

MC_Rack Tutorial: Cardiac Signals



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Printed: 05. 11. 2010

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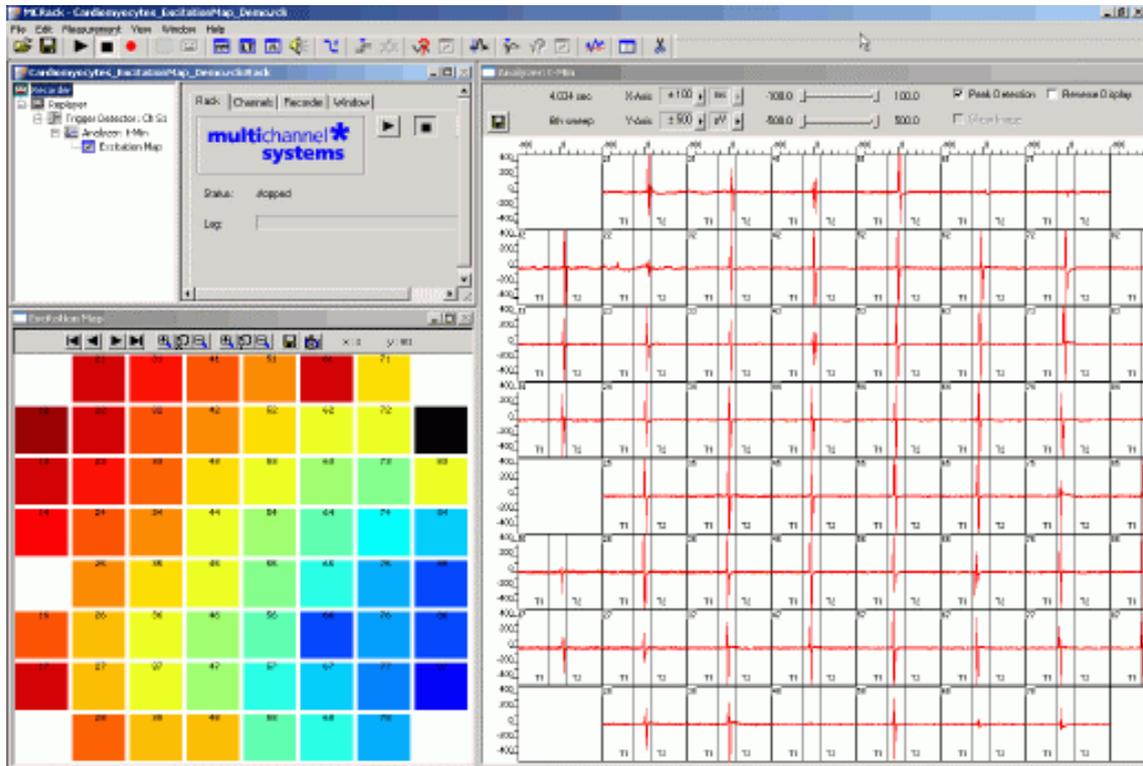
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1 Cardiac Signals

1.1 Aim of Experiment



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**).

MC_Rack Tutorial: MEA Application Examples

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.

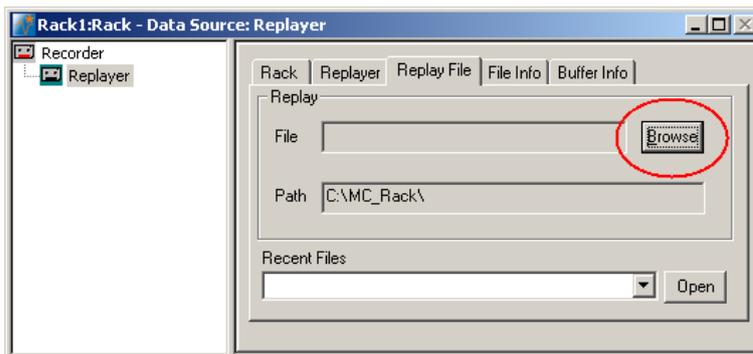
- Rack file: Cardiomyocytes_QT-Analysis_Demo.rck
- Data file: Cardiomyocytes_QT.mcd

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

1.2 Preparations

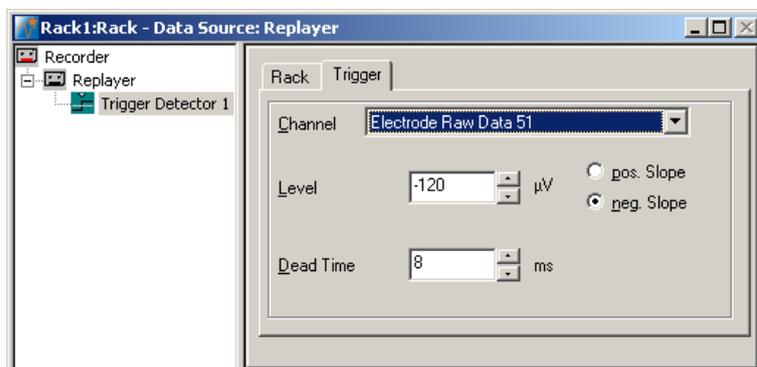
We recommend that you take some time for rebuilding the virtual rack for this application step by step in this tutorial, but if you prefer having a look at the completed rack or if you get stuck during the tutorial, you can also open the rack file "Cardiomyocytes_ExcitationMap_Demo.rck". Click **Open**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

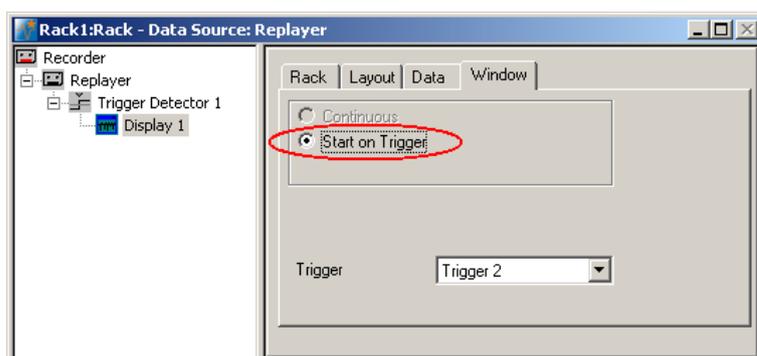


1.3 Monitoring Cardiac Activity

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).
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.



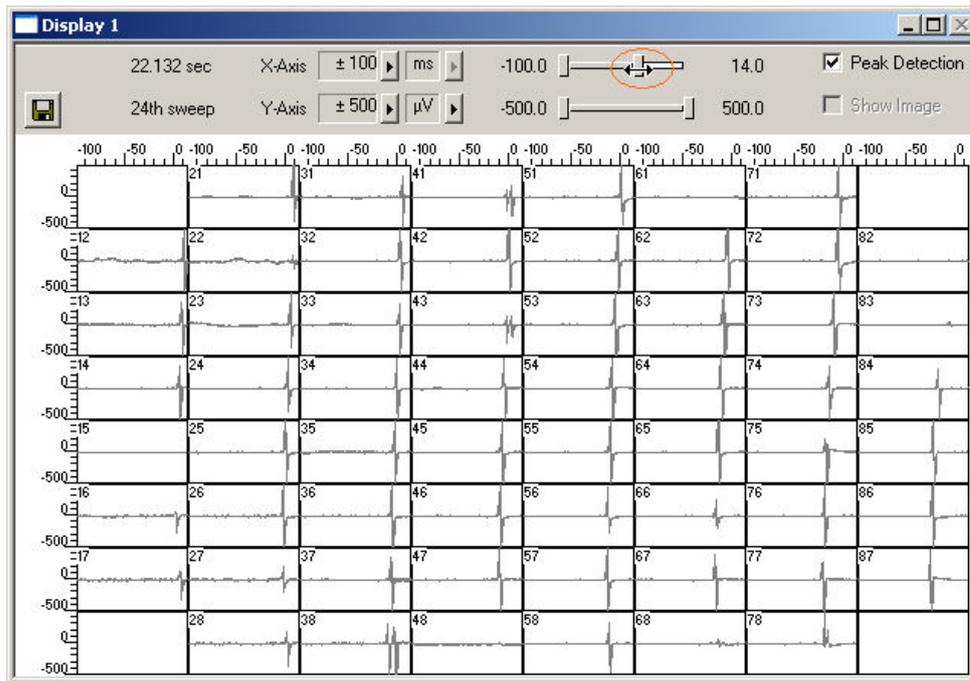
5. In the **Data Display**, select the option **Peak Detection**.
6. Click **Start**  (either on the **Measurement** menu, the toolbar, or the **Rack** tabbed page) to start the replaying of the data.

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.

MC_Rack Tutorial: MEA Application Examples

- Zoom in the x-axis to see the different kinetics on different channels.

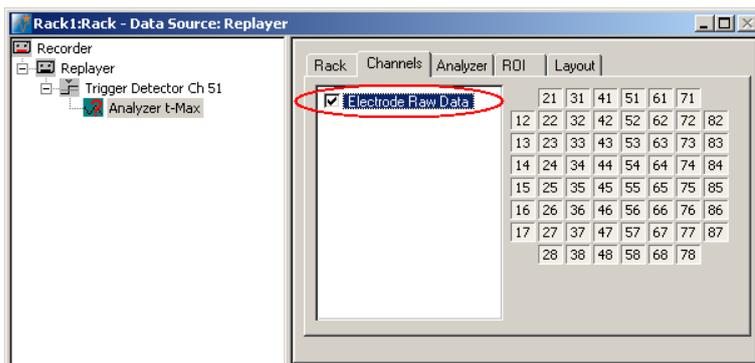


You can see that the signal originates from the bottom right corner of the MEA and propagates to the upper left. Relatively to the trigger event (on channel 51), the signal appears at time = 30 ms at, for example, electrode 77, and it takes about 40 ms until it appears at time = 10 ms at electrode 12.

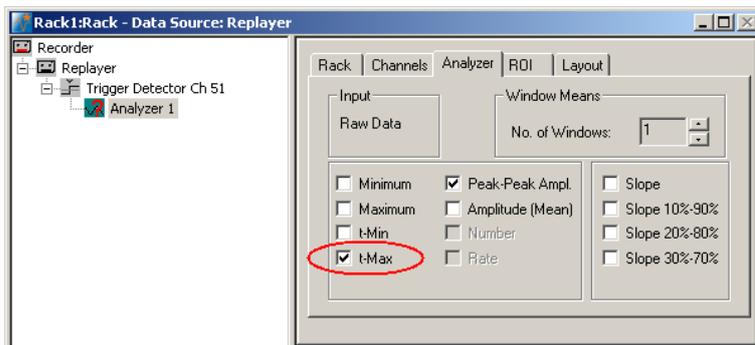
1.4 Signal Propagation

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.

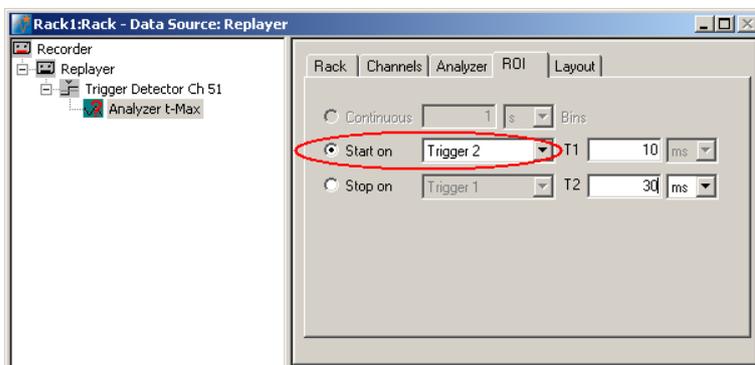
1. Remove the **Display 1** from the rack by clicking on the **Display 1** in the tree view of the virtual rack and pressing the DEL key or by clicking **Delete Instrument** on the **Edit** menu.
2. On the **Edit** menu, click **Add Analyzer** to add an **Analyzer** to your rack, again, in series with 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**).



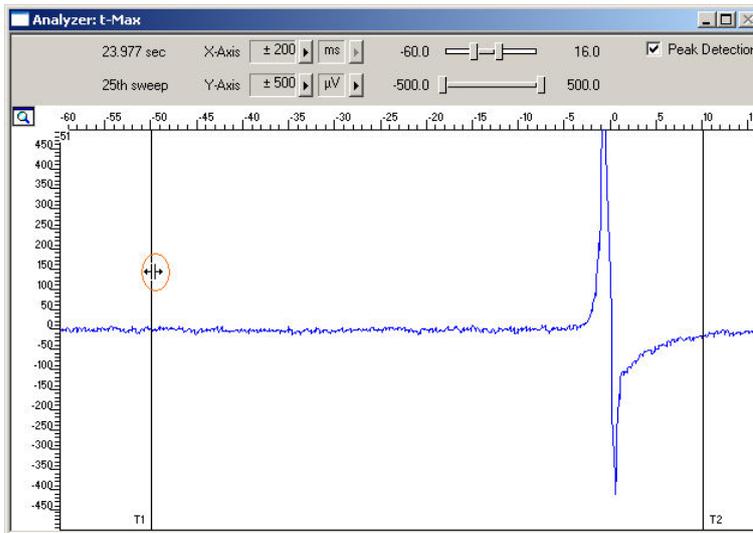
5. Click the **ROI** tab and select the option **Start On Trigger 1**. The **Analyzer** display opens. You can now define the region of interest (ROI) directly in the display or on the ROI page.



6. Click the start button and zoom in the display so that you can see the waveforms clearly on all channels.

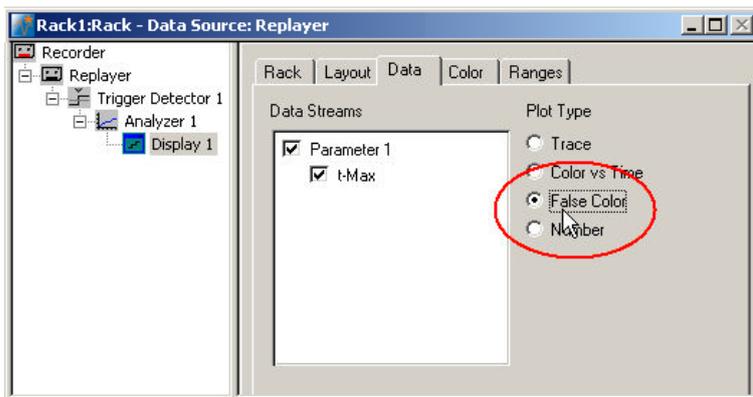
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7. Drag the **ROI** sliders such as that all signals from all electrodes are inside the ROI, or enter appropriate values into the T1 and T2 text boxes on the ROI page, for example T1 = 30 ms and T2 = 15 ms. T1 defines the left border, and T2 defines the right border of the ROI frame.



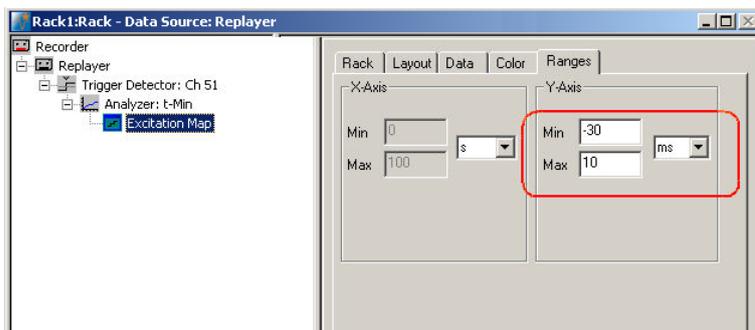
On the **Edit** menu, click **Add Parameter Display**. The **Parameter Display** can be configured as an excitation map.

8. In the tree view pane of the virtual rack, select the **Parameter Display** and click the **Data** tab.
9. Select the parameter **t-Max**. As plot type, select the **False Color** plot.

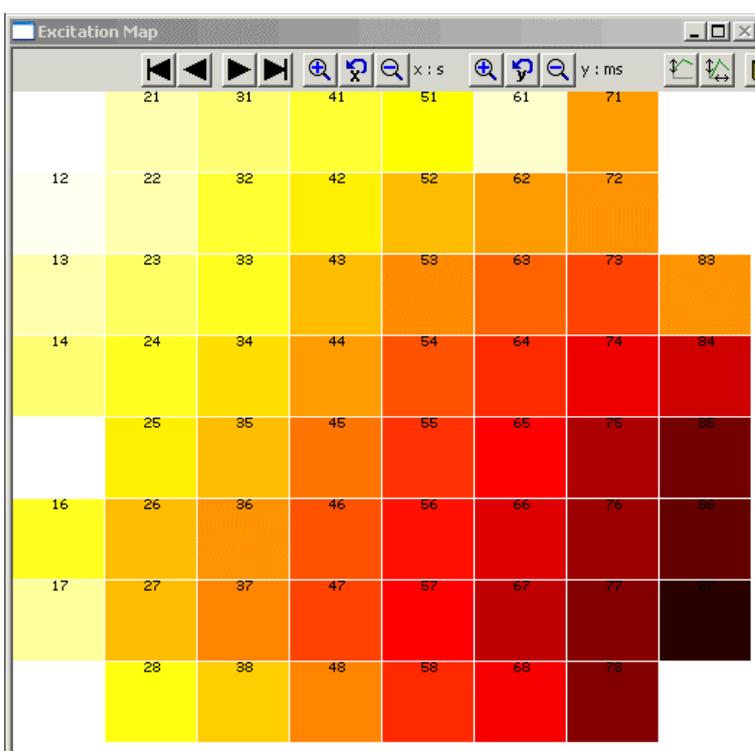


10. Click the **Color** tab. Select any color map, for example, the **Hot** color map. The y-value ranges of the plot are now assigned to the colors of the false color map. You have to select a proper range so that the color assignment makes sense, that is, if the range is too wide, you will not see any differences in the color; if the range is too narrow, the colors will get into saturation, that is, black.

- 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.



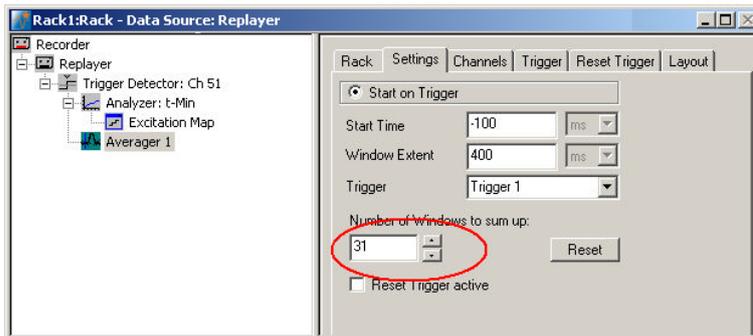
- On the **Measurement** menu, click **Start** to start the replaying of the data file.



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.

1.5 Averaging Cardiac Signals

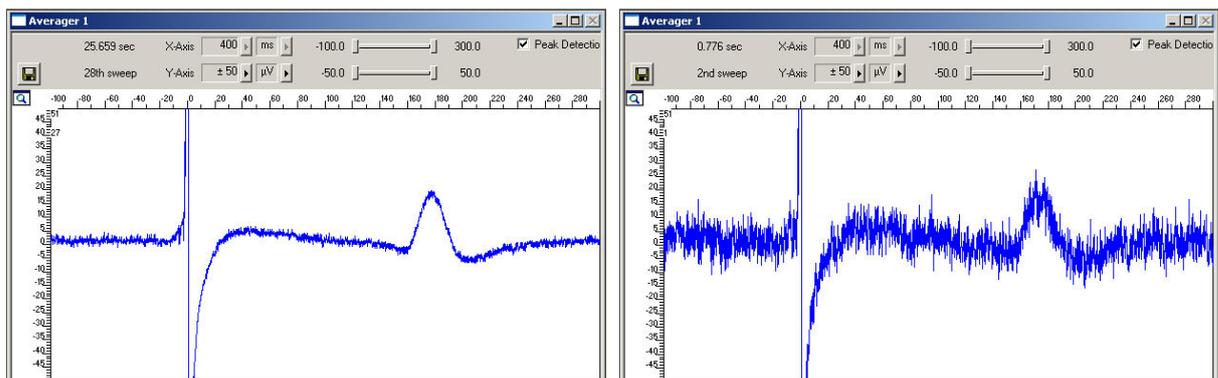
1. On the **Edit** menu, click **Add Averager** to add an **Averager** to the virtual rack (in series to the **Trigger Detector**).
2. In the tree view pane of the virtual rack, select the **Averager** and click the **Settings** tab. In this page, a trigger is defined for the **Averager**, and the window 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.



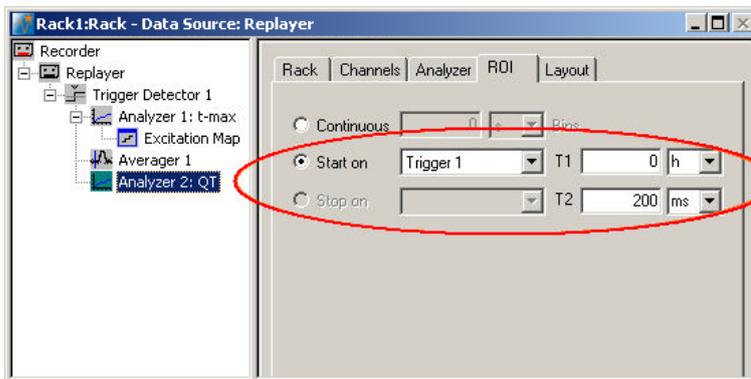
1.6 Automatic Extraction of the QT Interval

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.

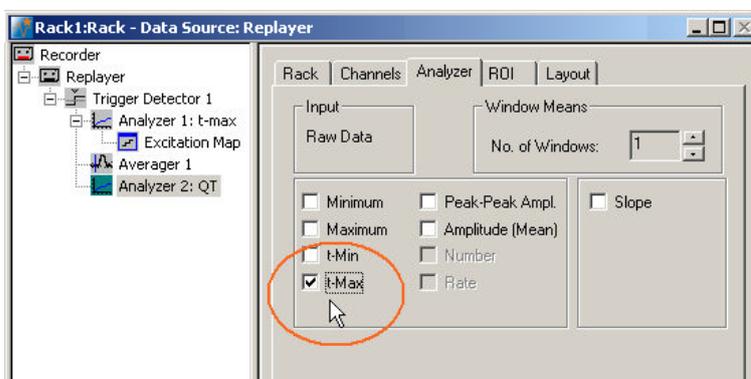
In triggered mode, the **Analyzer** extracts the parameters of interest from a distinct **time window** relative to the **trigger event**, the region of interest (**ROI**). As you need a different region of interest for extracting the time point of the IKr current peak than for the time point of the 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.
2. 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.

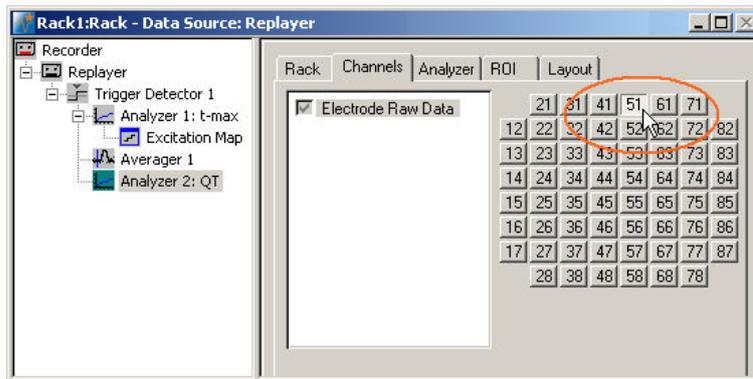


3. Click the **Analyzer** tab, and select the **t-Max** parameter for extracting the time point of the maximum inside the ROI.

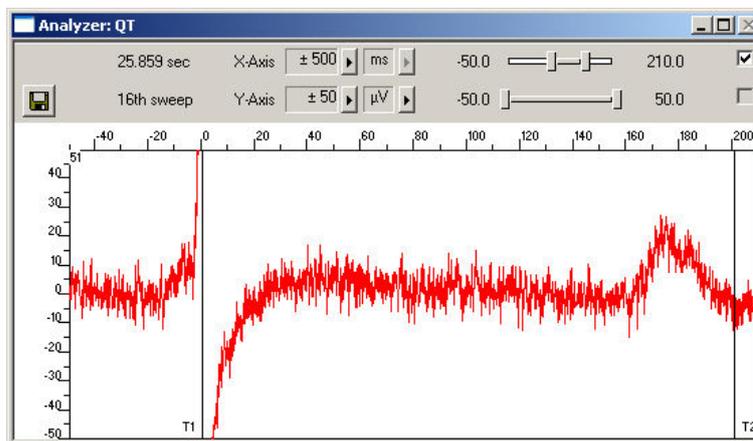


MC_Rack Tutorial: MEA Application Examples

- 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.

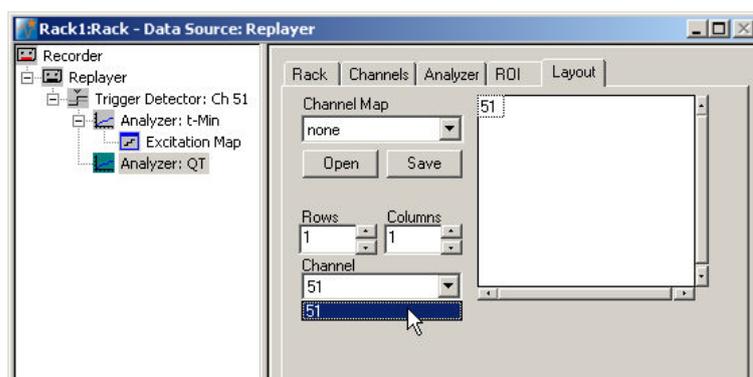


1.7 Customizing Displays

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.

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.



1.8 Monitoring the QT Interval

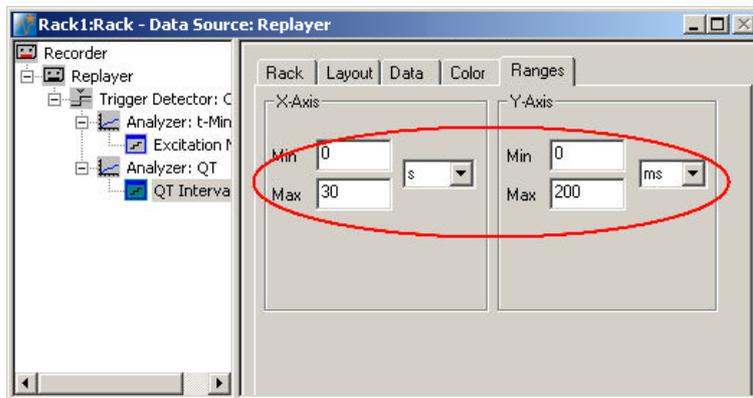
You can plot the extracted time point relative to T1 of the ROI. For plotting extracted parameters, we will need a **Parameter Display**, which features are optimized for **Parameter** streams.

- 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

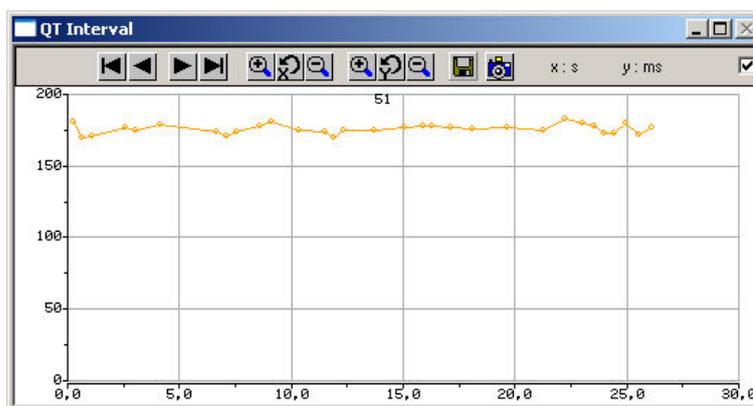
As this demo data file has about 30 s total recording time, 30 s will be fine for the x-axis range. As the duration cannot be longer than the ROI, that is, 200 ms, you can enter 200 ms for the y-axis.

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.

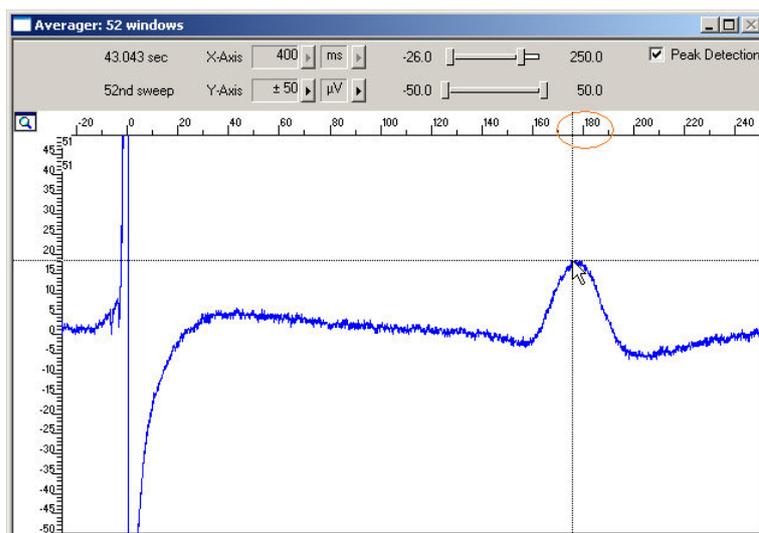
The QT interval is stable (in the range of 170180 ms) in this control experiment with no drug application.



1.9 Manual Measurement of the QT Interval

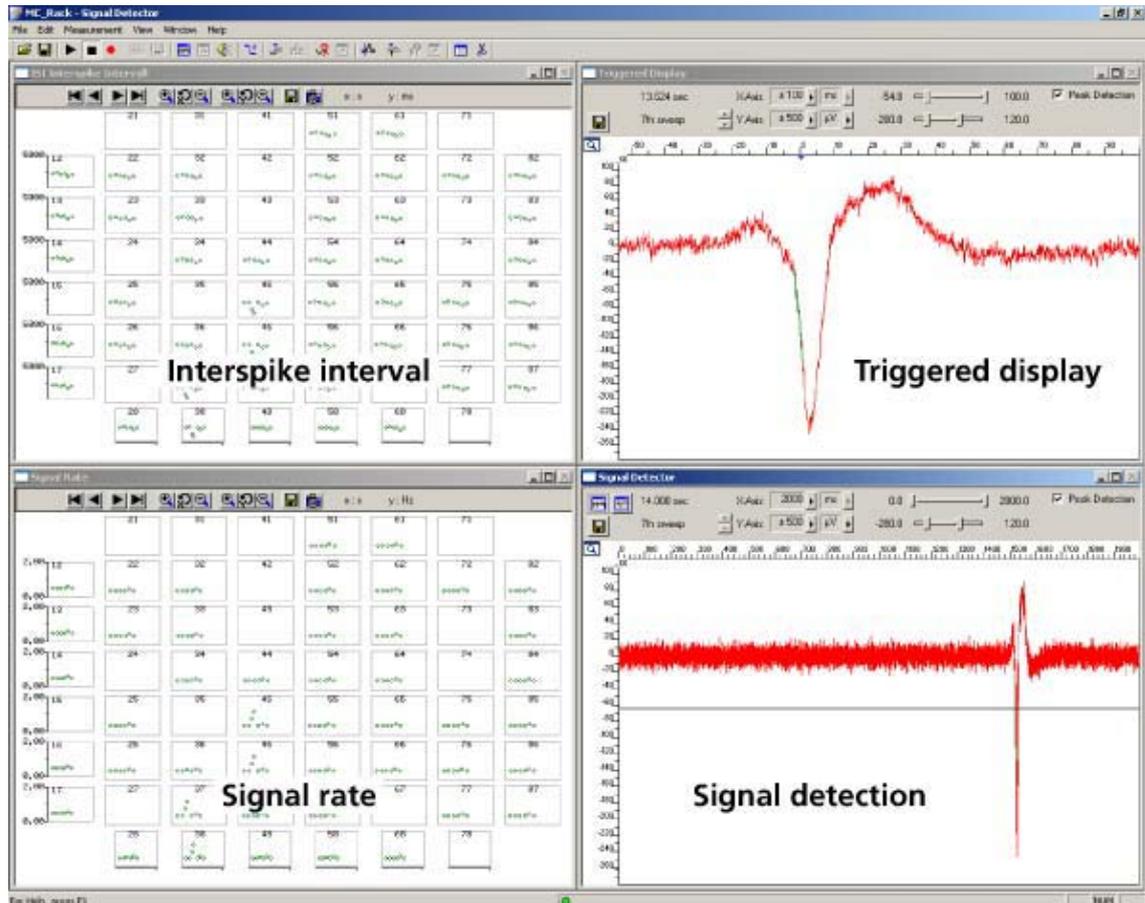
If you need the averaged data as the basis for the analysis, you can measure the parameter with the crosshair tool. As the Na^+ peak corresponds to the trigger event, the time point of the T wave is the relevant parameter.

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.



2 Event-Based Analysis of Cardiac Activity

2.1 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.

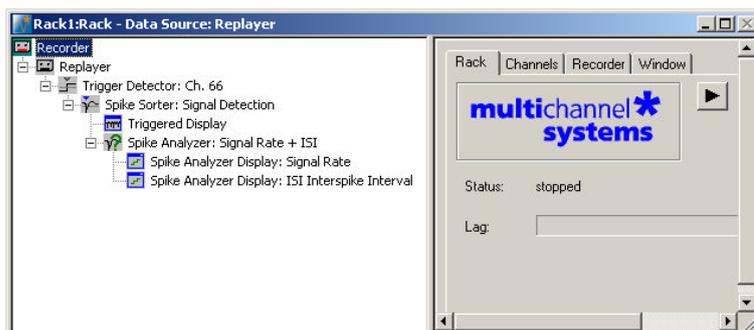
The demo data used in this part of the tutorial was recorded from a cardiomyocyte cell line from mouse that retains a differentiated cardiac myocyte phenotype: **HL-1** from Dr. W. Claycomb.

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 ...



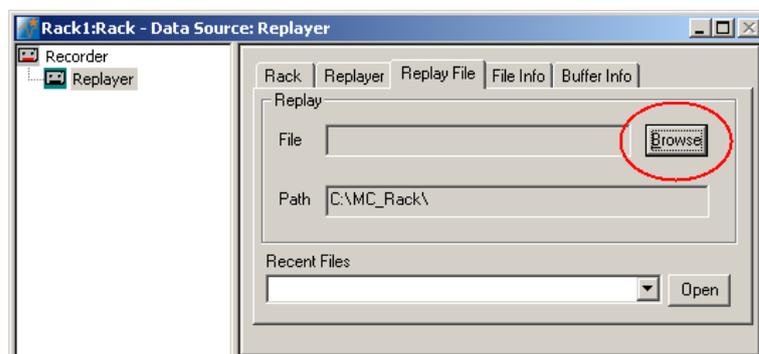
- How to define **basic functions** in your **rack configuration**:
 - Monitor cardiac activity (**Trigger Detector, Data Display**).
 - Signal detection (**Spike Sorter**).
 - Analyzing the signal rate and interspike interval (**Spike Analyzer**).
 - Monitoring the signal rate and interspike interval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

2.2 Preparations

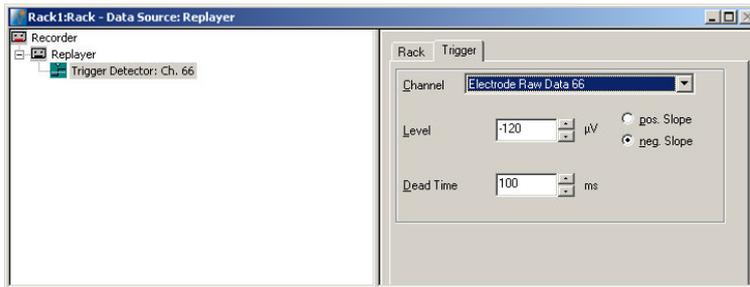
We recommend that you take some time for rebuilding the virtual rack for this application step by step in this tutorial, but if you prefer having a look at the completed rack or if you get stuck during the tutorial, you can also open the rack file "Cardiomyocytes_HL1_Demo.rck". Click **Open** on the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

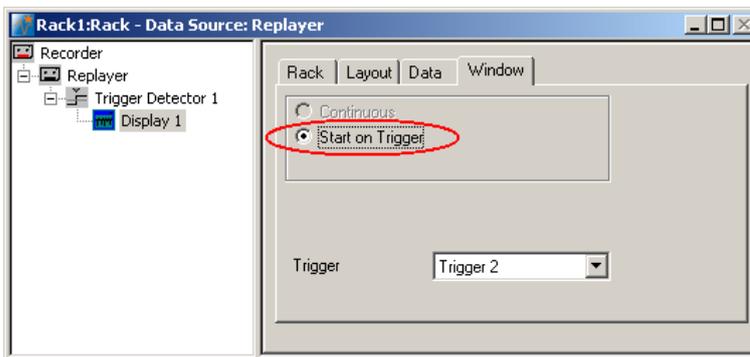


2.3 Monitoring Cardiac Activity (HL1)

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.



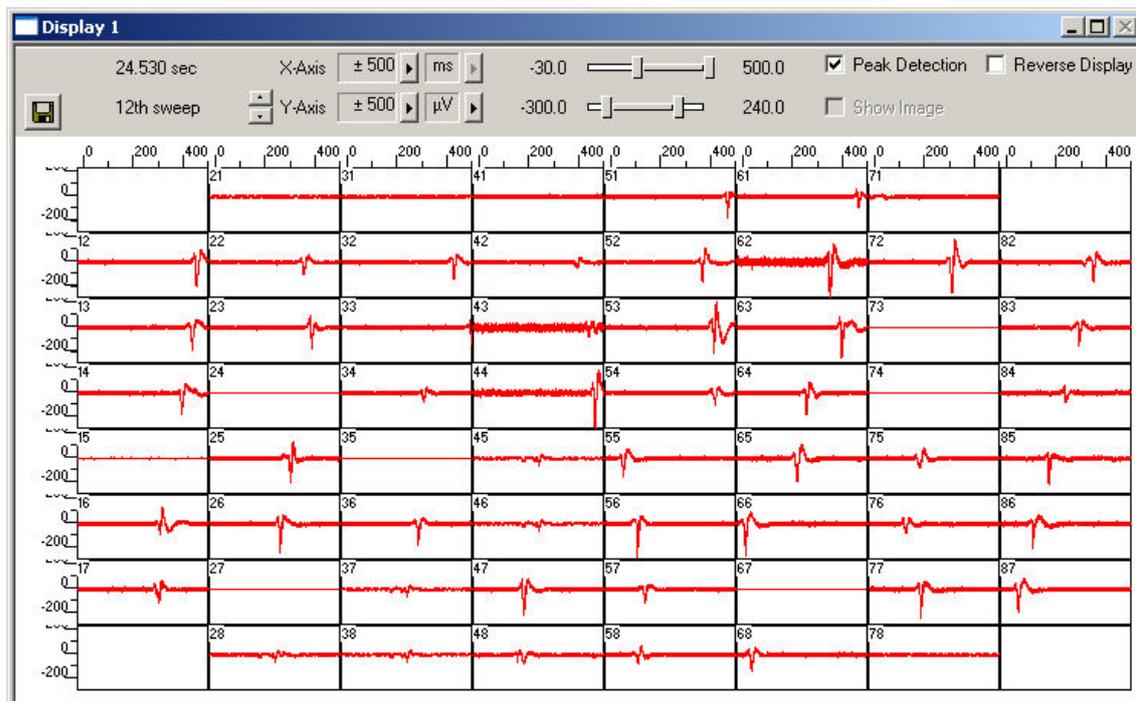
5. In the **Data Display**, select the option **Peak Detection**.

6. 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.

2.4 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).

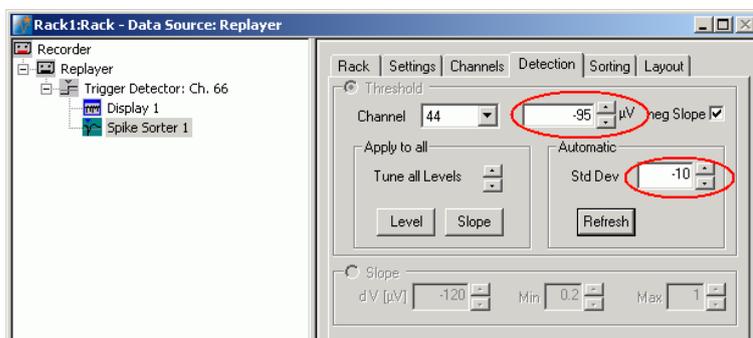
In contrast to the triggered display we used before, this display operates in a **continuous** (oscilloscope-like) mode: The displayed data is **not** synchronized to a trigger event. The **refresh rate** of the display is defined by the x-axis range selected from the drop-down list in the display header.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

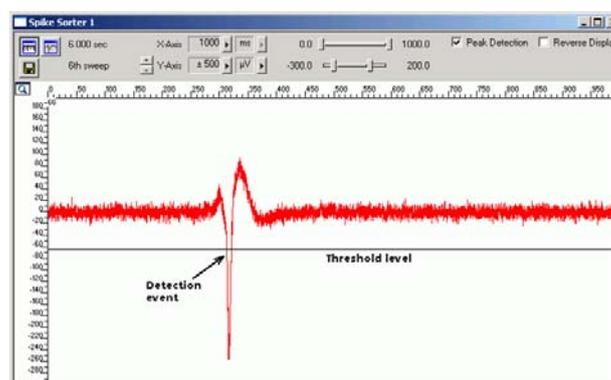
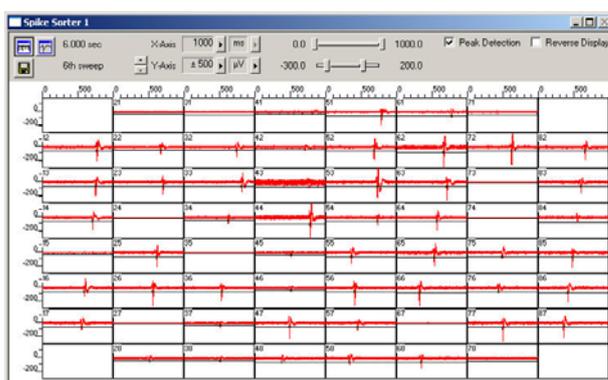
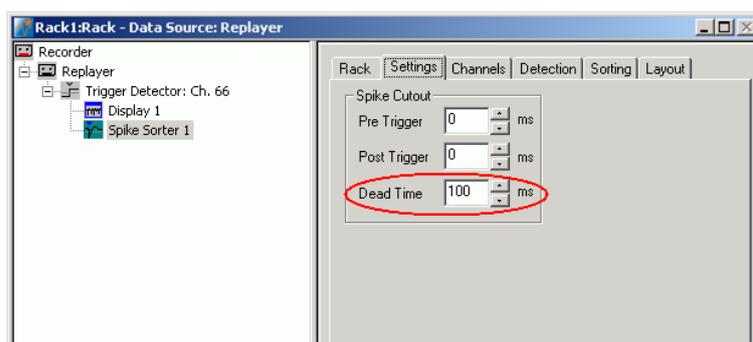
The detected waveform appears in a different color on the display. The detection level determined for a particular channel by the **Automatic** feature is visible as a horizontal straight line. For example, the threshold was set to 50 on channel 34, and to 95 on channel 44 (with a higher noise level).

- 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.

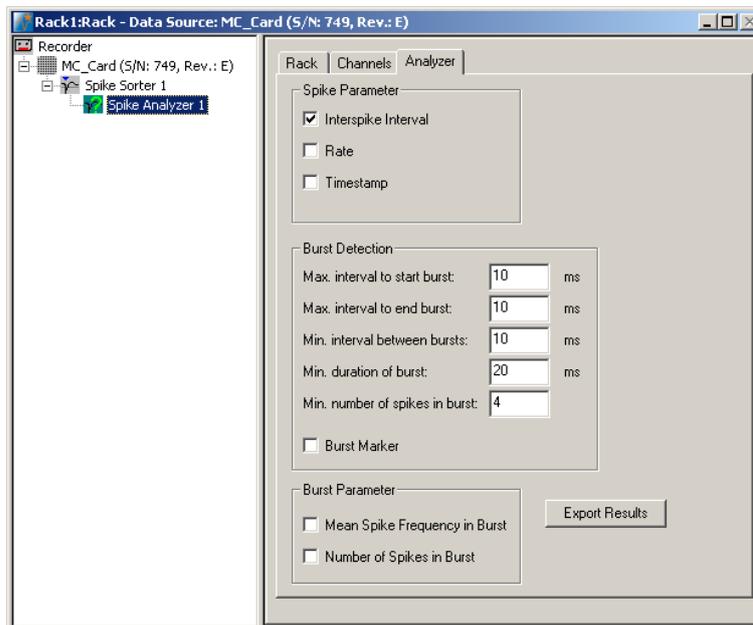


- In the tree view pane of the virtual rack, select the Spike Sorter and click the Settings tab. Enter an appropriate dead time, so that a single signal is not detected multiple times. As the waveform is about 100 ms long, 100 ms is an appropriate dead time. The Pre- and Post-Trigger times define the 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.)



2.5 Analyzing the Signal Rate

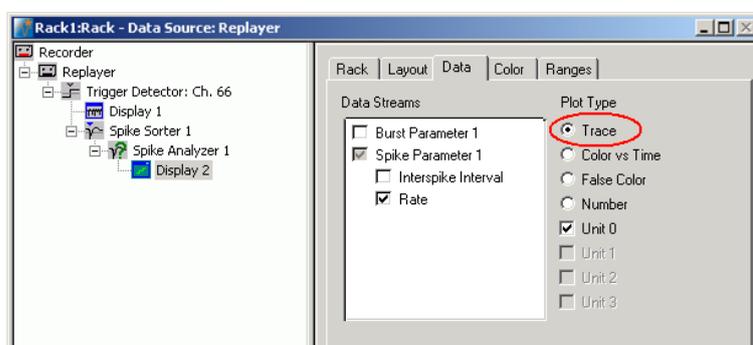
1. On the toolbar, click  to add a **Spike Analyzer** in **series** with the **Spike Sorter** to the virtual rack. (You cannot put the **Spike Analyzer** in parallel to the **Spike Sorter**, because virtual instruments can only use the output streams of other virtual instruments that are upstream in 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**.



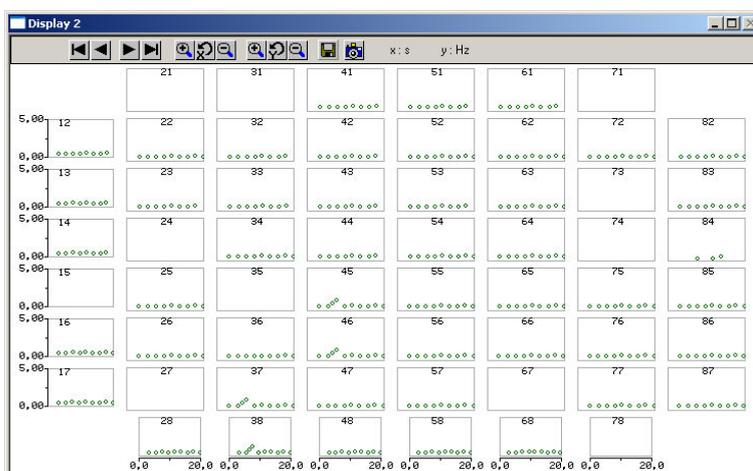
2.6 Monitoring the Signal Rate

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.)
2. In the tree view pane of the virtual rack, select the **Spike Analyzer Display**. Click the **Layout** tab to load the MEA Channel Map.
3. 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**.



4. Start the **Replayer**. The signal rate is plotted over time. You need to adjust the axes to see the data points.
5. 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.

3 Saving the Rack and Saving Data

3.1 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**.

3.2 Selecting Data Streams for Recording

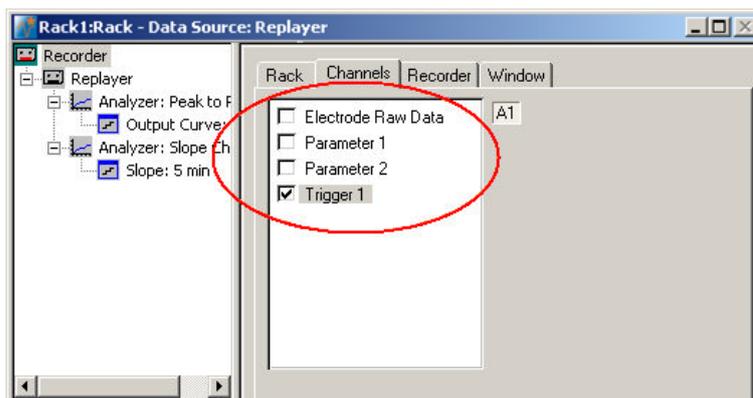
MC_Rack's philosophy is to **strictly separate** the actions of **all** virtual instruments in a rack. That means, that you could **record** to hard disk completely different data streams and channels than you **monitor** on the screen. This has the advantage that you can store exactly the channels you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

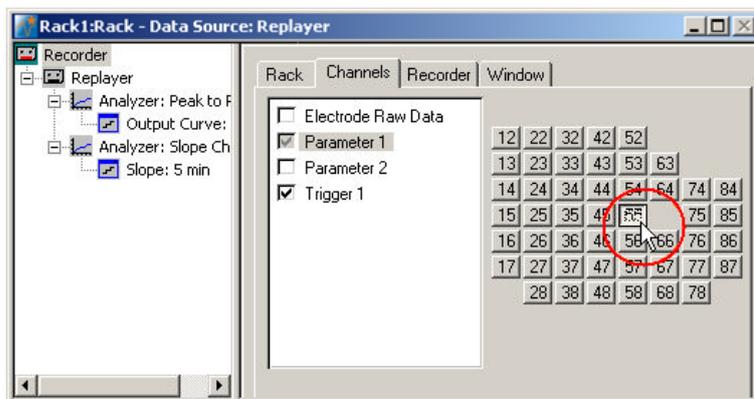
Selecting data streams and channels for recording

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.



- 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.

3.3 Creating a Data File

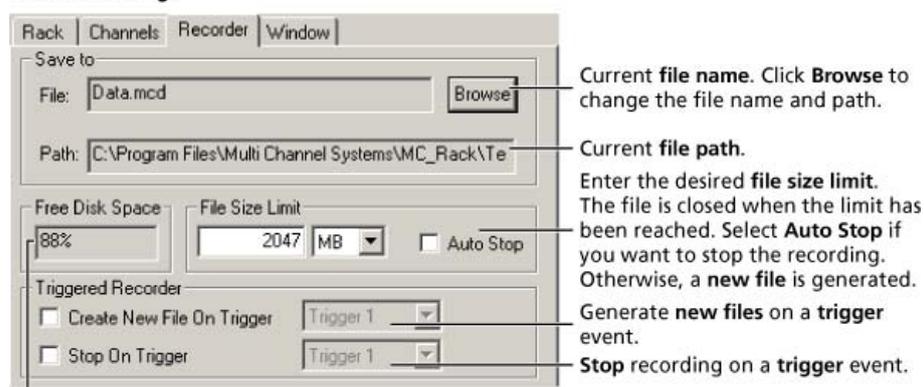
If you want to write the parameters that you will extract in an offline analysis to the hard disk, you have to specify the file name and path in the **Recorder**. (It is not possible to change or overwrite existing data files in MC_Rack, for example, adding the spike rate to an existing data file, but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

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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**.

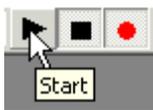
After you added a **data source**, you will see the electrode raw data streams provided by the data source (for example, electrode raw data, analog data, and digital data from the **MC_Card**, or the data streams included in the data file loaded into the **Replayer**). If the rack file contains virtual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

3.4 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.