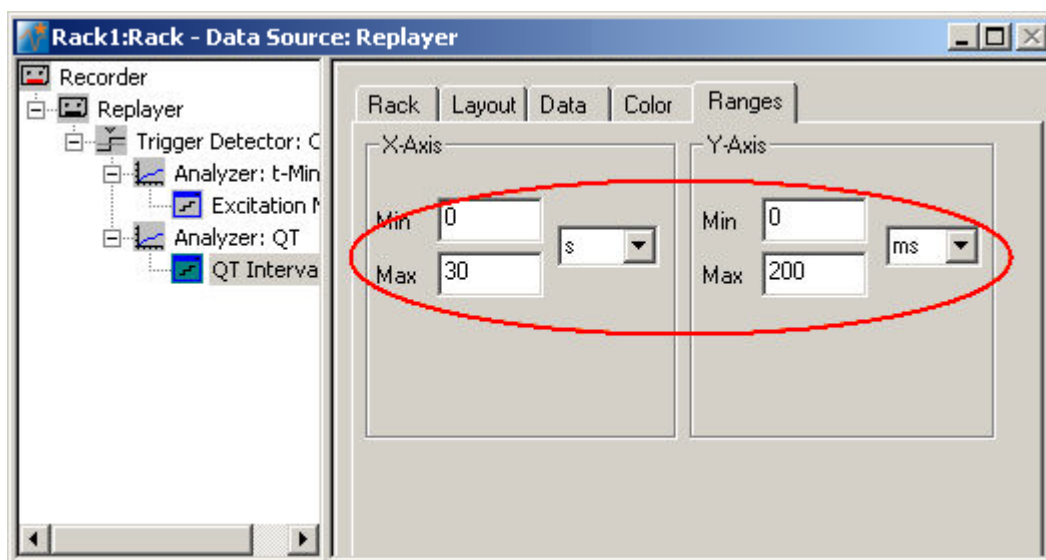
You can plot the extracted time point relative to T1 of the ROI. For plotting extracted parameters, we will need a **Parameter Display**, which features are optimized for **Parameter** streams.

- On the toolbar, click  to add a **Parameter Display** to your rack. You can rename it so that you will recognize it later.

Setting the window extent

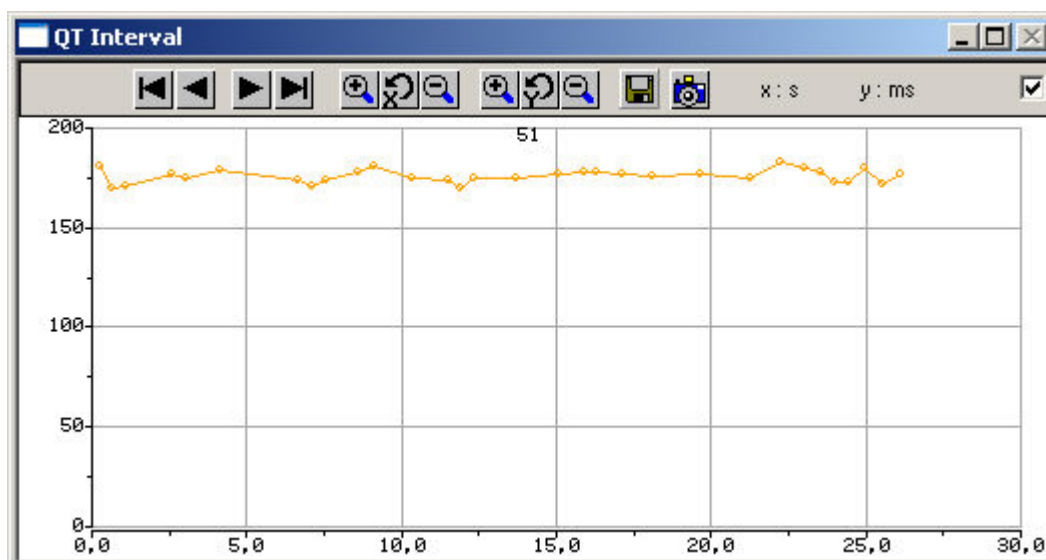
As this demo data file has about 30 s total recording time, 30 s will be fine for the x-axis range. As the duration cannot be longer than the ROI, that is, 200 ms, you can enter 200 ms for the y-axis.

1. In the tree view pane of the virtual rack, select the **Parameter Display**, and click the **Ranges** tab.
2. Enter 30 s for the x-axis range, and adjust the y-axis to 200 ms.



1. Click **Start**  to generate the plots.

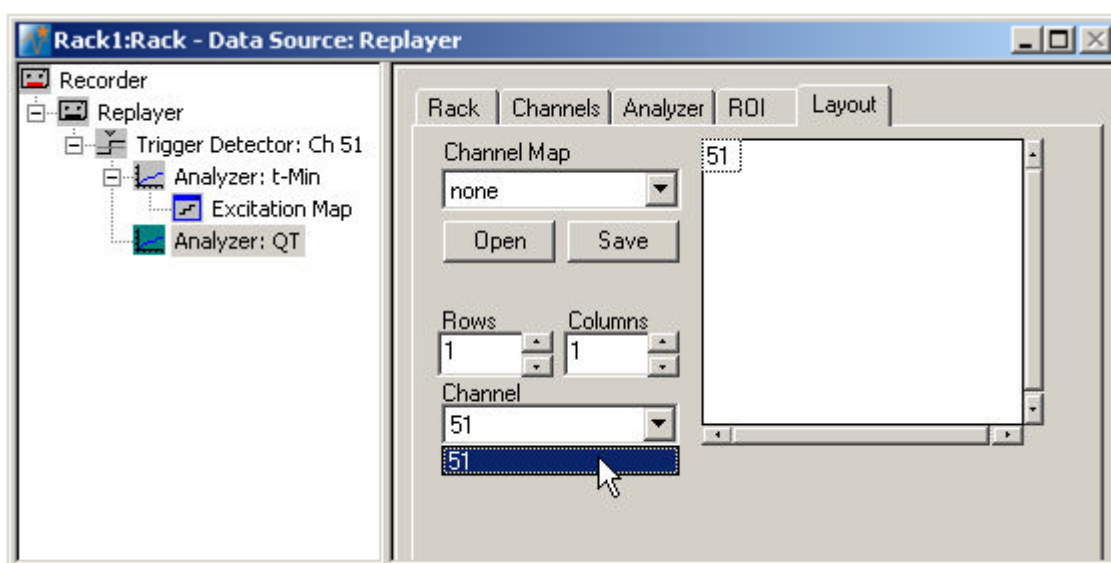
The QT interval is stable (in the range of 170180 ms) in this control experiment with no drug application.



11.3 Customizing Displays

As it may not be useful to extract parameters like the QT interval from all channels, you can set up your custom layout for the **Analyzer** and **Parameter Display** (this is true for all displays), to save space on the screen. You may be interested only in one or two channels. (Remember: You can save the raw data or extracted parameters of all channels in the **Recorder**.)

1. In the tree view pane of the virtual rack, select the **Analyzer** (or the display that you want to customize) and click the **Layout** tab. The default layout is loaded. In the **Rows** and **Columns** boxes, you can now modify the layout.
2. Type or select the number of **Rows**, for example 1, and the number of **Columns**, for example 1. On the right, a linear layout with two channels appears.
3. Assign the channel numbers to the empty slots, for example channel 51: Click to select the empty slot in the white layout pane, then pick 51 from the **Channel** list. The active slot is highlighted by a dotted line. The **Analyzer Display** is updated accordingly.



11.4 Automatic Extraction of the QT Interval

You can use the **Analyzer** to automatically extract the QT interval, measured from the fast sodium peak to the maximum (or minimum, depending on the polarity of the signals) of the IKr current peak. Select channels of interest that show a very clear repolarization waveform.

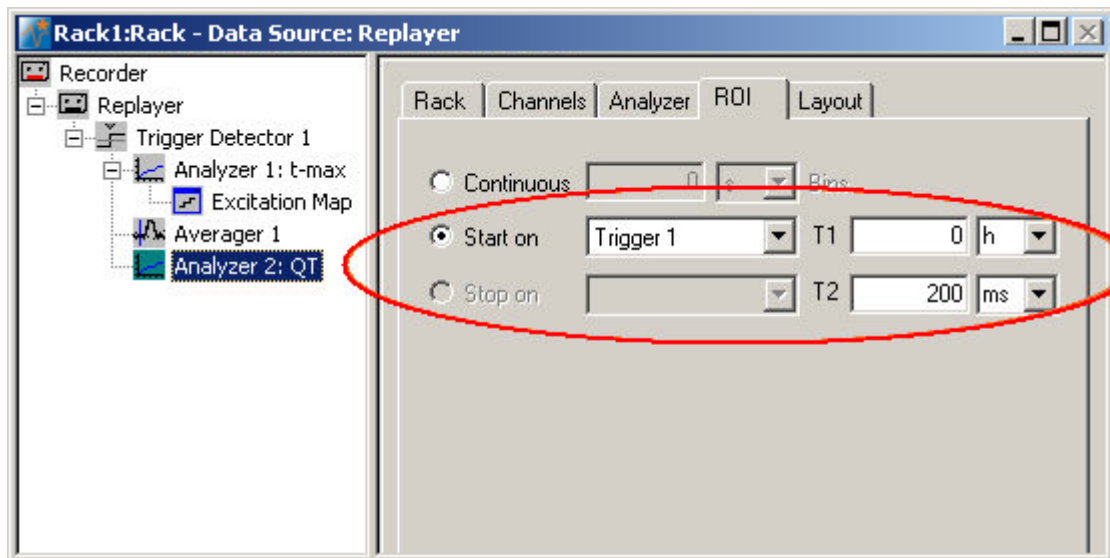
In triggered mode, the **Analyzer** extracts the parameters of interest from a distinct **time window** relative to the **trigger event**, the region of interest (**ROI**). As you need a different region of interest for extracting the time point of the IKr current peak than for the time point of the fast sodium peak, you need to set up a second **Analyzer**, based on the raw data stream. (You cannot use averaged data for an automated analysis in the current version of MC_Rack.)

As the cardiomyocytes are electrically coupled to each other, it does generally not make much sense to analyze the QT interval on more than one electrode. As the parameters of interest are extracted from the region of interest relative to the trigger event, it is ideal to extract the QT interval from the channel that triggers the **Analyzer** (channel 51 in this example). (If you want to extract the interval from multiple channels, there will be different time shifts on the other channels, as the signal arrives at different time points at different electrodes. You can of course also define multiple triggers on different channels and set up an individual **Analyzer** for each electrode, but this will be on the cost of computer performance.)

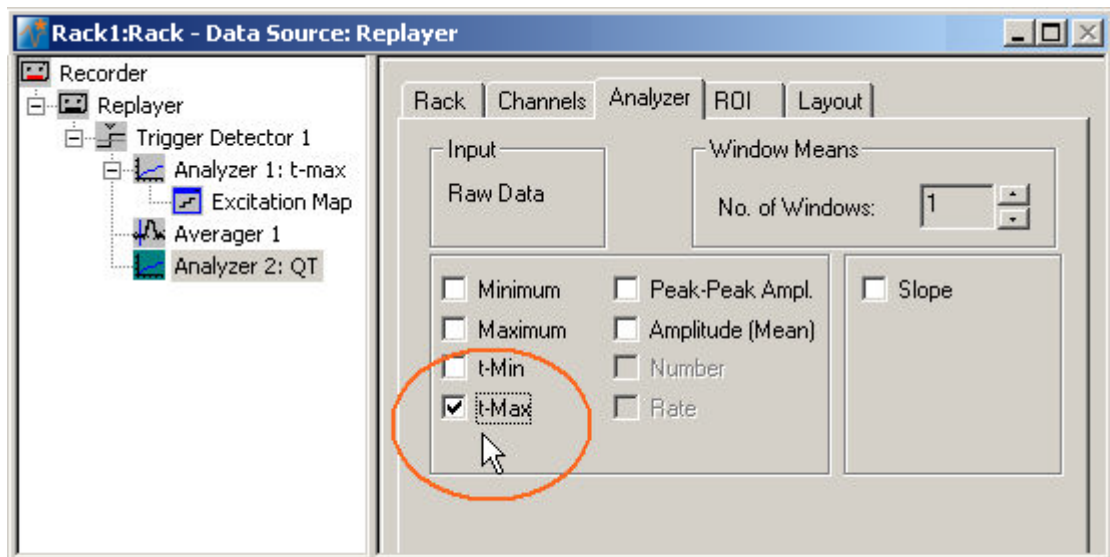
1. On the **Edit** menu, click **Add Analyzer** to add an **Analyzer** to your rack (in series with the **Trigger Detector**). You can rename the **Analyzer** to distinguish it from the other.

MC_Rack Tutorial: MEA Application Examples

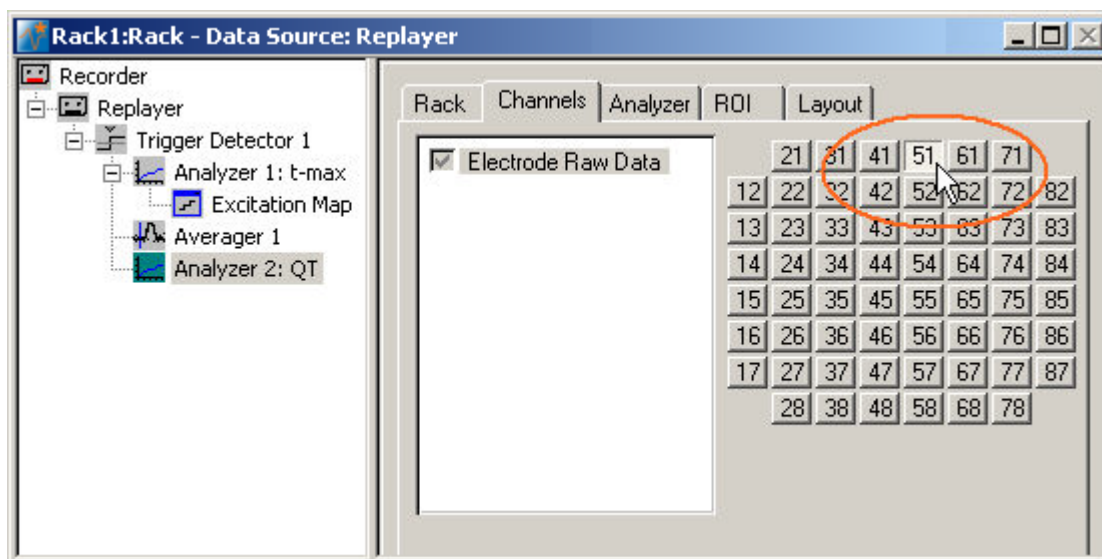
- In the tree view pane of the virtual rack, select the **Analyzer**, and click the **ROI** tab. Select **Start on Trigger 1**, and enter values for **T1** and **T2** so that the ROI will approximately include the complete waveform, for example 0 and 200 ms.



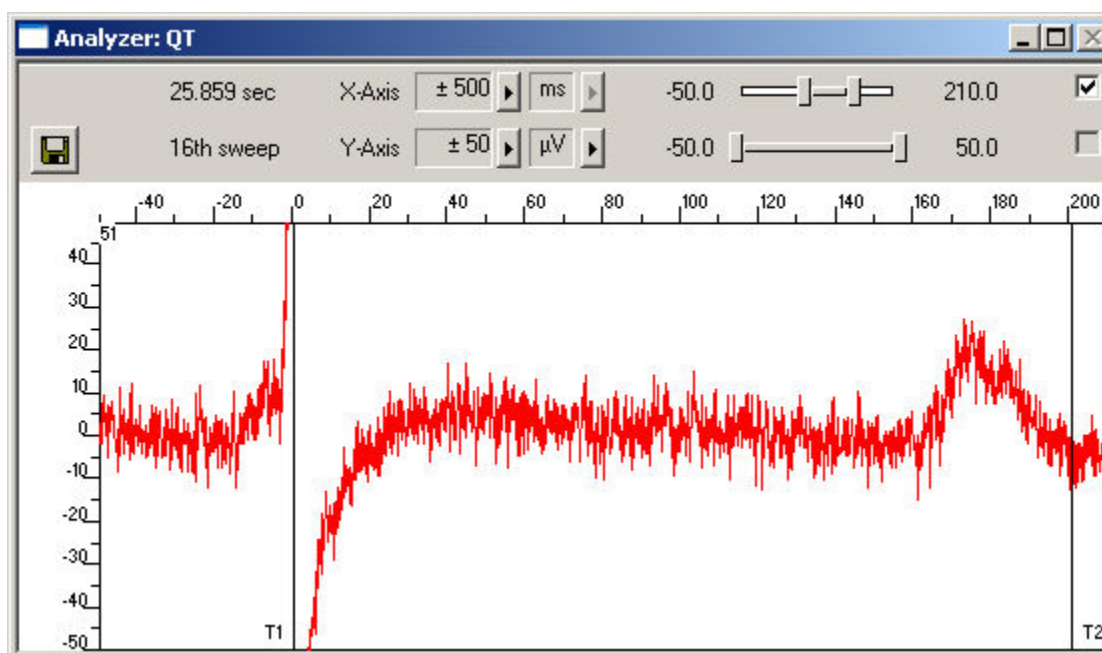
- Click the **Analyzer** tab, and select the **t-Max** parameter for extracting the time point of the maximum inside the ROI.



- Click the **Channels** tab. Deselect all electrodes by clearing the check box, and select the electrodes of interest, for example, electrode 51. Only the selected channel will appear in the **Analyzer** window. All other window slots appear empty. You may want to customize the display layout at this point.



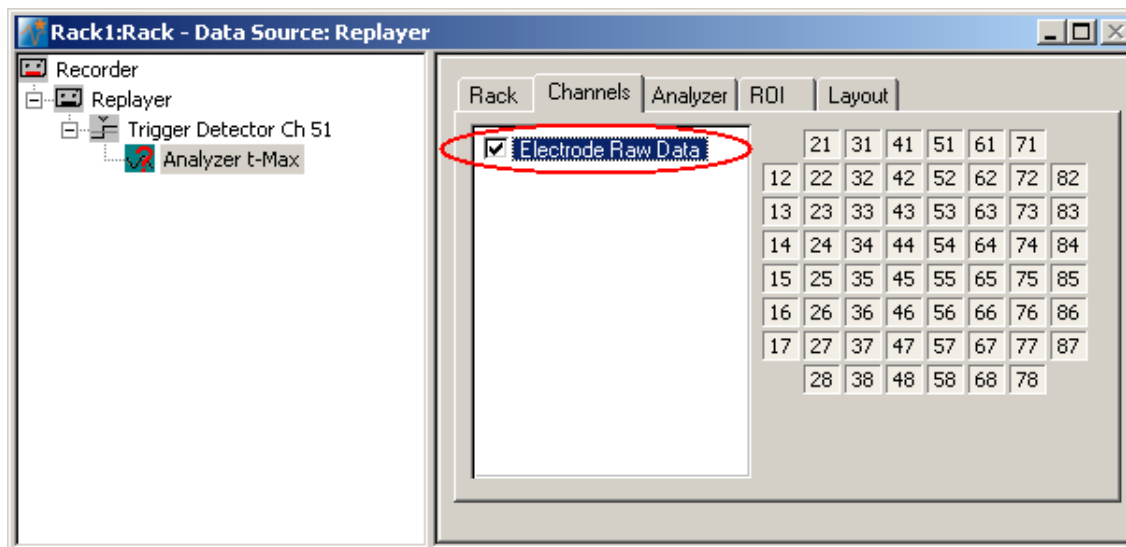
- Start the **Replayer** to view the traces, and zoom in the axes so that you can clearly see the waveform. Adjust the region of interest by dragging the T1 and T2 cursors, if necessary, so that the IKr peak is safely inside the ROI.



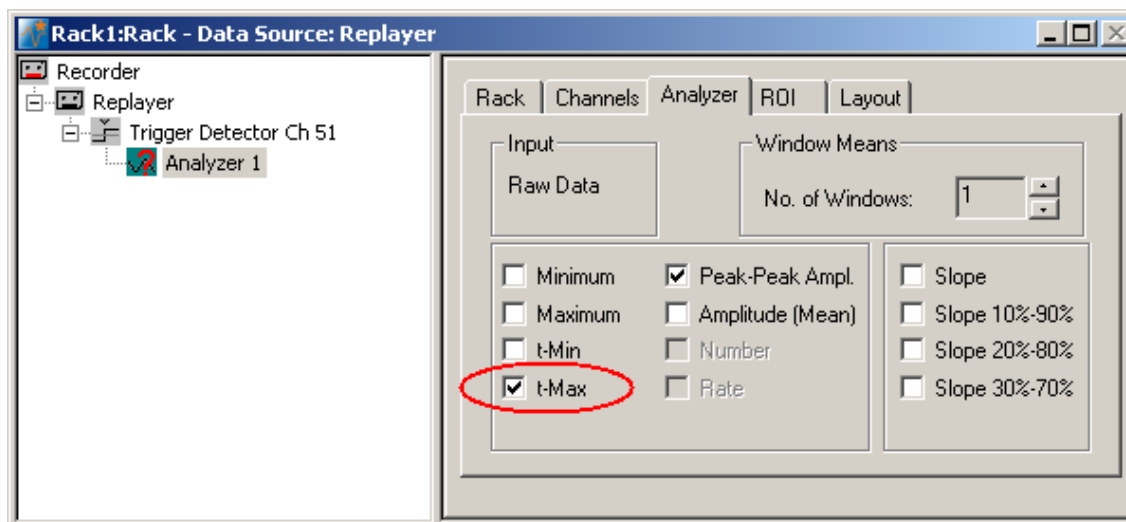
11.5 Signal Propagation

For measuring the signal propagation, you will extract the time point of the minimum (**t-Min**) or maximum (**t-Max**) of the fast sodium peak.

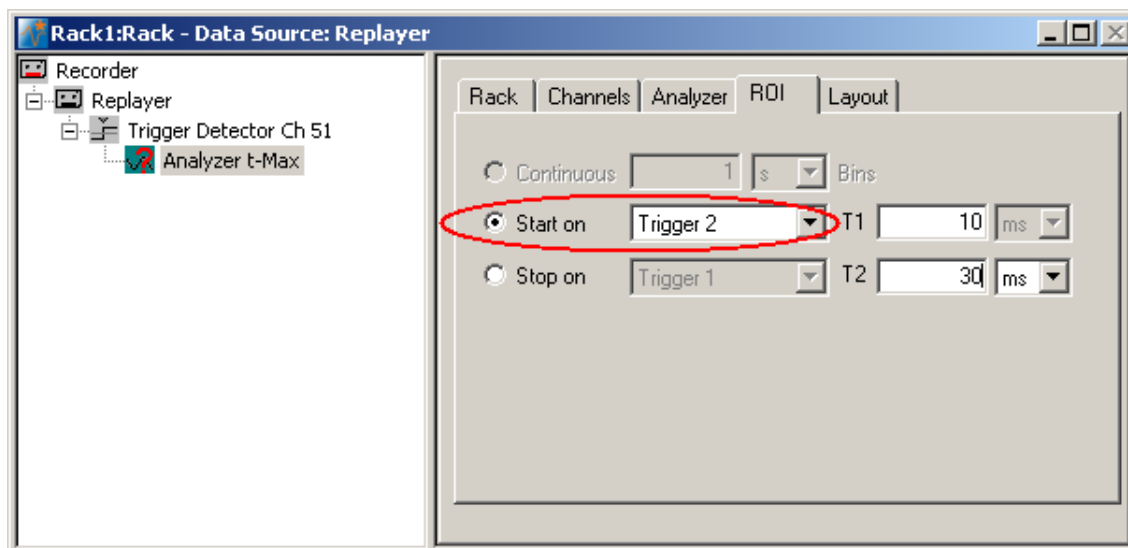
1. Remove the **Display 1** from the rack by clicking on the **Display 1** in the tree view of the virtual rack and pressing the DEL key or by clicking **Delete Instrument** on the **Edit** menu.
2. On the **Edit** menu, click **Add Analyzer** to add an **Analyzer** to your rack (again, in series with the **Trigger Detector**).
3. In the tree view pane of the virtual rack, select the **Analyzer**, and click the **Channels** tab. Select all **Electrode Raw Data** channels.



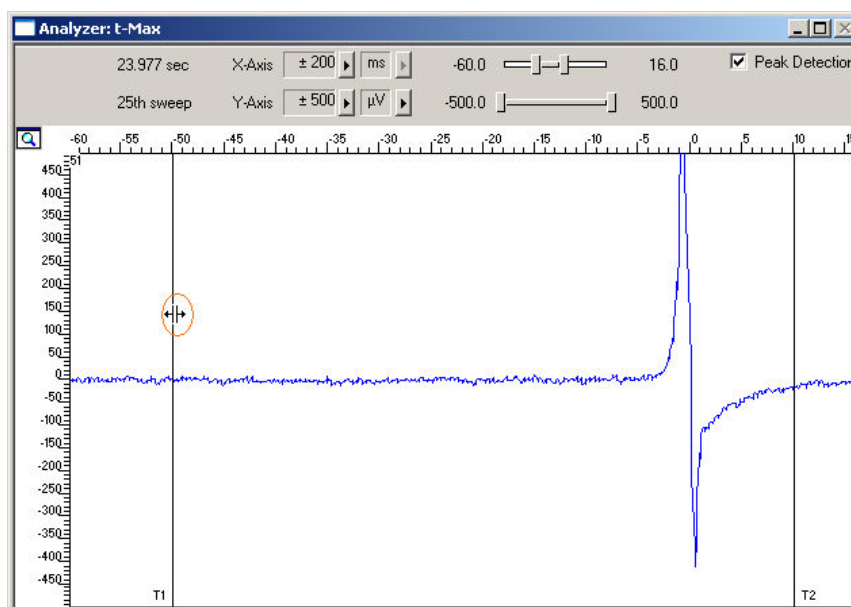
4. Click the **Analyzer** tab and select the parameter **t-Max** (or **t-Min**).



- Click the **ROI** tab and select the option **Start On Trigger 1**. The **Analyzer** display opens. You can now define the region of interest (ROI) directly in the display or on the ROI page.



- Click the start button and zoom in the display so that you can see the waveforms clearly on all channels.
- Drag the **ROI** sliders such as that all signals from all electrodes are inside the ROI, or enter appropriate values into the T1 and T2 text boxes on the ROI page, for example T1 = 30 ms and T2 = 15 ms. T1 defines the left border, and T2 defines the right border of the ROI frame.

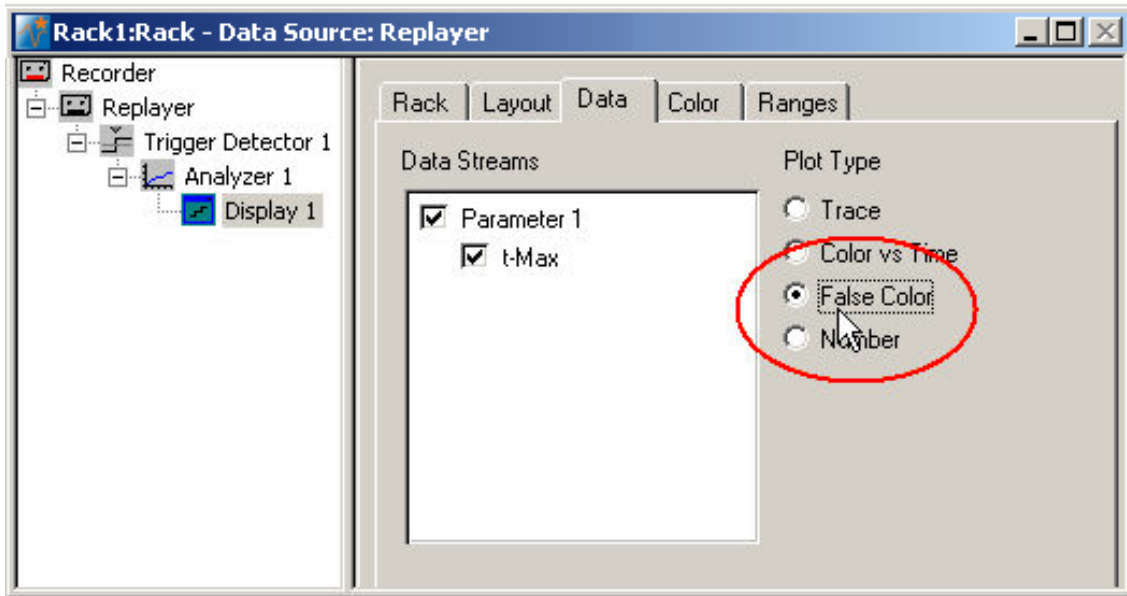


On the **Edit** menu, click **Add Parameter Display**. The **Parameter Display** can be configured as an excitation map.

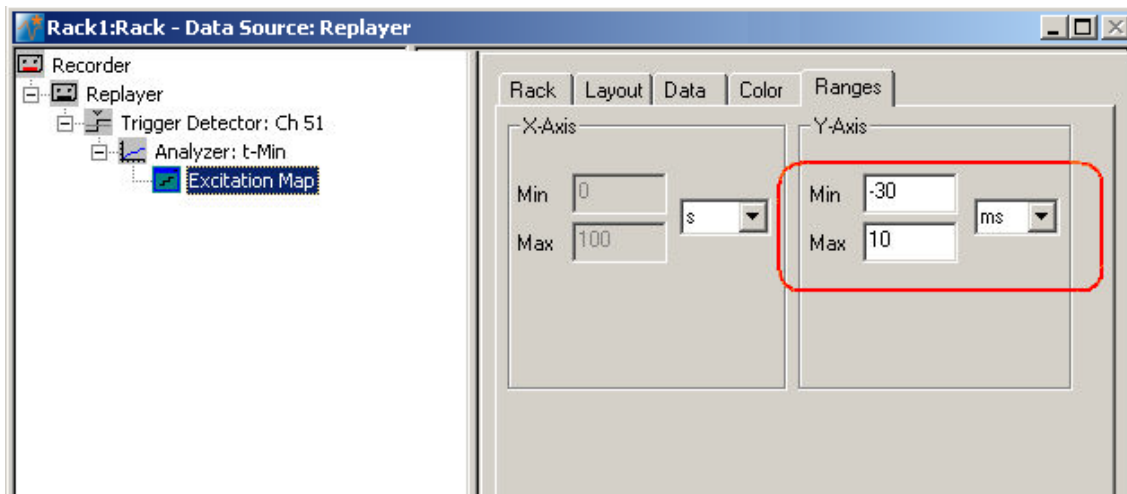
- In the tree view pane of the virtual rack, select the **Parameter Display** and click the **Data** tab.

MC_Rack Tutorial: MEA Application Examples

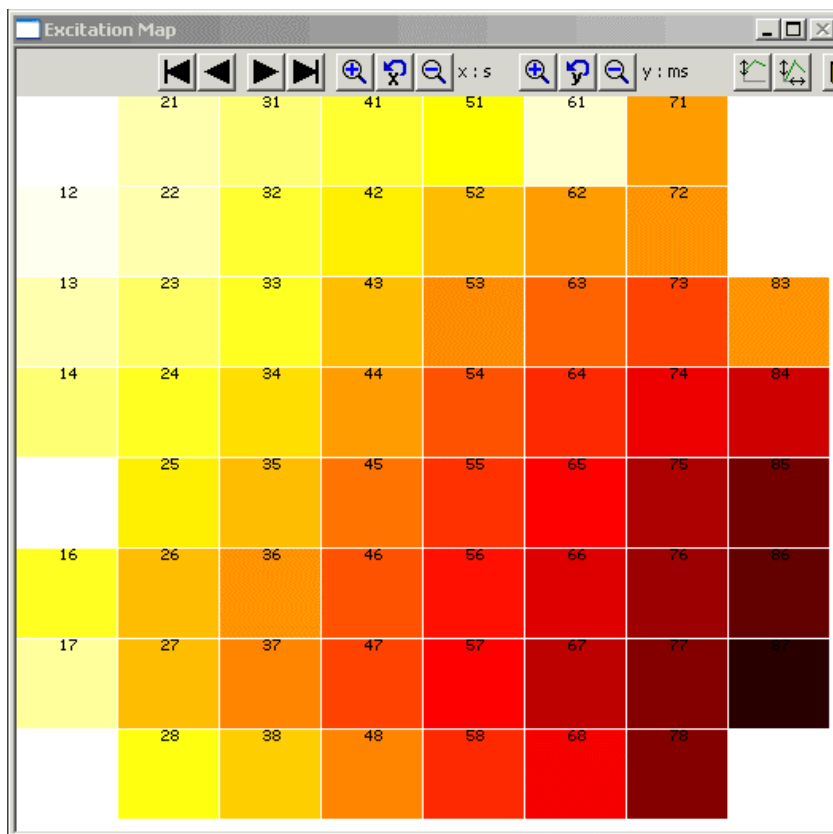
9. Select the parameter **t-Max**. As plot type, select the **False Color** plot.



10. Click the **Color** tab. Select any color map, for example, the **Hot** color map. The y-value ranges of the plot are now assigned to the colors of the false color map. You have to select a proper range so that the color assignment makes sense, that is, if the range is too wide, you will not see any differences in the color; if the range is too narrow, the colors will get into saturation, that is, black.
11. Click the **Ranges** tab. Enter an appropriate range that starts with the lowest value for the parameter of choice **t-Max** and that stops with the highest **t-Max** value on the MEA. As you see an excitation at about 30 to 10 ms (in relation to the trigger event) on different channels, a range from 30 to 10 ms is appropriate.

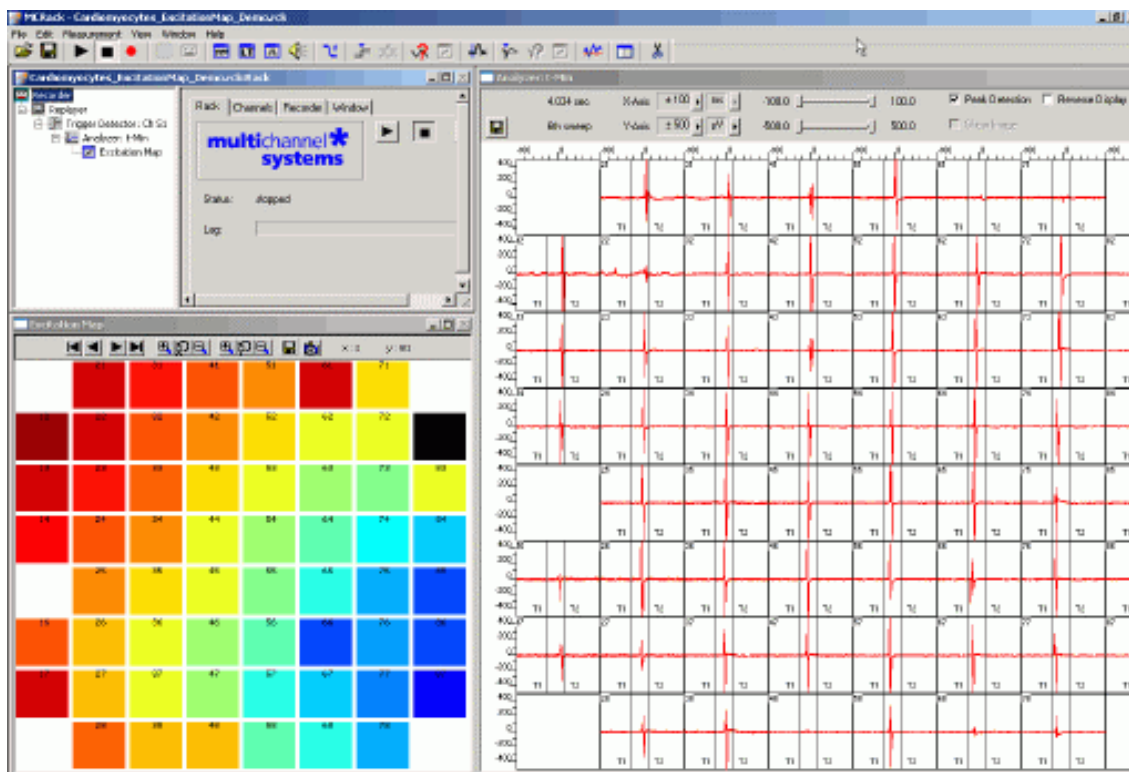


12. On the **Measurement** menu, click **Start** to start the replaying of the data file.



You can see the excitation spreading nicely from the lower right corner to the upper left corner. Low time values (= early signals) appear dark, the later a signal arrives at the electrode, the lighter gets the color, up to light yellow.

11.6 Aim of Experiment



MC_Rack Tutorial: MEA Application Examples

The MEA-System can be used for *in vitro* studies of the electrical properties of **cardiac myocytes** and, for example, effects of drug candidates on **field potential waveforms** and **kinetics**.

Primary cardiac myocytes can be easily harvested from fertilized chicken eggs, or from neonatal rat or mouse. It is also possible to obtain **murine** and **human embryonic stem cells** derived from embryoid bodies. There is also one cardiomyocyte cell line from mouse that retains a differentiated cardiac myocyte phenotype: **HL-1** from Dr. W. Claycomb.

Plated cells form a **syncytium** and start **beating spontaneously** after a couple of days, or contract after **electrical stimulation (pacing)**. The two-dimensional MEA layout is ideally suited for showing the **waveform propagation** and measuring the **conduction velocity** in a cardiomyocyte culture.

Simultaneous recordings of action potentials (with intracellular electrodes) and field potentials (with extracellular electrodes) have shown that there is a **correlation** between the rise time of the **cardiac action potential (AP)** and **field potential (FP)** as well as between AP and FP duration. A correlation between the **waveform components** and the **ion channel activities** was shown by using ion channel blockers or by depleting the medium of the respective ions (Reference: "Determination of Electrical Properties of ES Cell-derived Cardiomyocytes Using MEAs", Jürgen Hescheler et al., Journal of Electrocardiology, Vol. 37 Supplement 2004; "Estimation of Action Potential Changes from Field Potential Recordings in Multicellular Mouse Cardiac Myocyte Cultures", Marcel D. Halbach et al., Cell Physiol Biochem 2003;13:271 to 284).

The high and short peak of the extracellular field potential can be correlated to the rapid component of the depolarizing sodium current. The following plateau follows the time course of the slow calcium current; and the following positive or negative peak correlates to the slow rectifying K⁺ current (IKr). The polarity of this peak depends on several parameters, for example, the proximity of the cell layer to the measuring electrode, and cannot be predicted, but this fact does generally not matter for this assay.

The field potential duration corresponds to the action potential duration, which can be correlated to a **QT interval** in an electrocardiogram. It is measured from minimum of the Na⁺ peak to the maximum / minimum of the IKr current peak.

The effects of tested drugs on **QRS duration** and **amplitude, local activation time, T wave amplitude**, time of maximal **slope** of T wave, **QT interval** duration, and **ARI (activation refractory interval)** can be analyzed with the MEA-System.

Typical applications are:

- Drug testing for effects on cardiac signal waveforms, for example QT prolongation
- Analysis of changes in the signal propagation kinetics
- Cocultures of stem cells and primary cells for basic research and for gaining insights into transplantation options and techniques
- Characterizations of cells for cardiac properties, for example, of cardiomyocytes derived from stem cells

You will see in this tutorial how a virtual rack is set up for those purposes, and how typical signal waveforms look like. Please note that the amplifier filter settings affect the waveforms of recorded signals. Please see additional documentation, for example, the MEA1060 Manual or MEA Application Notes for more information on this matter.

The demo data shown here was recorded from chicken embryonic cardiomyocytes at a sampling rate of 10 kHz with a gain of 1200, and a bandwidth of 1 Hz to 3 kHz. The demo data **Cardiomyocytes_QT.mcd** was filtered with a digital 2 Hz high pass filter in MC_Rack.

You will learn in this chapter ...

- How to define **basic functions** in your **rack configuration**:
 - Monitoring cardiac activity (**Trigger Detector, Data Display**).
 - Generate an excitation map (**Analyzer, Parameter Display, False Color** plot).
 - Averaging data for increasing the signal to noise ratio (**Averager**).
 - Automatically extract the QT interval (**Analyzer**).
 - Monitor the QT interval (**Parameter Display**).
- How to **customize** the rack:
 - Modify the display layout (all display types).
- More **details**:
 - Manually measure the QT interval, for example, from averaged data (**Crosshair** feature of the **Data Display**).

Excitation map

The following rack and data file demonstrate the two-dimensional signal propagation.

- Rack file: Cardiomyocytes_ExcitationMap_Demo.rck
- Data file: Cardiomyocytes_60Ch.mcd

QT Prolongation


This rack and data file shows very prominent IKr peaks, and is therefore well suited for showing how to extract the QT interval from cardiac field potentials.

- Rack file: Cardiomyocytes_QT-Analysis_Demo.rck
- Data file: Cardiomyocytes_QT.mcd

Please see also the MEA Application Notes for more information on the preparation techniques and experiments.

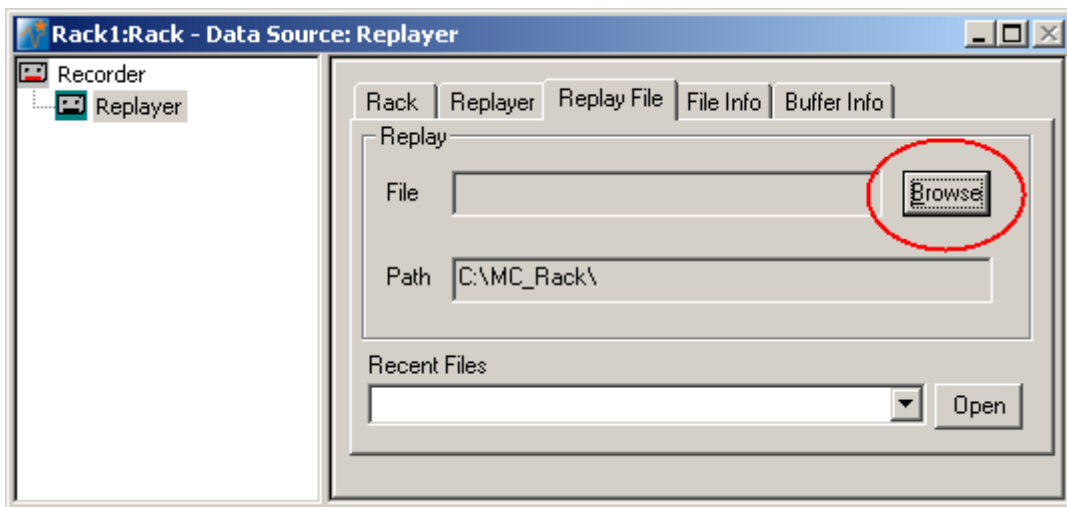
11.7 Preparations

We recommend that you take some time for rebuilding the virtual rack for this application step by step in this tutorial, but if you prefer having a look at the completed rack or if you get stuck during the tutorial, you can also open the rack file "Cardiomyocytes_ExcitationMap_Demo.rck". Click **Open** on the **File** menu to open the rack file.

1. Copy the **complete** MC_Rack **Tutorial** folder from the installation volume into the MC_Rack program directory with the following path "c:\Program Files\Multi Channel Systems\MC_Rack\"
2. Start MC_Rack or click **New** on the **File** menu to generate a new virtual rack file configuration.
3. Click  on the toolbar to add a **Replayer** to your virtual rack.

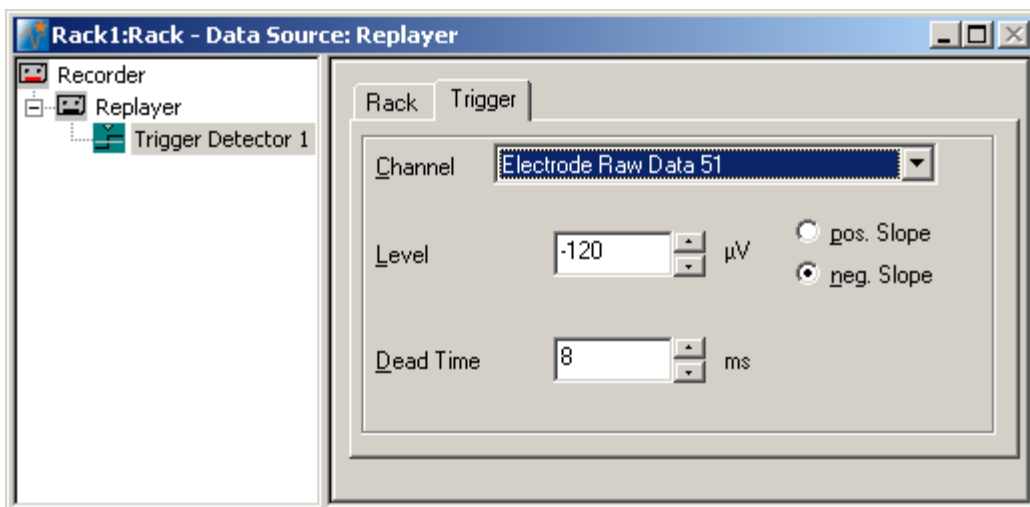
MC_Rack Tutorial: MEA Application Examples


4. In the tree view pane of the virtual rack, select the **Replayer** and click the **Replay File** tab. Click the **Browse** button and browse to the **Offline** subfolder of the **Tutorial** folder, and load the data file **Cardiomyocytes_60Ch.mcd** into the **Replayer**.



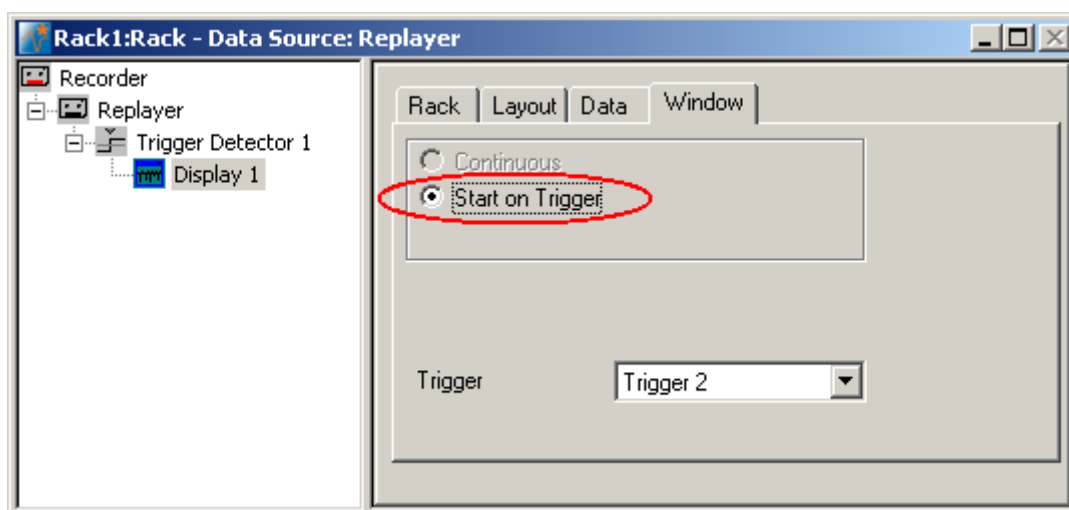
11.8 Monitoring Cardiac Activity


1. On the **Edit** menu, click **Add Trigger** to add a **Trigger Detector** to your virtual rack.
2. In the tree view pane of the virtual rack, select the **Trigger Detector**, and click the **Trigger** tab and select the channel that you want to use as a trigger, for example, channel 51. You can change the detection threshold level and the dead time on this page, but this is not necessary in the moment.



3. In the tree view pane of the virtual rack, select the **Trigger Detector** and click  on the toolbar to add an oscilloscope-like **Data Display** in **series** with the **Trigger Detector** to the virtual rack. (If you would put both instruments in parallel, you would not be able to use the trigger stream generated by the **Trigger Detector** for triggering the display, because virtual instruments can only use the output streams of other virtual instruments that are upstream in the virtual rack tree.) Have a look at the display settings (**Layout** tabbed page) and make sure an **8x8 MEA Channel Map** is loaded as the **Channel Map**, and **Electrode Raw Data** as the data input stream (see **Data** tabbed page).

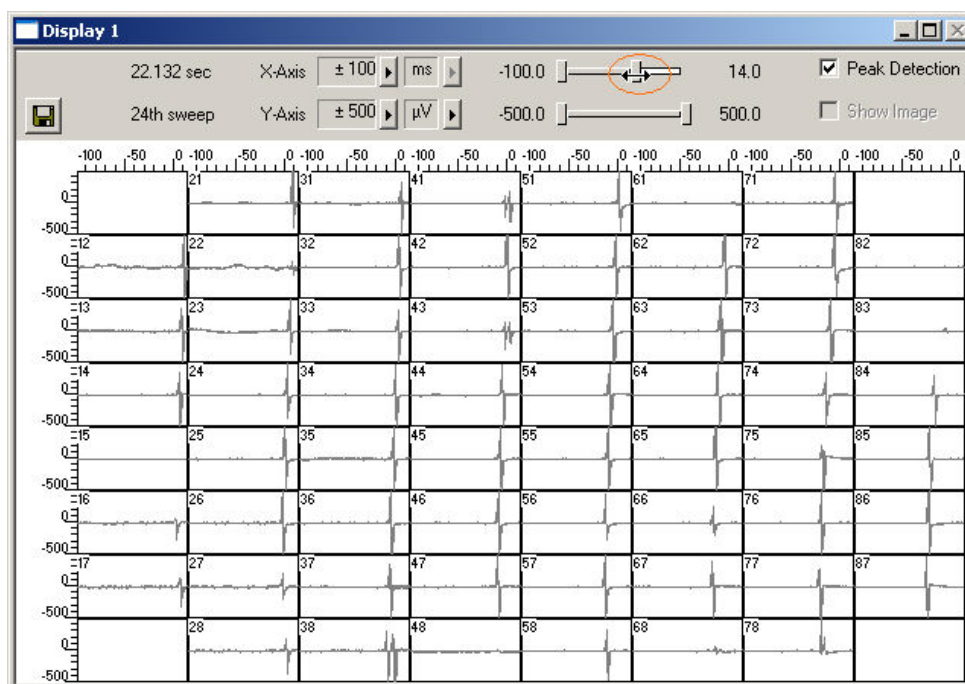
- In the tree view pane of the virtual rack, select the **Display 1**, click the **Window** tab, and click **Start on Trigger**. **Trigger 1** is preselected, as it is the only available trigger stream in the rack.



- In the **Data Display**, select the option **Peak Detection**.
- Click **Start**  (either on the **Measurement** menu, the toolbar, or the **Rack** tabbed page) to start the replaying of the data.

Each virtual instrument in the rack configuration starts to process the channels and data streams that were assigned to it. For example, data is graphed in the displays, and so on. You can adjust the replaying **speed** on the **Replayer** tabbed page of the **Replayer**.

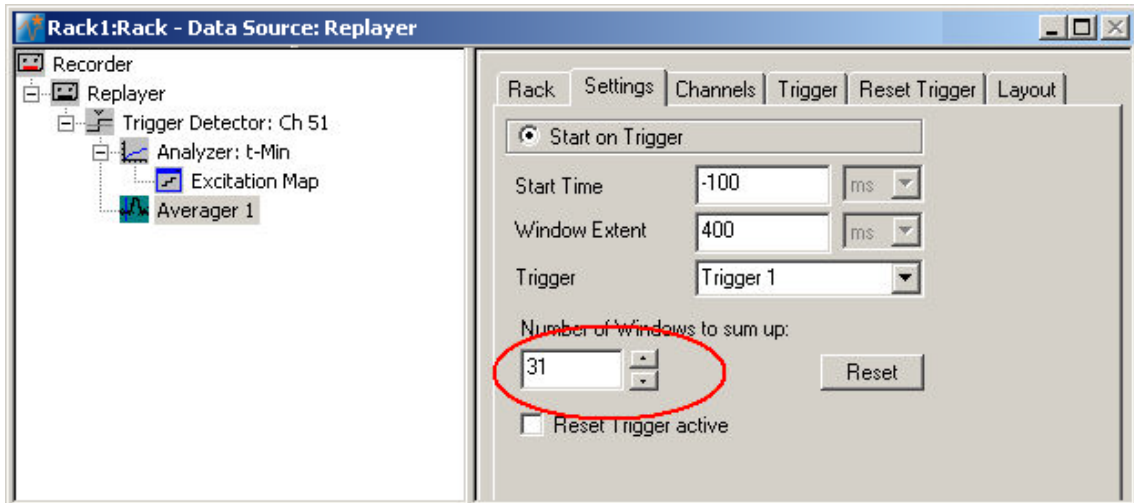
Sweeps triggered on the activity of channel 51 are plotted in the **Data Display**. You see cardiac activity on all channels. Zoom in the x-axis to see the different kinetics on different channels.



You can see that the signal originates from the bottom right corner of the MEA and propagates to the upper left. Relatively to the trigger event (on channel 51), the signal appears at time = 30 ms at, for example, electrode 77, and it takes about 40 ms until it appears at time = 10 ms at electrode 12.

11.9 Averaging Cardiac Signals

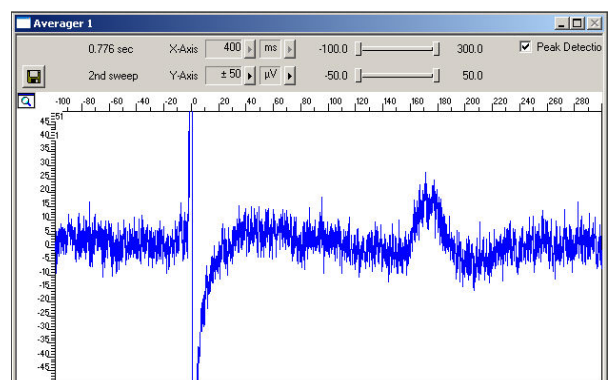
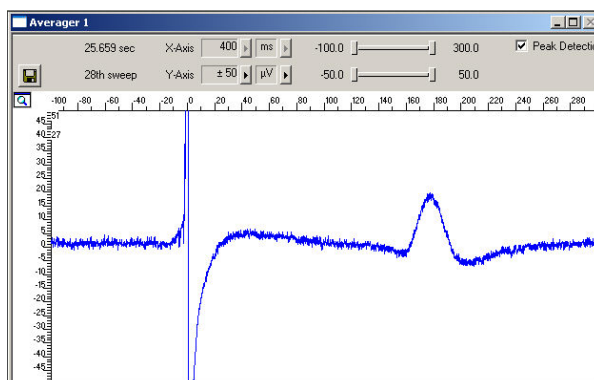
1. On the **Edit** menu, click **Add Averager** to add an **Averager** to the virtual rack (in series to the **Trigger Detector**).
2. In the tree view pane of the virtual rack, select the **Averager** and click the **Settings** tab. In this page, a trigger is defined for the **Averager**, and the window extent of the cutout relative to the trigger event.
3. Select the **Trigger 1** that is generated by the **Trigger Detector**. (It is also possible to set up a new trigger for the **Averager** on the **Trigger** tabbed page.) Select the number of windows that should be summed up, in the range of 30. Select an appropriate **Start Time** and **Window Extent** (relative to the trigger event) for the about 200 ms long signals, for example 50 ms and 400 ms.



4. On the **Measurement** menu, click **Start** to start the **Replayer**. Select **Peak Detection** in the **Averager** window and zoom in the axes.

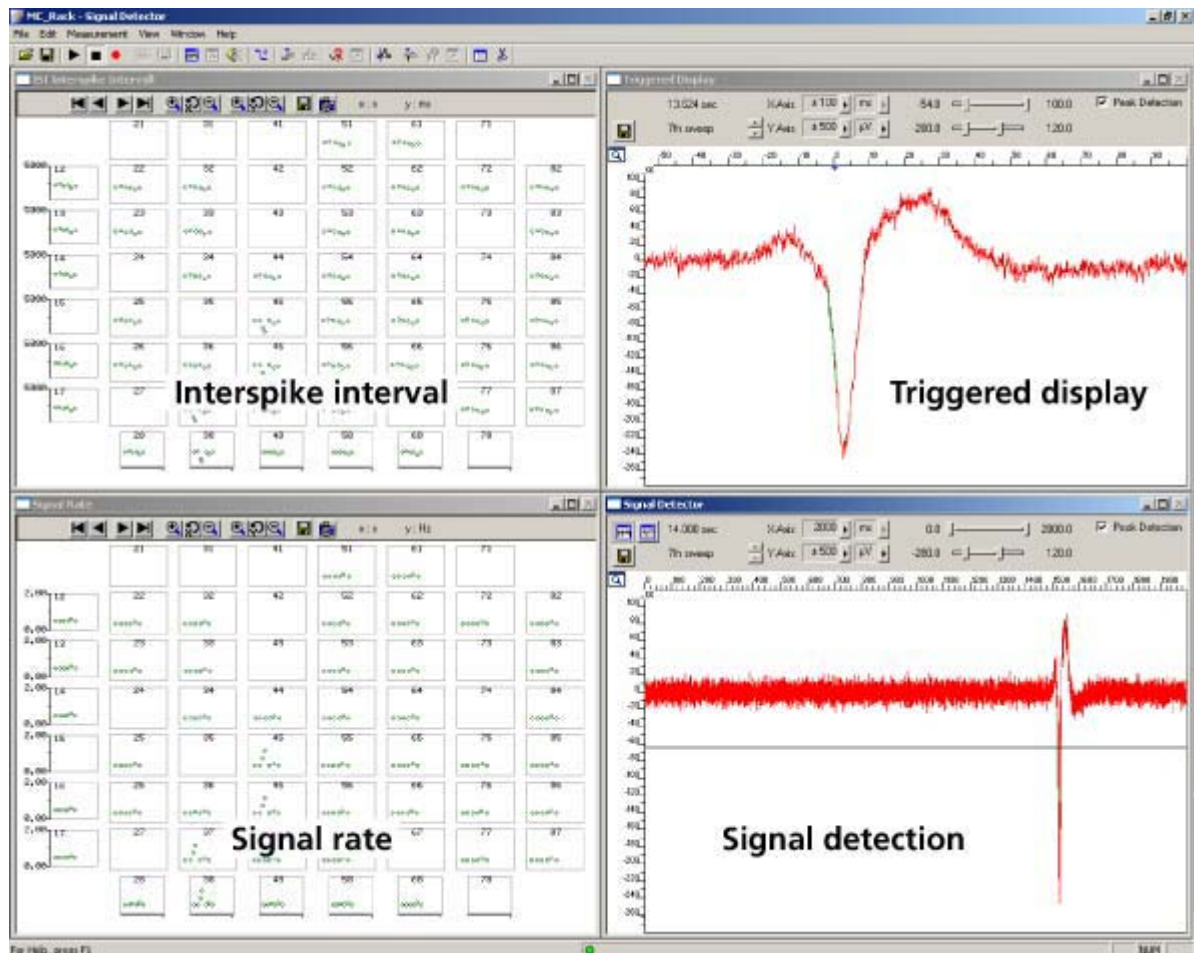
The data traces in each window are summed up until the target number of windows is reached. The summed up signal generates the data stream **Averager 1**, which can be saved with the **Recorder**.

You can see that the signal-to-noise ratio and signal quality improves significantly with each window that is summed up.



12 Event-Based Analysis of Cardiac Activity

12.1 Aim of Experiment



This chapter describes how cardiac signals can be automatically detected with the **Spike Sorter**. The **signal rate** and the **interspike interval (ISI)** as parameters that characterize the beating rhythm can be monitored online during the experiment, and can be saved together with the raw data. The export features of the event-based parameter displays allow a direct export of the extracted parameters in universal ASCII format, or as a graphics file.

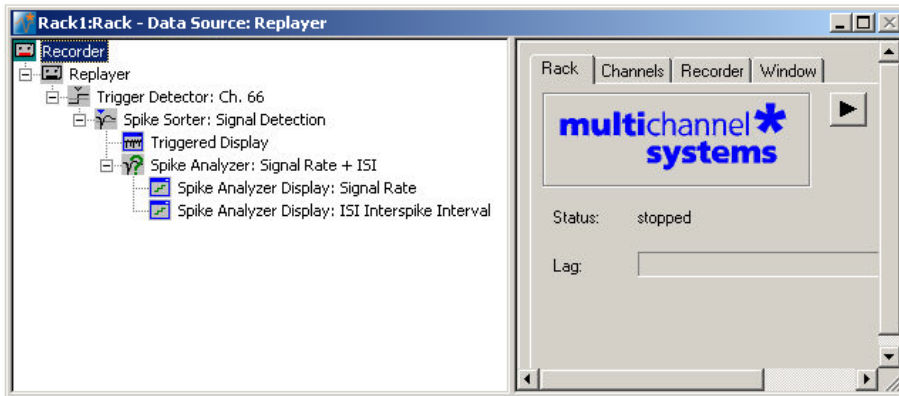
The demo data used in this part of the tutorial was recorded from a cardiomyocyte cell line from mouse that retains a differentiated cardiac myocyte phenotype: **HL-1** from Dr. W. Claycomb.

You will see in this part of the tutorial how a virtual rack is set up for those purposes, and how typical signal waveforms look like. Please note that the amplifier filter settings affect the waveforms of recorded signals. Please see additional documentation, for example, the MEA1060 Manual or MEA Application Notes for more information on this matter.

The demo data file **Cardiomyocytes_HL1.mcd** shown here was recorded from HL-1 cells at a sampling rate of 10 kHz with a gain of 1000, and a bandwidth of 1 Hz to 3 kHz.

- Rack file: **Cardiomyocytes_HL1_Demo.rck**
- Data file: **Cardiomyocytes_HL1.mcd**

You will learn in this chapter ...




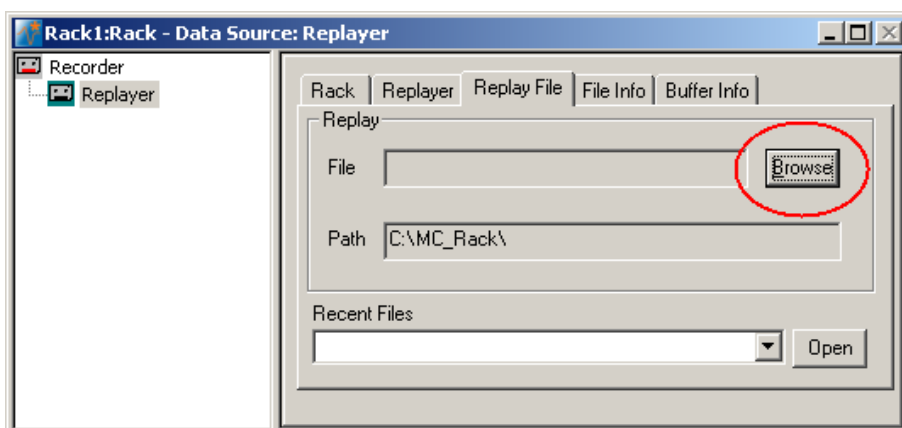
- How to define **basic functions** in your **rack configuration**:
 - Monitor cardiac activity (**Trigger Detector, Data Display**).
 - Signal detection (**Spike Sorter**).
 - Analyzing the signal rate and interspike interval (**Spike Analyzer**).
 - Monitoring the signal rate and interspike interval (**Spike Analyzer Display**).

Please see also the MEA Application Note "HL-1 Cardiac Cell Line by Dr. W. Claycomb" for more information on the preparation techniques and experiments available for download on the web site of Multi Channel Systems MCS GmbH.

12.2 Preparations

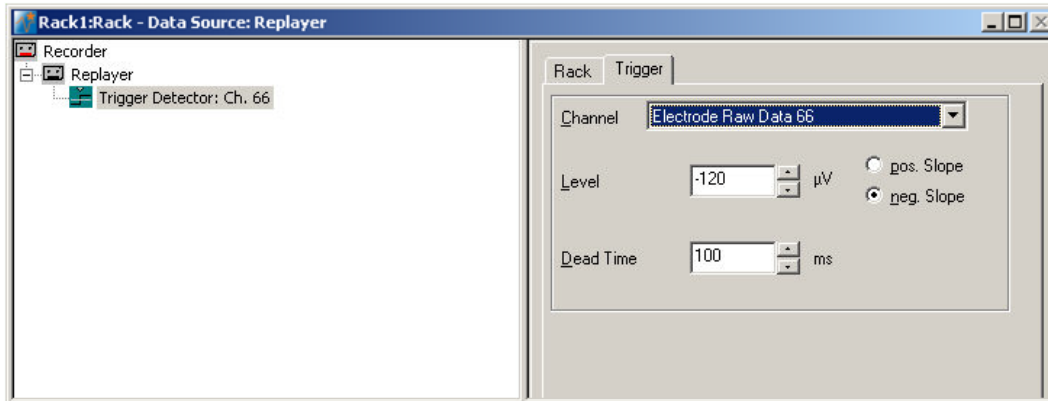
We recommend that you take some time for rebuilding the virtual rack for this application step by step in this tutorial, but if you prefer having a look at the completed rack or if you get stuck during the tutorial, you can also open the rack file "Cardiomyocytes_HL1_Demo.rck". Click **Open** on the **File** menu to open the rack file.


1. Copy the **complete MC_Rack Tutorial** folder from the installation volume into the MC_Rack program directory with the following path "c:\Program Files\Multi Channel Systems\MC_Rack\"
2. Start MC_Rack or click **New** on the **File** menu to generate a new virtual rack file configuration.
3. Click  on the toolbar to add a **Replayer** to your virtual rack.
4. In the tree view pane of the virtual rack, select the **Replayer** and click the **Replay File** tab. Click the **Browse** button and browse to the **Offline** subfolder of the **Tutorial** folder, and load the data file **Cardiomyocytes_HL1.mcd** into the **Replayer**.

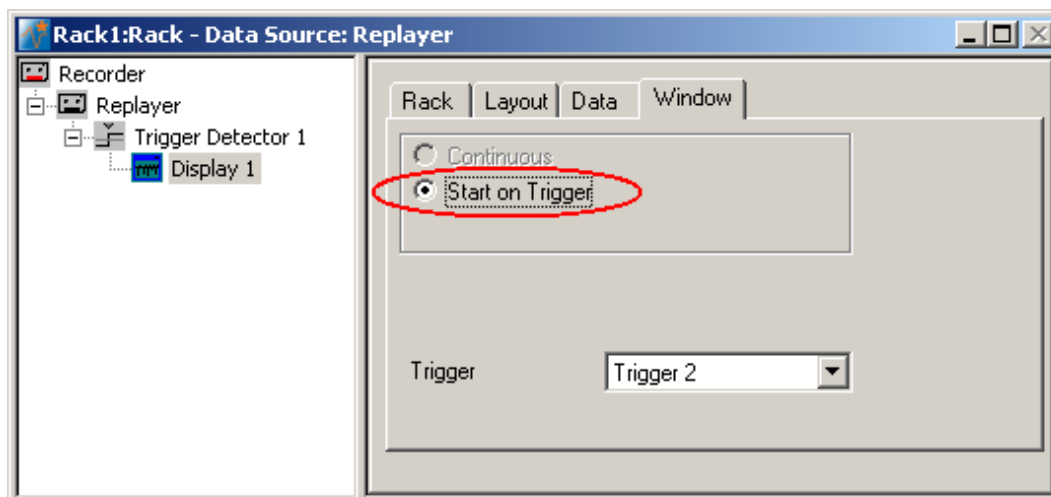


12.3 Monitoring Cardiac Activity (HL1)

1. On the **Edit** menu, click **Add Trigger** to add a **Trigger Detector** to your virtual rack.
2. In the tree view pane of the virtual rack, select the **Trigger Detector**, and click the **Trigger** tab and select the channel that you want to use as a trigger, for example, channel 66. You can change the detection threshold level as well on this page (if the signal size is smaller or the noise level higher), but this is not necessary in the moment. Increase the dead time to the expected waveform length (here about 100 ms), to make sure that one signal is detected only once, as a single event.



3. In the tree view pane of the virtual rack, select the **Trigger Detector** and click  on the toolbar to add an oscilloscope-like **Data Display** in **series** with the **Trigger Detector** to the virtual rack. (If you would put both instruments in parallel, you would not be able to use the trigger stream generated by the **Trigger Detector** for triggering the display, because virtual instruments can only use the output streams of other virtual instruments that are upstream in the virtual rack tree.) Have a look at the display settings (**Layout** tabbed page) and make sure an **8x8 MEA Channel Map** is loaded as the **Channel Map**, and **Electrode Raw Data** as the data input stream (see **Data** tabbed page).
4. In the tree view pane of the virtual rack, select the **Display 1**, click the **Window** tab, and click **Start on Trigger**. **Trigger 1** is preselected, as it is the only available trigger stream in the rack.



5. In the **Data Display**, select the option **Peak Detection**.

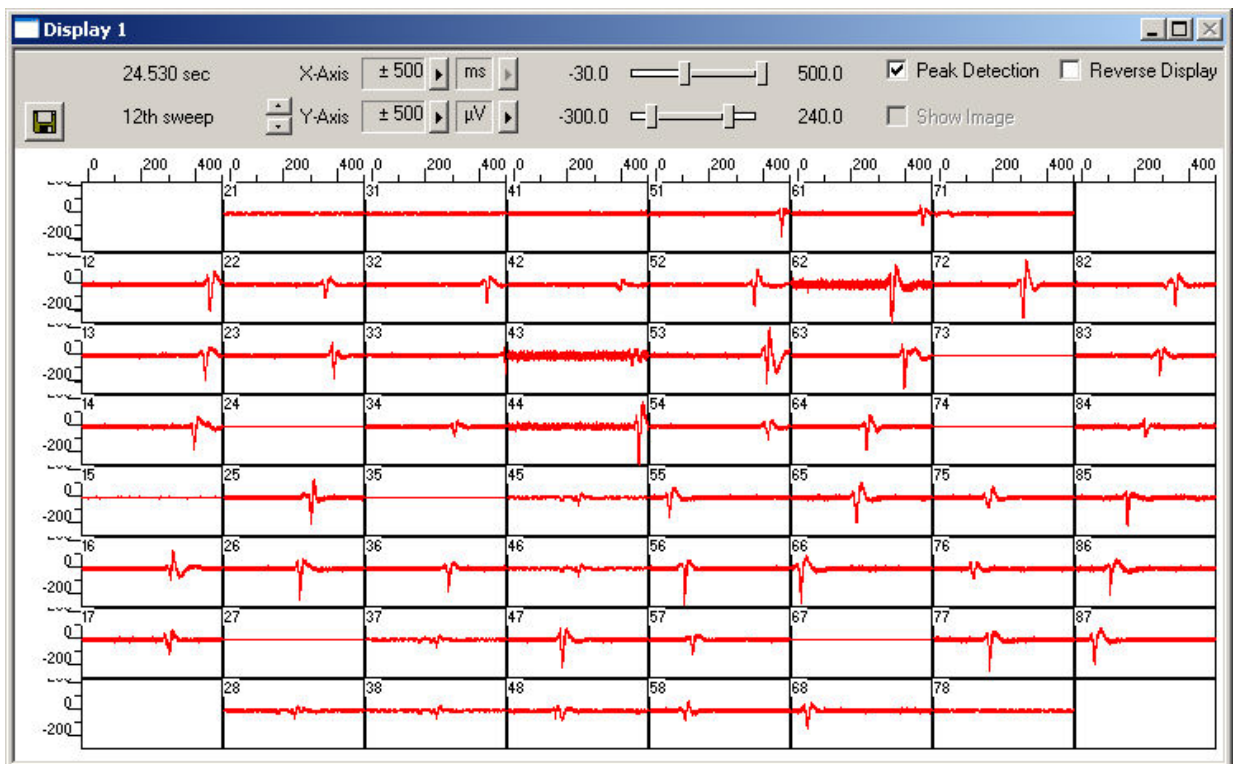
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- Click **Start**  (either on the **Measurement** menu, the toolbar, or the **Rack** tabbed page) to start the replaying of the data.

Each virtual instrument in the rack configuration starts to process the channels and data streams that were assigned to it. For example, data is graphed in the displays, and so on. You can adjust the replaying **speed** on the **Replayer** tabbed page of the **Replayer**.

Sweeps triggered on the activity of channel 66 are plotted in the **Data Display**. You see signals only on a few channels, because the conduction velocity of this cell type is very slow, and therefore, the time lag between the area, where the excitation starts to spread and other areas on the MEA is quite high. Therefore, you should increase the x-axis range to see the overall activity.

Zoom in the x-axis to see the different kinetics on different channels. You now see there is cardiac activity on a majority of channels.




The excitation starts at electrode 66 (which also triggers the display). Relatively to the trigger event (on channel 66), the signal appears at time = 50 ms at one of the neighboring electrodes # 55, and it takes about 460 ms until it appears at electrode 51. This documents a very different kinetic behavior as, for example, the chicken embryonic cardiomyocyte culture shown in chapter "Cardiac Signals", where the signal spread over the complete MEA in only 40 ms.

A false-color excitation map as shown in the demo rack **Cardiomyocytes_ExcitationMap_HL1.rck** illustrates the signal spreading very well. A detailed explanation how to set up an excitation map is given under "Signal Propagation" in chapter "Cardiac Signals". You should use channel 66 for triggering, as the signal starts to spread from here, and because the signal quality is very good on this channel.

12.4 Signal Detection (HL1)

The **Spike Sorter** is a general event detector for neuronal or cardiac applications that can either operate in two modes: One method uses a **threshold**, the other recognizes the **amplitude** and **slope** of a waveform. Both methods regard either the **rise** or the **fall** of a signal, depending on the settings. The **Spike Sorter** has a special display where you can adjust the spike detection level for each channel separately with the mouse. The **Spike Sorter** is called **Spike Sorter** because it also has a sorting functionality, which is used for spike analysis, but does not make much sense for cardiac applications. Therefore, it is not explained in this chapter.

There are two modes for **detecting spikes** with MC_Rack. The **Threshold** method is especially useful if the overall baseline is stable, and signals appear approximately on the same height. You can set the detection level for each channel separately (manually or automatically), or you can choose the same level for all channels. If you have an unstable baseline, signals will be missed, or noise signals may be detected as signals with the threshold method. In this case, the **Slope** method is more appropriate. As the baseline is very stable in this experiment, we will use the (easier) **Threshold** mode.

1. On the toolbar, click  to add a **Spike Sorter** to the virtual rack. The **Spike Sorter** display appears.
2. Select the option **Peak Detection** in the **Spike Sorter** display.
3. Click the **Channels** tab and assign the **Electrode Raw Data** stream to the **Spike Sorter**.
4. Click the **Start** button and zoom the display until you can see the signals clearly. You see that the baseline is on a similar level for all channels and that the spikes are quite small (approximately 10 to 20 μV).

In contrast to the triggered display we used before, this display operates in a **continuous** (oscilloscope-like) mode: The displayed data is **not** synchronized to a trigger event. The **refresh rate** of the display is defined by the x-axis range selected from the drop-down list in the display header. For the slow signal rate of cardiac signals, a refresh rate of 1 or 2 s is fine.

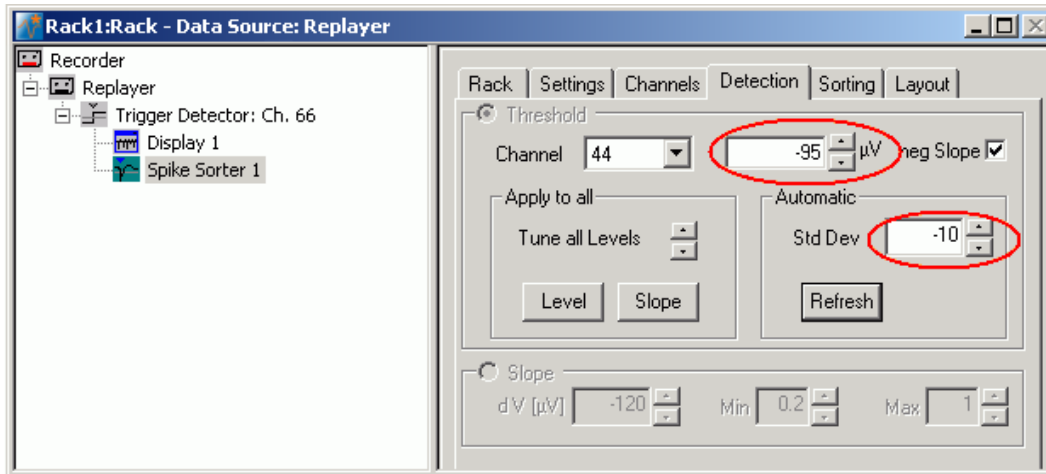
5. In the tree view pane of the virtual rack, select the **Spike Sorter**. Click the **Detection** tab. Make sure the **Threshold** method is selected. You can now set the detection threshold. Start with a value of 100 μV , for example.
6. Type in 100 in the text box. This value does apply now to the selected channel. Under **Apply to All**, click **Level** to apply this detection level to all channels. You see that this level is fine for most, but not for all channels, as the signal amplitude differs.
7. You could now adjust the threshold level for each channel separately. A more convenient alternative is to use the **Automatic** feature to automatically assign an **individual** detection level to **each** channel. The standard deviation of each data trace is used to estimate its signal threshold. A time interval of 500 ms is used to calculate the standard deviation. You set the factor, by which the standard deviation is multiplied. The sign of the factor determines whether the spike detection level is positive or negative. You generally will use a negative factor. A value between -10 and -20 is generally appropriate for larger cardiac signals. The smaller the signals, the lower you need to set the **StdDev** parameter. The higher you set the parameter, the more strict will be the signal detection. As there are also smaller signals here, you should start with a lower value. For example, enter a standard deviation of 10, and click **Refresh** to calculate the standard deviation and apply the result.

The detected waveform appears in a different color on the display. The detection level determined for a particular channel by the **Automatic** feature is visible as a horizontal straight line. For example, the threshold was set to 50 on channel 34, and to 95 on channel 44 (with a higher noise level).

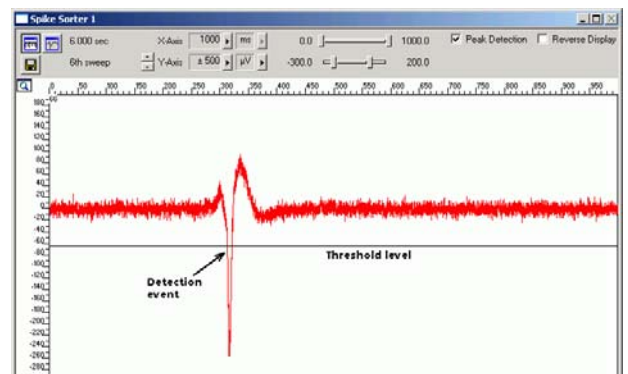
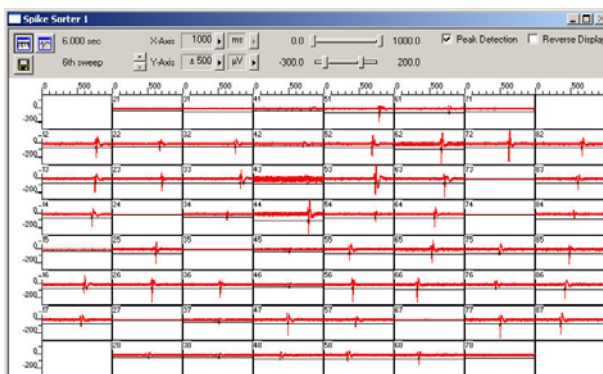
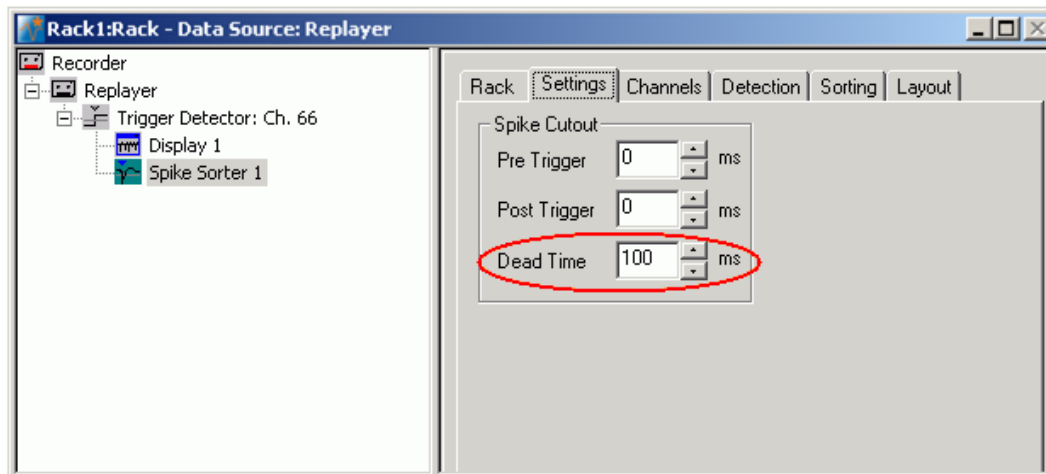
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- You can then fine tune the detection level by dragging the horizontal line of each channel in the **Spike Sorter** display with your mouse. This might be necessary, for example, on channel 43, where the signal to noise ratio is not so good.


As a single signal crosses the threshold several times, it is necessary to increase the dead time, that is, the time following a detection event during which the Spike Sorter will not accept a new signal.

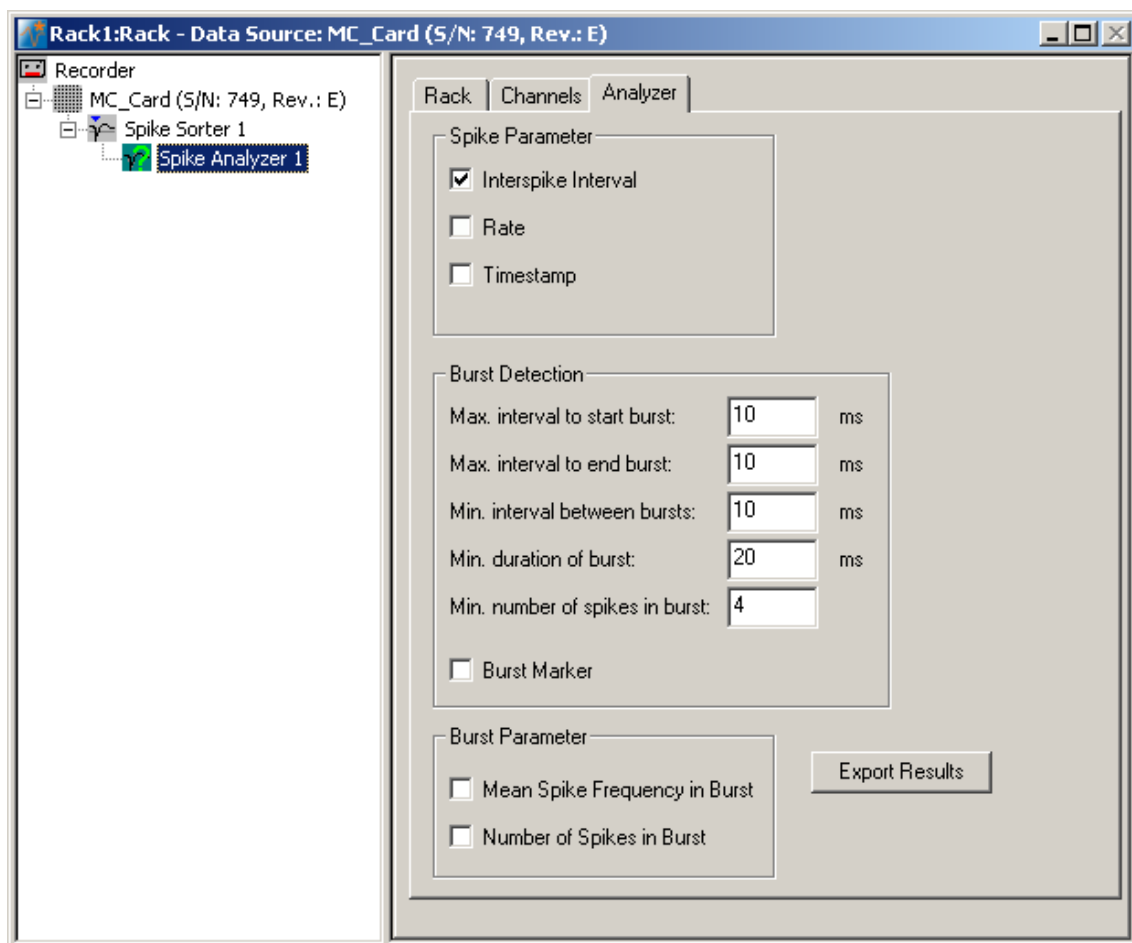


- In the tree view pane of the virtual rack, select the Spike Sorter and click the Settings tab. Enter an appropriate dead time, so that a single signal is not detected multiple times. As the waveform is about 100 ms long, 100 ms is an appropriate dead time. The Pre- and Post-Trigger times define the cutout length. As we are not interested in the cutouts, but only in the detection events, we can set the cutout times to zero. The generated Spike data stream will then contain only the time points of the detection events. (This is not important for monitoring, but only for recording to a data file.)




12.5 Analyzing the Signal Rate

1. On the toolbar, click  to add a **Spike Analyzer** in **series** with the **Spike Sorter** to the virtual rack. (You cannot put the **Spike Analyzer** in parallel to the **Spike Sorter**, because virtual instruments can only use the output streams of other virtual instruments that are upstream in the tree view as input streams.)
2. In the tree view pane of the virtual rack, select the **Spike Analyzer**. Click the **Channels** tab.
3. Select all channels of the **Spikes** data stream. (You could deselect channels for saving computer performance or hard disk space in a real experiment situation.)
4. Click the **Analyzer** tab.
5. Select the parameter of interest, or both the **Interspike Interval** and the **Rate**.



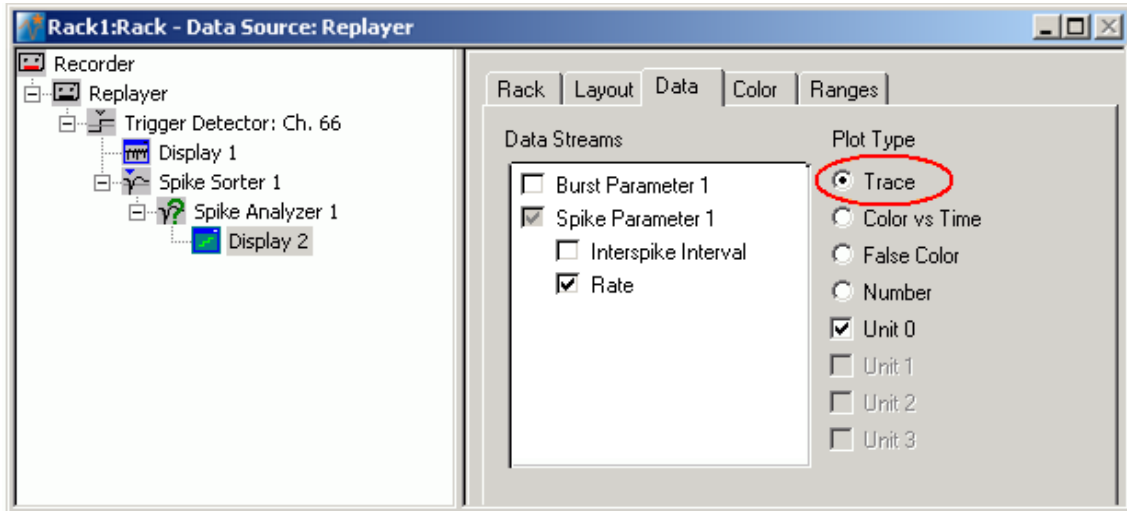
12.6 Monitoring the Signal Rate

For plotting parameters that were extracted by the Spike Analyzer, we will need a **Spike Analyzer Display**, which features are optimized for graphing **Parameter** streams. The **Spike Analyzer Display** can plot the **Interspike Interval** and the **Rate**. If you want to monitor both parameters in parallel, simply set up two **Spike Analyzer Displays** in your virtual rack.

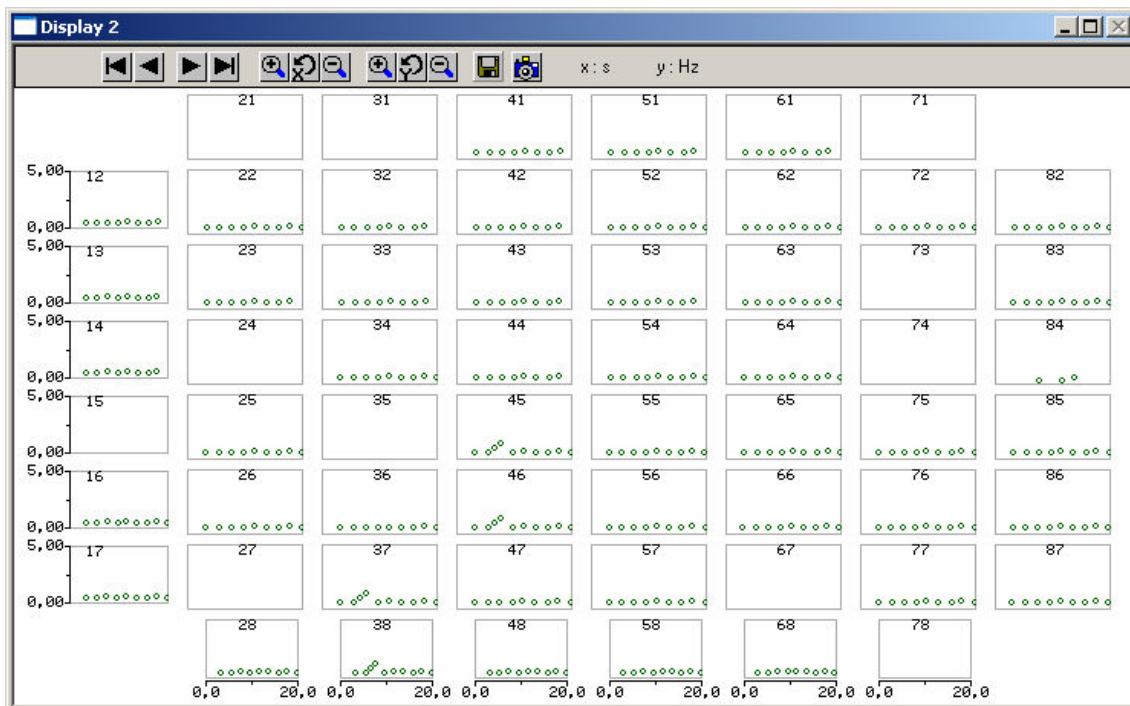
1. In the tree view pane of the virtual rack, select the **Spike Analyzer** and click  on the toolbar to add a **Spike Analyzer Display** in **series** with the **Spike Analyzer** to the virtual rack. (It would not be possible to add the **Spike Analyzer Display** in parallel to the **Spike Analyzer**, as the replayed data file does not contain any parameter streams as input streams for the display, and virtual instruments can only use the output streams of other virtual instruments that are upstream in the virtual rack tree.)

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- In the tree view pane of the virtual rack, select the **Spike Analyzer Display**. Click the **Layout** tab to load the MEA Channel Map.
- Click the **Data** tab and select the **Spike Parameter 1** stream. Select the parameter of interest, that is, either the **Interspike Interval** or the **Rate**.



- Start the **Replayer**. The signal rate is plotted over time. You need to adjust the axes to see the data points.
- Click the **Ranges** tab and adjust the ranges. For example, set the x-axis range to 25 s, and the y-axis range from 0 to 5 Hz. (You can see by subtracting the **Start** and **Stop** times on the **File Info** page of the **Replayer** that the total file length is 26 s.)



The signal rate is very stable on most channels at about 0.5 Hz. You easily detect a short arrhythmia on some electrodes, for example, # 45.

13 Saving the Rack and Saving Data

13.1 Saving a Rack

Save the virtual rack configuration if you like to keep it for future use, for example for an offline analysis of identical data recorded in another experiment.

1. On the **File** menu, click **Save As**.
2. The **Save As** dialog box opens.
3. Browse your folders and select a path.
4. Enter a file name and confirm by clicking **Save**.

The file extension for the rack files is **.rck**.

13.2 Selecting Data Streams for Recording

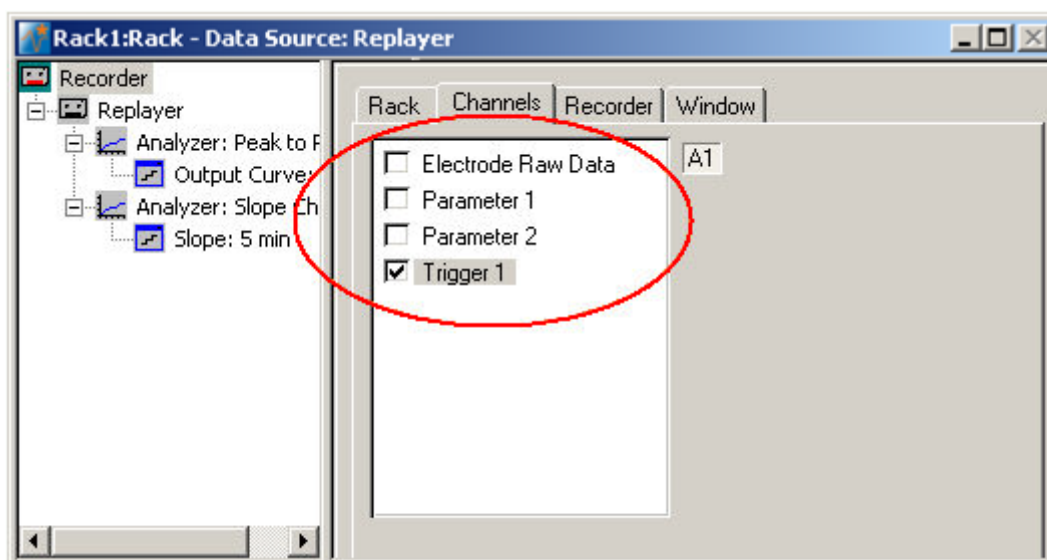
MC_Rack's philosophy is to **strictly separate** the actions of **all** virtual instruments in a rack. That means, that you could **record** to hard disk completely different data streams and channels than you **monitor** on the screen. This has the advantage that you can store exactly the channels you are interested in, but it also has the slight disadvantage that all virtual instruments have to be set up **separately**. Please be especially careful when configuring the **Recorder**, to avoid data loss.

When you have finished setting up the rack, you can select the data streams and channels that you want to save to the data file specified in the **Recorder**.

Selecting data streams and channels for recording

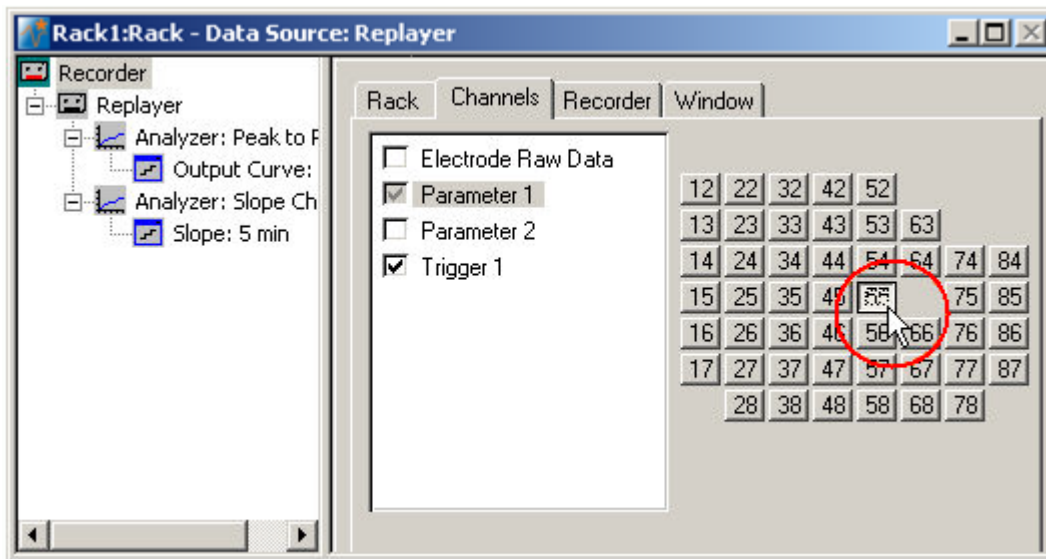
The fate of each single channel is **independent** from other channels. You can pick exactly the channels you like to save from all generated data streams. For example, you can decide to save only **one** channel of **raw data**, but the **peak-to-peak amplitude** results of **all**, or of a **specific selection** of channels.

1. Select the **Recorder** in the virtual rack tree view pane and then click the **Channels** tabbed page. On the white pane on the left of the **Channels** page, you see the data streams that are available with your rack configuration. When replaying data, you are generally more interested in the parameter data streams, but you can rerecord the raw data as well, in case that you want to save the raw data and the extracted parameters to the same data file.



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- Click the data stream that you are interested in, generally the parameter streams. The available electrode channels appear in a button array on the right side. Parameter stream 1 is generated by the first analyzer in the rack (here: for extracting the peak-peak amplitude), parameter 2 is generated by the second analyzer (here: for extracting the slope).



- You can now either select all channels by clicking the check box next to the data stream name, or you can pick single channels by clicking the corresponding buttons. For more information, please see "Channel Selection" in the MC_Rack Features section. Only data from the selected channels will be saved to the hard disk.

13.3 Creating a Data File

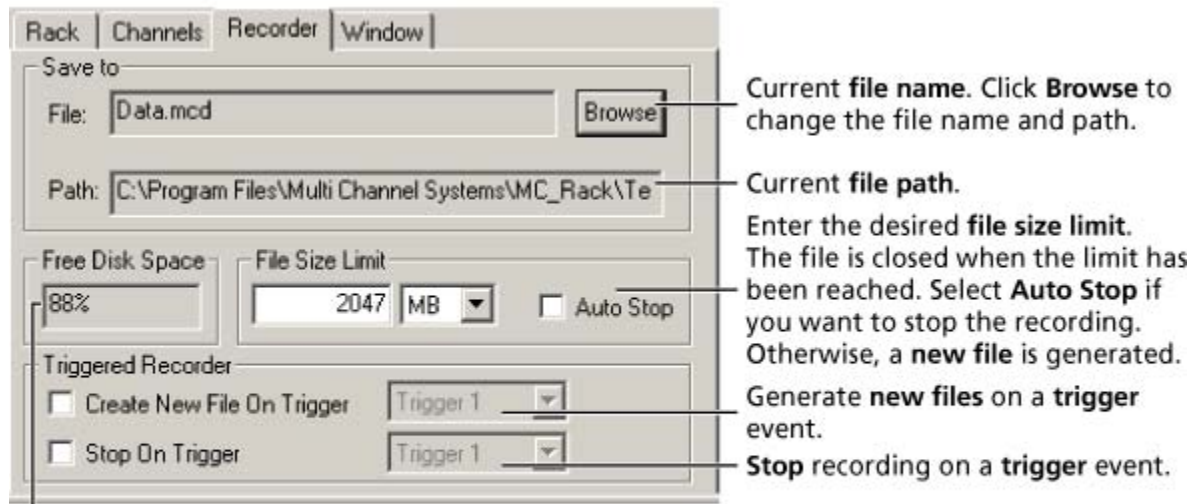
If you want to write the parameters that you will extract in an offline analysis to the hard disk, you have to specify the file name and path in the **Recorder**. (It is not possible to change or overwrite existing data files in MC_Rack, for example, adding the spike rate to an existing data file, but you can record the raw data together with the spike rate stream to the same file.)

Choosing the file name and path

- Click the **Recorder** tab.
- Browse** your folders and select a path.
- Type a file name into the text box.
- Confirm by clicking **Save**.

The data file is then generated automatically when you start MC_Rack in **recording** mode. The file extension for the data files is ***.mcd**.

Recorder settings



Displays available disk space on the currently selected hard disk.

File size limit

When the maximum file size specified by the user has been reached, a new file is generated automatically. The file name is extended by four digits, counting up, for example LTP-Parameters0001.mcd, LTP-Parameters0002.mcd, and so on.

If you rather prefer that the recording is completely stopped when a file has reached the maximum size, please select the option **Auto Stop**.

For information on more options, please see "Generating Data Files" in the MC_Rack Features section.

Selecting data streams and channels

As has been said before, the fate of each single channel is **independent** from other channels. You can pick exactly the channels you like to save from all generated data streams. For example, you can decide to save only **one** channel of **raw data**, but the **peak-to-peak amplitude** results of **all**, or of a **specific selection** of channels.




→ Click the **Channels** tab.

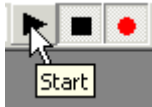
As long as the rack is still empty, you see an empty box. There are no channels available at this point, because you have not chosen a data source yet (**MC_Card** for online data acquisition or **Replayer** for replaying data files). Without a data source, there are no data streams available for recording. Remember later, when you have completed the rack, to assign the channels that you like to save to the data file to the **Recorder**.

After you added a **data source**, you will see the electrode raw data streams provided by the data source (for example, electrode raw data, analog data, and digital data from the **MC_Card**, or the data streams included in the data file loaded into the **Replayer**). If the rack file contains virtual instruments that **generate** data streams such as Spikes from a **Spike Sorter** or Parameter streams from an **Analyzer**, these data streams will be available for recording as well.

13.4 Starting Data Acquisition and Recording

Now that you have completed the virtual rack, you are ready to start the rack.

- Click **Start**  (either on the **Measurement** menu, the toolbar, or the **Rack** tabbed page) to start the data acquisition. Each virtual instrument in your rack starts to process the channels and data streams that were assigned to it.
- Click first **Record**  and then **Start**  to write data to the hard disk. The data from the electrodes selected in the **Recorder** is saved to the file and location specified in the **Recorder**.



- Click **Stop**  to stop the data acquisition.



Warning: **Only** data of the channels and data streams that were **selected** in the **Recorder** are saved in your data file when you start a recording. Data is **only** saved to the hard disk when the red **Record** button is pressed **in**. Make always sure that you have selected all channels of interest, and that the **Record** button is active before starting an experiment to avoid data loss.

14 Glossary

A

aliasing: Frequencies (noise) that are above half the sampling rate (for example above 2.5 kHz at a 5 kHz sampling rate) will be transformed into lower frequencies.

anti-aliasing: Using an analog Low Pass filter for removing high frequency noise, which would lead to aliasing if a sampling frequency lower than twice the bandwidth of the input signal is used.

B

Bandstop Resonator: In signal processing, a bandstop filter is a filter that passes most frequencies unaltered, but attenuates those in a specific range to very low levels. A bandstop filter with a high Q factor has a narrow stopband.

Bessel Filter: A Bessel filter is a type of linear filter with a maximally flat group delay (maximally linear phase response). Analog Bessel filters are characterized by almost constant group delay across the entire passband, thus preserving the wave shape of filtered signals in the passband.

bit: A bit is a binary digit, taking a value of either 0 or 1. The digital data stream of the MC_Card has 16 input and 16 output bits. A single bit can be used, for example, to trigger the recording (based on a TTL output of a stimulator) or an external device. Multiple bits can be used to set up more complex trigger patterns (where several requirements need to be met) or to encode a decimal number, for example, from an external measuring instrument. In a number, bits are always counted starting with 0, i. e. the 16 bits are numbered from 0 to 15.

Bitmap (BMP): Standard Windows bitmap image format, file extension *.bmp

Butterworth Filter: The Butterworth filter is one type of filter design. The frequency response of the Butterworth filter is maximally flat (has no ripples) in the passband, and rolls off towards zero in the stopband. For a second-order Butterworth filter, the response decreases at -12 dB per octave.

C

Chebyshev Filter: Chebyshev filters are analog or digital filters having a steeper roll-off than Butterworth filters. Chebyshev filters have the property that they minimize the error between the idealized filter characteristic and the actual over the range of the filter, but with ripples in the passband. Because of the passband ripple inherent in Chebyshev filters, filters which have a smoother response in the passband but a more irregular response in the stopband are preferred for some applications.

continuous recording: Opposite of triggered recording. Recording is started once and the data is saved continuously to the hard disk until the recording is stopped. Note that a triggered data analysis or display (online or offline) is possible independent of the recording mode.

D

Device Independent Bitmap (DIB): Windows clipboard format, , file extension *.dib

Digital Data: The Digital Data stream is the input data stream from the 16-bit digital input of the MC_Card. In a standard MEA- or ME-System, only three of the 16 digital input bits are available via BNC connectors. A digital in/out extension is available as an accessory for accessing all 16 input and output bits. The Digital Data stream can only have values of 0 and 1. It can be displayed with the Digital Data Display.

Downsampling: The downsampling feature offers the possibility to generate raw and filtered data streams with different sampling frequencies to avoid huge amounts of resulting data when recording more than one data stream.

E

Enhanced Meta File (EMF): Intermediate format for exchanging vector graphics data, file extension *.emf

EPSP: Excitatory postsynaptic potential, an electrical change (depolarisation) in the membrane of a postsynaptic neuron caused by the binding of an excitatory neurotransmitter from a presynaptic cell to a postsynaptic receptor.

F

fEPSP: Field excitatory postsynaptic potential

G

Graphics Interchange Format (GIF): Indexed-color bitmap graphics, LZW-compressed format designed to minimize file size, binary transparency, file extension *.gif

H

High: Logical active state of a bit, also known as state 1 or On. For TTL pulses, a High state means a voltage level of usually 5 Volts.

high pass filter: A filter that passes high frequency waveforms. For example, a 300 Hz high pass filter passes spikes, but no LFPs.

I

ICO: Bitmap format used for Windows icons (in programs and on desktop), file extension *.ico

J

Joint Photographic Experts Group (JPEG): Bitmap format, compressed by selectively discarding data, no transparency, file extension *.jpg

L

LFP: Local Field Potentials are sum field potentials of the activity within a volume of tissue. The amplitude reflects the number of active neurons that are contributing to the LFP. In comparison with single spiking activity, it is a lower frequency signal. To obtain only LFPs (without single spikes), a 300 Hz low pass filter can be used.

Low: Logical inactive state of a bit, also known as state 0 or Off. For TTL pulses, a low state means 0 Volts.

low pass filter: A filter that passes low frequency waveforms. For example, a 300 Hz low pass filter passes LFPs, but no spikes.

lower cutoff frequency: Between the lower cutoff frequency and the upper cutoff frequency of a frequency band is the resonant frequency, at which the gain of the filter is at its maximum. Signal components with a lower frequency than the lower cutoff frequency of a band pass filter are attenuated.

LTD: Long Term Depression

LTP: Long Term Potentiation

M

MB (megabyte): One megabyte is equal to 1,048,576 bytes, or 1,024 kilobytes.

O

OTC: Organotypic Culture: An MEA experiment where the slice is cultured on the MEA for weeks or months, in contrast to an acute slice experiment.

P

peak-peak: Interval from maximum to minimum signal

PPD: Paired Pulse Depression

PPF: Paired Pulse Facilitation

R

Real-time Feedback: The real-time feature allows the user to generate TTL signals in response to defined signal patterns detected by one or several electrodes. These TTL signals in turn can control a stimulus generator (STG) to deliver feedback stimulation to the biological sample within a time frame of one millisecond after the actual event..

ROI: Region of interest: In triggered operation mode, the Analyzer extracts parameters from a user defined region of interest.

S

Savitzky Golay Filter: The parameters of the Savitzky-Golay filter are the order (2 or 4) and the number of points which are included from the right and the left side of a data point for averaging the calculated data point (2, 4, 8, 16, 32, 48). This filter can be used to smooth a noisy signal. However, it is possible to accidentally filter out fast signals, like spikes those in a specific range to very low levels. A bandstop filter with a high Q factor has a narrow stopband.

spike: A field potential that is the extracellular representation of an action potential. The spike rate reflects the intensity of activity. In comparison with LFPs, it is a higher frequency signal. To obtain only spikes (without LFPs), a 300 Hz high-pass filter can be used.

T

triggered recording: A non-continuous recording of cutouts around a trigger event, for example, for recording evoked responses. The cutout size is selected in the Recorder. Note that the data in-between the cutouts is lost permanently.

TTL: Transistor-Transistor Logic. Digital signal for communication between two devices. A voltage between 0 V and 0.8 V is considered as a logical state of 0 (LOW), and a voltage between 2 V and 5 V means a 1 (HIGH).

U

upper cutoff frequency: Between the lower cutoff frequency and the upper cutoff frequency of a frequency band is the resonant frequency, at which the gain of the filter is at its maximum. Signal components with a higher frequency than the upper cutoff frequency of a band pass filter are attenuated.

W

Windows Meta File (WMF): Intermediate exchange format for 16-bit Windows applications, limited vector graphics support, file extension *.wmf

