multichannel systems

MC_Rack Tutorial: Spike Activity (OTC)

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Printed: 08. 11. 2010

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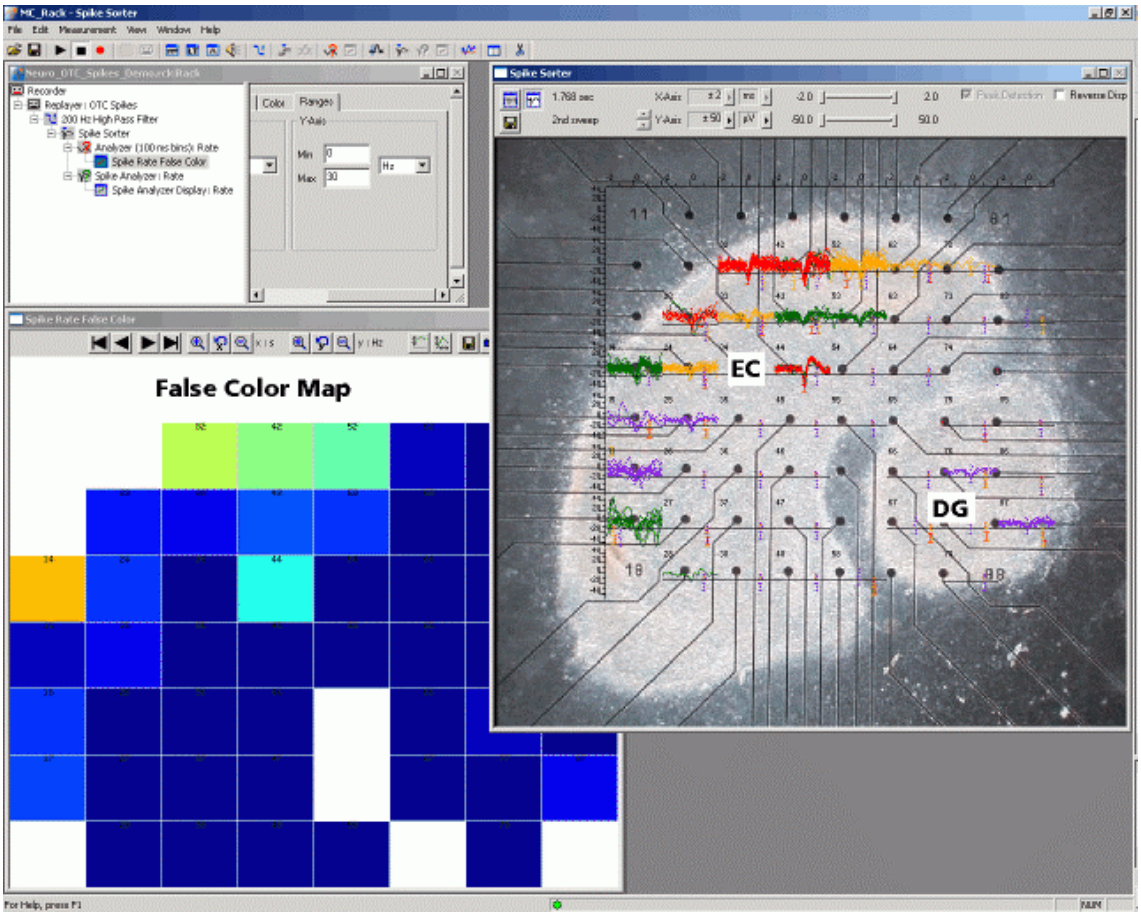
1 Spike Activity (Organotypic culture)

1.1 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.

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

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 "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.

1.3 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.

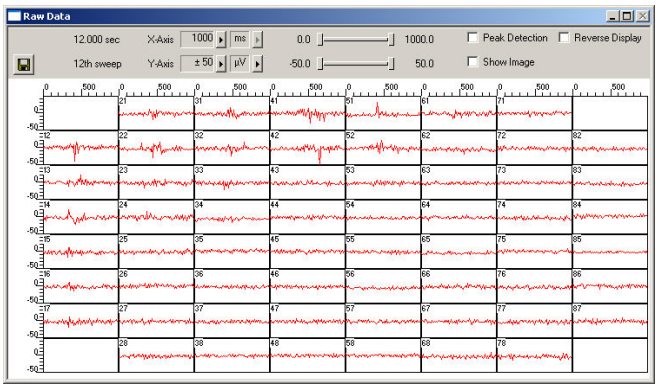


Fig. 1 Without Peak Detection

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

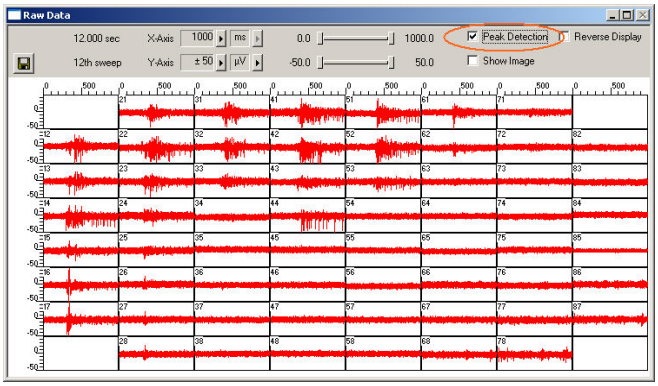
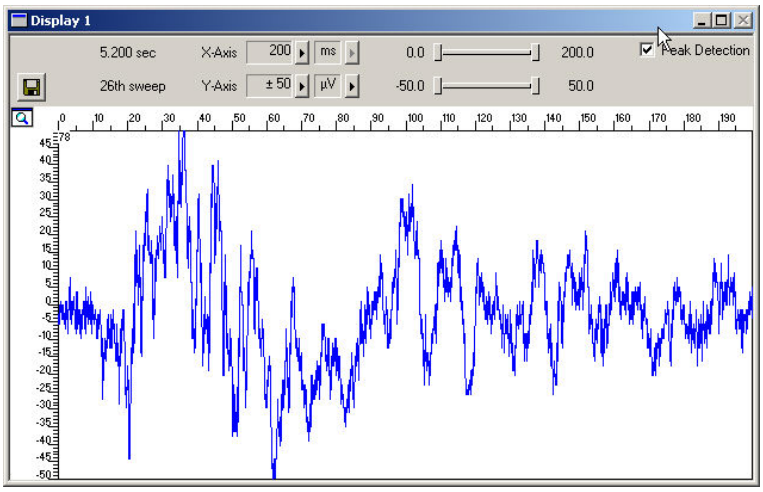


Fig. 2 With Peak Detection

*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.*

1.4 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.

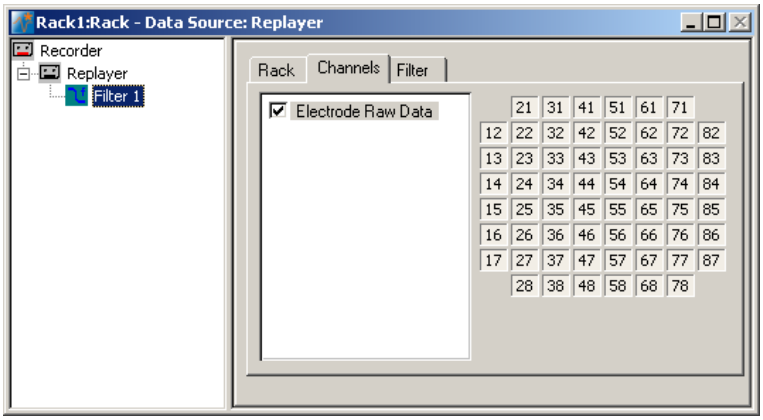


If you would try to detect peaks by a **threshold**, you would miss most of the peaks. To circumvent this problem, you can use a digital filter that removes slow signals, that is, field potentials. In the current version of MC_Rack, a second order Butterworth filter is available. Please be aware that 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.)

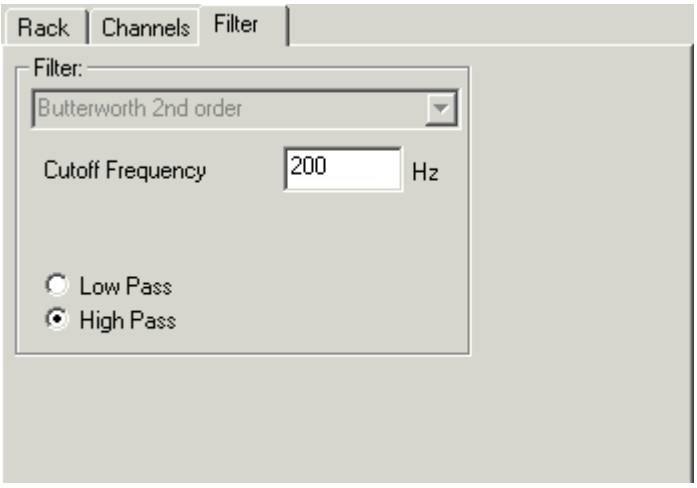
2. Click the **Delete Instrument** button to remove the **Data Display** again to save computer performance.

3. On the toolbar, click to add a **Filter** to your rack.

4. Click the **Channels** tab and select all channels.



5. Click the **Filter** tab.

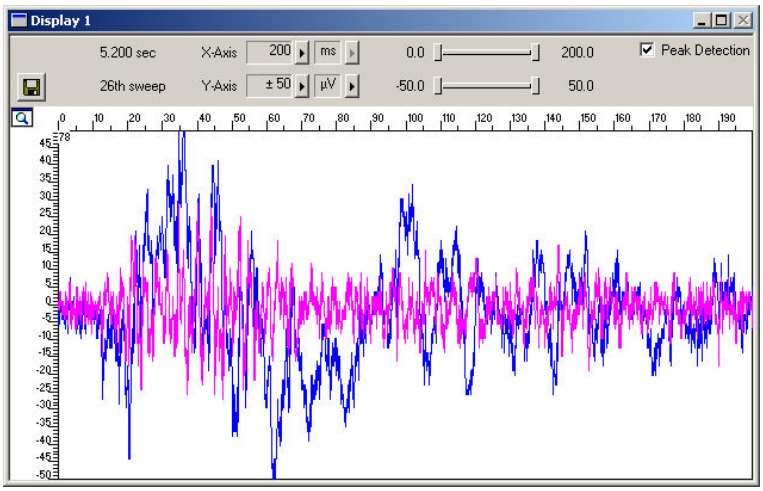


6. Select **High Pass**.
   You can choose between two filter types: **Low Pass** and **High Pass**. As the name suggests, a low pass filter passes the lower frequency components but attenuates the higher frequency components of signals. To remove slow components (with a low frequency) and to let pass high frequency signals, you need a high-pass filter.

7. 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.

8. 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.

9. 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.



1.5 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.

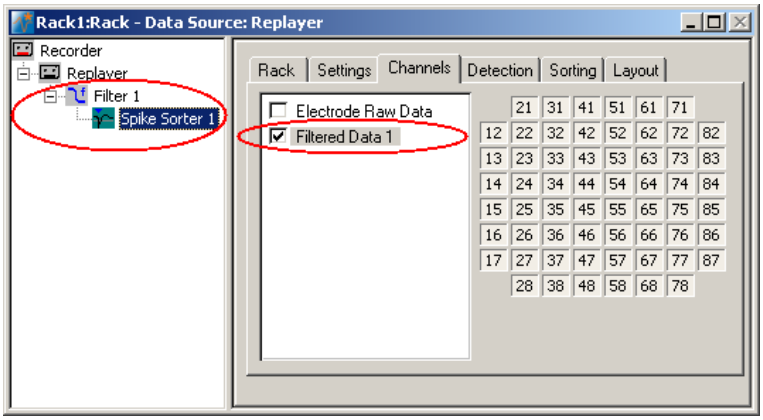
The **threshold** method is especially useful if the overall baseline is stable, and spikes appear approximately on the same height. If local field potentials are underlying the spikes, or if you have an unstable baseline, spikes will be missed, or noise signals may be detected as spikes with the threshold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

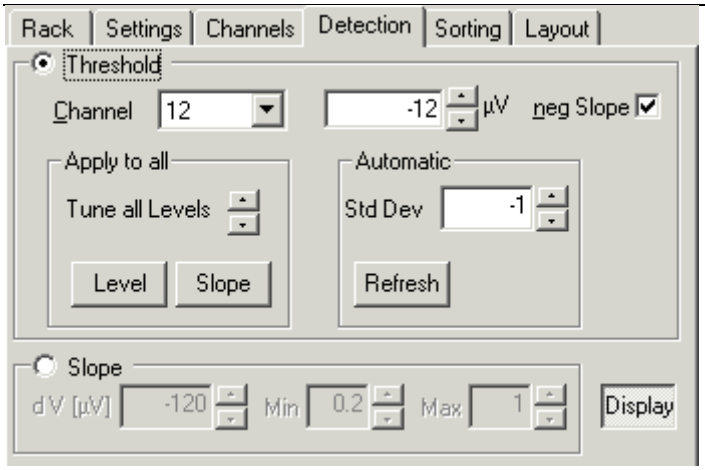
2. Click the **Channels** tab and assign the **Filtered Data** stream to the **Spike Sorter**.



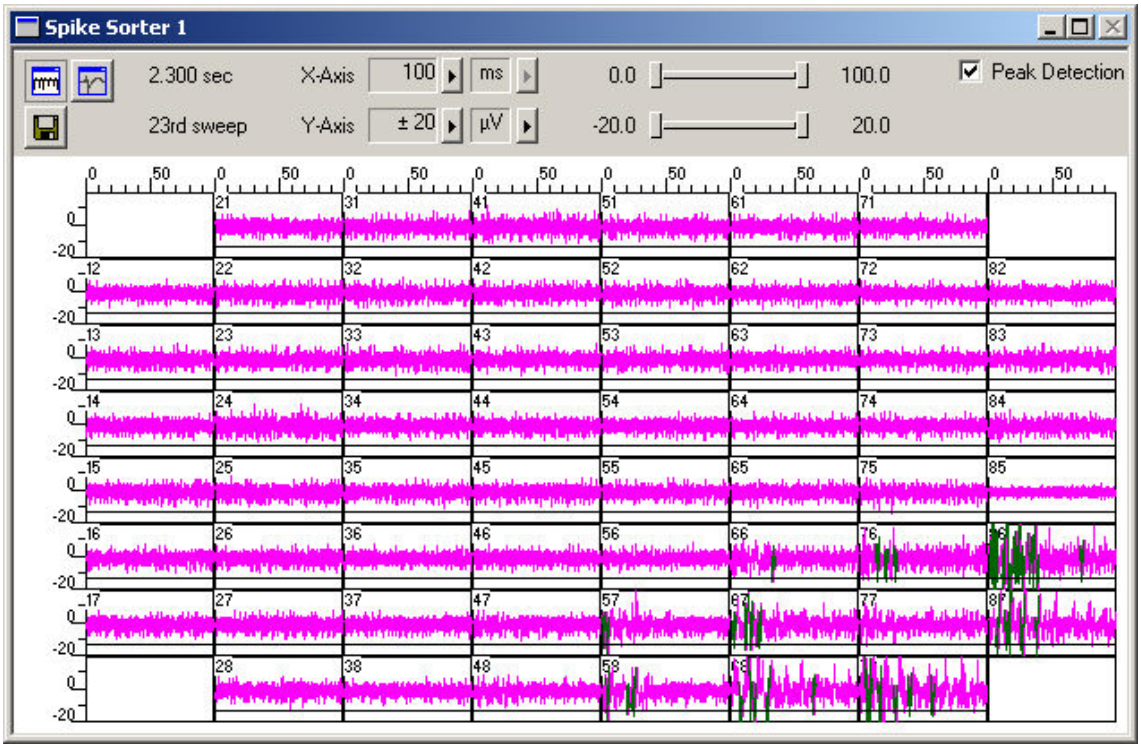
3. Select the **Peak Detection** option of the display. Click the **Start** button and zoom the display until you can see the signals clearly. You see that the baseline is on a similar level for all channels and that the spikes are quite small (approximately 10 to 15 µV).

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.



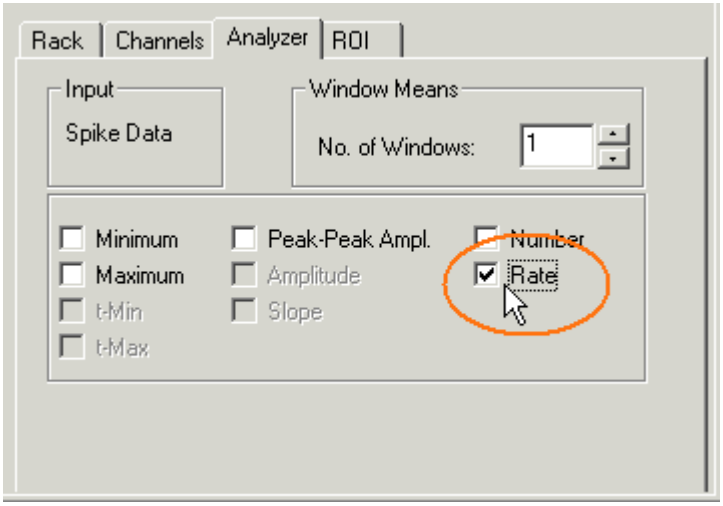
6. Use the option **Tune all levels** to modify the level of all channels until the detection level seems to be optimized (approximately to -12 µV). You can also click and drag the detection level (black line) of each channel in the **Spike Sorter** display with your mouse.



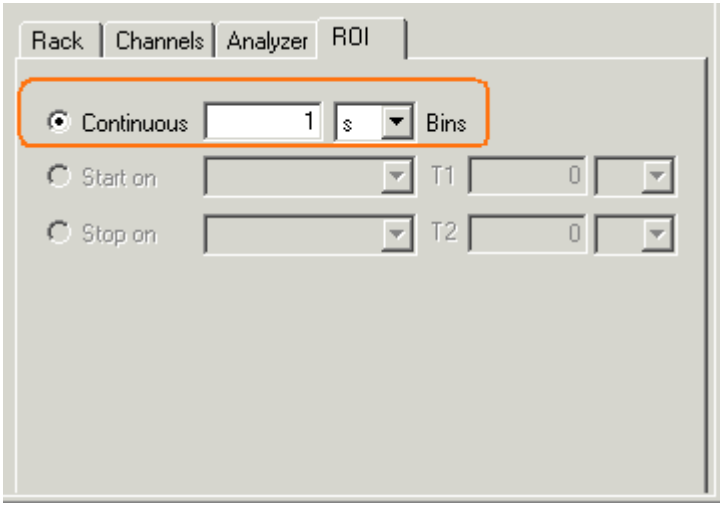
1.6 Analyzing the Spike Rate

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.

1. 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.)
2. In the tree view pane of the virtual rack, select the **Analyzer**. Click the **Channels** tab.
3. Select all channels of the **Spikes** data stream.
4. 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.



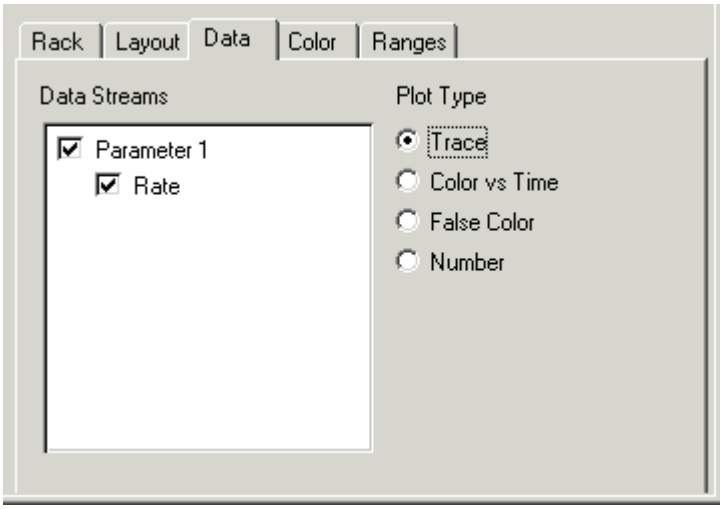
1.7 Plotting the Spike Rate

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

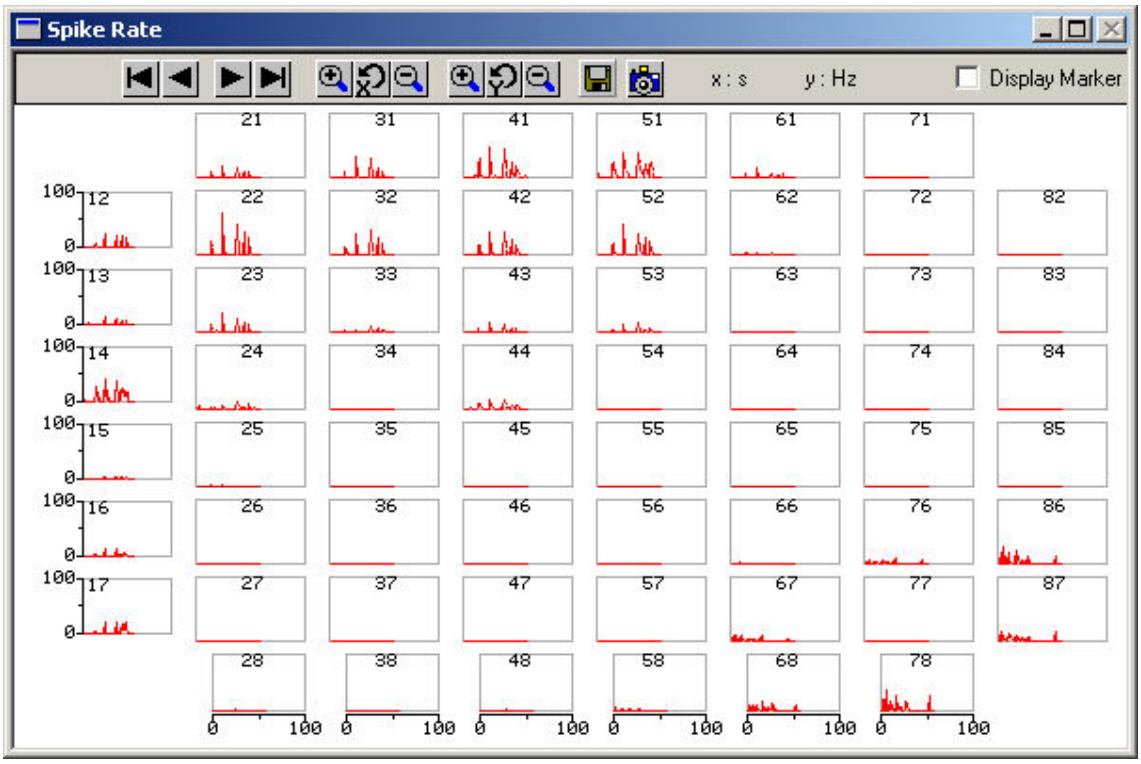
1. In the tree view pane of the virtual rack, select the **Analyzer** and click on the toolbar to add a **Parameter Display** in **serial** with the **Analyzer** to the virtual rack. (It would not be possible to add the **Parameter Display** in parallel to the **Analyzer**, as the replayed data file 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.

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



4. 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.



1.8 Visualizing the Spatial Distribution

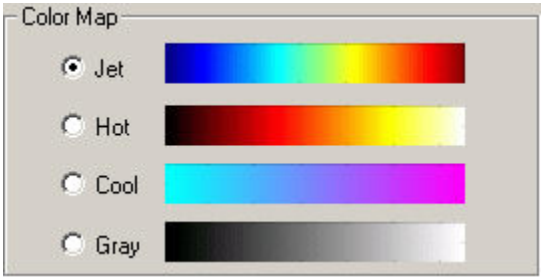
The **Trace** plot is good for monitoring the spike rate over the complete recording time. A **False Color** plot is the better choice for getting an idea of the spatial distribution and whether two regions fire simultaneously or independently. In a **False Color** plot, a specific color map is assigned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

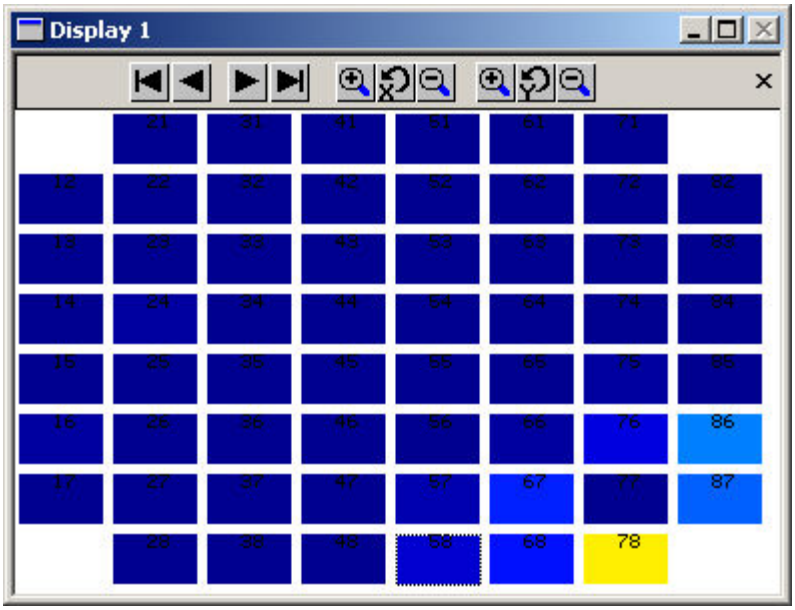
Example: If the range was set from min = 0 Hz to max = 64 Hz, each color of the map would correspond to 1 Hz steps. Spike rates lower than 1 Hz would then be plotted as the first (most left) color of the color map, spike rates between 1 Hz and 2 Hz would be plotted as the second color of the color map,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

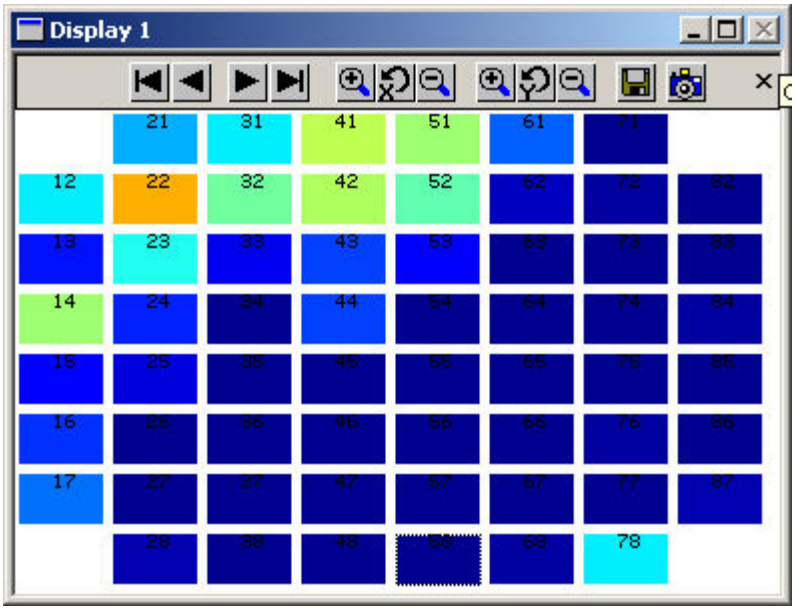
That means, for example, for the **Gray Color Map**, the lighter the color becomes, the higher is the spike rate.



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**.

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.





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

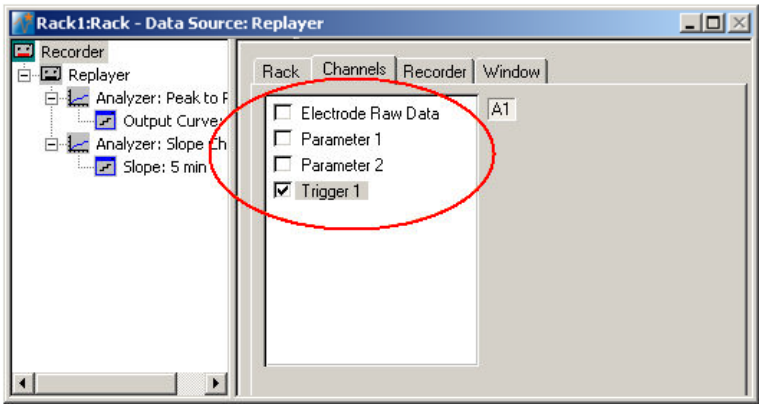
MC_Rack's philosophy is to **strictly separate** the actions of **all** virtual instruments in a rack. That means, that you could **record** to hard disk completely different data streams and channels than you **monitor** on the screen. This has the advantage that you can store exactly the channels you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

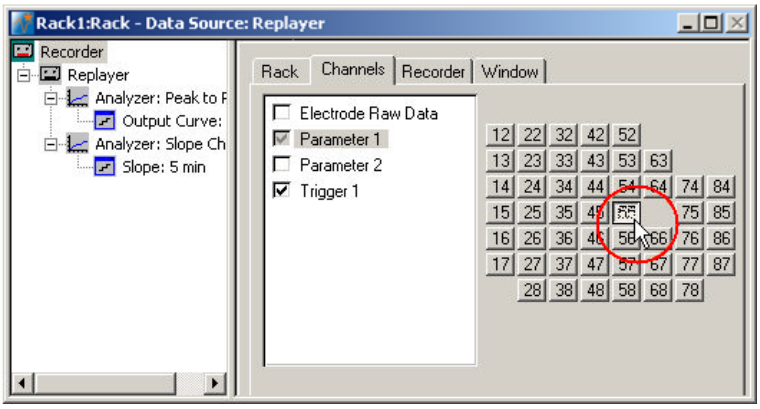
Selecting data streams and channels for recording

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.



2. 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).



3. 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.

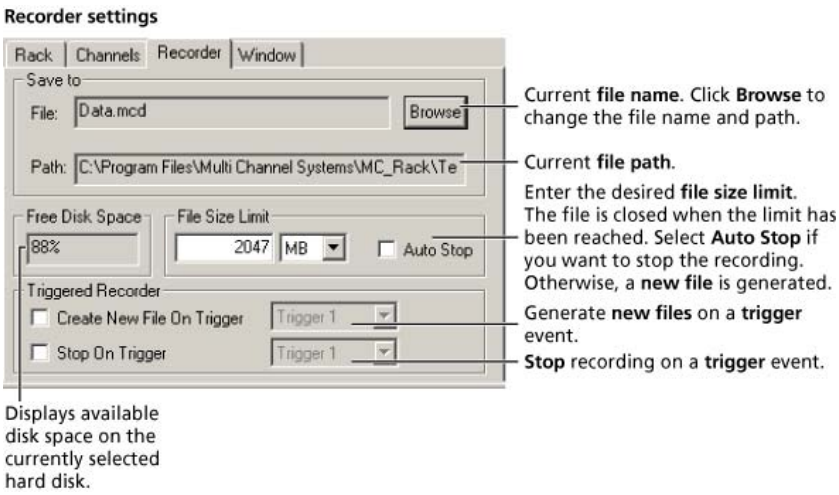
2.3 Creating a Data File

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

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**.



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**.

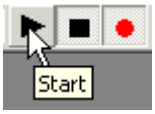
After you added a **data source**, you will see the electrode raw data streams provided by the data source (for example, electrode raw data, analog data, and digital data from the **MC_Card**, or the data streams included in the data file loaded into the **Replayer**). If the rack file contains virtual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