

**MC_Rack Tutorial:
Retina: MicroERG + Spike Recordings**



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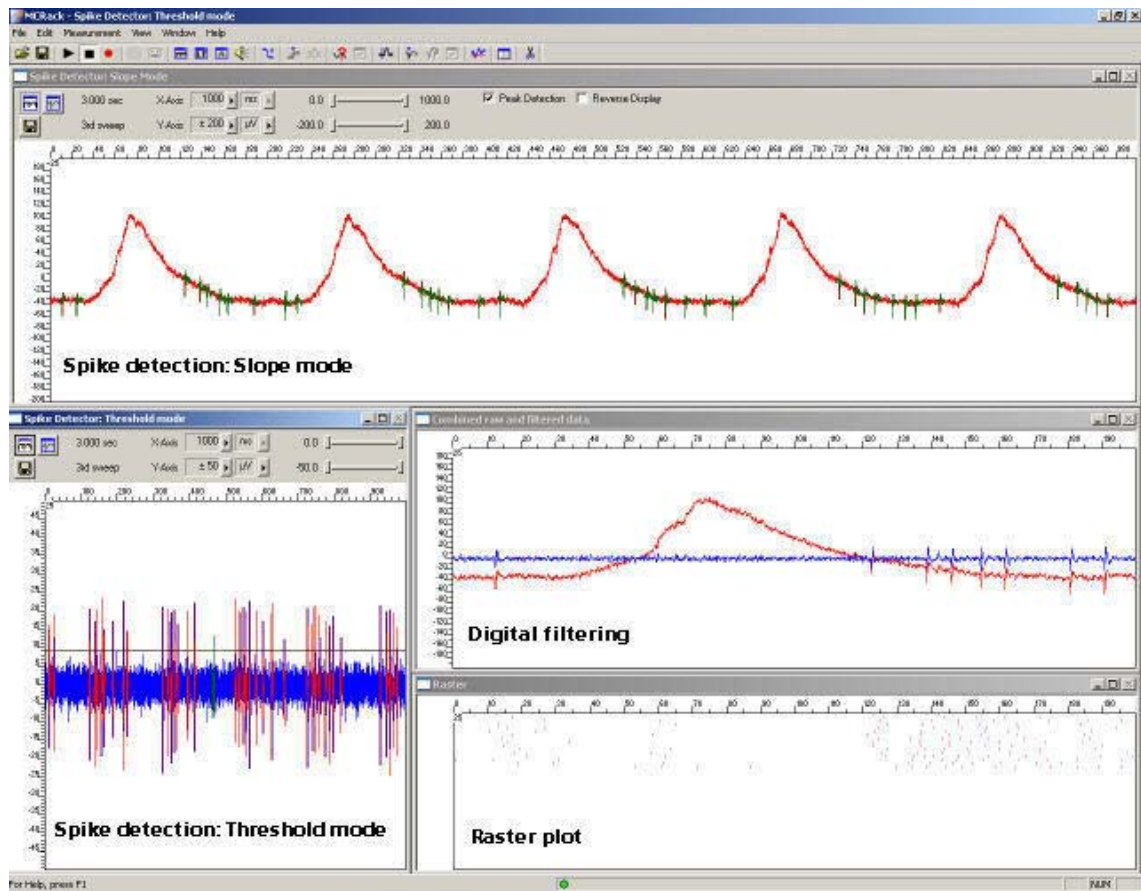
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1 Retina: MicroERGs and Spikes

1.1 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**.

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**

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.

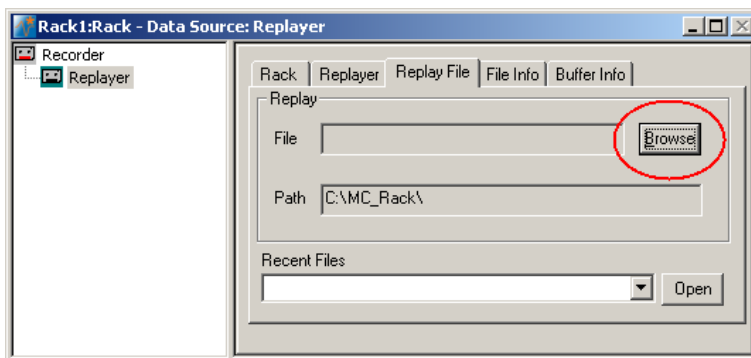
You will learn in this chapter ...

- 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).

1.2 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**.



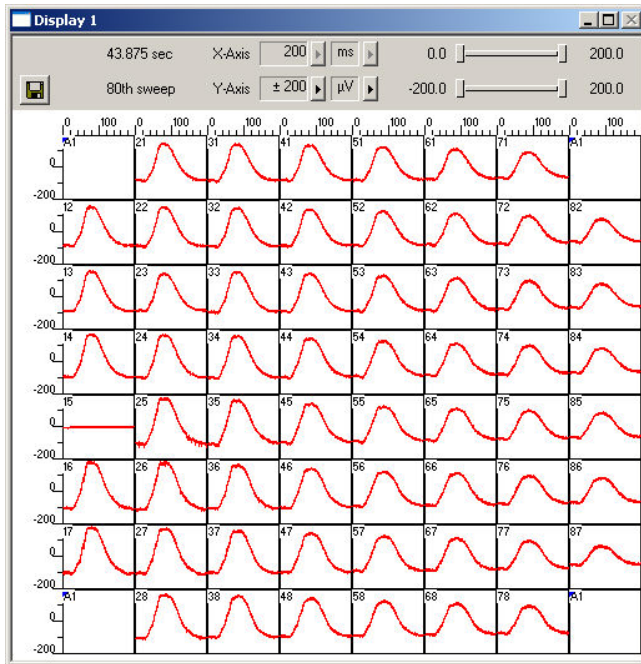
1.3 Monitoring Field Potentials and Spikes

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

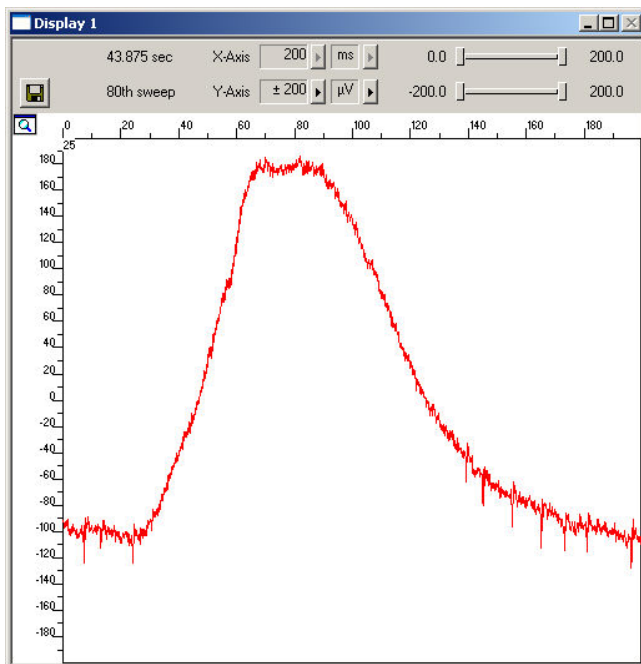
1. Click  on the toolbar to add a **Data Display** to the virtual rack.
2. Click **Start**  (either on the **Measurement** menu, the toolbar, or the **Rack** tabbed page) to start the replaying of the data.
Each virtual instrument in the rack configuration starts to process the channels and data streams 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.




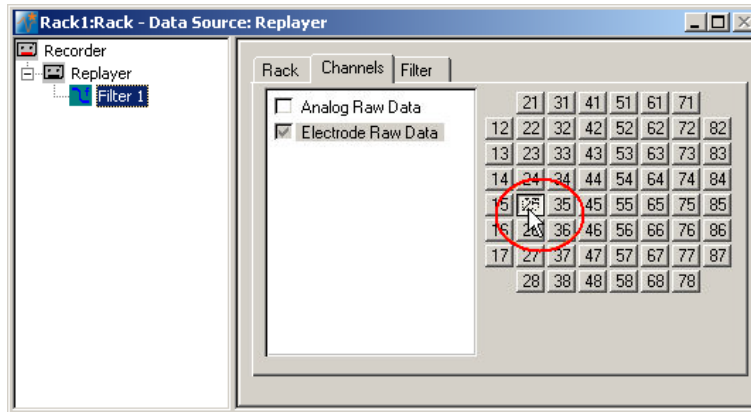
You see spike signals superimposed on the large field potential. It is impossible to detect all the spikes by defining a threshold. To circumvent this problem, you can use a filter that removes slow signals, that is, field potentials. In the current version of MC_Rack, a second order Butterworth filter 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.

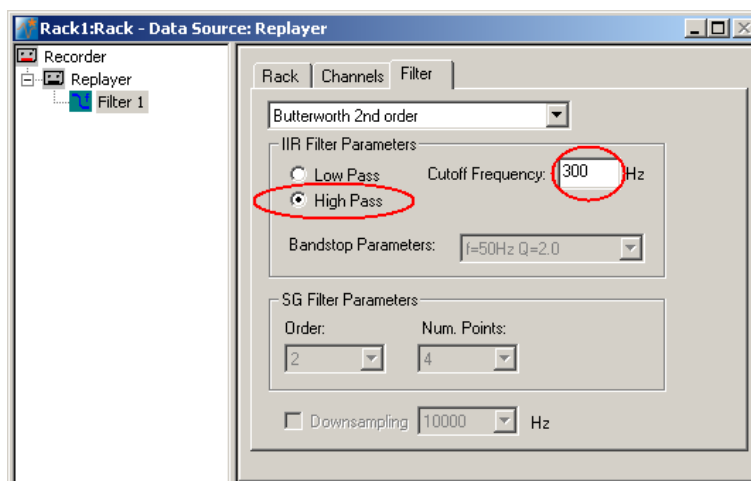
- Click the **Delete Instrument**  button to remove the **Data Display** again to save computer performance.

1.4 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.




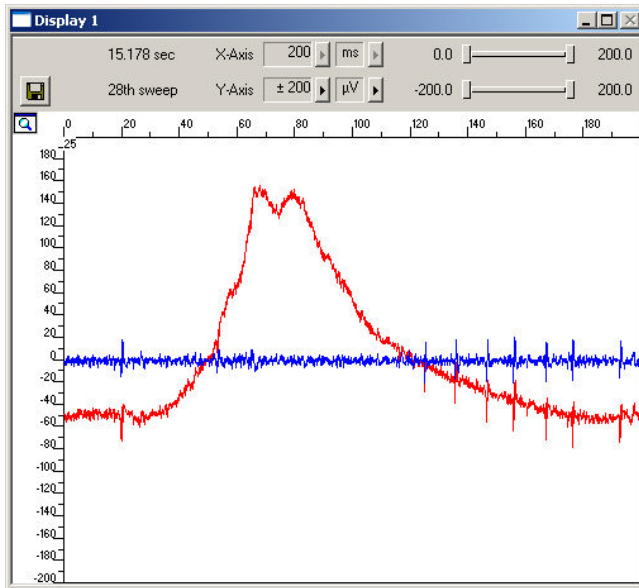
3. Click the **Filter** tab.
4. Select **High Pass**.
You can choose between two filter types: **Low Pass** and **High Pass**. As the name suggests, a low pass filter passes the lower frequency components but attenuates the higher frequency components of signals. To remove slow components (with a low 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.



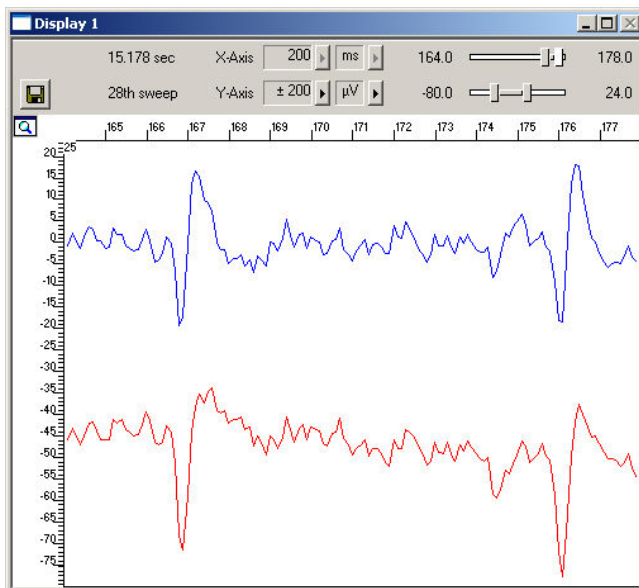
6. Add a **Data Display** to your rack. Load the 8x8 MEA **Channel Map**. On the **Data** tabbed page, select the **Electrode Raw Data** and the **Filtered Data** streams.

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- 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.



- 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.




1.5 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.

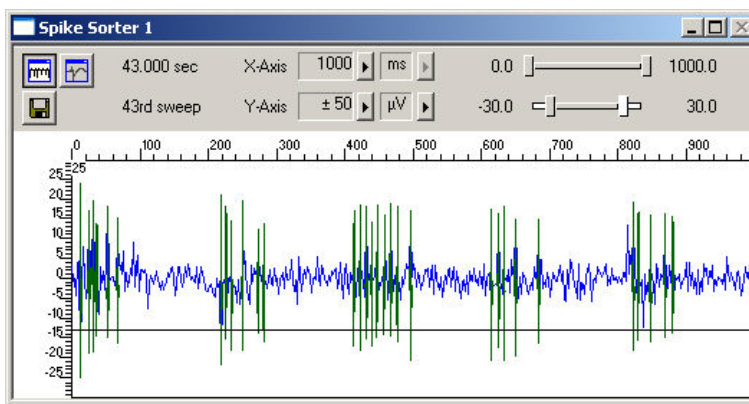
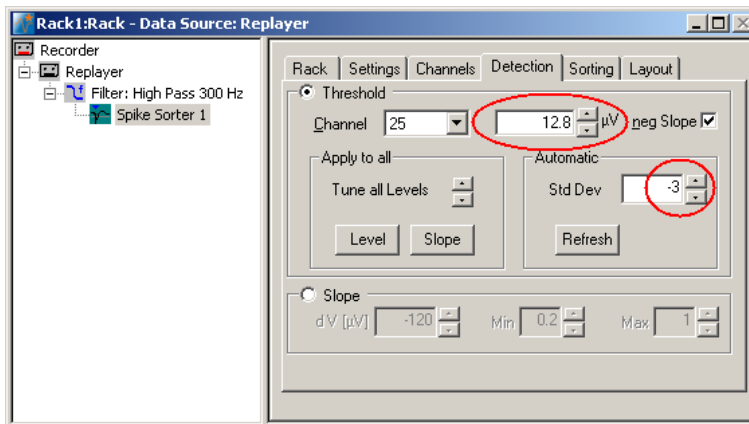
The **Threshold** method is especially useful if the overall baseline is stable, and spikes appear approximately on the same height. You can set the spike detection level for each channel separately (manually or automatically), or you can choose the same level for all channels. If local field potentials 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.

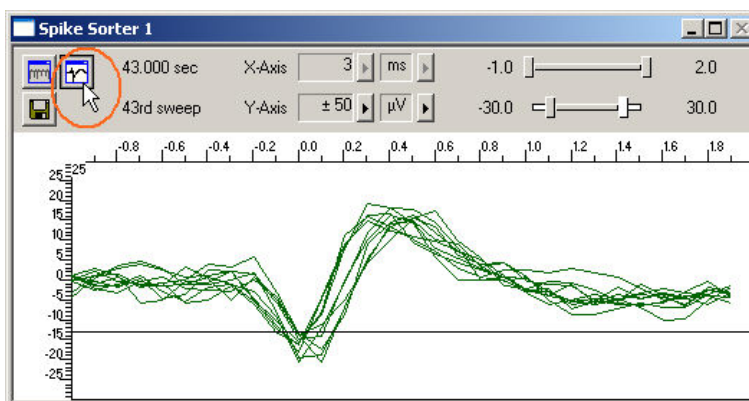
1. On the toolbar, click  to add a **Spike Sorter** in **series** with the **Filter** to the virtual rack. If you put the **Spike Sorter** in parallel to the **Filter**, it would not be possible to use the filtered data stream as an input stream for the **Spike Sorter**, because virtual instruments can 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.



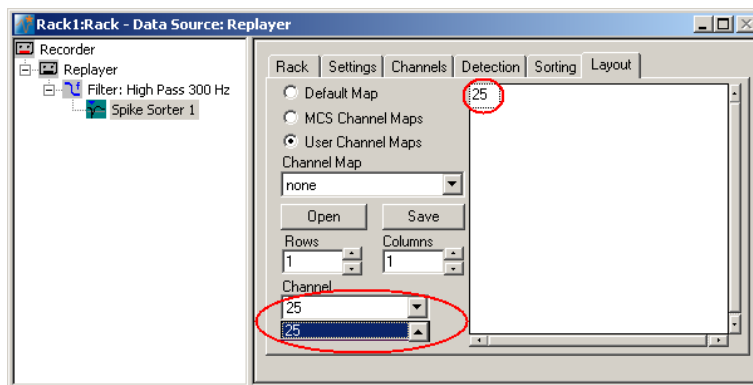
- You can then switch to the spike **overlay** mode of the display by clicking the  button on the display toolbar, and switch back again to the other mode with the  button. Depending on the application, you could then use the spike sorting feature for discriminating signals from ON and OFF ganglion cells,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



1.6 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**.)

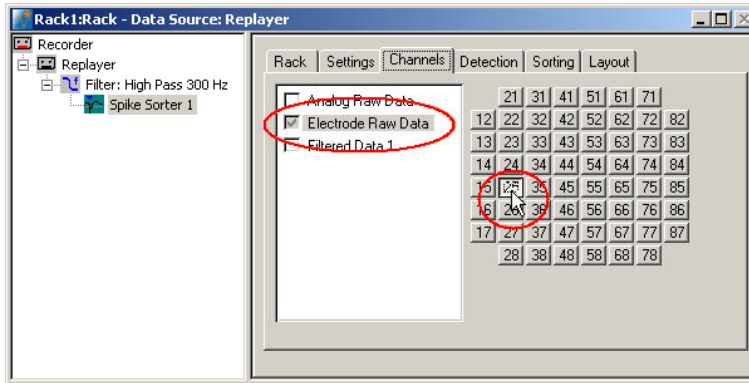
1. In the tree view pane of the virtual rack, select the **Spike Sorter** (or the display that you want to customize) and click the **Layout** tab. The default layout is loaded. In the **Rows** and **Columns** boxes, you can now modify the layout.
2. Type or select the number of **Rows**, for example 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.



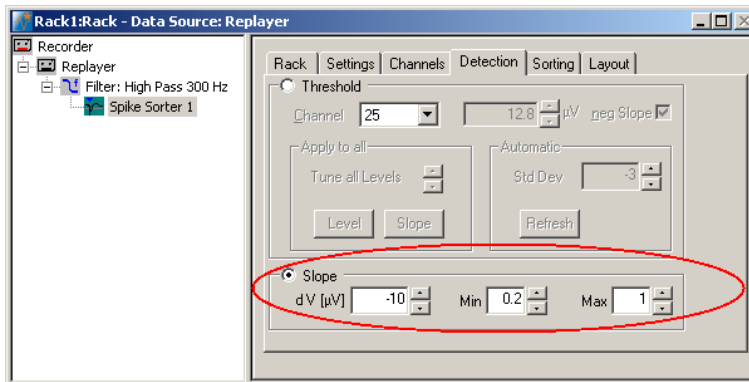
1.7 Detecting Spikes (Slope Mode)

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.

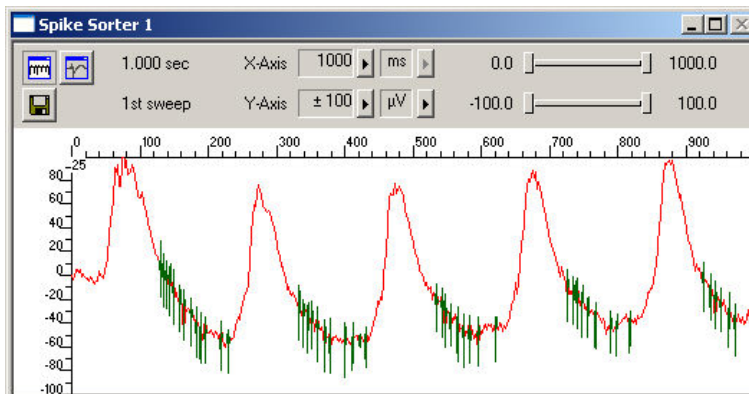
1. In the tree view pane of the virtual rack, select the **Spike Sorter**, and click the **Channels** tab.
2. Click the **Electrode Raw Data** stream, and then select channel 25.



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.



2 Saving the Rack and Saving Data

2.1 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**.

2.2 Selecting Data Streams for Recording

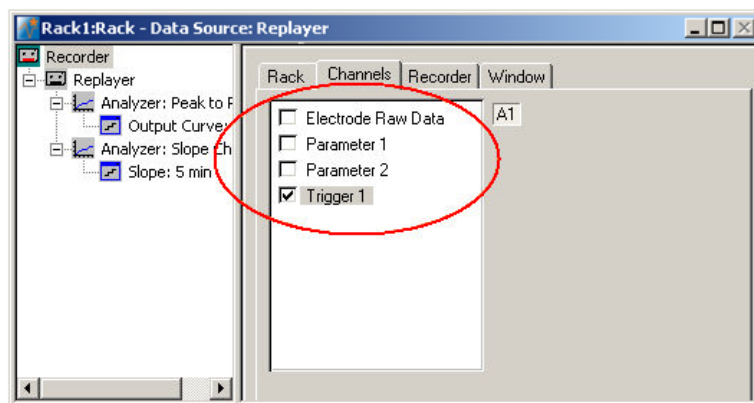
MC_Rack's philosophy is to **strictly separate** the actions of **all** virtual instruments in a rack. That means, that you could **record** to hard disk completely different data streams and channels than you **monitor** on the screen. This has the advantage that you can store exactly the channels you 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

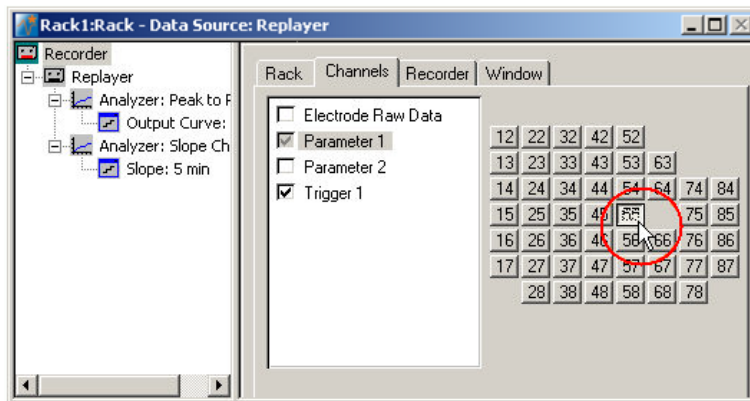
The fate of each single channel is **independent** from other channels. You can pick exactly the channels you like to save from all generated data streams. For example, you can decide to save only **one** channel of **raw data**, but the **peak-to-peak amplitude** results of **all**, or of a **specific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

2.3 Creating a Data File

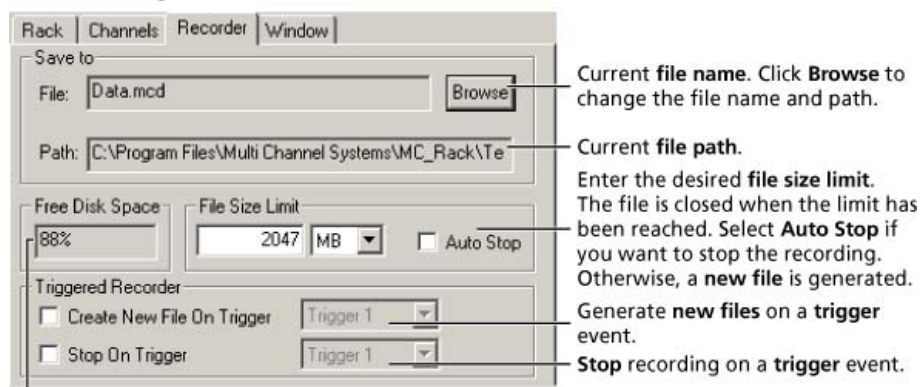
If you want to write the parameters that you will extract in an offline analysis to the hard disk, you have to specify the file name and path in the **Recorder**. (It is not possible to change or overwrite existing data files in MC_Rack, for example, adding the spike rate to an existing data file, but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

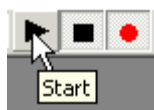
After you added a **data source**, you will see the electrode raw data streams provided by the data source (for example, electrode raw data, analog data, and digital data from the **MC_Card**, or the data streams included in the data file loaded into the **Replayer**). If the rack file contains virtual 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.

2.4 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.