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Multi Channel Analyzer Manual

Imprint

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1 Welcome to the Multi Channel Analyzer

The Multi Channel Analyzer is the offline data analysis software of the Multi Channel Suite package. The Multi Channel Experimenter is for online data acquisition, and the Multi Channel DataManager allows to export Multi Channel Experimenter Data to 3rd party file formats. Please read the following chapters to understand the general concept of the Multi Channel Suite before starting.

2 Before Your Start

2.1 Terms of Use

You are free to use Multi Channel Analyzer for its intended purpose. You agree that you will not decompile, reverse engineer, or otherwise attempt to discover the source code of the software.

2.2 Limitation of Liability

Multi Channel Systems MCS GmbH makes no guarantee as to the accuracy of any and all tests and data generated by the use the Multi Channel Analyzer software. It is up to the user to use good laboratory practice to establish the validity of his findings. To the maximum extent permitted by applicable law, in no event shall Multi Channel Systems MCS GmbH or its suppliers be liable for any special, incidental, indirect, or consequential damages whatsoever (including, without limitation, injuries, damages for data loss, loss of business profits, business interruption, loss of business information, or any other pecuniary loss) arising out of the use of or inability to use Multi Channel Analyzer or the provision of or failure to provide Support Services, even if Multi Channel Systems MCS GmbH has been advised of the possibility of such damages.

2.3 Important Safety Advice

Warning: Make sure to read the following advices prior to install or to use Multi Channel Analyzer. If you do not fulfill all requirements stated below, this may lead to malfunctions or breakage of connected hardware, or even fatal injuries. Obey always the rules of local regulations and laws. Only qualified personnel should be allowed to perform laboratory work. Work according to good laboratory practice to obtain best results and to minimize risks. Make always sure to validate your findings. Prepare backup copies on a regular basis to avoid data loss.

The operator is obliged to ensure that Multi Channel Analyzer is only be used for its intended purpose and that it is only used by qualified personnel.

Warning: Multi Channel Analyzer software is developed for Multi Channel Systems MCS devices. MCS devices are not intended for medical uses and must **not be used on humans**, especially not for uses that could impair health. MCS assumes no responibility in any case of contravention.

3 Installation and Updates

One of the following Microsoft Windows ® operating systems is required: Windows 10, 8.1 or Windows 7 (English and German versions supported) with the NT file system (NTFS). Other language versions may lead to software errors. If a computer was acquired from MCS, the Multi Channel Suite will be preinstalled. **Updates** are available for free download on a regular basis from the MCS web site. It is recommended always to install the latest software version. To install the software, download and start the respective *.exe file and follow the instructions on the screen.

3.1 Recommended Operating System Settings

The following automatic services of the Windows operating system interfere with the data storage on the hard disk and can lead to severe performance limits in Multi Channel Analyzer. These routines were designed for use on office computers but are not very useful for a data acquisition computer.

- Turn off Screen Saver.
- Turn off Windows Indexing Service. Only important for data hard drive.
- Manual management of automatic Windows Update.
- Power Options: Power scheme: Never turn off monitor, hard disk and system standby.

It is also not recommended to run any applications in the background when using Multi Channel Analyzer. Remove all applications from the Autostart folder. Be careful when using a Virus Scanner. If possible, exclude Multi Channel Suite data files (*msrd) from being scanned.

3.2 Compatible Data Files

The Multi Channel Analyzer is free to download and install on an unlimited number of computers. However, the software will operate only with data generated by the Multi Channel Experimenter. There are no import options for 3rd party data files.

4 General Software Features

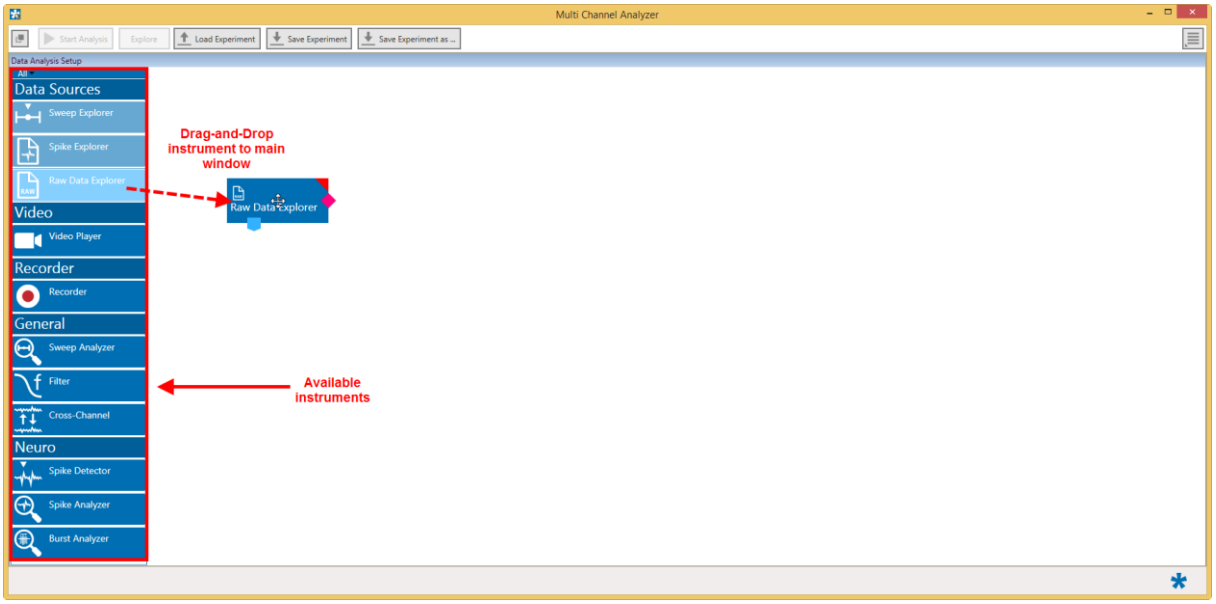
The Multi Channel Suite consists of four parts: The **Multi Channel Experimenter** for online data acquisition, the **Multi Channel Analyzer** for offline data analysis, the **Multi Channel DataManager** for data conversion, and the **Multi Channel VideoControl** for controlling a video camera. Many features and concepts apply to all parts.

4.1 Functional Principle

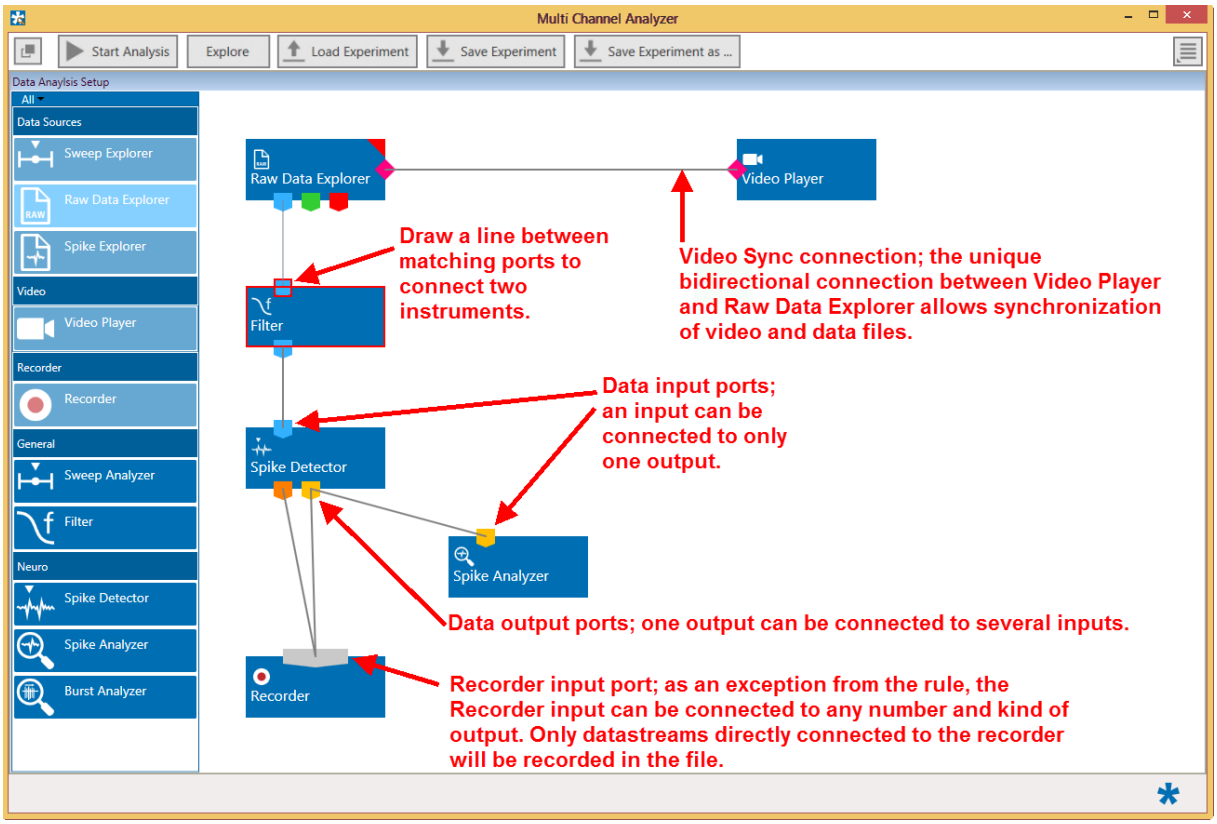
Open the software with a double click on the Multi Channel Analyzer icon, or select it from the start menu.

The Multi Channel Analyzer operates with virtual instruments, which can be combined and saved as so called **Experiments** for later use. When opening the software, all available instruments are shown as blue icons on the left hand side of the screen. Each icon can be dragged and dropped in the main window.

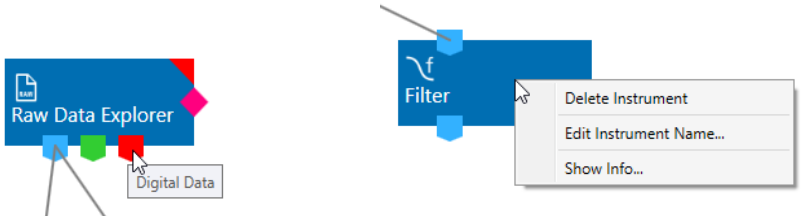
4.1.1 Designing an Experiment



Most instruments have color coded **data ports**. Ports on the upper side represent **data input** to the instrument, ports on the lower side represent **data outputs** of the instrument. As an exception, the Raw Data Explorer, the Spike Explorer and the Video Player also have a bidirectional port on the side. Only ports with matching colors can be connected by **drawing a connection line** between a data output and one or more data inputs. Data flows along those lines from the data source through the instruments. An instrument without connected data inputs is not functional.

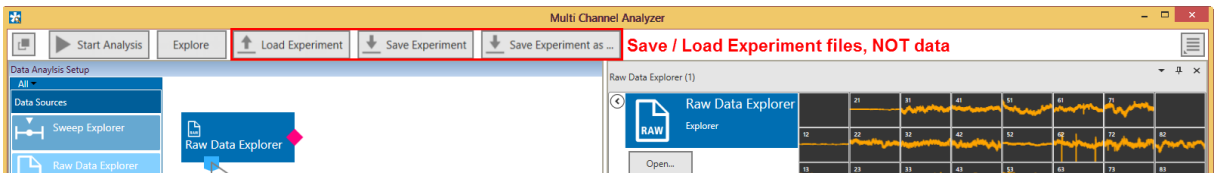


Hovering the mouse pointer above an Output Port will give you a **Tooltip** with the data type the port provides. A right click on a data connection allows to delete the connection. A right click on an instrument brings up a menu which allows to **delete or rename** the instrument.



4.1.2 Save and Load an Experiment

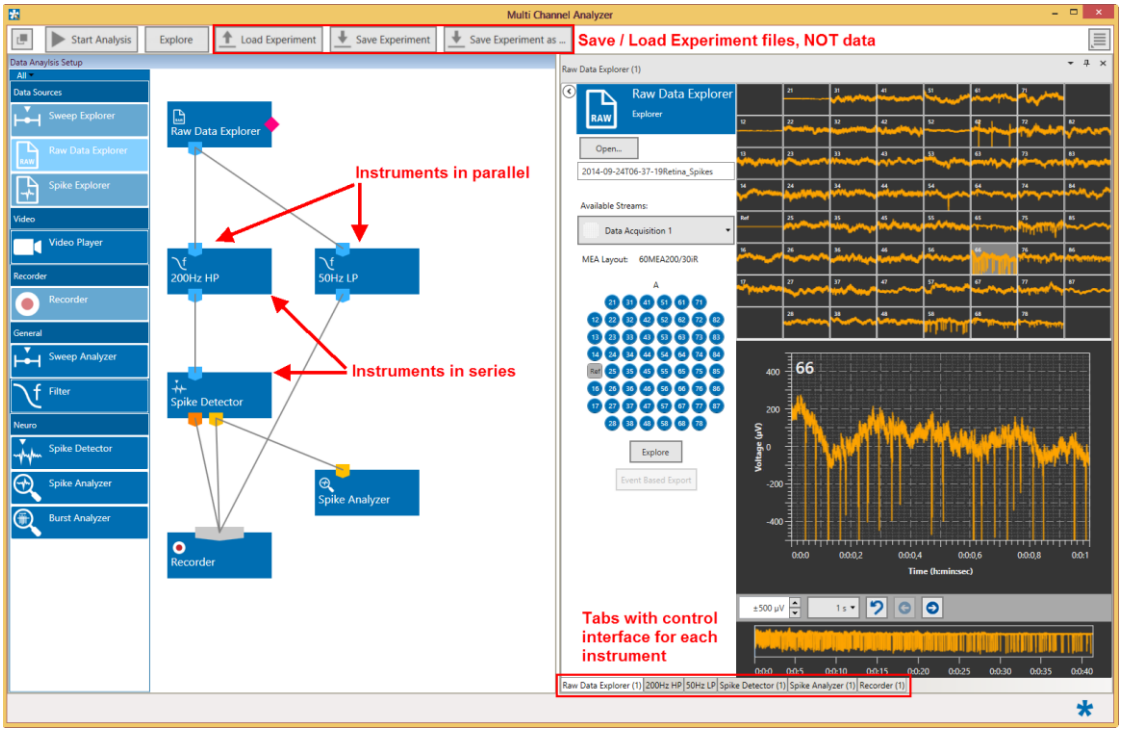
Once a configuration of instruments has been designed, it can be saved for later use. Such a configuration is called **Experiment**, and can be saved and loaded from the main menu bar. The file extension for an Experiment file is ***.msa**. The Save and Load Experiment functions do not save or load data, just the combination of instruments.



4.1.3 Instrument Control Windows

A double click on each instrument opens a tab with the control interface for that instrument. Each instrument has its' own **control window tab**. The tab of the selected data source always shows the raw data. Most instruments can be selected **more than once**, and be connected in parallel or in series. Exceptions are the data source and the recorder which can be selected only once.

Once a configuration of instruments has been designed, it can be saved for later use. Such a configuration is called **Experiment**, and can be saved and loaded from the main menu bar. The file extension for an Experiment file is ***.msa**. The Save and Load Experiment functions do not save or load data, just the combination of instruments.



The connecting lines between the instruments represent the flow of data. In the example above, there is a stream of raw data coming from the data source Raw Data Explorer through a 50 Hz low pass filter into the recorder directly and will consequently be recorded in the file. The same raw data stream is processed through a 200 Hz high pass filter, and the filtered data is further processed by Spike Detector. The generated spike and cutout data is then connected to the recorder and will generate two more data streams in the recorded file.

Instruments operate as independent units. They process the data from the respective input port and generate one or more data streams in the output port(s). Settings have to be done separately for each instrument in the respective control window tab.

4.1.4 Workflow for Analyzing Data

To analyze data files with the Multi Channel Analyzer it's mandatory to follow a **specific workflow**. After designing an experiment (Chapter **4.1.1,** Design an Experiment) and opening a file (Chapter **4.6** Opening Data Files) there are several options to review the data (Chapter **5.1.1**, Navigating in Data Files). It is recommended to first use the **Explore** function (Chapter **5.1.2**, Exploring Data Files) to optimize the settings of the analysis instruments, before analyzing the whole file with the **Start Analysis** function.

4.2 Data Port Types

The types of data occurring in the Analyzer, as indicated by the color-coded ports are:

- **Blue:** Electrode data, raw or filtered, cross channel data. These data types have the same structure and the same compatibility with instruments, hence they use the same data port color.
- **Green:** Auxiliary analog data, from the additional analog inputs on the interface board.
- **Red:** Digital data, from the 16 bit digital data stream from the Interface board.
- **Orange:** Spike cutouts generated by the Spike Detector instrument, a fragment of data around a detected spike.
- **Yellow:** Spike time stamps generated by the Spike Detector Instrument, just the time stamp of a detected spike.
- **Cyan:** Sweep data, as generated by the Sweeps tool, a fragment of data around a trigger event.
- Purple: Averaged Sweeps, as generated by the Sweeps tool.
- **Magenta:** The bidirectional synchronization connection between Raw Data or Spike Explorer and Video Player.
- Pink: Trigger events.

4.3 File Types

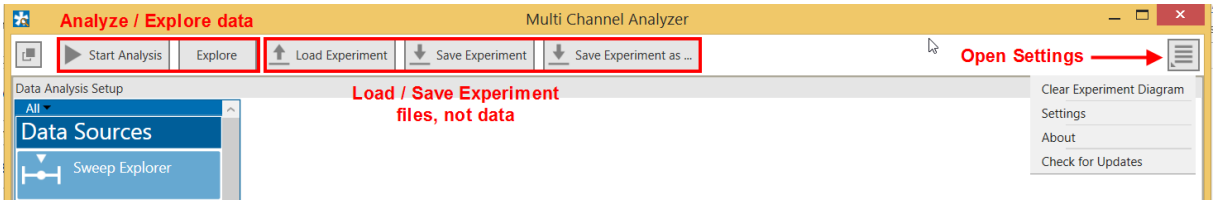
The types of files occurring in the Analyzer are:

- ***.msa**: Experiment file, a configuration of instruments. Created manually by the user.
- ***.msrd**: Data file, generated by the Recorder, contains the raw data of all data streams.
- ***.msrs**: Settings file, generated by the Recorder, must also be present to open a data file.
- ***.html**: Instrument settings, one *.html file is generated by the Recorder with each data file. Each *.html is linked to the *.xml files of the individual instruments.
- ***. xml**: One *.xml per instrument is generated for each recording. It contains all settings of the respective instrument. Stimulation patterns in the Stimulator instrument can also be saved as *.xml files by the user.

All files generated during a recording have the **same name**, in case of the *.xml files with an extension representing the instrument name ([file name][instrument name].xml).

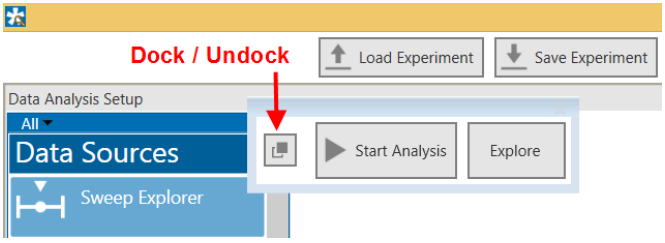
4.4 Main Menu

The main menu bar contains the major control functions for starting the **data analysis,** as well as the **Save and Load** options for Experiment files. Furthermore, there is a button to open the general settings.

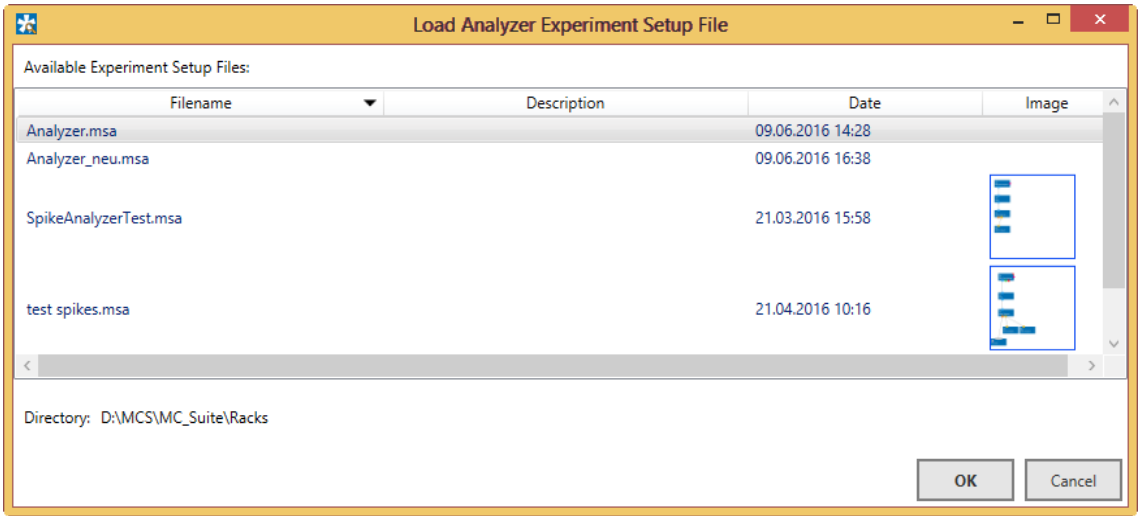


The **Start Analysis** function will process the complete loaded data file through all analysis instruments (see chapter 0, Analyzing Data Files). **Explore** will only analyze the segment of data currently visible in the Explorer instrument (see chapter 5.1.2 Exploring Data Files).

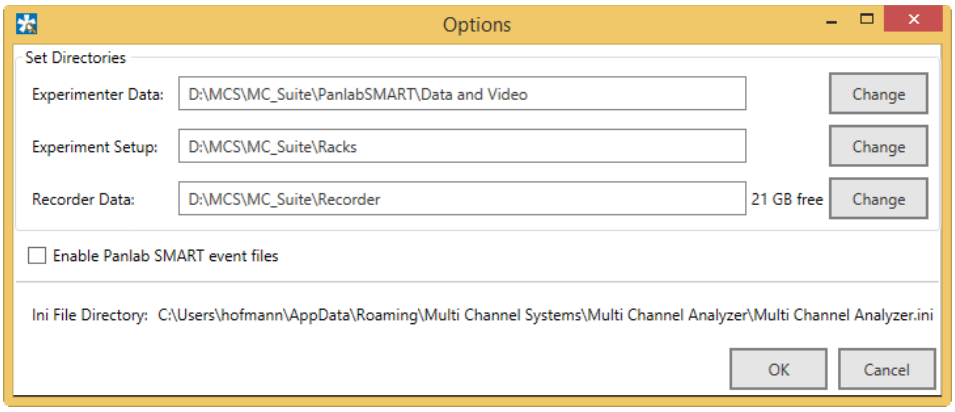
The Analyze / Explore controls can be **undocked** from the main Menu and be dragged anywhere. They will always stay on top of all other windows.



The **Save / Load Experiment** function will open a dialog to safe the current configuration of instruments as a *.msa file, or open a previously saved configuration.

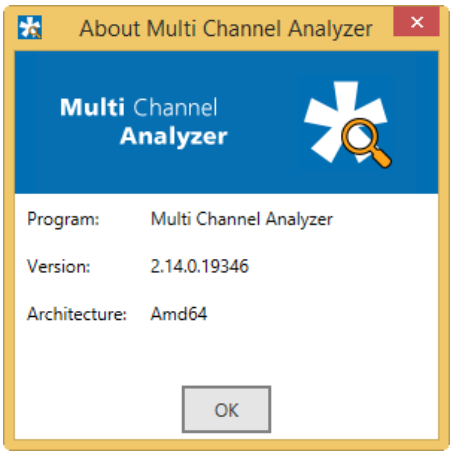


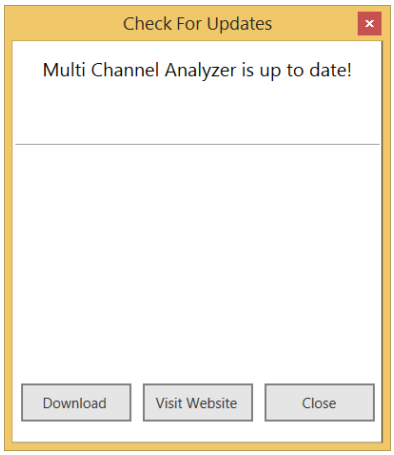
The **Settings** allow to change the default source path for the data files to replay, the data files generated by the Recorder, and the directory where the experiment files are stored.



The use of the **Panlab SMART events** is described in chapter 6.

It's also possible to delete all instruments currently in the main window with the **Clear Experiment Diagram** command. **About** will show the software version, while **Check for Updates** will connect to the MCS web site download section and automatically search for newer versions of the Multi Channel Analyzer.

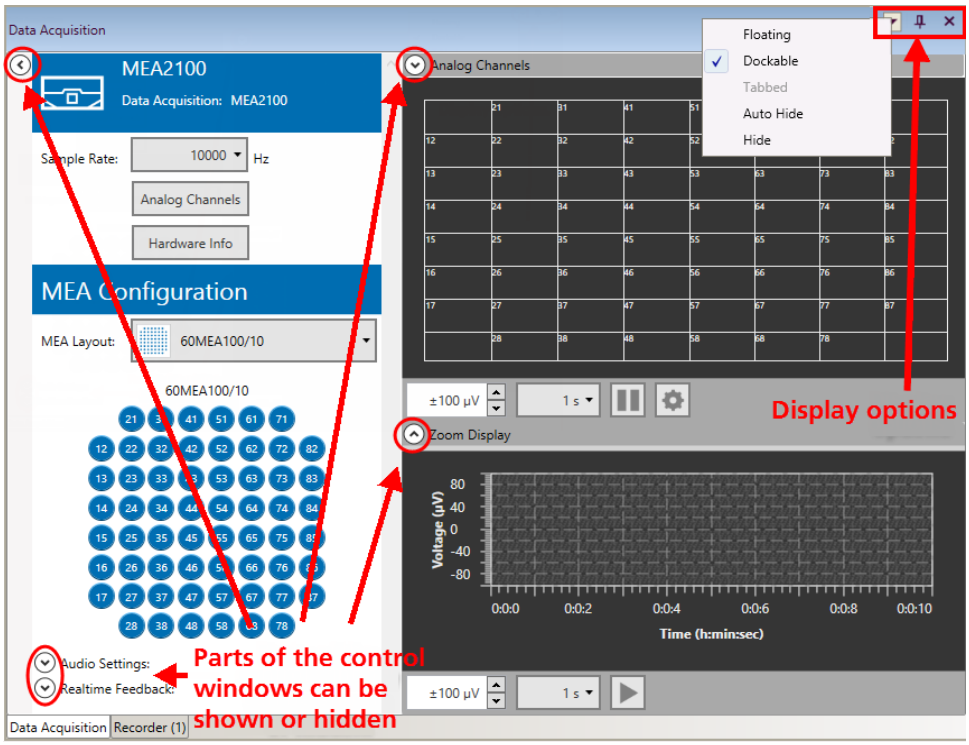




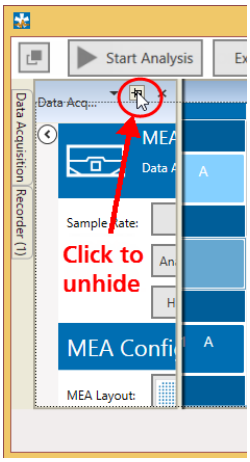
The lower menu bar shows the MCS icon in the right corner. The icon will rotate while an analysis is underway. The amount of free data space on the hard drive selected for data storage is shown next to it.

4.5 General Display Features

In most control windows, there are additional display or control functions which can be hidden or shown, as need be. By default, if more than one control window is open, they are shown as tabs, and only one window is visible at a time. This option is called **Dockable**.



However, there is also the option to make individual control windows **Floating**. A floating window can be individually resized and dragged anywhere, also out of the main Experimenter window and onto a second monitor. All control windows can also be hidden, either with the pin icon, or from the drop down menu (**Auto Hide**). Hidden windows go to a sidebar on the left side of the Experimenter window. To make them visible again, hover the mouse over the sidebar. A part of the control window will pop up and you can click the pin icon to unhide it.



In all control windows, **all channels** of the data acquisition are shown in the layout which was selected during recording. One channel can be selected by mouse click to be shown in the **Zoom Display**. All Displays share similar scaling and zoom functions. Under each display the x- and y-axis scaling can be changed.

In the Zoom Display, it is possible to zoom in further by dragging a frame around the region of interest. A click on the arrow button will reset the zoom. The data from the Zoom Display can be exported as ASCII (*.txt file).

The **Apply to Overview** button will transfer the currently selected scaling for the zoom display to the overview display. To indicate this, the scaling display of the overview will become yellow. The reset arrow will switch the overview back to the selected scaling. For most displays, this will apply only to the y-axis. In some cases, like in the Sweeps Tool, both axes are affected.

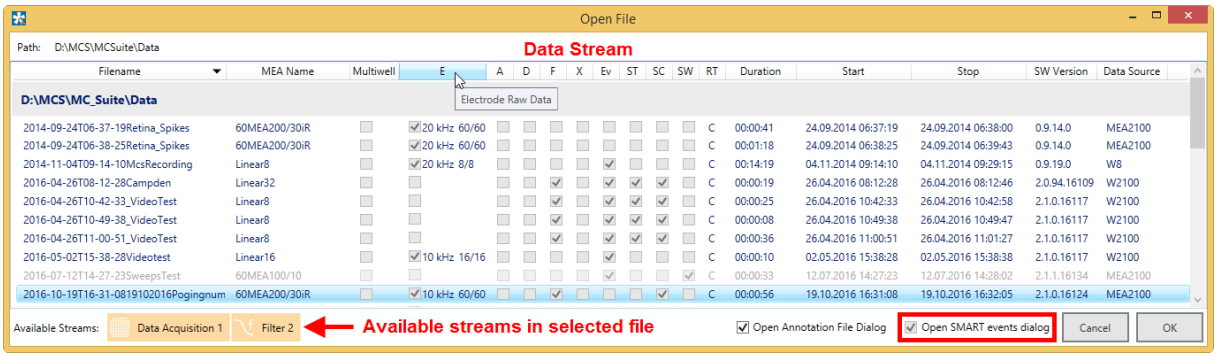
4.6 Opening Data Files

At the moment, the Multi Channel Analyzer has three different instruments which can be used to open different types of data, the **Raw Data Explorer**, the **Spike Explorer** and the **Sweep Explorer**. These instruments will be described in detail in the later chapters. Each instrument opens only files which contain a **specific type of data**. More than one type of data can be present in a single file, depending on what was recorded. If the file browser is opened in any of those three instruments, files containing a compatible data stream are shown solid, all other data files are grey and not accessible.

The Open File window shows detailed information about each *.msrd file in the selected data folder:

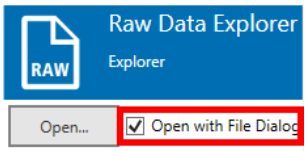
- Layout
- Data streams in file
- Recording type
- Duration
- Recording time
- Software version used during recording

Hover over the data streams with the mouse pointer to get a tooltip with the full name of the stream. If a file is selected, the streams compatible with the currently used Explorer instrument are shown at the bottom.



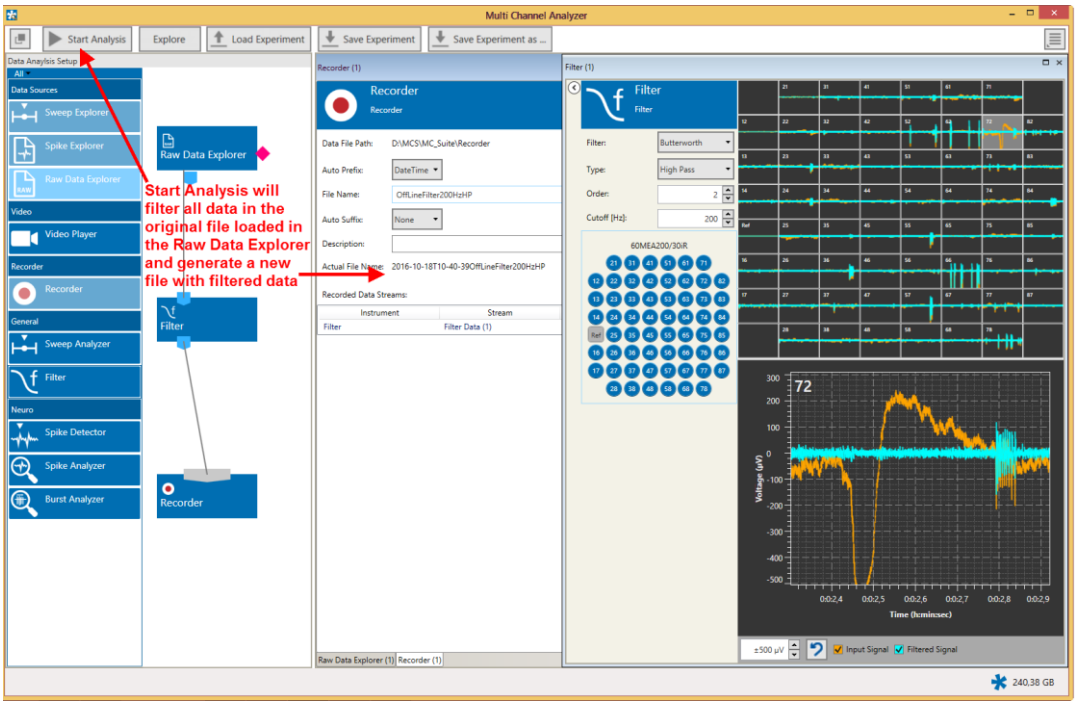
The Raw Data Explorer opens files with electrode **raw data (E), filtered data (F)** and **cross channel data (X)**. The Spike Explorer reads files with spike **time stamps (ST)** and/or **spike cutouts (SC)**. The Sweeps Explorer reads only files containing **sweeps (SW)**. The SMART events are described in detail in chapter 6.

The **Open…** function usually opens the data **Experiment Data Folder** selected in the Settings (see chapter 4.4). If you want to use the regular **Windows file explorer** instead, select the **"Open with File Dialog"** option. The Windows file dialog will show all files (compatible or not) and won't contain any additional meta-information.



4.7 Re-recording Data

The Multi Channel Analyzer also features a **Recorder instrument**. It is possible to generate a new data file with an existing file as data source just as well as with live recorded data in the Multi Channel Experimenter. For example, it's possible to replay a raw data file, process it through a filter instrument, and record the filtered data as a **new file**. It' also possible to write only a time segment of the original file into a new file, see chapter 5.2.4.3.

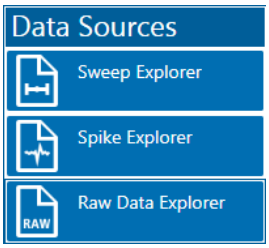


5 Instruments

In the following chapters, all instruments of the Multi Channel Analyzer are described individually. Instruments operate as **independent units**. They process the data from the respective input port and generate one or more data streams in the output port(s). Settings have to be done separately for each instrument in the respective control tab. Instruments without a connection to the respective input ports are not functional. Data generated by an instrument is only recorded if the output port is connected to the recorder (see chapter **4.7,** Re-recording Data).

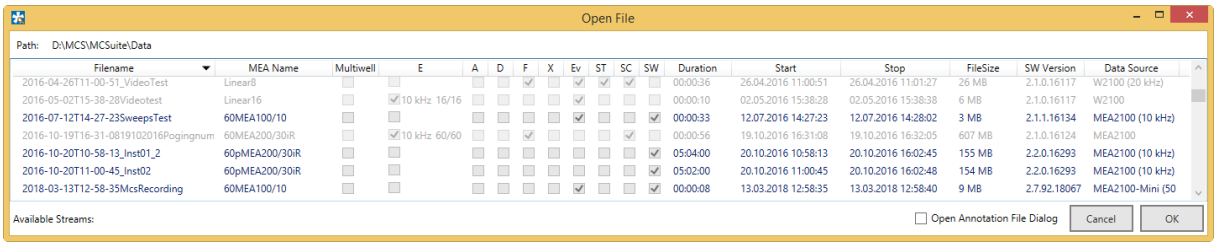
5.1 Data Sources General

At the moment, the Multi Channel Analyzer has three different Data Source instruments, which can be used to open different types of data files generated with the Multi Channel Experimenter, the **Raw Data Explorer**, the **Spike Explorer** and the **Sweep Explorer**.



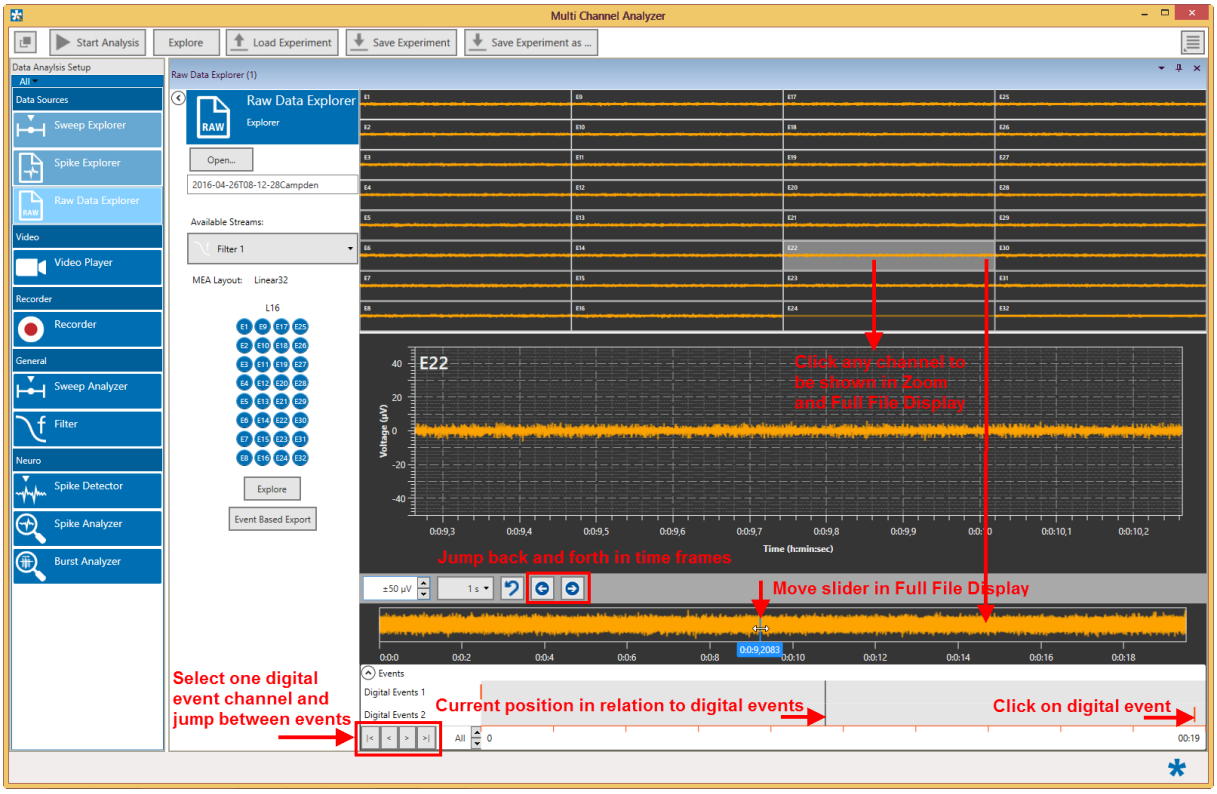
These instruments will be described in detail in the later chapters. Each instrument opens only files which contain a **specific type of data** (see chapter **4.6,** Opening Data Files).

In the Open File dialog, only files compatible with the respective explorer instrument are shown solid, all other data files are greyed out.



5.1.1 Navigating in Data Files

Each Explorer instrument allows to jump back and forth in the respective data file. There are different options to navigate the file. It is possible to go through the file **frame by frame** with the arrow buttons. Each click will move forward one selected time window. The second option is to use the **Full File Display**, which shows the complete length of the loaded file for any one selected channel. A blue slider allows to move to any point in time on that display, the data shown for all channels will jump to that point.



The third option is available for files containing **trigger events**. These trigger events will be displayed in a separate display as red markers. One can either click on any of those trigger markers to move the whole file to that point or jump from one trigger to the next using the arrow buttons. A black slider in the digital event display marks the current position of the data display in relation to the digital events. This slider cannot be moved actively.

5.1.2 Exploring Data Files

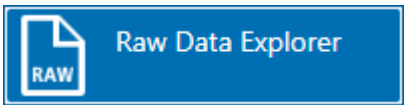
Each of the Explorer instruments contains an Explore button. The Explore function is supposed to **test** and **optimize** an analysis setup before processing the whole file. This can save time, especially for larger files. The Explore button will send only the fragment of data **currently visible** in the Explorer display through the attached analysis instruments. This allows to adjust parameters, as spike detection level or filter parameters, before proceeding with the analysis of the complete file. The Explore function is similar for all Explorer instruments, only the **visible segment of data is analyzed**, no matter whether it contains raw data, spikes or sweeps.

If the "**Auto Explore**" function is active, analysis of every new data segment is automatically started, when navigating through the file by arrow keys, slider, or by events.

5.1.3 Analyzing Data Files

Finally, the Start Analysis function in the main bar will process the **complete file** which has been loaded in the Explorer instrument through the attached analysis instruments. During the analysis, the MCS icon in the lower main bar will rotate. The already analyzed time is displayed next to the icon.

5.2 Data Source: Raw Data Explorer

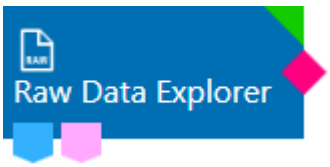


5.2.1 Description and Purpose

The Raw Data Explorer can open any file which contains a **raw data, filtered data, or cross channel data** stream. If more than one compatible stream is available in the file, the stream to analyze can be selected from a drop down list. The Raw Data Explorer cannot open files containing only Spike or Sweeps data. **Analog data** can be reviewed with the Raw Data Explorer, too, but only if there is also raw, filtered, or cross channel data in the file.

5.2.2 Data Ports and Export Options

The Raw Data Explorer has a **blue output port for data** and a **pink port for trigger events**. The data can be electrode raw data, filtered data or cross channel data. As with all data sources, there are no input ports. The pink port is only operational if trigger events are present in the opened file. The Raw data explorer can be connected directly with any number of other instruments with blue input port. In addition, there is the bidirectional **synchronization connection port** on the side of the explorer icon. This port is used exclusively to synchronize electrophysiological data in the Raw Data Explorer and behavioral video files opened in the Video Player instrument. An event based export of raw data is possible, if the file contains trigger events. The Export function in the Zoom display can be used to export a segment of a single channel as ASCII data (*.txt).

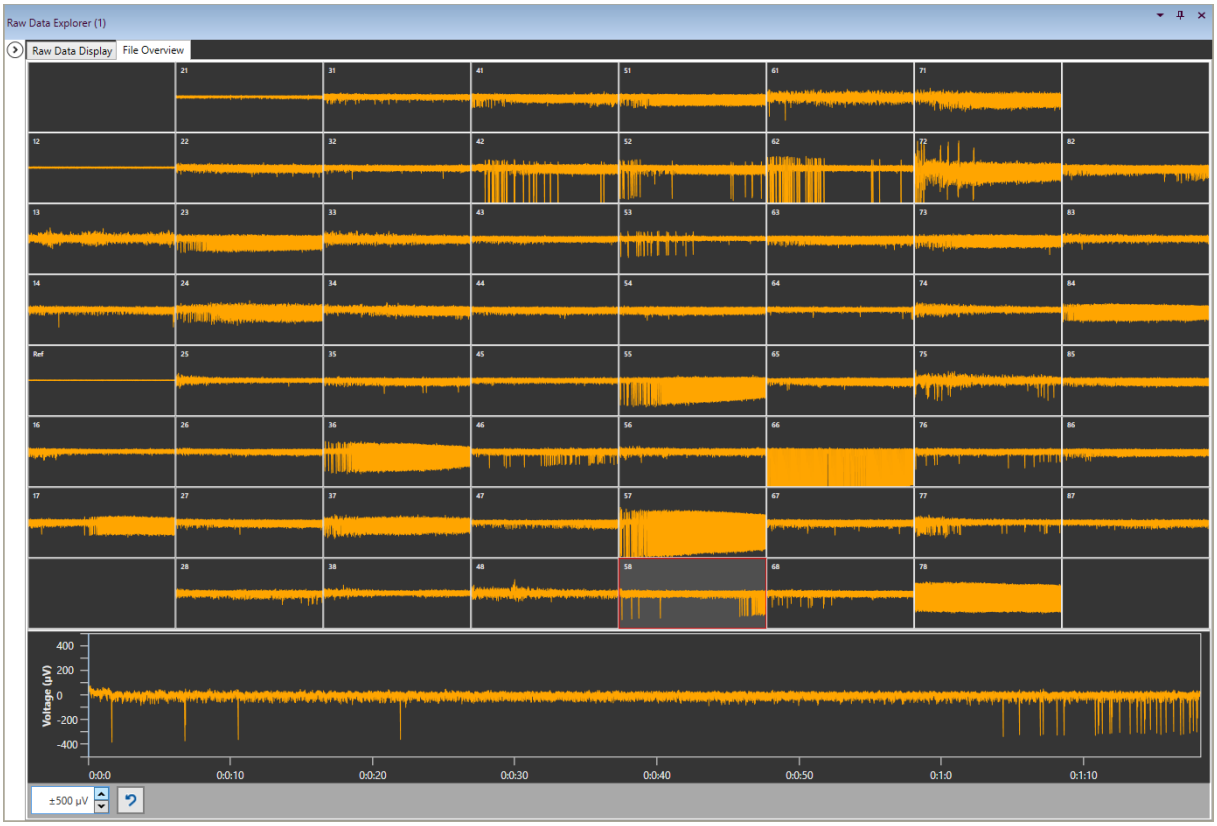


5.2.3 Operation

The Raw Data Explorer data source control window contains an Open file dialog. The **Open…** function will bring up a file browser for the folder selected in the general settings (see chapter 4.4, Main Menu). All *.msrd data files will be shown, but only files containing a data stream **compatible with the Raw Data Explorer** can be opened (see chapter 4.6, Opening Data Files). If you want to use the regular **Windows file explorer** instead, select the **“Open with File Dialog”** option. The Windows file dialog will show all files (compatible or not) and won’t contain any additional meta-information. If more than one compatible data stream is present, the stream to be shown can be selected from a drop down menu.

All available data channels of the selected stream in the loaded file will be shown in the layout which was selected during recording. Individual channels can be **toggled** by clicking on them. Deactivated channels will no longer be shown, and not be transmitted to the connected analysis instruments. This can speed up the analysis process.

A usual, there is an additional Zoom Display for a single channel. Just click on any channel to select it to be shown the Zoom Display. An additional display at the bottom, the **Full File Display**, shows the data for the full length of the file for the selected channel. A blue slider in that display allows to move back and forth in the file (see chapter **5.1.1**, Navigating in Data Files). The **File Overview** tab will show data for the full file duration of for all channels. The **Explore** button will send the currently visible segment of data through the attached analysis instruments.

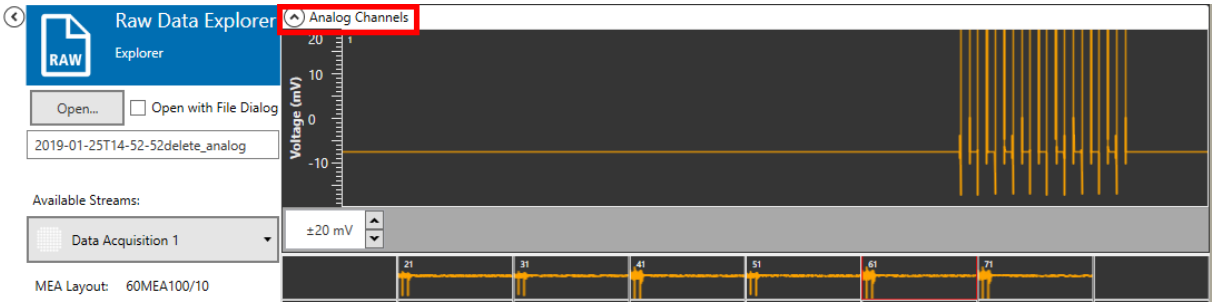


5.2.3.1 Annotation File Dialog

In the Open File menu, there is a tick box to open an annotation file dialog. **Annotation files** are used in combination with synchronized behavioral videos, and contain comments made by the user during the experiment.

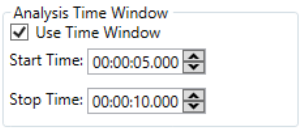
5.2.3.2 Analog Data Display

If a file contains data from one or more **analog channels** (green port in Multi Channel Experimenter), and also raw, filtered or cross channel data, this file will open in the Raw Data Explorer and the analog data will be available in an extra display, similar to the Data Sources in the Experimenter.



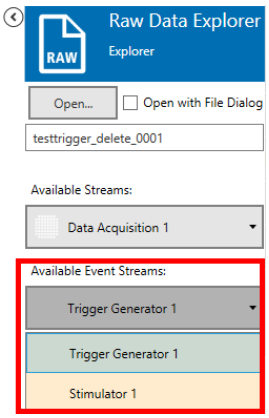
5.2.3.3 Analysis Time Window

Instead of analyzing the whole file with the Start Analysis function (see **0**), in the Raw Data Explorer it's also possible to select only a **specific time segment** for analysis. If active, only the data in the selected time window will be analyzed. This function can also be used to generate a new file with only a subset of the data.



5.2.3.4 Files with Trigger Events

If one or more **trigger event streams** are present in an open file, the pink trigger port of the Raw Data Explorer becomes operational. An additional drop down menu appears with all available event streams in the file. The user can decide which of the will become available for other instruments from the pink trigger port.

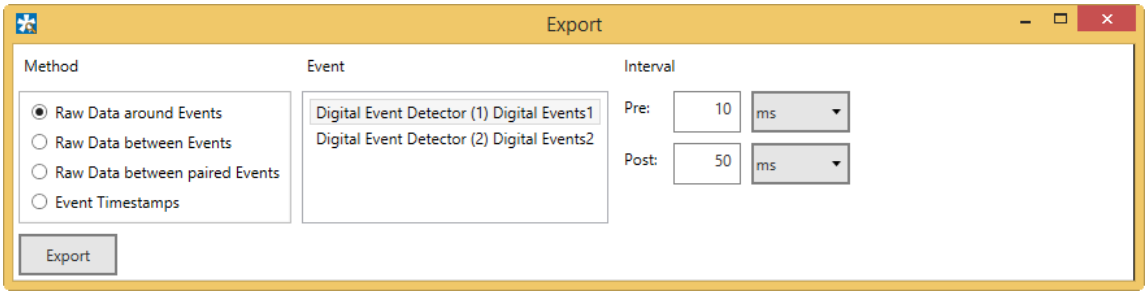


The selected stream is sent through the pink port to connected instruments with a trigger input, like a **Sweeps** or **PSTH** tool.

5.2.3.5 Event Based Export

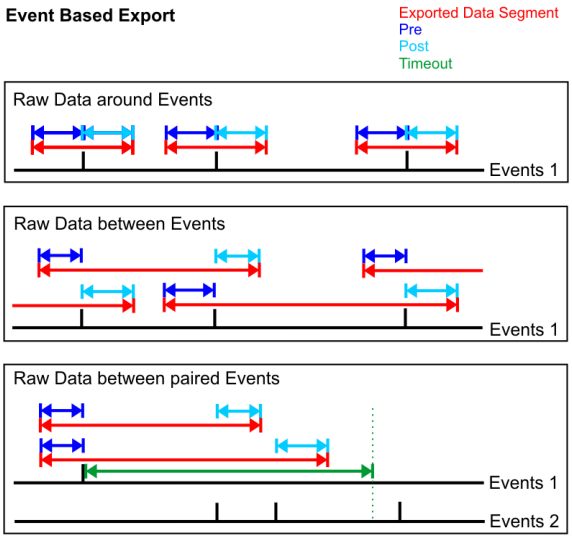
The **Event Based Export** can export raw data in ASCII format based on digital events (Triggers). **Raw Data around Events** will generate a segment of data with selectable length around each event of the selected event channel. Pre is the length of data before, and Post the length of data after each event.

Raw Data between Events will split the raw data at each event of the selected event channel. Pre and Post time can be added to each data segment. This will lead to overlapping segments of data.



Raw Data between paired Events operates with trigger events on two different channels. Data segments starting from the first event on Event channel 1 to each following event on event channel 2 will be generated, till the time distance between event on channel 1 and event on channel 2 is higher than the selected Timeout. Pre and Post time can be added to each data segment. This will lead to overlapping segments of data. Then the same procedure will be repeated for the second event on event channel 1 and so on.

In all cases, all segments of ASCII data will be exported to a single file. Segments are separated by empty lines. Event Time stamps will export the time stamps of all events on the selected event channel to an ASCII file.

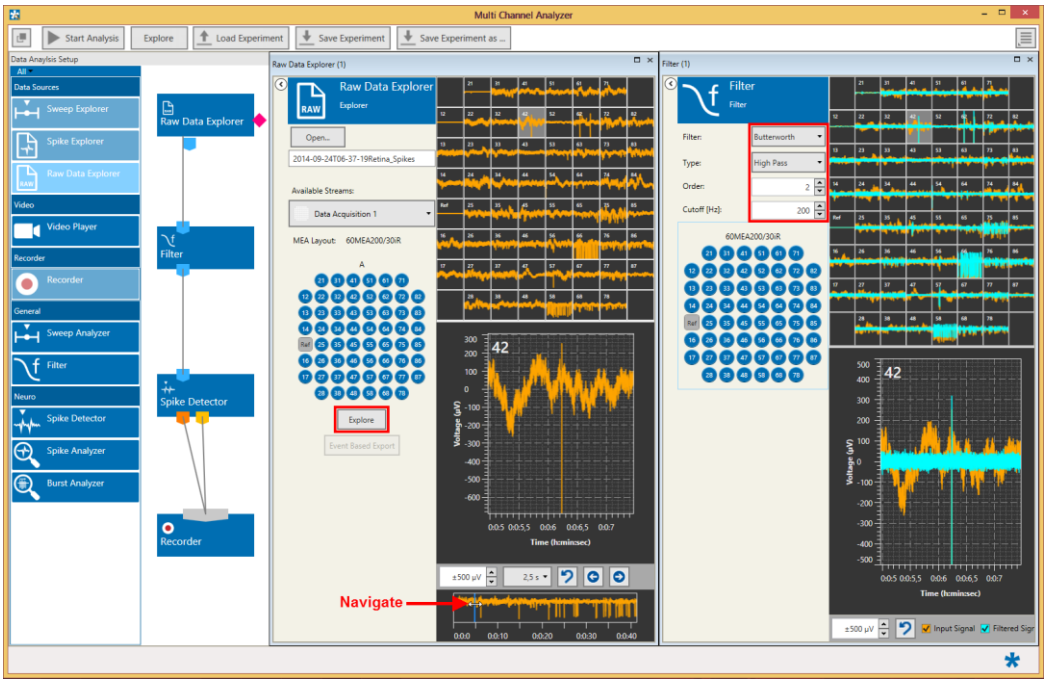


5.2.4 Examples: How to …

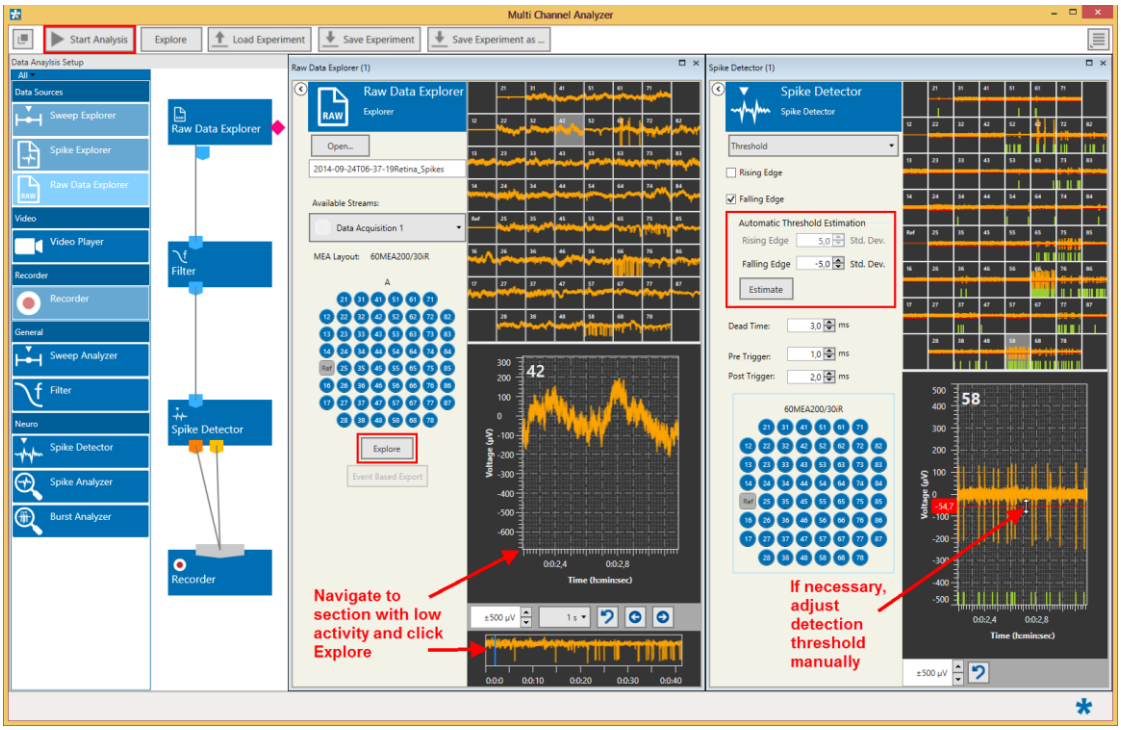
This section explains how to open and analyze raw data files with the Raw Data Explorer.

5.2.4.1 Extract Spikes from a Raw Data File

In the experiment below, a raw data file with sixty channels is loaded by the Raw Data Explorer. The data contains many slow frequency components, which make spike detection difficult.

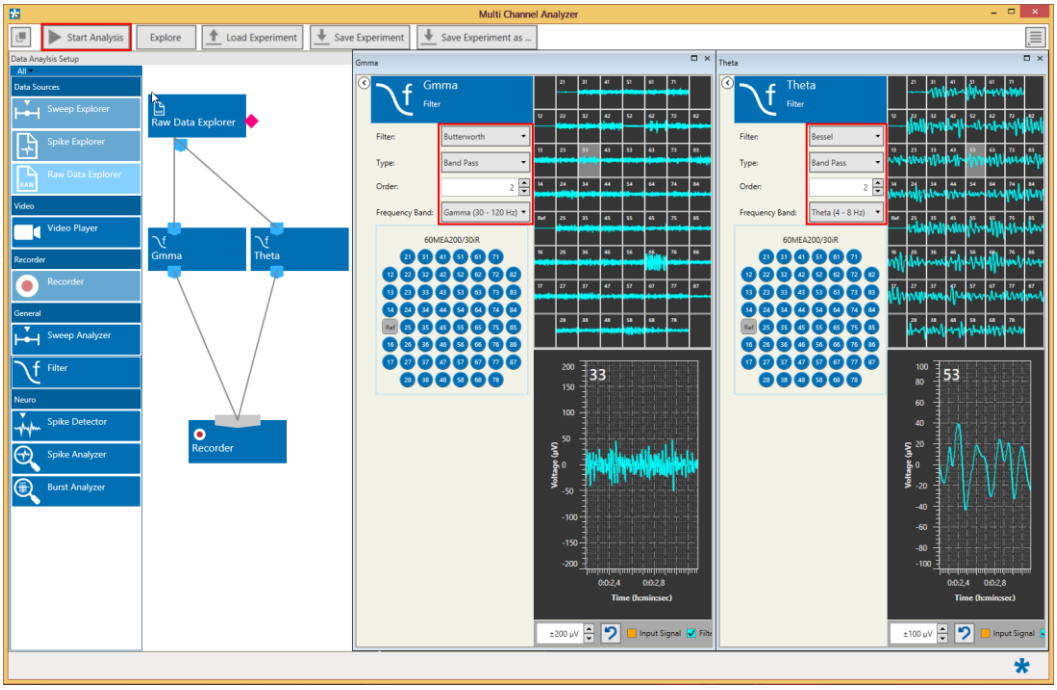


Therefore, the raw data is first filtered at 200 Hz high pass, and then processed by a Spike Detector instrument. The spike time stamps and spike waveforms (Spike Data) are recorded in a new file by the Recorder. Ideally, move to a segment of data without spike activity, either using one arrow keys, or the blue slider in the full file display of the selected channel. Use the Explore function to send the visible segment of data through the Filter and Spike Detector. The Spike Detector sets the detection threshold based on the standard deviation of the noise. Actual Spike activity increases the SD, hence it makes sense to use a data segment without low spike activity spikes to optimize the spike detection levels in the Spike Detector.
A threshold of five times SD of the noise usually works well. To fine tune of the detection levels of individual; channels, select the channel as zoom channel, and drag the detection threshold with the mouse. In the Explore mode, no new file is recorded yet. Once the filter and spike detection settings are optimized, use the Start Analysis function to process the complete file. This will generate a new file, containing only time stamps and spike data. The original file is not affected. The spike data can now be exported to a third party application with the Multi Channel DataManager, or processed by the Spike Explorer.



5.2.4.2 Generate Files with Different Filter Bands from a Raw Data File

Data containing only certain frequency bands can be extracted from a wide band raw data file using one or more filters. In the example below, the wide band data is processed by two filters in parallel to extract Theta and Gamma frequency components. If the whole file is analyzed with the Start Analysis function, a new file with two filtered data streams will be generated by the Recorder.



5.2.4.3 Generate a new File with only a Time Segment of the Original Data

With the Analysis Time Window option, it's possible to analyze only a specific time segment out of a raw data file, but also to split a larger data file into smaller segments. To generate a new file with only a fraction of the original data, open the original file and select the data stream you want in the new file. Only one stream at a time can be processed. Connect the Raw Data Explorer to the Recorder (with or without additional instruments in between) and select the time window you want to be in the new file. Start Analysis, and the new file will be generated in the Data File Path folder set for the Recorder.
In the example below, a file containing filtered data of minute 1 to 2 of the original raw data will be generated.

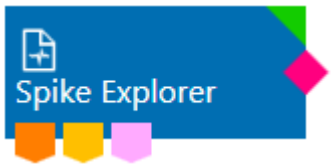
5.3 Data Source: Spike Explorer

5.3.1 Description and Purpose

The Spike Explorer can open any file which contains a **spike data or spike time stamp** stream. If more than one compatible stream is available in the file, the stream to analyze can be selected from a drop down list. The Spike Explorer cannot open files containing only Raw or Sweeps data.

5.3.2 Data Ports and Export Options

The Spike Explorer has a **yellow and/or orange output port for spike time stamps and cutouts, and a pink trigger port,** depending which data streams are present in the open file. As with all data sources, there are no input ports. The Spike Explorer can be connected directly with any number of other instruments with yellow or orange input port. In addition, there is the bidirectional **synchronization connection port** on the side of the explorer icon. This port is used exclusively to synchronize electrophysiological data in the Spike Explorer and behavioral video files opened in the Video Player instrument. The Export function in the Zoom displays can be used to export time stamps or cutouts of a single channel as ASCII data (*.txt). Events (spike time stamps, triggers, SMART events) can be exported with the “Event Export”.



5.3.3 Operation

The Spike Explorer data source control window contains an Open file dialog. The **Open…** function will bring up a file browser for the folder selected in the general settings (please see chapter 4.4, Main Menu). All *.msrd data files will be shown, but only files containing a data stream compatible with the Spike Explorer can be opened (see chapter 4.6, Opening Data Files). If more than one compatible data stream is present, the stream to be shown can be selected from a drop down menu.

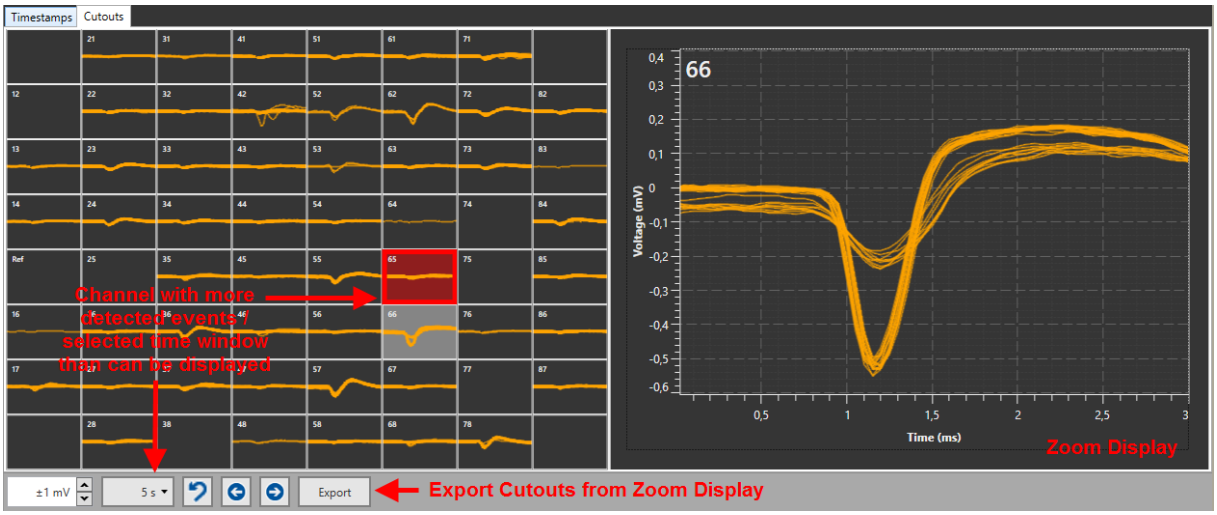
All available data channels of the selected stream in the loaded file will be shown in the layout which was selected during recording. Individual channels can be **toggled** by clicking on them. Deactivated channels will no longer be shown, and not be transmitted to the connected analysis instruments. This can speed up the analysis process. There are two tabs in the Spike Explorer, one for the **Time Stamps** and one for the **Cutouts**. Both tabs contain one overview display for all channels, and one Zoom display for any one selected channel. Just click on any channel to select it to be shown the Zoom Display.

An additional display at the bottom shows the **spike rate** as **histogram** and also as **false color plot** for the full length of the file for all channels. The channels are shown in rows, as they appear in the respective layout. For example, for the regular 8x8 layout for 60 channels, it’s row one first, electrodes 21, 31, 41, …71 then 12, 22, 32, … 82 and so on.

A **black slider** in that display allows to move back and forth in the file. Then second slider indicates the currently visible segment of data in the cutout and time stamp displays above. The color scheme and scaling of the false color plot can be changed.

Data from the Zoom Display can be **exported as ASCII** (*.txt file), from the Cutouts as well as from the Time stamps tab.

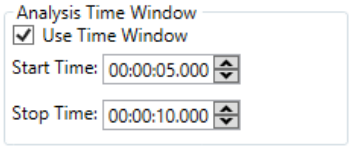
The Cutouts tab shows all detected events for the currently selected segment of data in overlay, up to a maximum of one hundred. If more than hundred events are in the segment, the overlay window will be **labeled red**.



As with all Explorer instruments, the **Explore** button will send the currently visible segment of data through the attached analysis instruments, while the Start Analysis button will analyze the whole file.

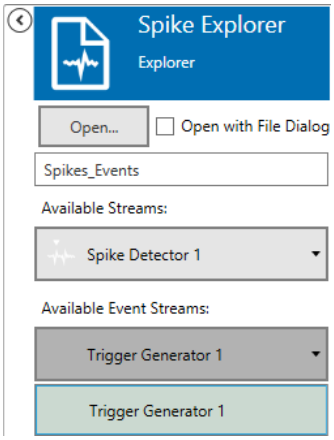
5.3.3.1 Analysis Time Window

Instead of analyzing the whole file with the Start Analysis function (see 0), in the Spike Explorer it's also possible to select only a **specific time segment** for analysis. If active, only the data in the selected time window will be analyzed. This function can also be used to generate a new file with only a subset of the data.



5.3.3.2 Files with Trigger Events

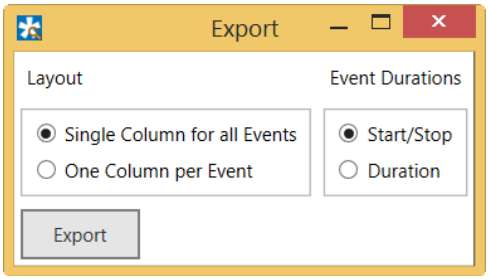
If one or more **trigger event streams** are present in an open file, the pink trigger port of the Spike Explorer becomes operational. An additional drop down menu appears with all available event streams in the file. The user can decide which of the will become available for other instruments from the pink trigger port.



The selected stream is sent through the pink port to connected instruments with a trigger input, like a **PSTH** tool.

5.3.3.3 Event Export

The **Event Export** function can export all events in a file as ASCII data. Spike time stamps, triggers and all other events can be exported in a single column, or individual columns. This is actually an ASCII export of the overview raster plot Events with a duration can be shown with their respective start and stop, or duration.



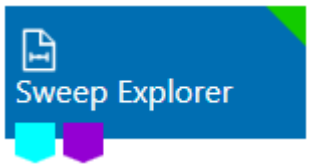
5.4 Sweep Explorer

5.4.1 Description and Purpose

The Sweep Explorer can open any file which contains a **sweep data** stream. If more than one compatible stream is available in the file, the stream to analyze can be selected from a drop down list. The Sweep Explorer cannot open files containing only Raw or Spike data.

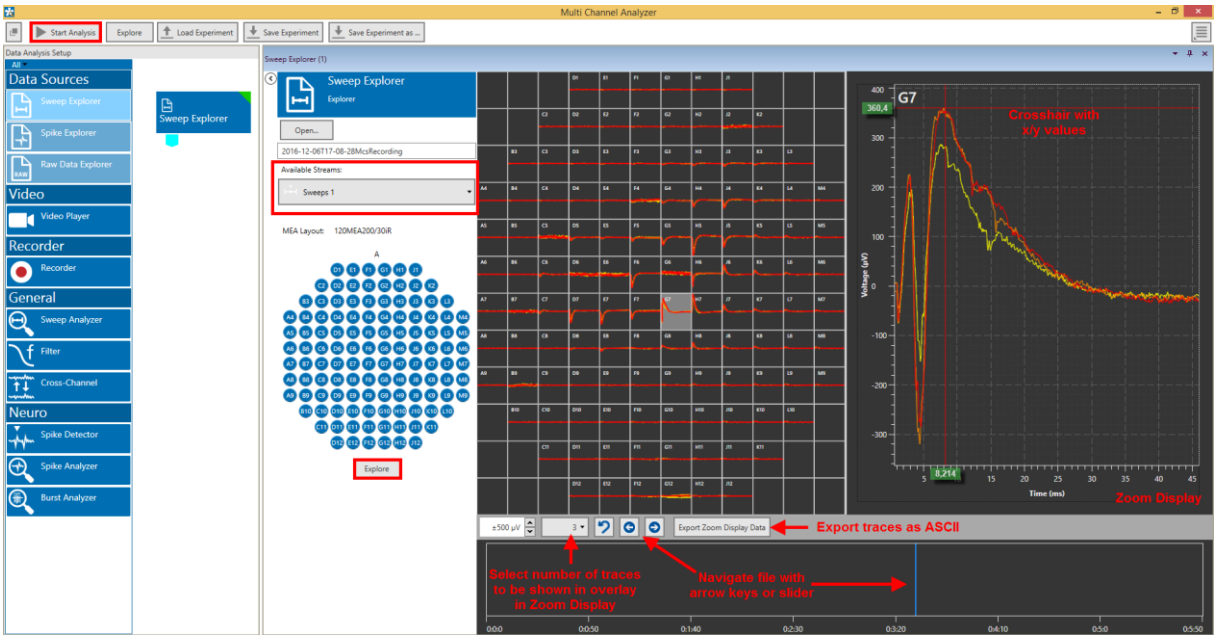
5.4.2 Data Ports and Export Options

The Sweep Explorer has a **cyan output port for sweep data** and a **purple output port for averaged sweeps.** As with all data sources, there are no input ports. The Sweep Explorer can be connected directly with any number of other instruments with cyan or purple input port. The Export function in the Zoom display can be used to export sweep traces of a single channel as ASCII data (*.txt).



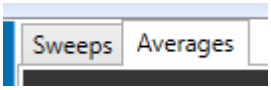
5.4.3 Operation

The Sweep Explorer data source control window contains an Open file dialog. The **Open…** function will bring up a file browser for the folder selected in the general settings (please see chapter 4.4, Main Menu). All *msrd data files will be shown, but only files containing a data stream **compatible with the Sweep Explorer** can be opened (see chapter 4.6, Opening Data Files). If more than one compatible data stream is present, the stream to be shown can be selected from a drop down menu.



All available data channels of the selected stream in the loaded file will be shown in the layout which was selected during recording. Individual channels can be **toggled** by clicking on them. Deactivated channels will no longer be shown, and not be transmitted to the connected analysis instruments. This can speed up the analysis process.

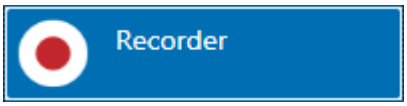
Two individual tabs show **Sweeps** and **Averages**, respectively, in case they are present in the file. Sweeps are individual signal after each trigger, while averages are already processed by the Sweeps Tool averager function.



As usual, there is an additional Zoom Display for a single channel. Just click on any channel to select it to be shown the Zoom Display. In the Zoom Display, either a single sweep or up to ten sweeps can be shown in overlay. Select the number of sweeps to be shown from the drop down menu. A crosshair displays the x- and y-values for any point in the Zoom Display.

An additional display at the bottom shows only a time line for the full length of the file. A blue slider in that display allows to move back and forth in the file (see chapter **5.1.1**, Navigating in Data Files). The **Explore** button will send the currently visible segment of data through the attached analysis instruments.

5.5 Recorder



5.5.1 Description and Purpose

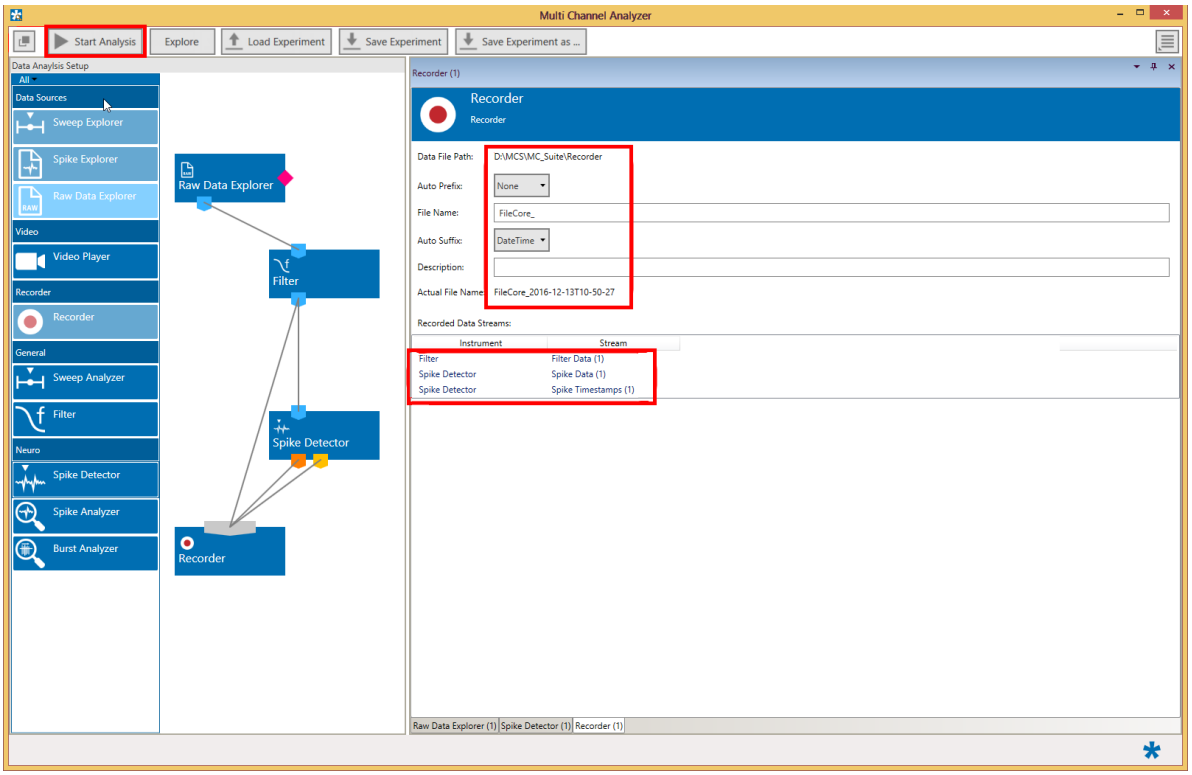
The Recorder allows to generate new files with different data streams out of a raw data file (see chapter **4.7**, Re-recording Data). Only one recorder can be used in an experiment. All data streams which are **directly** connected to the Recorder will be recorded.

5.5.2 Data Ports and Export Options

The Recorder has a **unique input port** which accepts connections from all other ports in unlimited number. One file is generated containing all connected data streams. There are no direct export options.

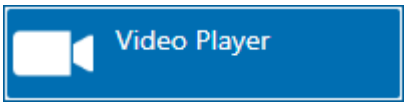
5.5.3 Operation

The Recorder can be used in offline mode to extract spike data filtered data from any file with a raw data stream. The default **file path** for the source file and the new file is set in the Main Window settings (see chapter **4.4**, Main Menu). A **file name** is generated from a random file core, and an optional pre and/or postfix. If the same file name is used repetitively, nothing will be overwritten. Instead, the second, third and so on file with the same name will additionally be labeled with 0001, 0002…at the end of the file name. A number of files with meta information, like instrument settings, will be generated with each recording. The actual data is in the *.msrd file. (Please see chapter **4.3**, File Types). All recorded data streams are listed in the Recorder control window.



A new file is created **only if the Start Analysis** function is used. The Explore function in the Raw Data Explorer will not generate a new file. The original source file is not changed in any way.

5.6 Video Player

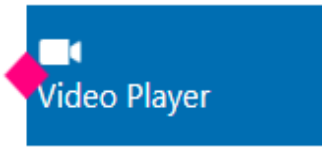


5.6.1 Description and Purpose

The Video Player can open any **video file** created with the W2100-System. If connected to the Raw Data or Spike Explorer with the synchronization connection, the video can be synchronized frame by frame with the electrophysiological data. Other windows compatible video files can be opened, too, but synchronization is only possible under certain conditions. Please contact support@multichannelsystems.com for details.

5.6.2 Data Ports and Export Options

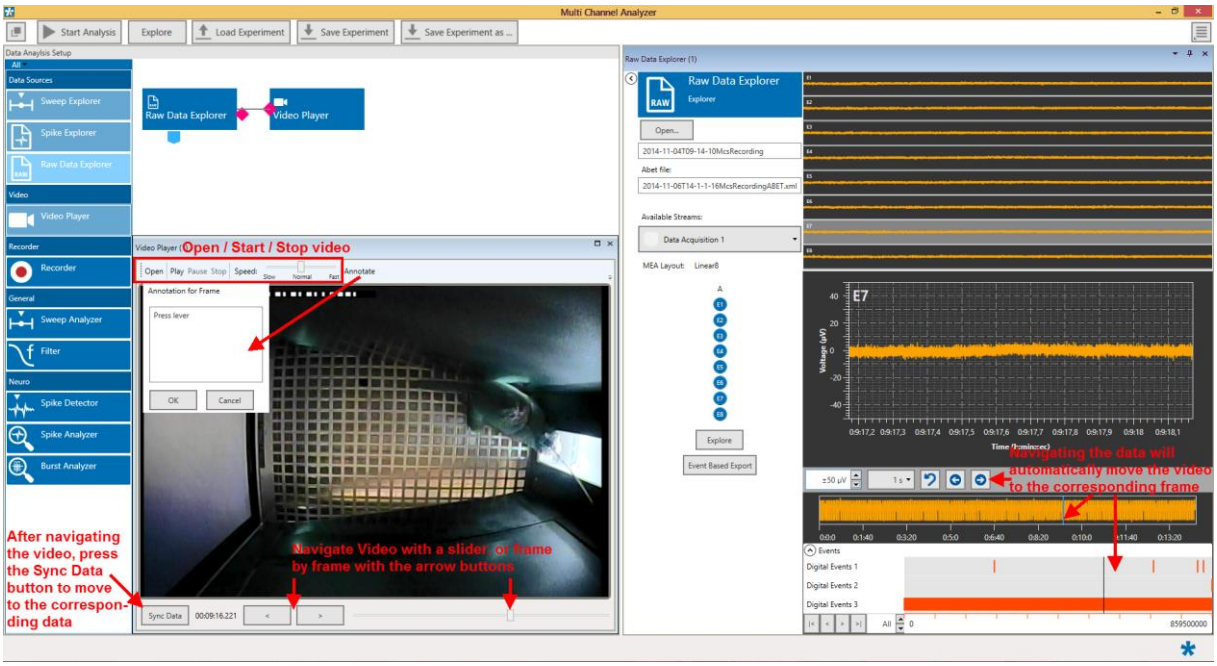
The Video Player can be connected with a Raw Data Explorer or a Spike Explorer via the bidirectional **synchronization connection port** on the side of the explorer icon. This port is used exclusively to synchronize electrophysiological data in the Explorer and behavioral video files opened in the Video Player. There are no export options.



5.6.3 Operation

The Video Player control window contains an Open file dialog. The **Open…** function will bring up a file browser to select a video file. All compatible video files will be shown. Precise synchronization between video and electrophysiological data only works if both files have been created together with the W2100-Video-System.

The Raw Data or Spike Explorer need to be connected via the Synchronization port with the Video Player.



Video files can be played continuously with different speeds, navigated with a slider, or frame by frame with arrow keys. The **Annotate** button will bring up a text field which allows entering a comment to each video frame. Navigating the data in the Explorer (see chapter 5.1.1, Navigating in Data Files) will automatically move the video to the corresponding frame. After navigating the video, the **Sync Data** button will show the corresponding data.

5.7 Sweeps

5.7.1 Description and Purpose

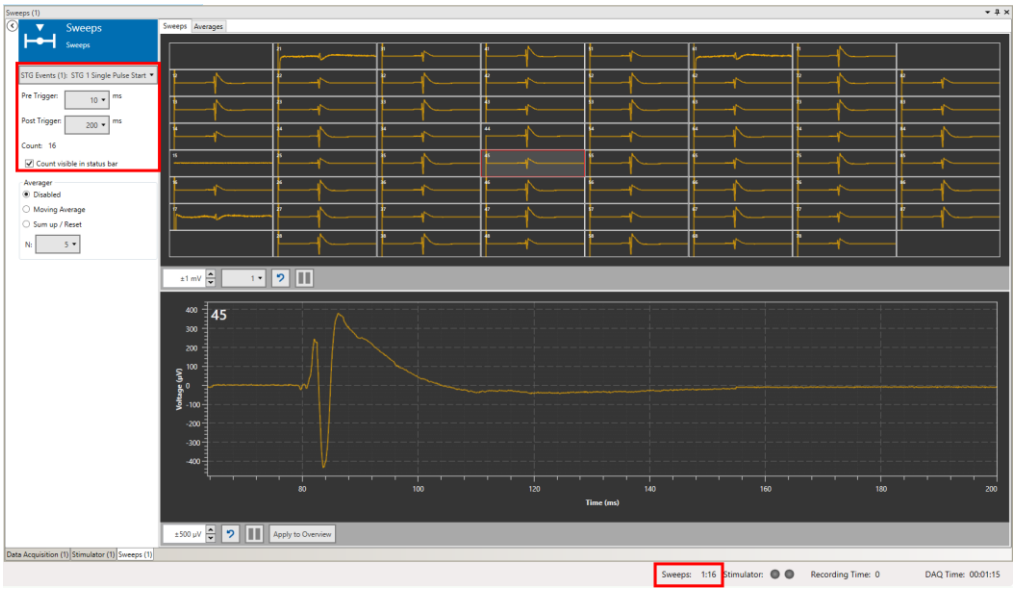
The Sweeps instrument can be used to extract **short segments of data (sweeps)** around a trigger event from a raw data file including also trigger events. More than one Sweeps instrument can be used in one experiment and more than one instrument can be connected to each Sweeps tool. The Sweeps tool is most often used to extract the response to an **electrical or optical stimulation**, where the trigger marks the time stamp of stimulation.

5.7.2 Data Ports and Export Options

The Sweeps instrument has a blue Electrode Raw Data input and a pink Trigger input. The Electrode Raw Data input provides the data, and the trigger is the reference signal to generate the data segment. Without both inputs, the instrument is not functional. The output is a cyan Sweeps data stream and a purple Averages stream, both of which can be connected to a **Sweep Analyzer and the Recorder**. There are no direct export options.

5.7.3 Operation

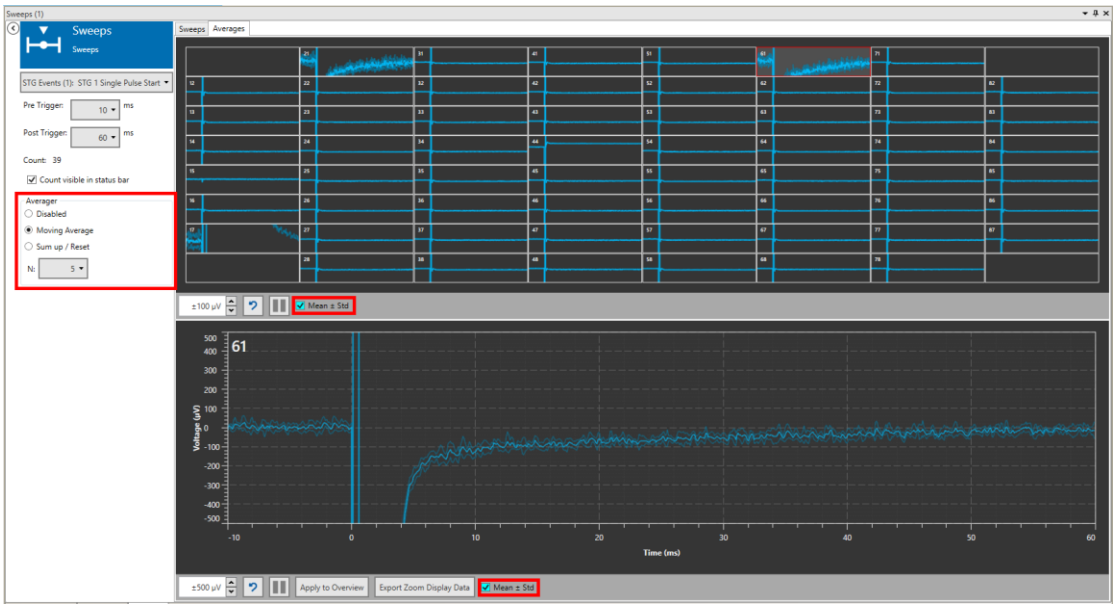
The Sweeps tool has two tabs, Sweeps and Averages, corresponding to the two output ports. The **Sweeps tab** shows the selected data segment around each trigger event for all channels. The maximum size of a sweep can be selected in a range from **50 ms before till 1000 ms** after the trigger. The **trigger signal** which should be used as reference point can be selected from a drop down menu. The Stimulator tool for example will provide different Trigger options, while the Trigger Generator will provide only one.



The time base (time 0) is always the selected trigger event. The number of sweeps generated since start of the data acquisition is counted, optionally also in the status bar.

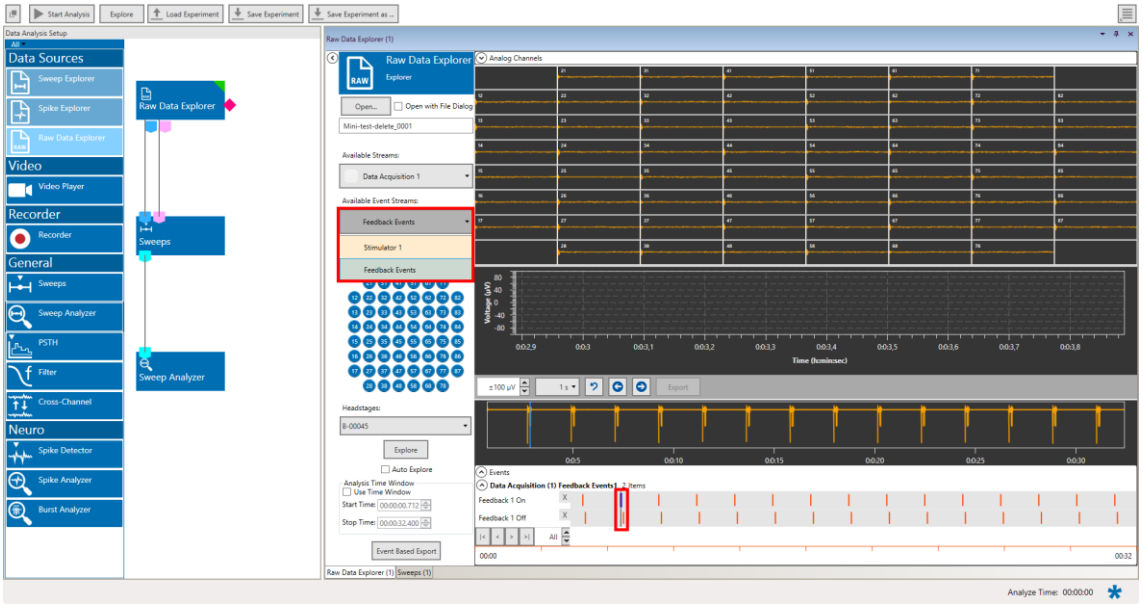
The **Averages tab** allows to automatically average a number of sweeps (N), which can be selected between 2 and 20 from a drop down menu. The result is shown just as an averaged trace, or as Mean +/- SD (standard deviation). Two options are available for the average function, **Moving Average** or **Sum up / Reset.**
Moving Average means that the selected number of sweeps will be averaged and shown. If more sweeps come in, then always the latest sweep will be added to the average, and the oldest one will be removed instead. If for example N is five, then the first average will be from sweeps 1 - 5, the second one from 2 - 6, then 3 - 7 and so on.

In Sum up / Reset mode, N sweeps are averaged, then the averaging starts new with sweep N+1. Again, with an N of five, the first average will be from sweeps 1 - 5, the second one from 6 - 10, then 11 - 15 and so on.

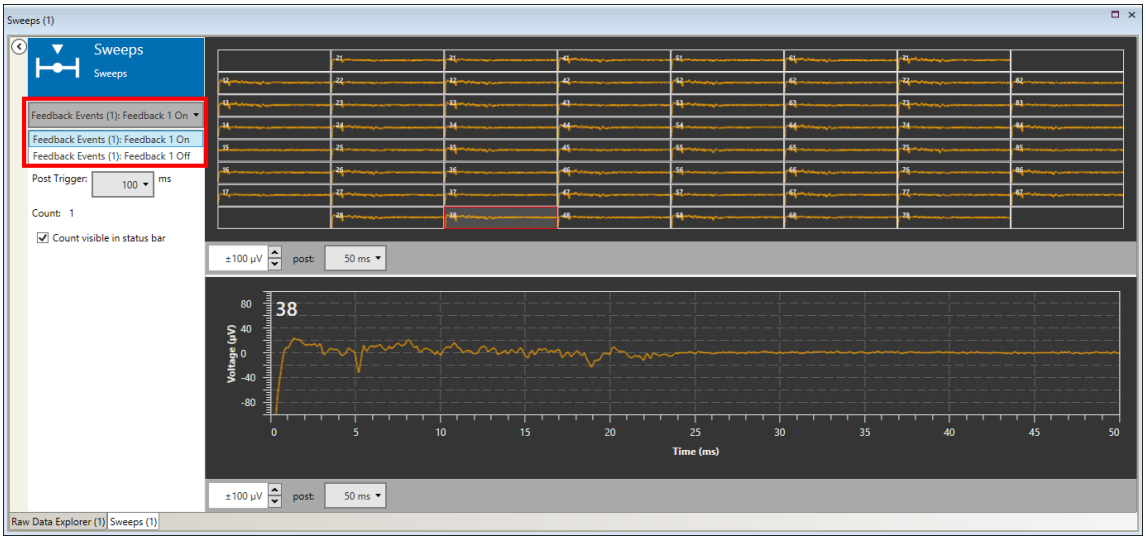


5.7.4 Example: How to Extract Sweeps from a Raw Data File

If a raw data file also contains an event (Trigger) data stream, the Sweeps tool can be used to extract and analyze activity in relation to the trigger events. Open the respective file and connect the Raw Data and Trigger port of the Raw Data Explorer with the Sweeps tool. A sweep Analyzer can be added for analysis, see next chapter, **5.8**, Sweep Analyzer.



If more than one Trigger data stream is present, select one from the drop down menu Make sure the position in the file is around a trigger before using Explore, or the Sweep window will be empty. If the selected Trigger stream contains more than one type of event, you can define the exact event to be used as trigger source in the Sweeps tool.



Sweeps extracted that way can also be recorded, to generate a new file containing only the sweeps, see chapter **4.7** Re-recording Data.

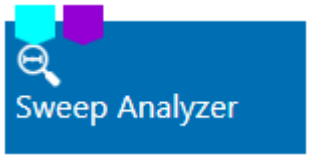
5.8 Sweep Analyzer

5.8.1 Description and Purpose

The Sweep Analyzer instrument can analyze the sweep data replayed by the Sweep Explorer. The Sweep Analyzer is most often used to analyze the response to an **electrical or optical stimulation**, where the trigger marks the time stamp of stimulation.

5.8.2 Data Ports and Export Options

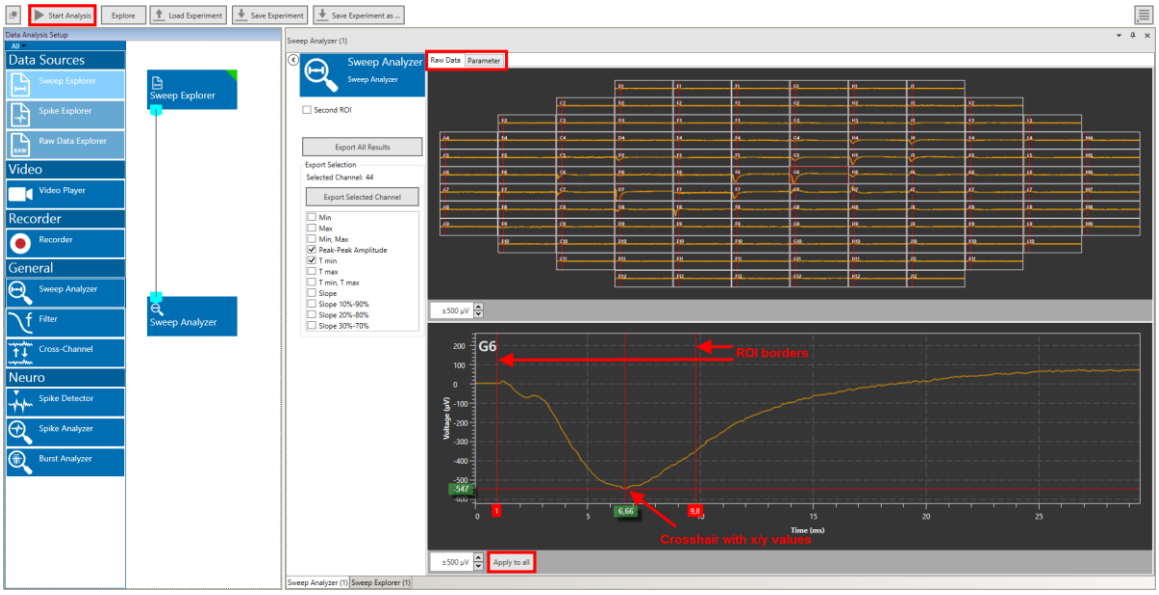
The Sweep Analyzer has a cyan sweep data input, a purple averages input and no outputs. Only **one of the inputs** can be connected at a time. The results of the analysis can be extracted directly as **ASCII data**.



5.8.3 Operation

5.8.3.1 ROI Definition

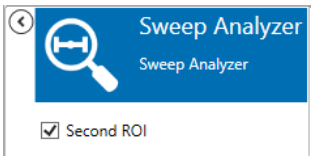
The Sweep Analyzer has two tabs, Raw Data and Parameter. The **Raw Data** tab shows the sweeps send from the Sweep Explorer with the **Explore** function and allows to define a **region of interest (ROI)** for the analysis. To define a ROI, click on any channel. The selected channel will show up on the Zoom Display below, with the borders of the ROI. Drag the ROI borders to the desired position. This procedure can be repeated for as each channel individually, or the ROI settings of the selected channel can be **applied to all** others. It is possible to apply a ROI to all and modify it afterwards individually for a few selected channels. For example, the analysis of an EPSP requires different ROI settings than the analysis of a population spike.



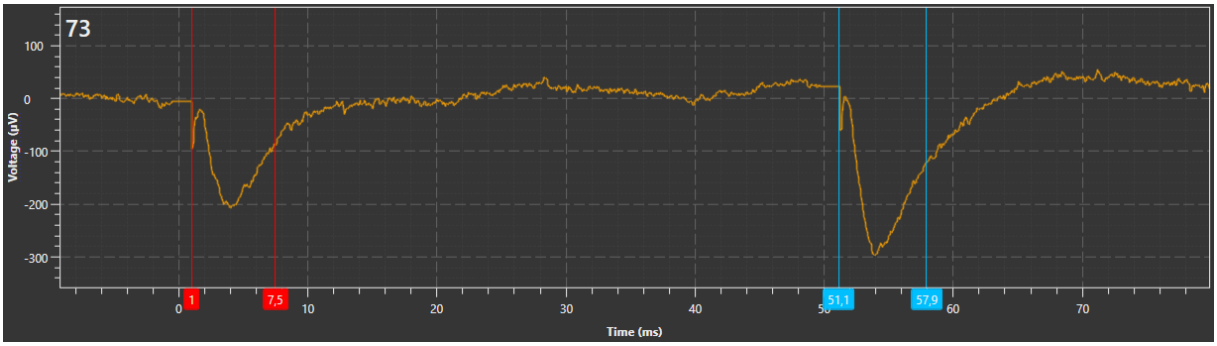
A cross hair is available, which can show x and y values of any specific point in the plot. Once the ROIs are set for all relevant channels, the **Start Analysis** function can start the processing of all sweeps in a file.

5.8.3.2 Paired Pulse Analysis

If a sweep contains two signals, usually as the result of a paired pulse stimulation, a **second ROI** can be activated.

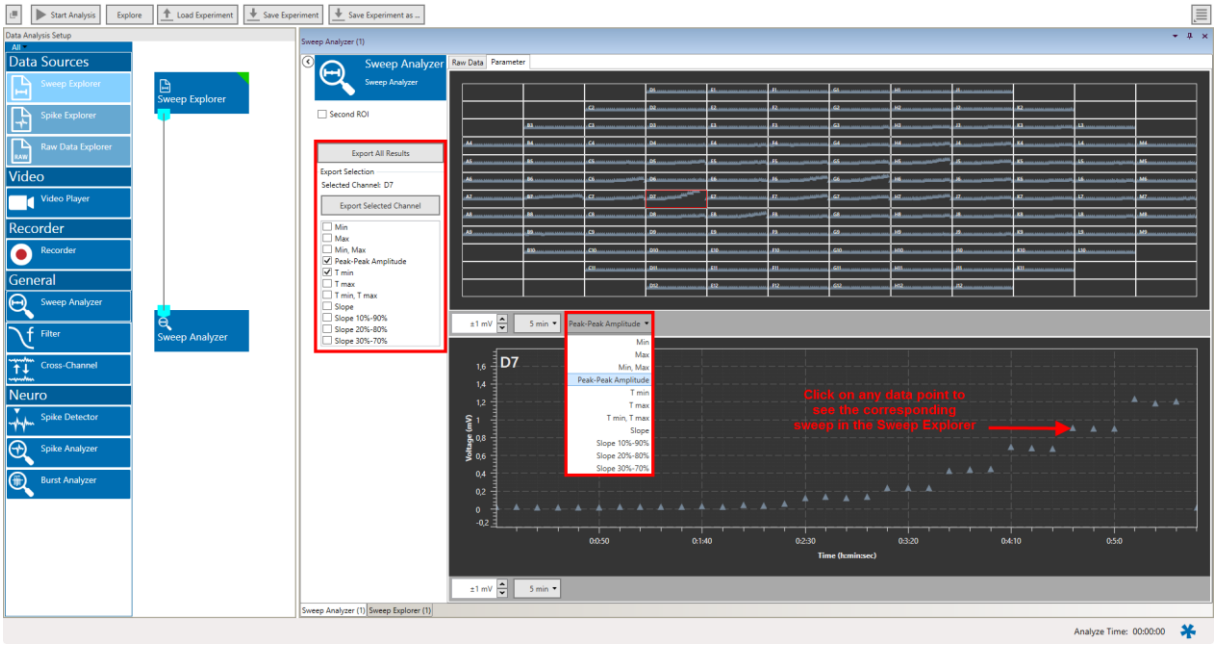


Both ROIs can be changed independently. The analyzed parameters of the second ROI are displayed as ratio relative to the first ROI.



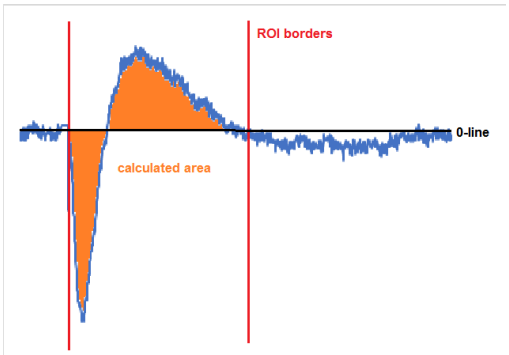
5.8.3.3 Parameter Display and Extraction

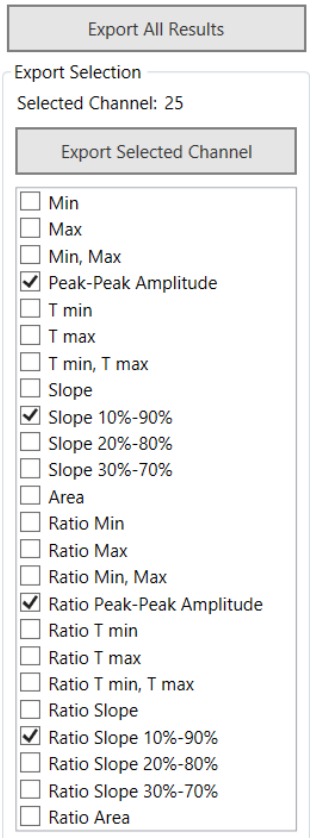
Within the ROIs, a number of parameters will be calculated: Minimum, Maximum, Peak to Peak Amplitude, Time of Min and Max, and Slopes. The analyzed parameters are displayed in the **Parameter** tab. If a second ROI is active, for each parameter also the **ratios** between ROI one and two are available.



Select the desired parameter to display from the drop down menu. All parameters are analyzed simultaneously, so it's possible to switch back and forth between different parameters online. A **click on any data point** in the parameter display will show the corresponding **raw data sweep** in the Sweep Explorer.

The parameters are defined as follows:

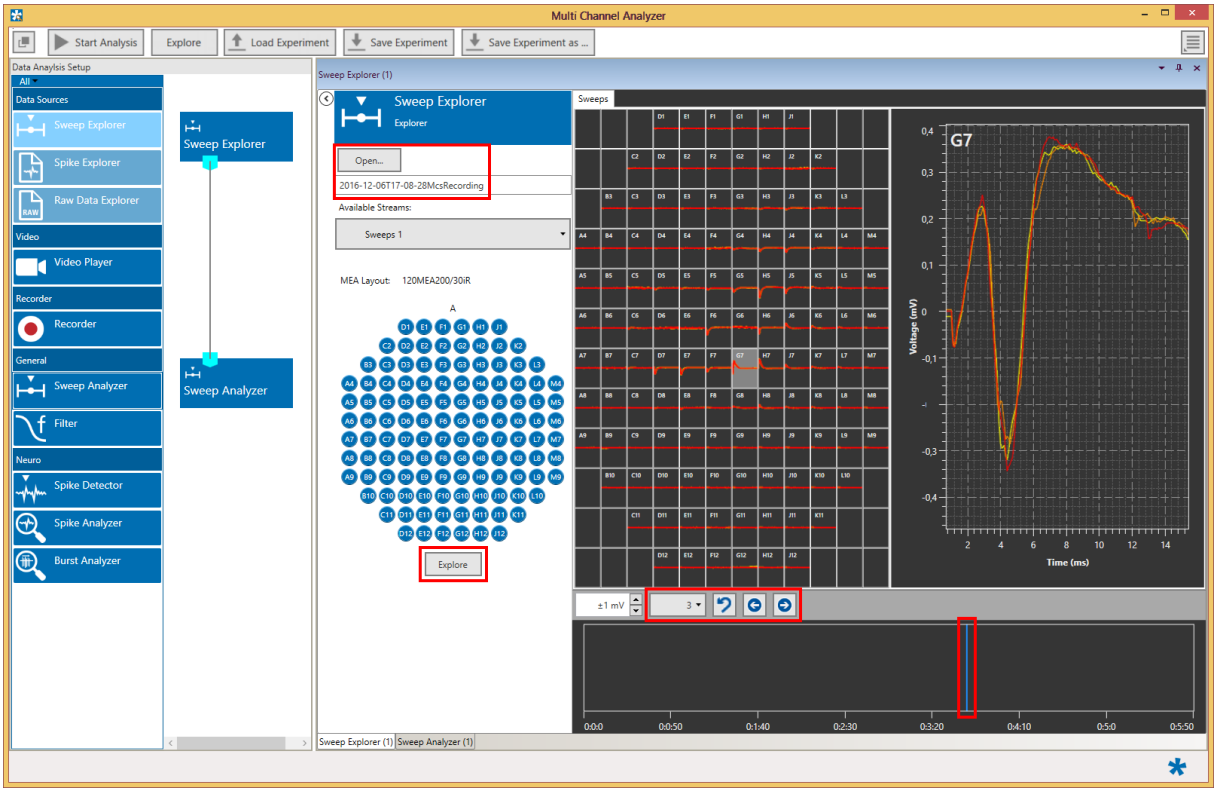
Max	Highest value in ROI
Min	Lowest value in ROI
T max	Point in time when maximum occurs, relative to trigger event
T min	Point in time when minimum occurs, relative to trigger event
Peak to Peak Amplitude	Amplitude, maximum minus minimum
Slope	Slope of linear fit regression line (Least Square Algorithm): A straight line is fitted through the data points in the region of interest. The slope of the straight line is then extracted.
Slope X % to X %	The 10 % – 90 % (or 20/80 or 30/70) interval of the peak-peak amplitude (stretching from minimum to maximum) in the region of interest is detected. Only data points in this interval are used for the linear regression fit (Least Square Algorithm). The slope of the resulting straight line is extracted as the slope.
Area	 Within the ROI, absolute value of the area under the curve is calculated.
Ratio	For each parameter, the ratio parameter of ROI 2 divided by the parameter of ROI 1



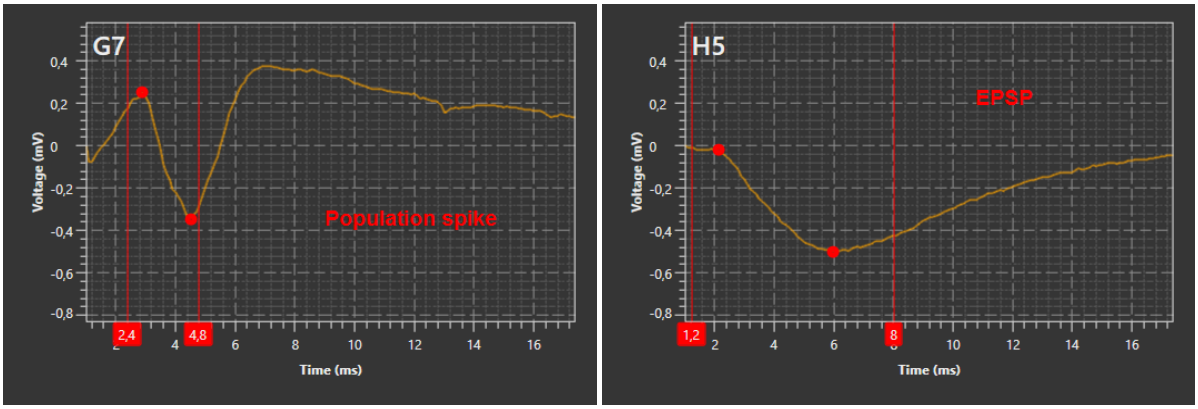
All analyzed parameters can be exported as ASCII data. The **Export All Results** button will extract all parameters from all channels as one ASCII (*.txt) file. Alternatively, only the parameters selected in the parameter list from **one selected channel** can be exported. The selected channel is the one also shown in the zoom display.

5.8.4 Example: How to Analyze EPSPs and Population Spikes from a Hippocampal Slice

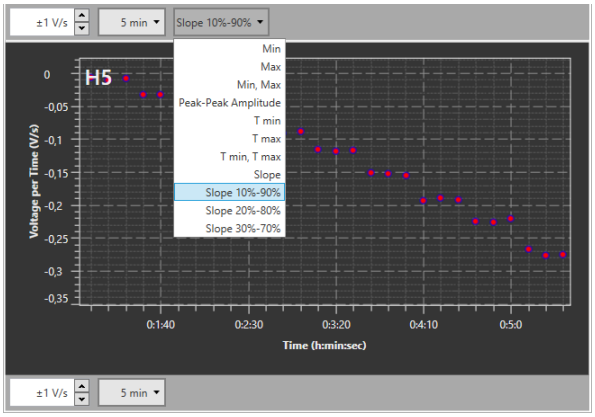
This example illustrates the most common use of the Sweeps Explorer and Sweep Analyzer instrument, the recording and analysis of evoked activity. The displayed file contains sweeps recorded from a hippocampal slice in response to electrical stimulation with increasing stimulation strength. Three pulses were applied for each stimulation amplitude, from 500 mV to 300 mV, in steps of 250 mV.



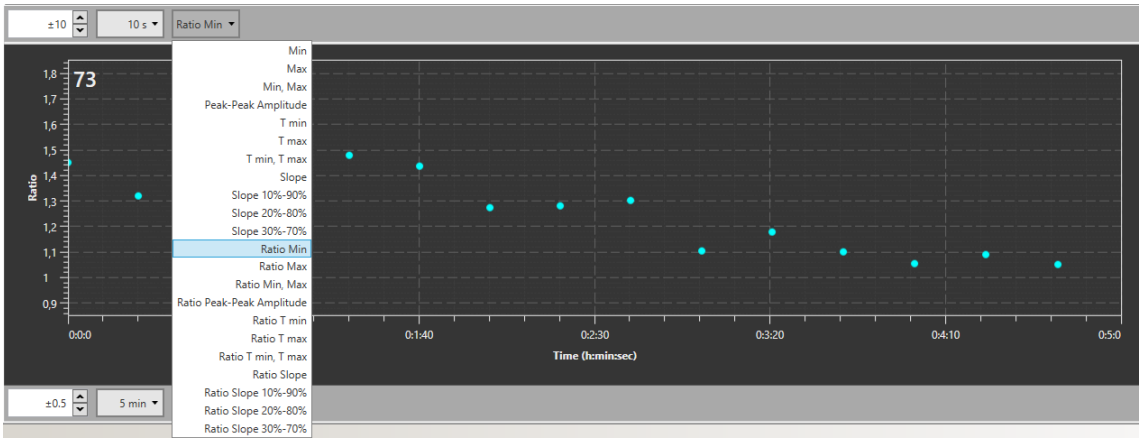
Open the file and display the sweeps in steps of three, as every three sweeps correspond to one stimulation strength. This will show whether the response to the same stimulation is constant or not. The blue slider in the lower display shows the position of the visible data along the time axis of the file. Select representative traces and send the data to the Sweep Analyzer with the Explore function.



In the Raw Data tab of the Sweep Explorer, adjust the ROIs on the channels showing EPSPs and population spikes to cover the minimum and maximum of the respective waveform. Start the Analysis for the whole file. All available parameters will be analyzed, select the parameter to display from the drop down menu in the Parameter tab. Adjust the scaling of the x- and y-axis to show the full file length and all data points. You can change the displayed parameter back and forth. The Export function will export either all results or only selected parameters as ASCII (*.txt) file.



If paired pulse stimulation is used, the second ROI needs to be activated, to be able to analyze also the ratio of each parameter between ROI one and two.



5.9 Filter

5.9.1 Description and Purpose

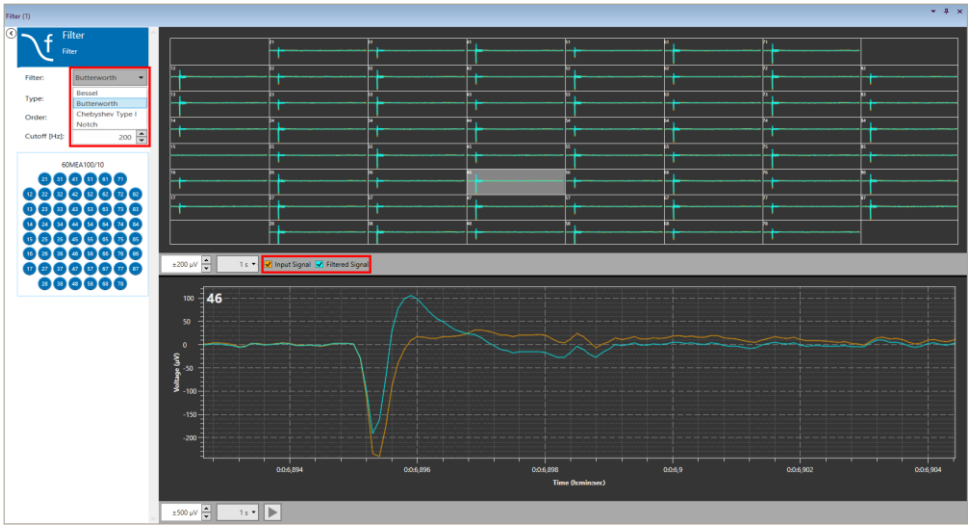
The Filter can apply time based filters with different characteristics to the raw data. More than one Filter can be used in one experiment, the filters can be used in series or in parallel. Please also see movie for illustration.

5.9.2 Data Ports and Export Options

The Filter has a blue Electrode Raw Data input and a similar blue Filter Data output. The input data can be directly from the Raw Data Explorer, or from another instrument with a blue output. Likewise, the Filter Data output is compatible with any other blue input port. There are no direct export options.

5.9.3 Operation

The Filter shows raw data and filtered data for all channels in overlay. Both traces can be toggled with tick boxes. Different filter characteristics are available, Bessel, Butterworth, Chebyshev and Notch.



5.9.3.1 Filter Characteristics

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

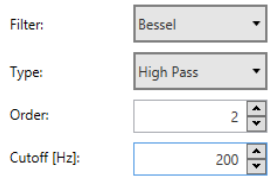
The **Butterworth** filter is designed to have a frequency response which is as flat as mathematically possible in the passband.

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

A **Notch** filter is designed to remove a certain frequency. 50 Hz or 60 Hz are available, corresponding to the power net frequencies (Americas 60Hz, Europe, Asia, Australia 50Hz).

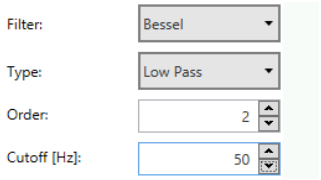
5.9.3.2 High Pass

A High Pass filter will remove all frequencies below the Cutoff. For High Pass filters, just select the Order and the Cutoff frequency from the respective drop down menus.



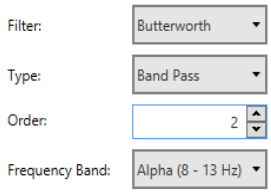
5.9.3.3 Low Pass

A Low Pass filter will remove all frequencies above the Cutoff. For Low Pass filters, also select the Order and the Cutoff frequency from the respective drop down menus.



5.9.3.4 Band Pass

Band Pass filters remove all frequencies outside the selected frequency band. Fixed Band Pass options are available. Select Alpha, Beta, Gamma or Theta band from the drop down menu.



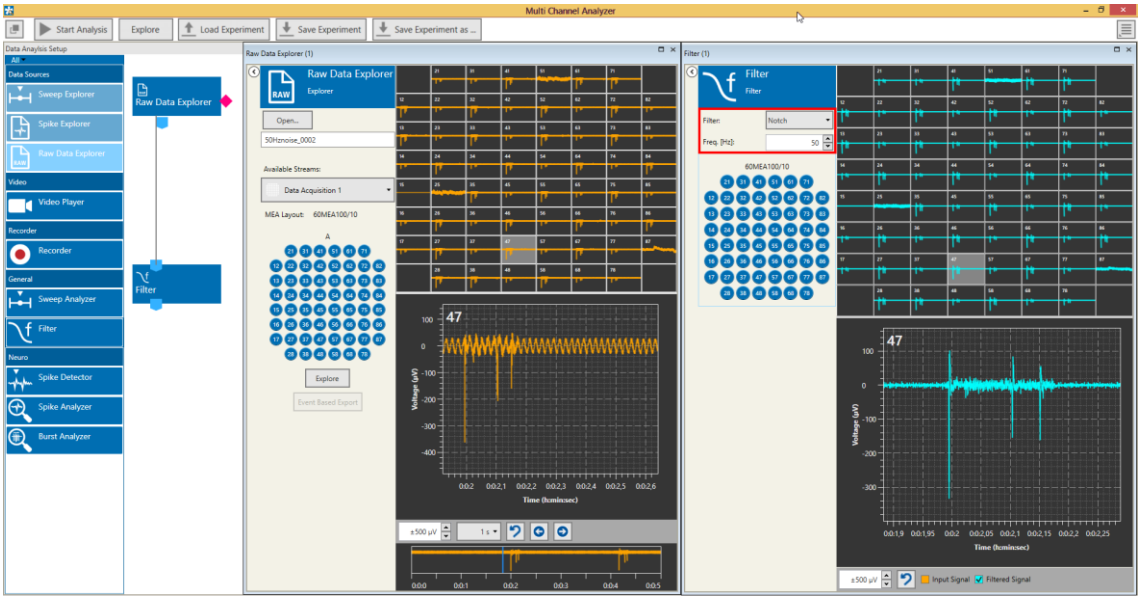
5.9.4 Examples: How to …

This section highlights a few common uses of the Filter.

5.9.4.1 Differentiate Theta Waves and Spike Activity from a Raw Data Signal

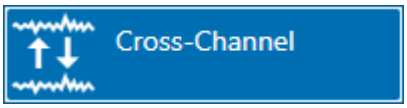
In the example above, Theta activity is extracted with a Band Pass filter and recorded as a separate data stream. A 200 Hz High pass filter is used to facilitate spike detection by a Spike Detector (see chapter **5.11**, Spike Detector), and the detected spikes are also recorded. The Theta filter control window shows only the filtered data, the High Pass filtered data is shown in overlay with the raw data. The newly generated file will contain spike waveforms, time stamps and Theta frequency filtered raw data.

5.9.4.2 Remove 50 Hz Noise



If problems with 50 Hz noise occur, the best option is to find the source of the noise. However, if data contaminated with 50 Hz noise was recorded, a Notch filter can be used to remove the noise. Periodic noise is very often caused by other electronic instruments. Europe/Asia/Australia usually has an AC frequency of 50 Hz, while in the Americas it is 60 Hz. Adjust the filter accordingly.

5.10 Cross-Channel



5.10.1 Description and Purpose

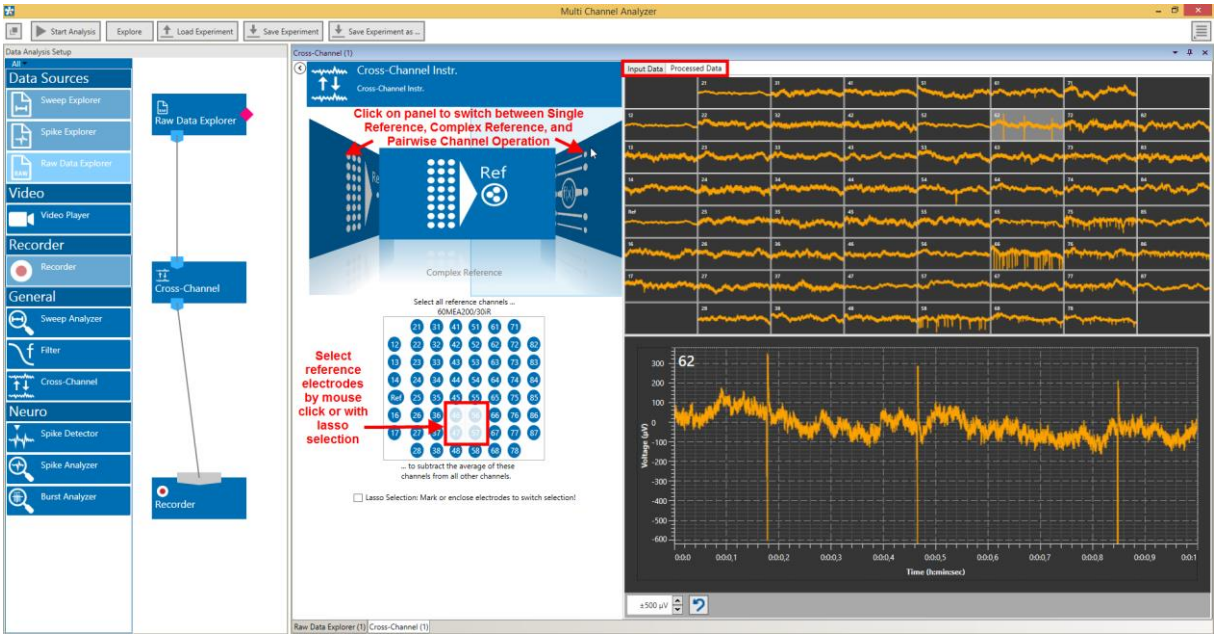
The Cross-Channel instrument can do calculations **between data channels**, for example to remove common noise. More than one Cross-Channel can be used in one experiment, and more than one instrument can be connected to a Cross-Channel data output.

5.10.2 Data Ports and Export Options

The Cross-Channel Instrument has a blue Electrode Raw Data input and a similar blue Cross-Channel Data output. The input data can be directly from the Raw Data Explorer, or from another instrument with a blue output, like a Filter. Likewise, the Cross-Channel Data output is **compatible with any other blue input port**. There are no direct export options.

5.10.3 Operation

The Cross-Channel has a tab which shows the input data, and one which shows the processed data. Different options are available, Simple Reference, Complex Reference, and Pairwise Channel Operation.



5.10.3.1 Simple Reference

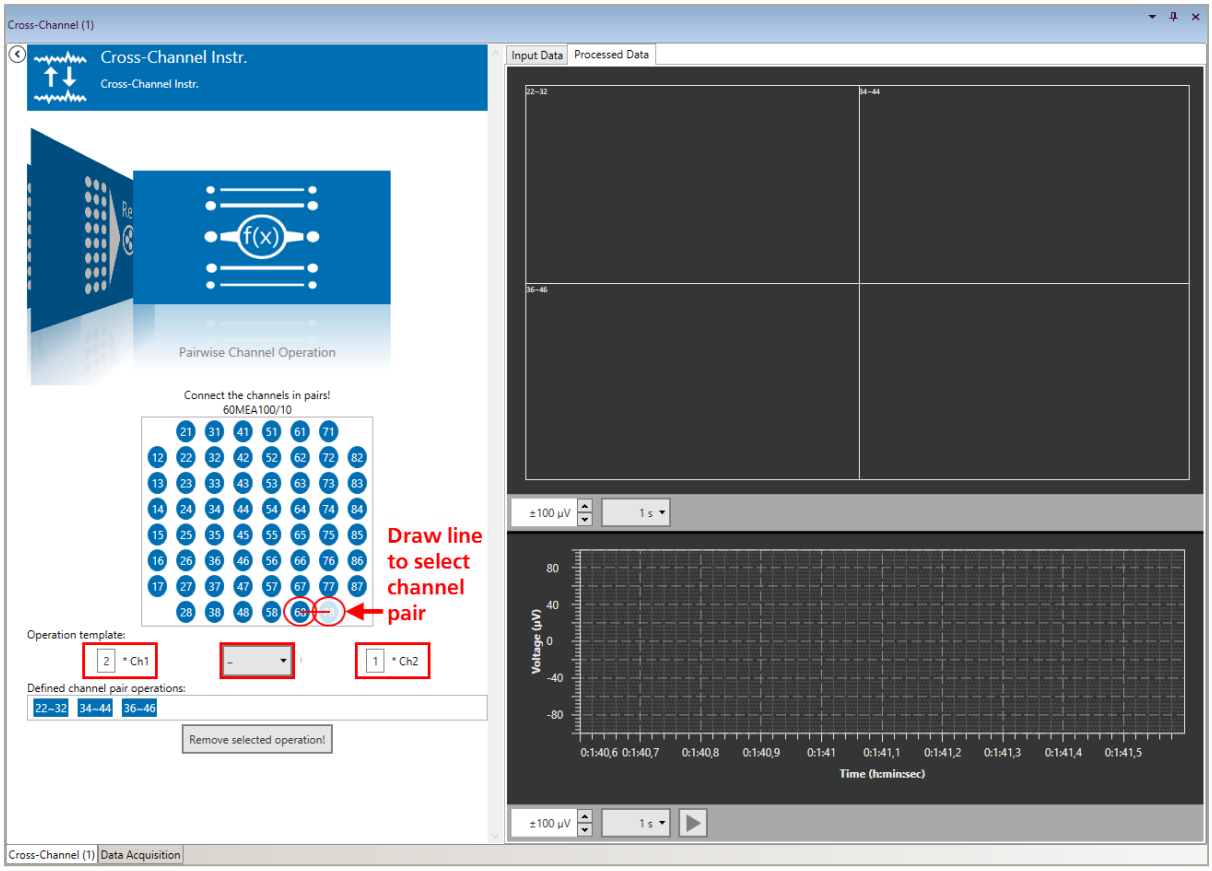
In Simple Reference mode, the signal **one selected reference** channel is subtracted from all others. The number of output channels in the Cross-Channel Data output is identical to the number of input channels.

5.10.3.2 Complex Reference

In Complex Reference mode, the signal of **all selected reference channels is averaged**, and then subtracted from all others. The number of output channels in the Cross-Channel Data output is identical to the number of input channels.

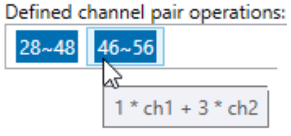
5.10.3.3 Pairwise Channel Operation

In Pairwise Channel Operation mode, pairs of channels can be selected by drawing a line between them, and a **new signal is calculated** from the signals of each of these channel pairs.



Channels can be added, or subtracted from each other. Each channel signal can be multiplied with an individual factor before the operation. The number of output channels in the Cross-Channel Data output is identical to the number of channel pairs.

The operation can be set **individually for each pair of electrodes**. The operation for each pair can be seen in a tooltip. First select the operation, then connect the electrode pair.



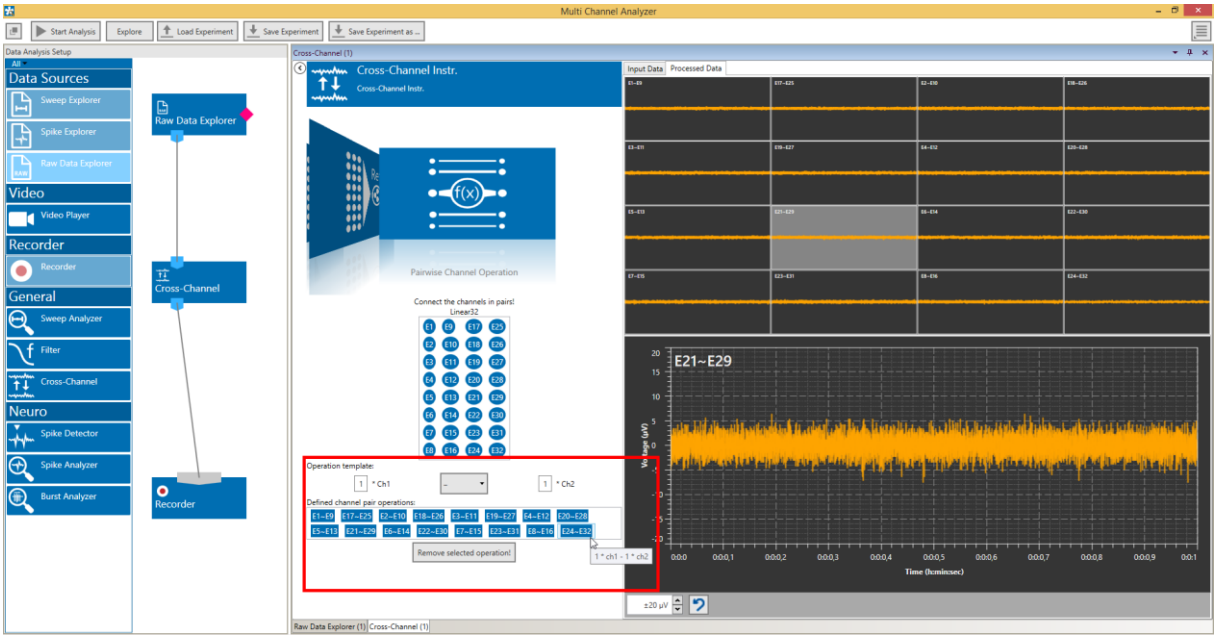
5.10.4 Examples: How to …

This section highlights a few common uses of the Cross-Channel Tool.

5.10.4.1 Remove Common Artefacts from a Wireless Recording

In the example above, a single electrode is selected as Simple Reference, which shows common artefacts as all other channels (breathing, heartbeat), but no actual signals. In the processed data, the common noise is removed. The referenced signal will be recorded as new file.

5.10.4.2 Do Differential Measurements between Pairs of Electrodes



In the example above, sixteen pairs of electrodes are selected out of 32 input channels and subtracted from each other, to generate 16 differential signals as Cross-Channel Data output. The differential signal is recorded as a new file.

5.11 Spike Detector

5.11.1 Description and Purpose

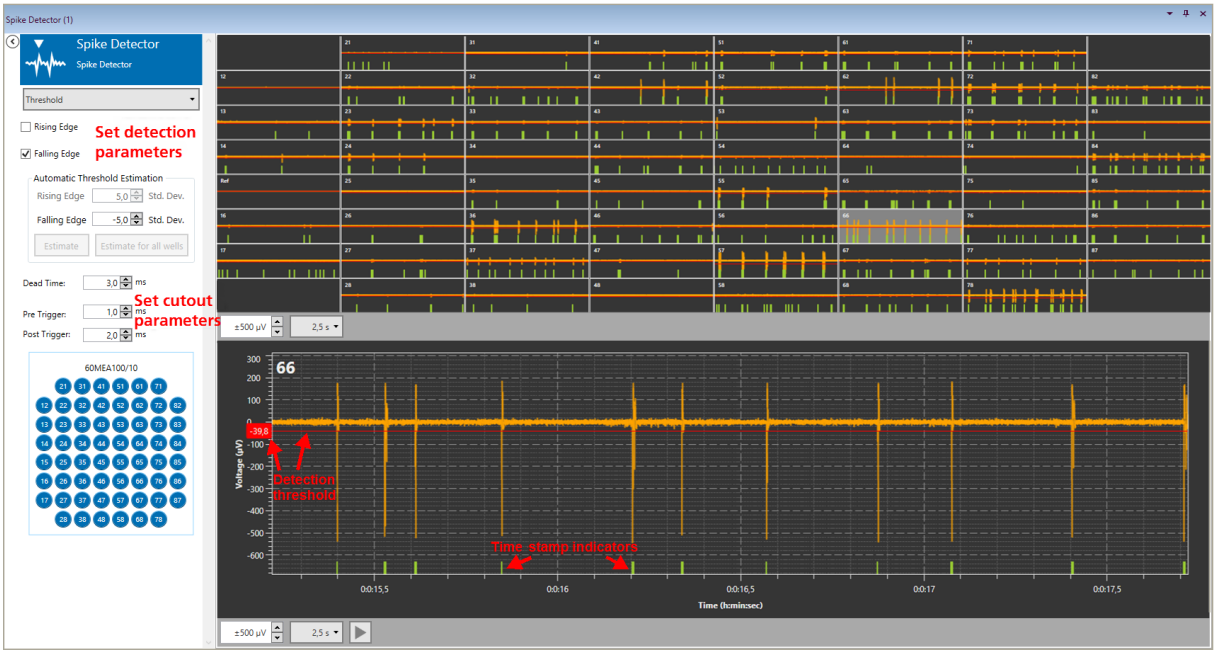
The Spike Detector can extract spikes from a raw data stream by different methods, and generates time stamps and cut outs with spike waveforms. Please also see movie for illustration.

5.11.2 Data Ports and Export Options

The Spike Detector has a blue Electrode Raw Data input and orange Spike Data and yellow Spike Time Stamps output. The input data can be directly from the Raw Data Explorer, or from another instrument with a blue output, like a Filter. There are no direct export options.

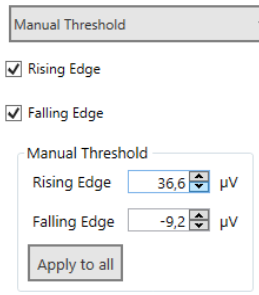
5.11.3 Operation

The Spike Detector shows the input data and indicates the **time stamps**. Spikes can be detected by different methods, Threshold, Manual Threshold and Slope. Within each method, the detection threshold of each individual channel can be **manually adjusted**. Click on the respective channel to open it in the Zoom window. Drag the detection threshold to the desired position. The threshold value is shown with an accuracy of 0.1 µV.



5.11.3.1 Detection by Manual Threshold

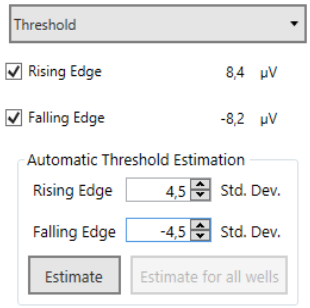
If Manual Threshold is selected from the drop down menu, the user needs to either set the detection threshold manually for all channels, or apply an identical threshold to all. Threshold values can either be changed with the updown box, or the threshold can be dragged with the mouse in the Zoom window. Thresholds can be positive, negative, or both. The **Apply to all** button will set the current value of the zoomed channel for all channels.



5.11.3.2 Detection by Threshold

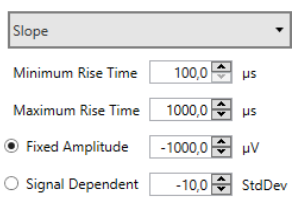
If Threshold is selected from the drop down menu, the thresholds are calculated individually for each channel by multiplying a user defined factor with the standard deviation (SD) of the noise on each specific channel. The SD is calculated from 500 ms of data every time the **Estimate** button is pressed. The **Estimate for all wells** button become active if a multi well layout is selected. Thresholds can be positive, negative, or both.

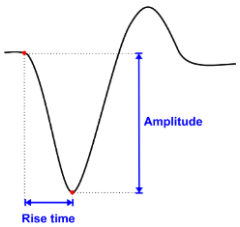
The Threshold detection takes into account small differences in noise level between the channels, and is usually superior to a manually selected threshold value The SD is strongly depending on the 500 ms of data used for calculation. If a lot of spiking activity occurs within these 500 ms, the SD will increase, and also the detection threshold. If possible, hit the Estimate button during a period without activity.



5.11.3.3 Detection by Slope

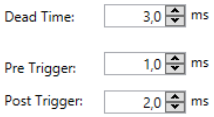
Spikes can also be detected using a combination of minimum and maximum **slope**, and a minimum **amplitude**. This method is sometimes more effective in detecting small signals close to the noise, without risking too many false detections caused by random peaks. Amplitudes can be fixed, or dependent on the standard deviation of the noise on each channel (see above).





5.11.3.4 Spike Cutouts

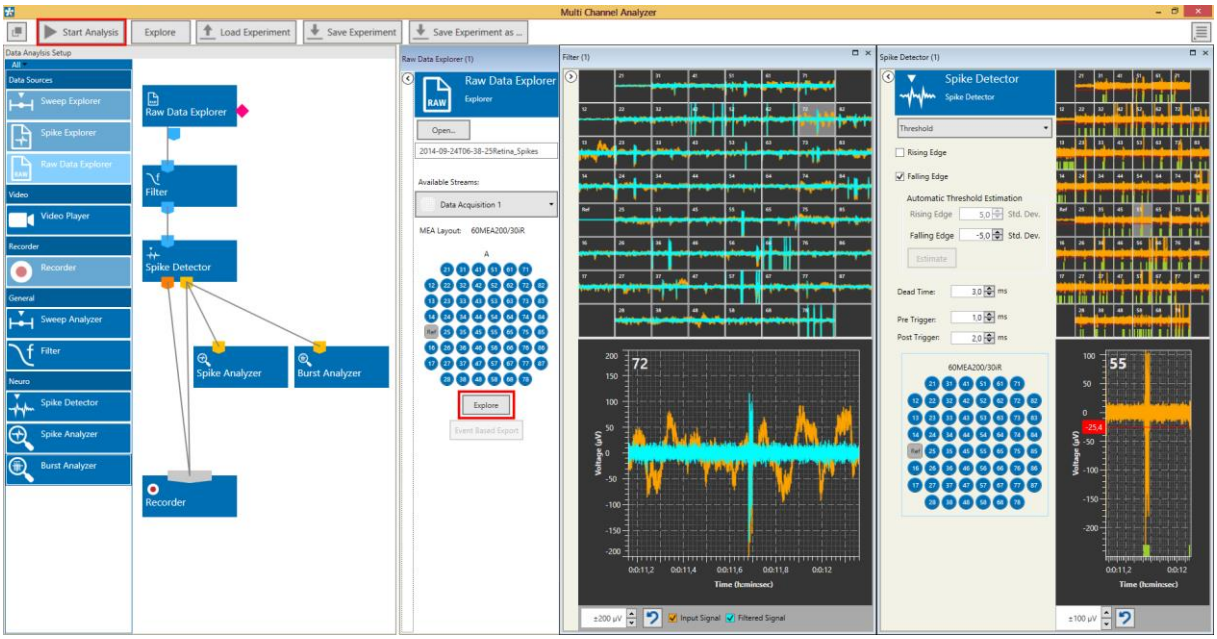
The Spike Data stream consist of cutouts, short segments of data around each detection time stamp which contain the spike waveform. The **length of this cutout** in relation to the detection time stamp (Trigger) can be adjusted. The Dead Time is the time after each detection time stamp after which a second detection event will be ignored. The Dead Time should be as long as the expected signal length, to avoid double detections.



5.11.4 Example: How to Extract Spikes from a Raw Data File

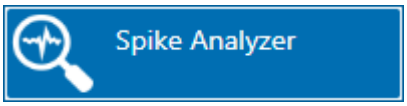
The sole purpose of the Spike Detector is to extract spikes from a raw data signal. The instrument works identical **online** (Experimenter) or **offline** (Analyzer). Usually it helps to improve spike detection if the data is filtered before the spike detection. If the raw data signal contains low frequency components, the baseline **drifts through the detection threshold** and leads to false detections. High pass filters with cut off frequencies between 100 and 300 Hz are commonly used to remove the low frequency components. Similarly, contamination with 50 Hz noise can be eliminated with a notch filter before applying the spike detection.

The resulting spike data and spike time stamps can be re-recorded with a Recorder, to be exported to a 3rd party software for further analysis with the Multi Channel Data Manager (see chapter 4.7, Re-recording Files). It can also be analyzed straight away with the Spike Analyzer and/or Burst Analyzer or PSTH instruments (see chapters 5.12, 5.13, 5.14).



Open the raw data file and navigate to an area with low activity. Send data fragment to the Filter and Spike Detector with **Explore**. Adjust the detection threshold, manually or with "Estimate". Hit Explore again, to see the adjusted thresholds. **Start Analysis** to analyze the whole file with the given thresholds. In the example above, a new file with spike data and time stamps will be generated by the recorder.

5.12 Spike Analyzer

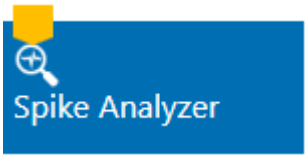


5.12.1 Description and Purpose

The Spike Analyzer can analyze spike time stamps. Analysis of spike numbers and frequencies can be event based, or in discrete time bins.

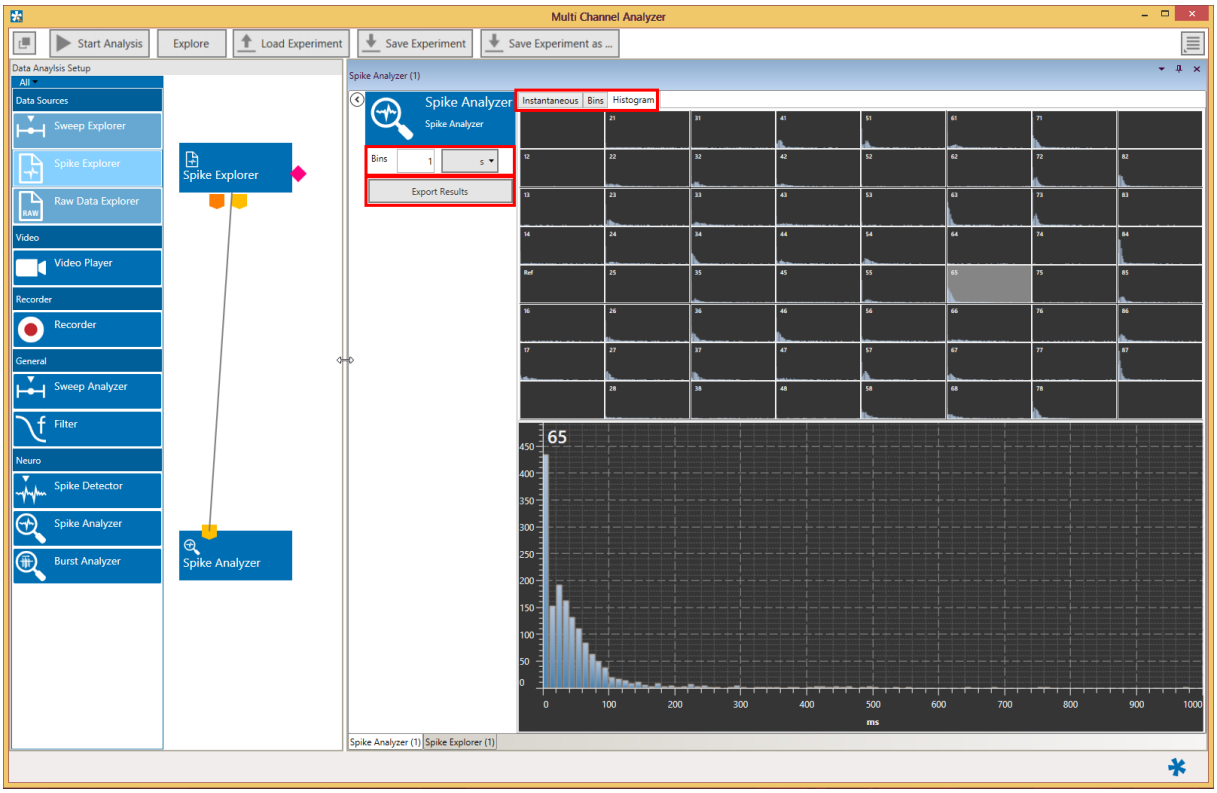
5.12.2 Data Ports and Export Options

The Spike Analyzer has a yellow Spike Time Stamps input only. The input data can either be directly from a Spike Explorer, or extracted from a raw data stream offline by the Spike Detector. Analysis results can be exported in ASCII format.



5.12.3 Operation

The Spike Analyzer analyzes the inter spike intervalls (ISI) between the time stamps and generates ISI, number and frequency plots, as well as ISI histograms. Spike frequencies are either generated event based (Instantaneous) or for discrete time bins. All analysis options are excecuted simultaneously and displayed on individual tabs. All results can be exported as ASCII data.

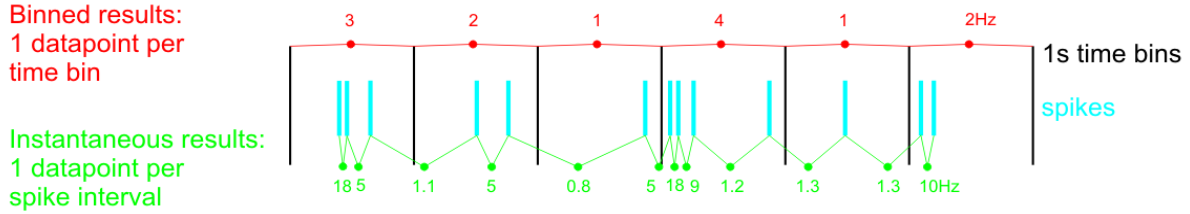


5.12.3.1 Instantaneous Versus Binned Analysis

The **Bin wise** analysis creates one data point for each time bin. Number per time bin or frequency (Hz) is available as parameters. For 1 second time bins, number and frequency are identical. For other time bins, the frequency is the averaged frequency/s. In the example shown below, the results for 3 s bins would be:

(3+2+1)/3=2.0 and (4+1+2)/3=2.3

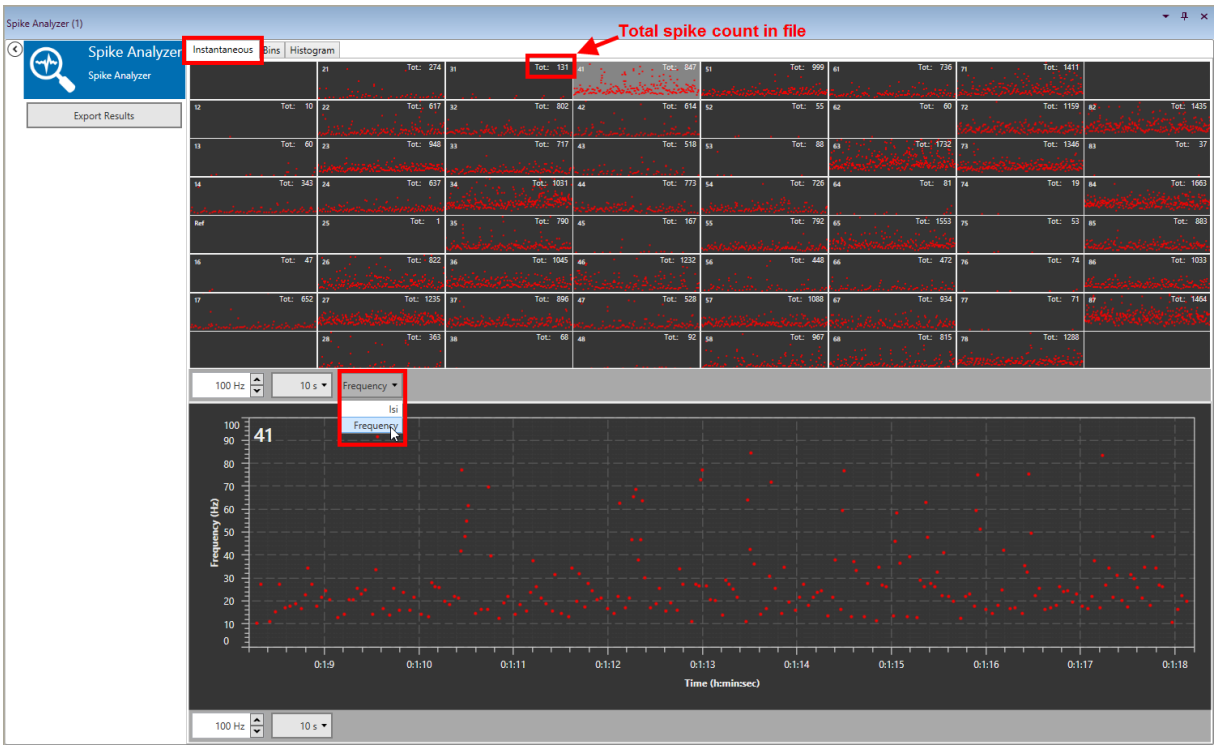
Bin wise analysis allows averaging of spike activity over longer time periods.



The **Instantaneous** (event based) analysis always works from one time stamp to the next, and creates one data point for each interval. Inter Spike Interval (ISI) and frequency are available as parameters.

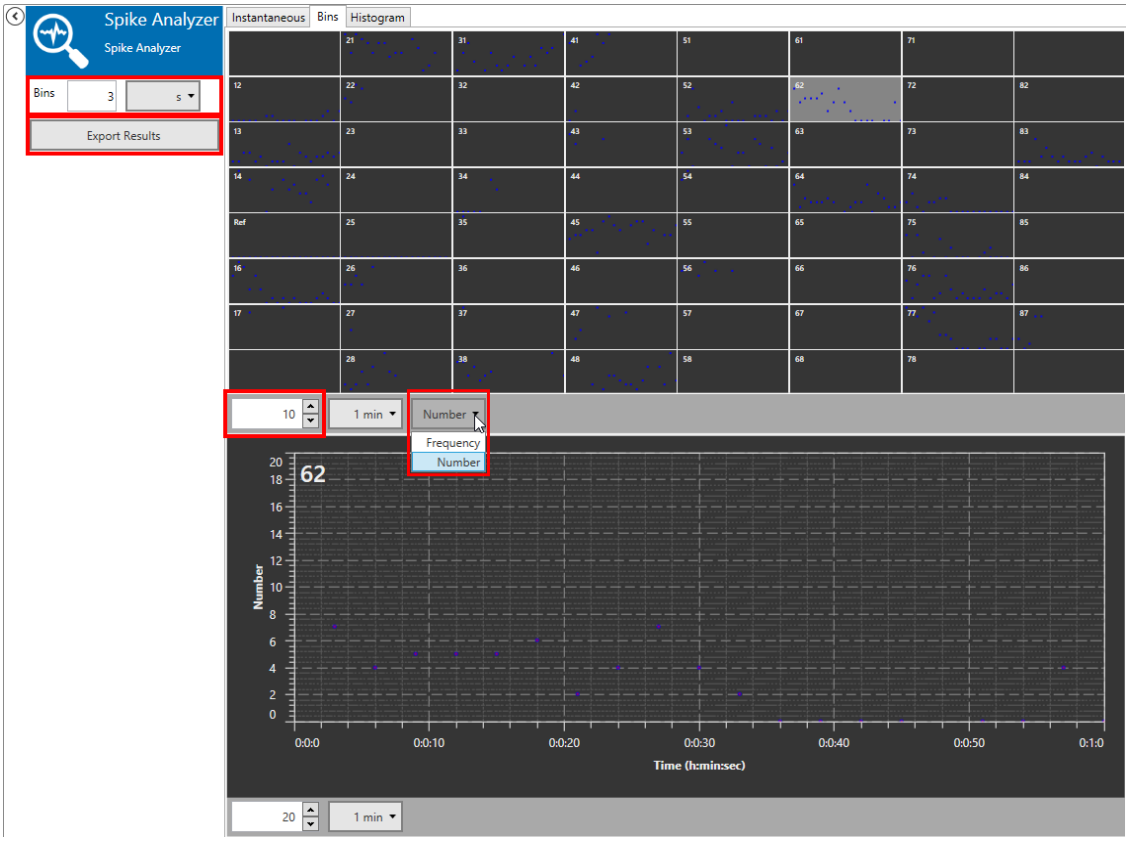
5.12.3.2 Instantaneous

The **Instantaneous analysis** generates one frequency and one ISI data point for each time interval between two detected time stamps. Both parameters are calculated, and can be selected from a drop down menu. If no data points show up at first glance, try adjusting the y-axis. The Bin length parameter has no influence on the Instantaneous results. The Export button will export all parameters for all channels. After analyzing the file, the **total spike count** for each channel is displayed.



5.12.3.3 Bins

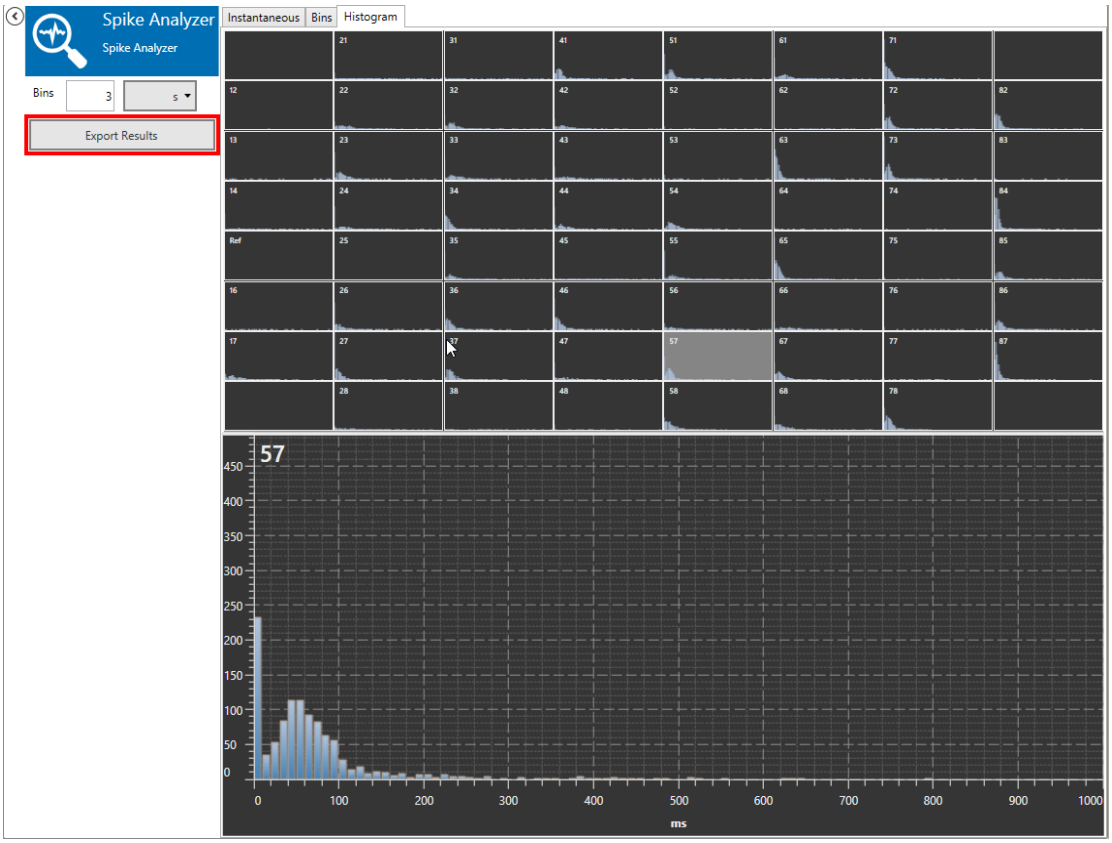
The **Bins analysis** generates one frequency and one number data point for each time bin. Number will show the number of detected time stamps per time bin, frequency will show the averaged frequency in the time bin. The length of the time bins can be adjusted. Analysis has to be restarted after changing the bin length to refresh the results. Both parameters are calculated, and can be selected from a drop down menu. If no data points show up at first glance, try adjusting the y-axis. The Export button will export all parameters for all channels.



5.12.3.4 Histogram

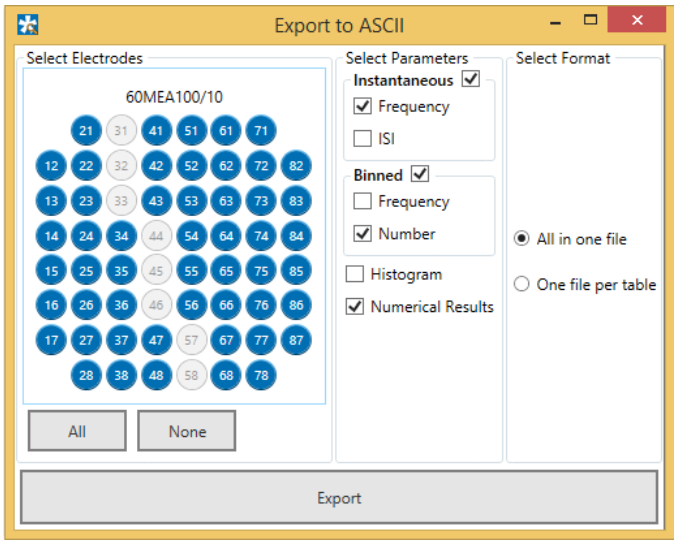
The **Histogram** will show the distribution of inter spike intervals in the file (or the analyzed segment of the file) in fixed 10 ms bins for each channel. The maximum ISI displayed is 1 s. The bin length parameter has no influence on the Histogram. The Export button will export all parameters for all channels.

In the example below, the results for the zoomed channel 57 mean that an ISI of 0 - 10 ms occurred on channel 57 around 230 times in the complete file, an ISI of 11 - 20 ms occurred around 35 times, 21 - 30 ms around 55 times, and so on.



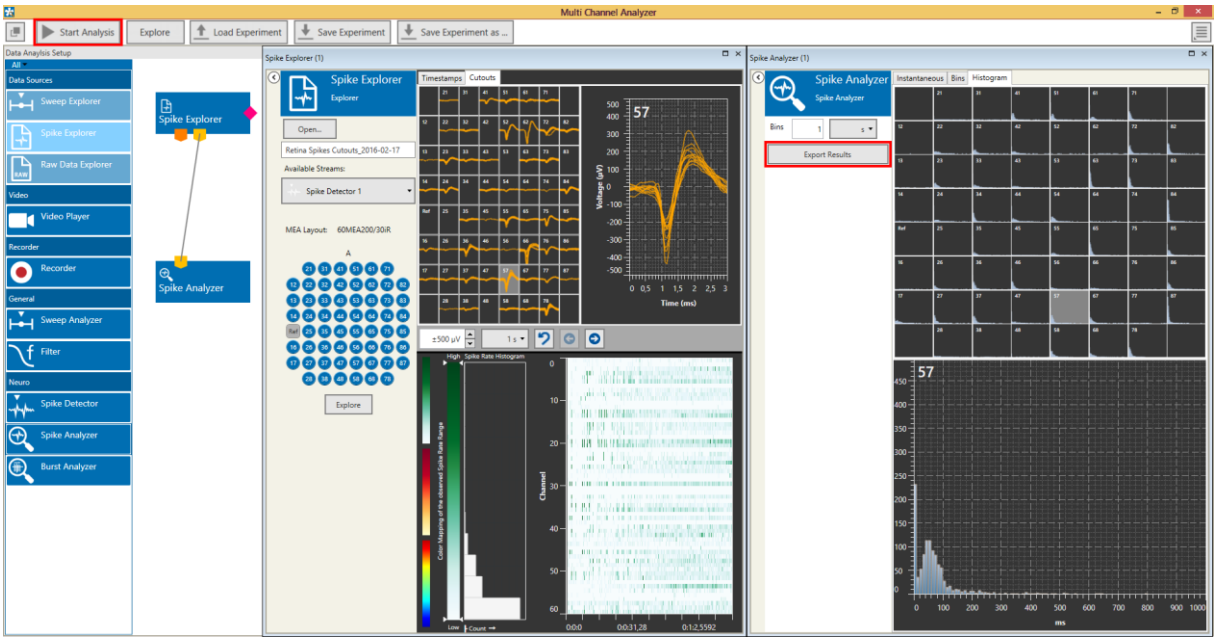
5.12.3.5 Export Results

All result data can be exported as ASCII. The export dialog offers the option to select any number of electrodes and/or parameters to be exported in a single file, or individual files per parameter.

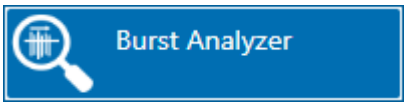


5.12.4 Example: How to Analyze Spikes

See chapter 5.11.4 for extracting the spikes from a raw data file. The spike data could come just as well from a Spike Explorer. With the **Start Analysis** function, all parameters are generated event based, and based on time bins, as described above in chapter 5.12.3, Operation of the Spike Analyzer. The **Export Results** function will produce one ASCII file with all parameter data.



5.13 Burst Analyzer

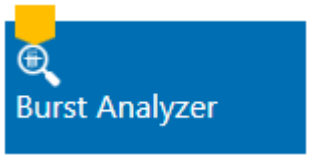


5.13.1 Description and Purpose

The Burst Analyzer can analyze spike time stamps and detect spike bursts based on the MaxInterval method.

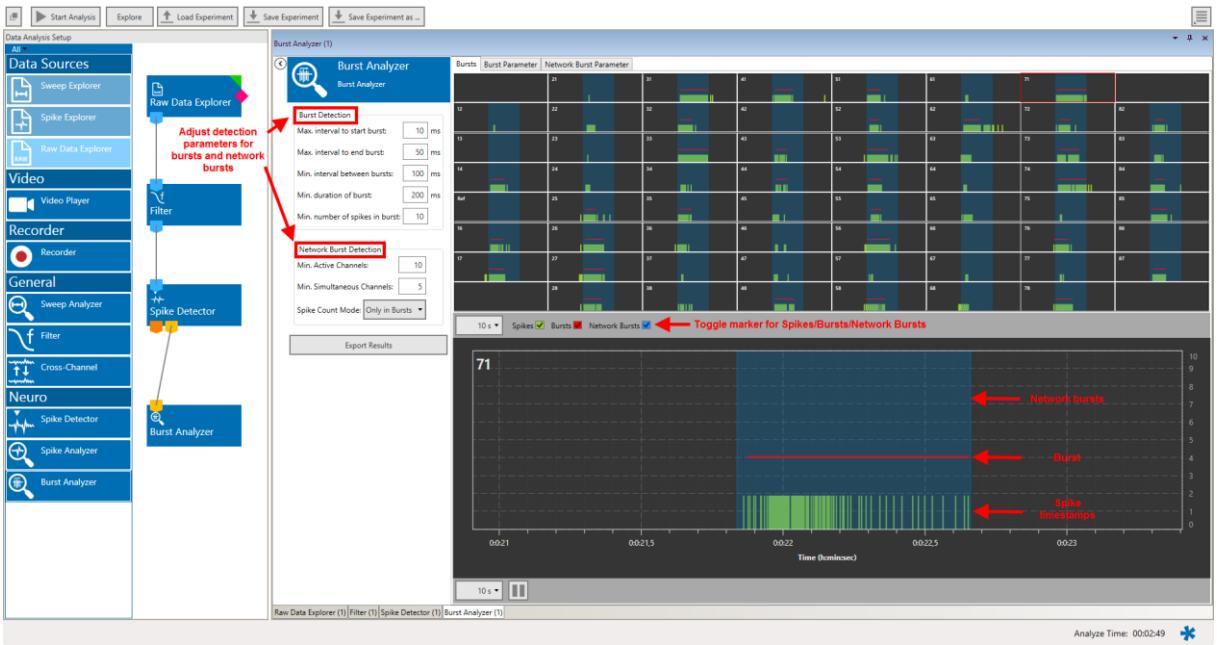
5.13.2 Data Ports and Export Options

The Burst Analyzer has a yellow Spike Time Stamps input only. The input data can either be directly from a Spike Explorer, or extracted from a raw data stream offline by the Spike Detector. Analysis results can be exported in ASCII format.



5.13.3 Operation

The Bust Analyzer analyzes the inter spike intervals (ISI) between the time stamps and applies the Max. Interval algorithm to detect **Spike Bursts** individually on all channels. All results can be exported as ASCII data. Simultaneous bursts on several electrodes can be detected as **Network Bursts**, to characterize synchronization in a neuronal network.



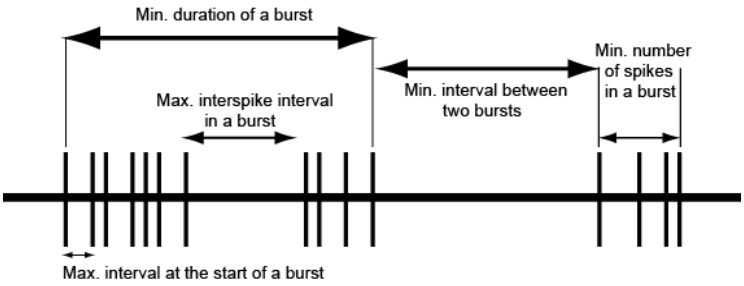
5.13.3.1 Burst Detection Parameters

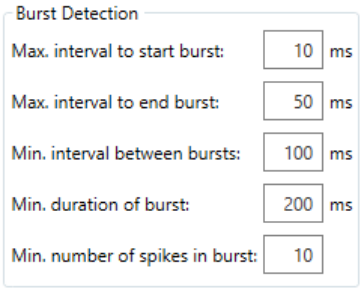
The Burst Analyzer uses the **MaxInterval Method** for detecting bursts:

- Scan the spike train until an inter spike interval is found that is less than or equal to Max. Interval.
- While the interspike intervals are less than Max. End Interval, spikes are included in the burst.
- If the inter spike interval is more than Max. End Interval, the burst ends.
- Merge all the bursts that are less than Min. Interval between Bursts apart.
- Remove the bursts that have duration less than Min. Duration of Burst or have fewer spikes than Min. Number of Spikes.

(Reference: Legendy C.R. and Salcman M. (1985): Bursts and recurrences of bursts in the spike trains of spontaneously active striate cortex neurons. J. Neurophysiology, 53 (4: 926-939)

See illustration of the parameters below:





These parameters can be adjusted in the Burst Analyzer control window. Bursts are labeled in the Bursts tab with **red lines**.

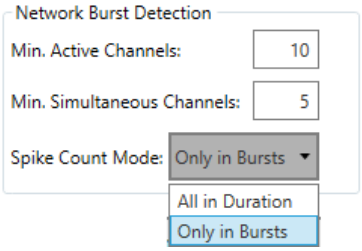
5.13.3.2 Network Burst Detection Parameters

The network burst detection algorithm identifies time regions with simultaneously occurring bursting activity on multiple channels. A Network Burst is characterized as a sequence of temporally overlapping bursts, detected by the burst detection algorithm on individual channels.

To qualify as a network burst,

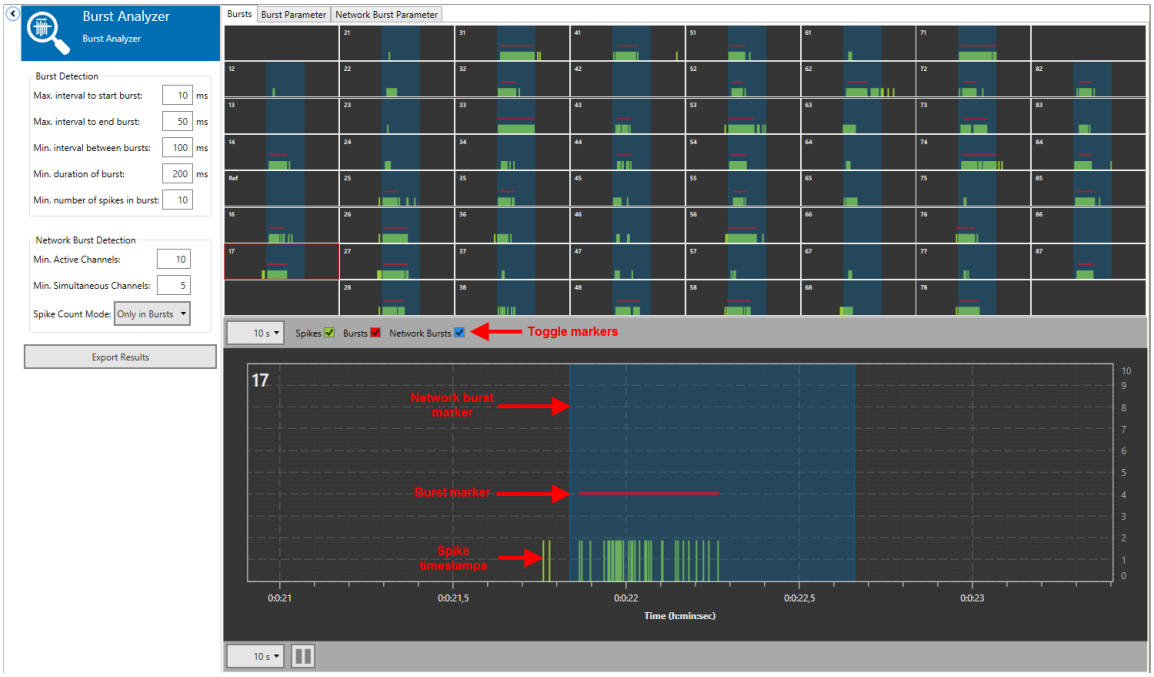
- the number of distinct bursting channels in the sequence needs to be at least the number defined for **Min. Channels Participating** and
- at some point during the sequence, there need to be at least the **Min. Simultaneous Channels** bursting at the same time.

The third parameter, **Spike Count Mode**, determines which spikes are counted as part of the network burst. This has an effect when computing the spike rate and the percentage of spikes in a network burst. If this parameter is set to **All in Duration**, all spikes in the duration of the network burst are counted for all channels, whether the individual channel was bursting at the time or not. If the Spike Count Mode is set to **Participating Bursts**, only spikes within bursts on the individual channels within the Network Burst are counted. The network bursts **starts** with the first overlap of two participating bursts, and **ends** with the end of the last participating bursts. Network bursts are labeled in the Bursts tab with **blue background**.



5.13.3.3 Bursts Tab

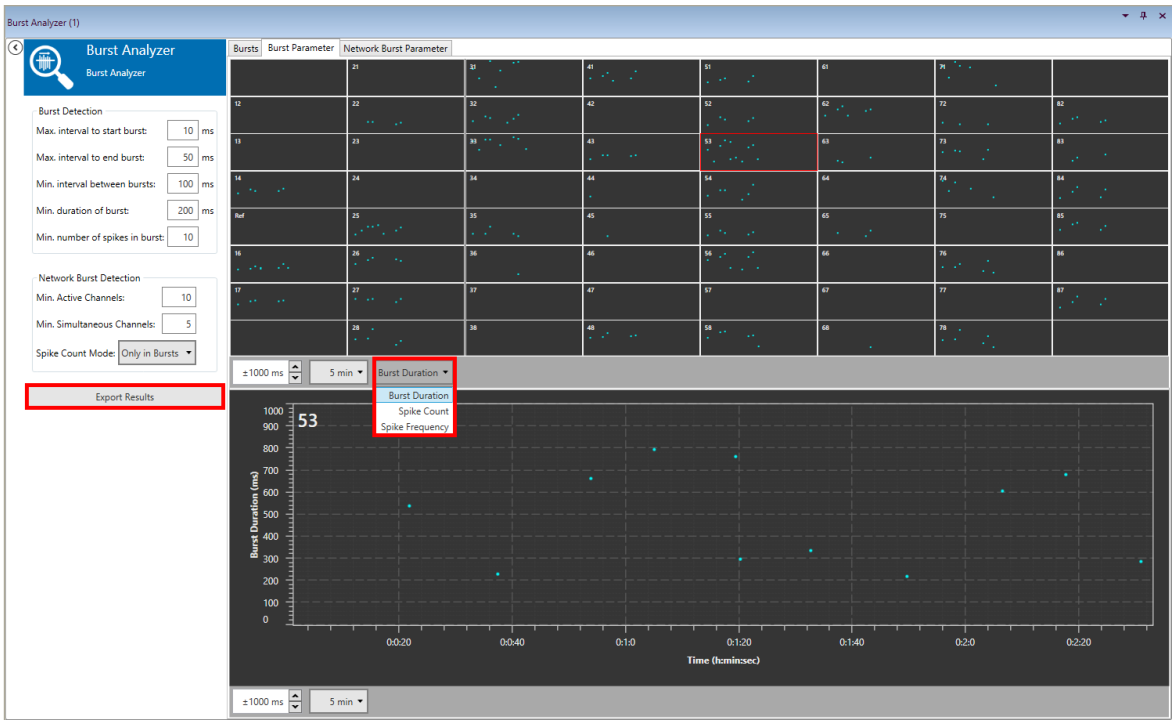
The Bursts tab shows the spike time stamps and the detected bursts as a red line on top of the events. If a network burst is detected, it's shown on all channels as a blue background.



5.13.3.4 Burst Parameter Tab

The Burst Parameter tab shows all analyzed parameters for all channels. Select the parameter to display from a drop down menu. For each detected burst, three parameters are plotted:

- Count: Number of spikes in burst
- Duration: Duration of burst
- Frequency: Mean frequency of spikes in burst



Some of the parameters the Burst Analyzer produces are displayed **continuously**, as they can be calculated for each individual burst: spike count, burst duration and mean spike frequency inside the burst. These parameters describe changes in bursting behaviour, like bursts getting longer or shorter. If the results are exported as ASCII data, all continuous parameters are sown for all channels underneath each other.

t	32	32	32
	Duration	Count	Frequency
[s]	[ms]	[-]	[Hz]
688,040	157,95	15	88,636
4,888,730	166,65	15	84,008
7,048,010	160,15	14	81,174
9,839,835	128,80	13	93,168
12,311,145	154,80	13	77,519
13,403,760	117,55	16	127,605
t	33	33	33
	Duration	Count	Frequency
[s]	[ms]	[-]	[Hz]
687,920	173,40	17	92,272
2,935,490	108,25	12	101,617
4,889,535	158,60	15	88,272
8,306,255	106,60	14	121,951
9,840,205	114,05	15	122,753
12,311,740	170,65	16	87,899
13,404,060	105,05	16	142,789
15,429,655	127,95	17	125,049

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

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

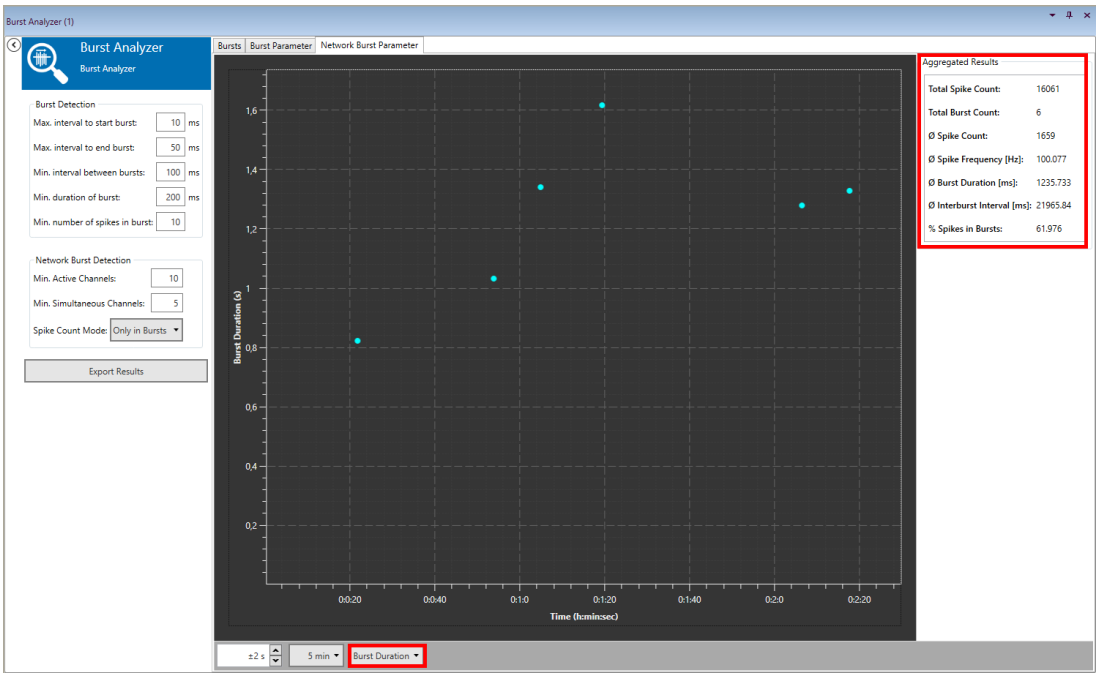
The Interburst Interval indicates changes in burst frequency. This requires that different experimental conditions, which need to be compared, were recorded in individual files, or different time segments are analysed consecutively (see 5.2.3.3). Bursting behaviour is well described by the parameter **% Spikes in Bursts**. A normal baseline value for a fairly active electrode in a culture with not synchronized, random activity is 10 - 20 %. In a bursting culture, this can go up to 90 - 100 %.

5.13.3.5 Network Burst Parameter Tab

The Network Burst Parameter tab shows all continuous parameters for all network wide bursts in a single display. Select the parameter to display from a drop down menu. For each detected network burst, three parameters are plotted:

- Burst Count: Number of spikes in network burst
- Burst Duration: Duration of network burst
- Spike Frequency: Mean frequency of spikes in network burst

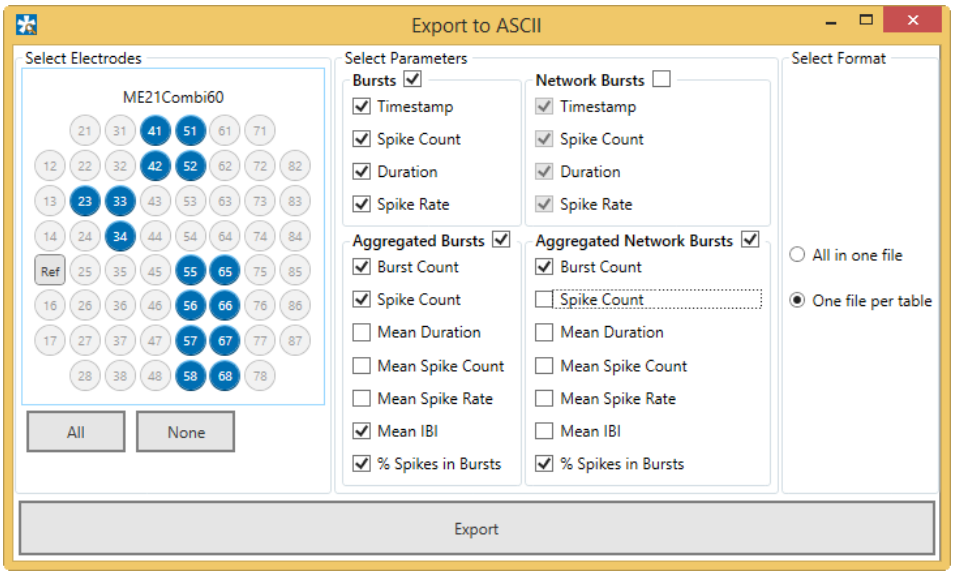
Note that the **Spike Count Mode** setting (see 5.13.3.2) influences these results.



The **Aggregated Results** for all network bursts detected in the analyzed file (or file segment) are shown as numerical results. The percentage of spikes in network bursts is an excellent parameter to characterize the degree of synchronization in a network. This parameter is also influenced by the **Spike Count Mode** setting in the network burst detection parameters.

5.13.3.6 Export Results

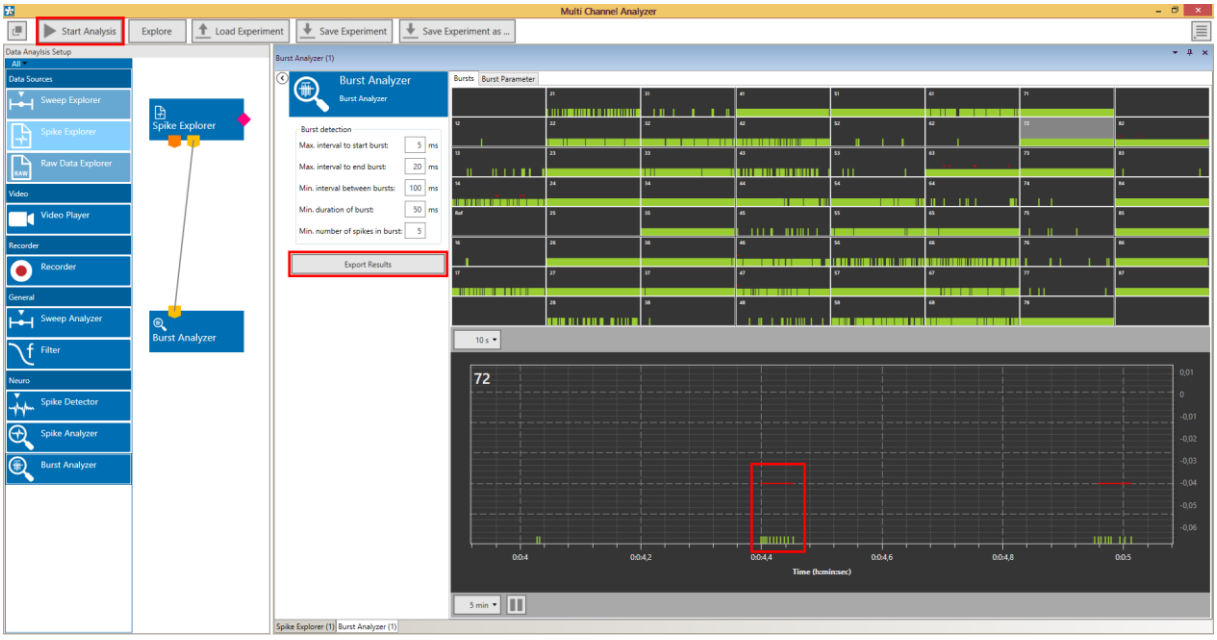
All burst and network burst parameters results can be exported as ASCII data with the **Export Results** function. The export dialog allows to select all or only individual channels to be exported, as well as toggling any parameter set or individual parameter. Results can be exported all in one file, or each parameter set (Bursts, Aggregated Bursts, …) in an individual file.



5.13.4 Example: How to …

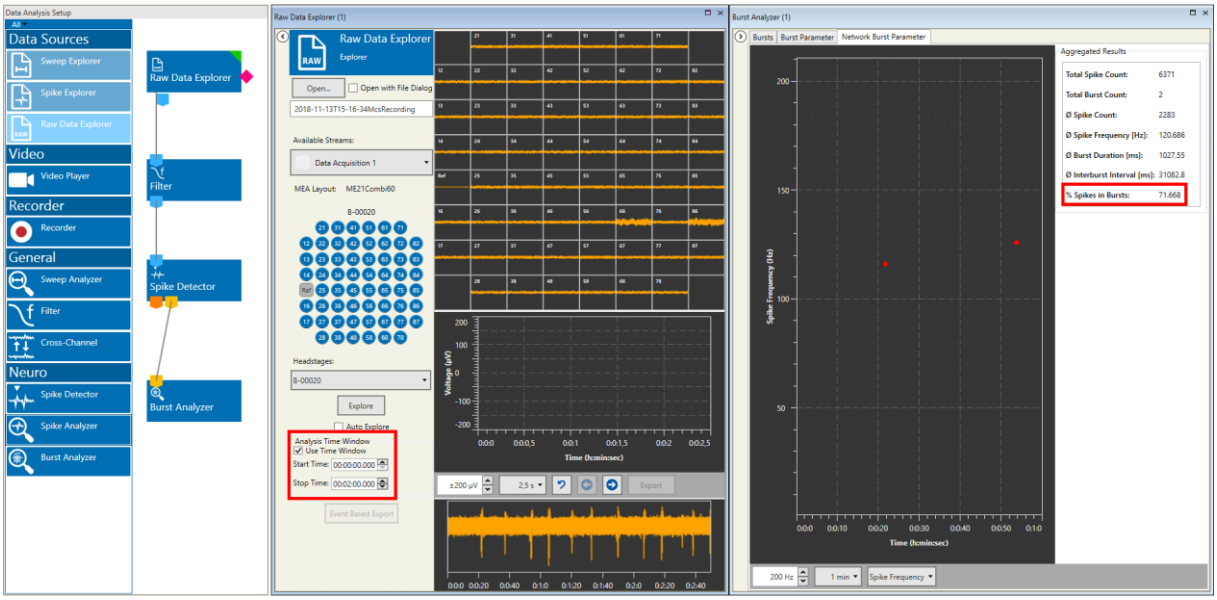
5.13.4.1 Analyze Spike Bursts

See chapter 5.11.4, for extracting the spikes from a raw data file. The spike data could come just as well from a Spike Explorer. With the **Start Analysis** function all burst parameters are generated as described above (see chapter 5.13.3, Burst Analyzer Operation). Detected bursts are indicated with a red line above the spike cutouts. The **Export Results** function will produce ASCII files with all selected burst results parameters.



5.13.4.2 Compare Network Synchronization before and after Substance Application

If a **raw data** file has been recorded, and a substance was applied at some time during the recording, network synchronization can be compared before and after application. Open the file and set the spike detection, burst detection and network burst detection parameters as required. Set the **Analysis Time Window** in the Raw Data Explorer from the start of the file till before the substance application. Analyze the segment and export the network burst parameters. Repeat the process with a Time Segment, ideally with the same duration, from after substance application till end of the file.



5.14 PSTH

5.14.1 Description and Purpose

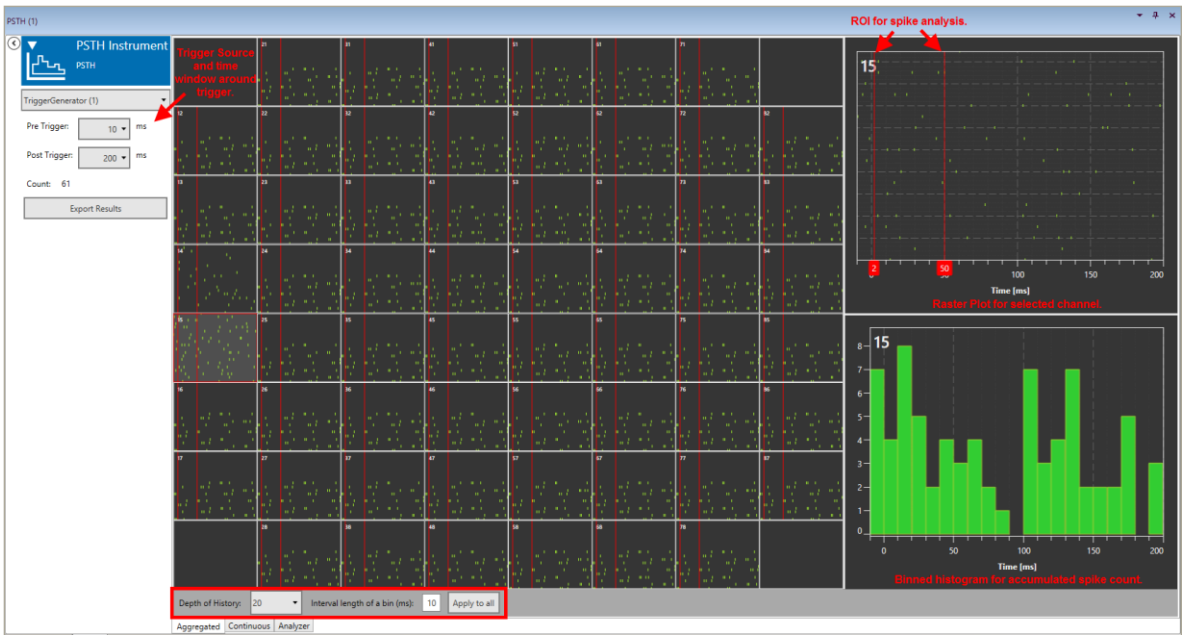
The PSTH (Peri Stimulus Time Histogram) tool can display spike time stamps arranged in relation to a Trigger input, usually a stimulation timepoint.

5.14.2 Data Ports and Export Options

The PSTH has a yellow Spike Time Stamp and a pink Trigger input. There are no outputs. Analysis results can be exported as ASCII data.

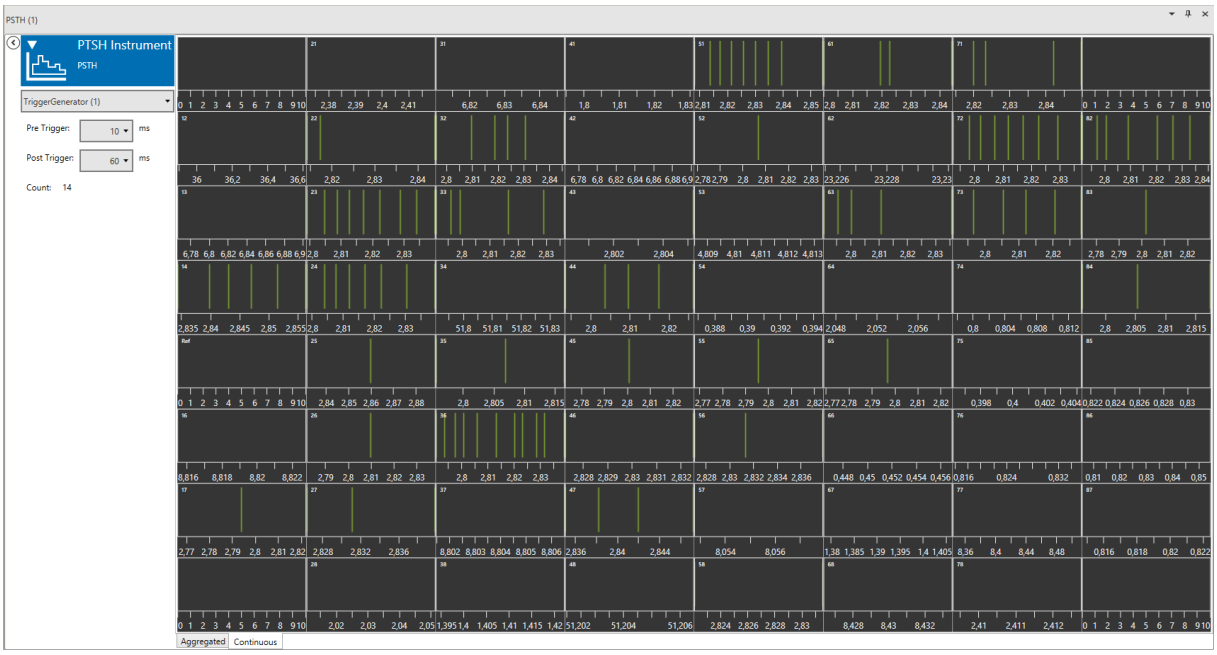
5.14.3 Operation

The PSTH instrument can display spike time stamps in relation to a trigger continuously and aggregated. In the **Aggregated** tab, spike time stamps for a selected number of episodes around consecutive triggers are displayed in a **cascading raster plot**. The duration of the episode around each trigger (**Pre- and Post Trigger**) and the number of episodes to be shown (**Depth of History**) can be selected. Two zoom windows show the rater plot for one selected channel, and the accumulated spike counts as histogram, with a selectable **bin size**.

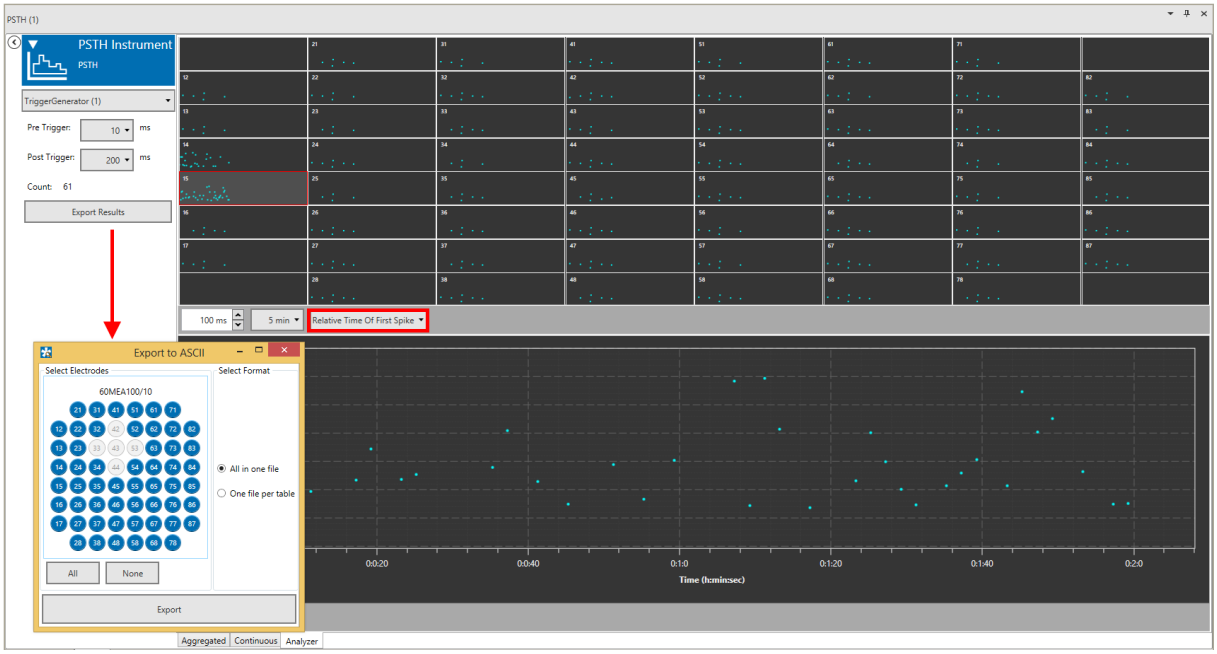


In the zoomed raster plot, a **Region Of Interest (ROI**) can be set by dragging the red lines. The same ROI can be **applied to all** channels, or the ROIs can be set individually channel by channel. Within the ROI, the number, average frequency, and relative time of first spike is analyzed and plotted in the **Analyzer** tab.

In the **Continuous** tab, spike time stamps for the most recent episode are shown continuously, with actual DAQ time on the x-axis.



The **Analyzer** tab shows the analyzed results for all spikes within the ROI. Three parameters are shown, the **Number of Spikes** in each ROI, the **Mean Spike Frequency** (if there is more than one spike in the ROI), and the **Relative Time of First Spike**, in relation to the trigger.

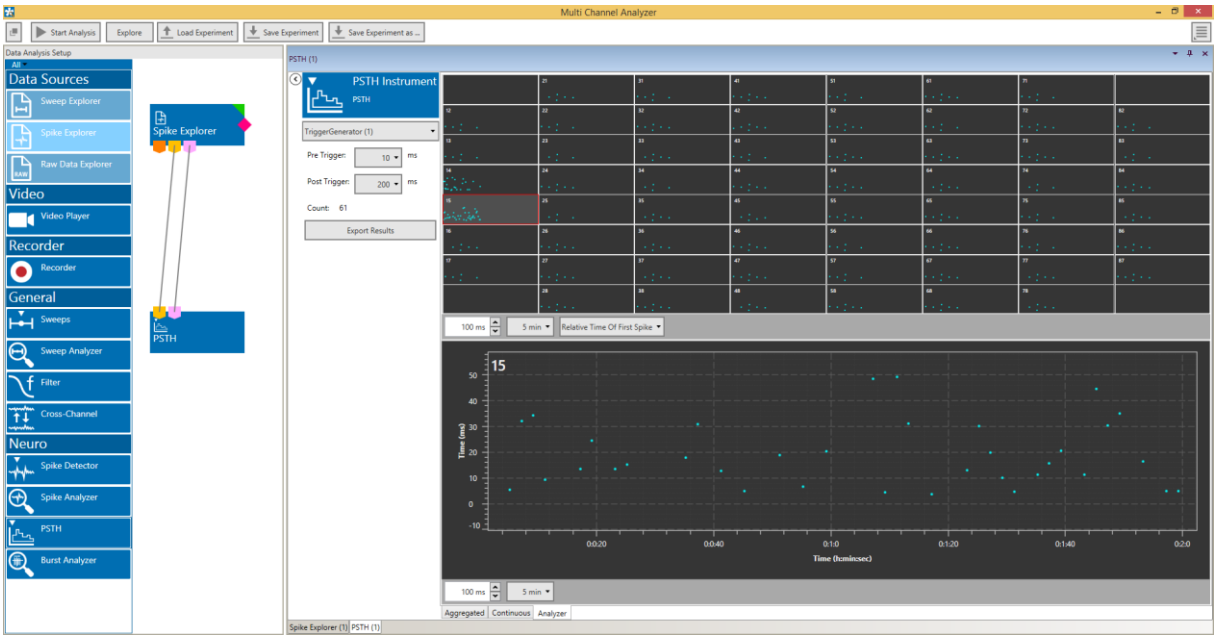


Results can be exported as ASCII data, for all or only for selected channels, into a single file or individual files for each parameter.

5.14.4 Example: How to Analyze Evoked Spikes

To be able to analyze evoked spikes, a file containing trigger events from a stimulator and raw data and/or spike data is needed. The spikes (from the datafile or generated from raw data, see 5.2.4.1) and the trigger stream must be connected to the PSTH tool. Set the pre- and post-trigger time and adjust the ROI after each stimulation you want to be analyzed. To avoid detecting the stimulation artefact, the ROI should start a few milliseconds after the stimulation.

As a control, it is also possible to analyze an equally long segment of data before the trigger, to account for the probability of spontaneously occurring spikes after stimulation. After analyzing the file, the Number of Spikes in each ROI, the Mean Spike Frequency (if there is more than one spike in the ROI), and the Relative Time of First Spike will be plotted and available for ASCII export.



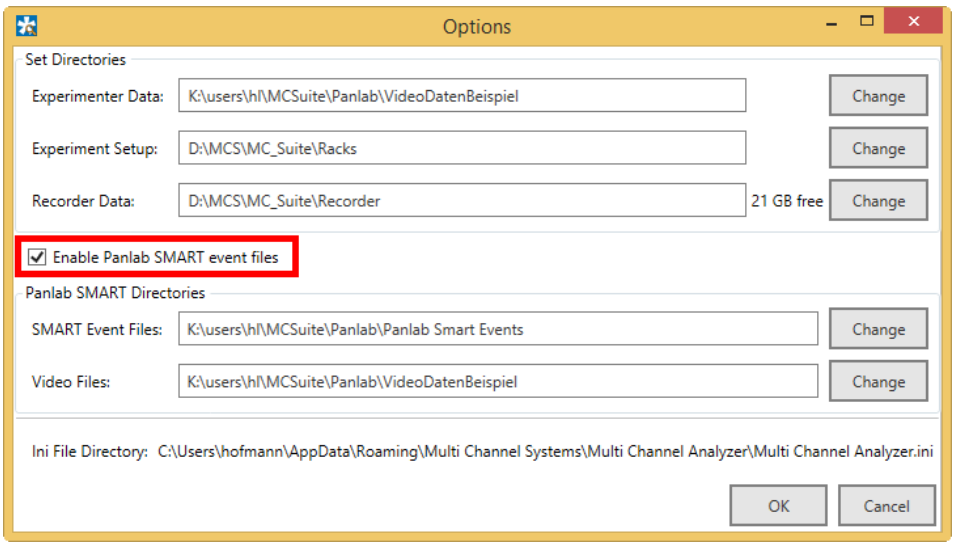
6 Combining the Analyzer with Panlab SMART Video Tracking Software

SMART is a powerful **video tracking** software provided by the company Panlab. This software tracks a number of behavioral parameters in combination with a recorded video file. These parameters are stored as events. If electrophysiological data was recorded with the W2100 system synchronized to such a video file. It is possible to open the data, the video file and the SMART events of an experiment in the Analyzer. All navigation, export, and video synchronization capabilities of the Analyzer are available also for these events.

Important: Currently the Panlab SMART event feature is only available for data files created with W2100 hardware.

6.1 Enabling the SMART Events

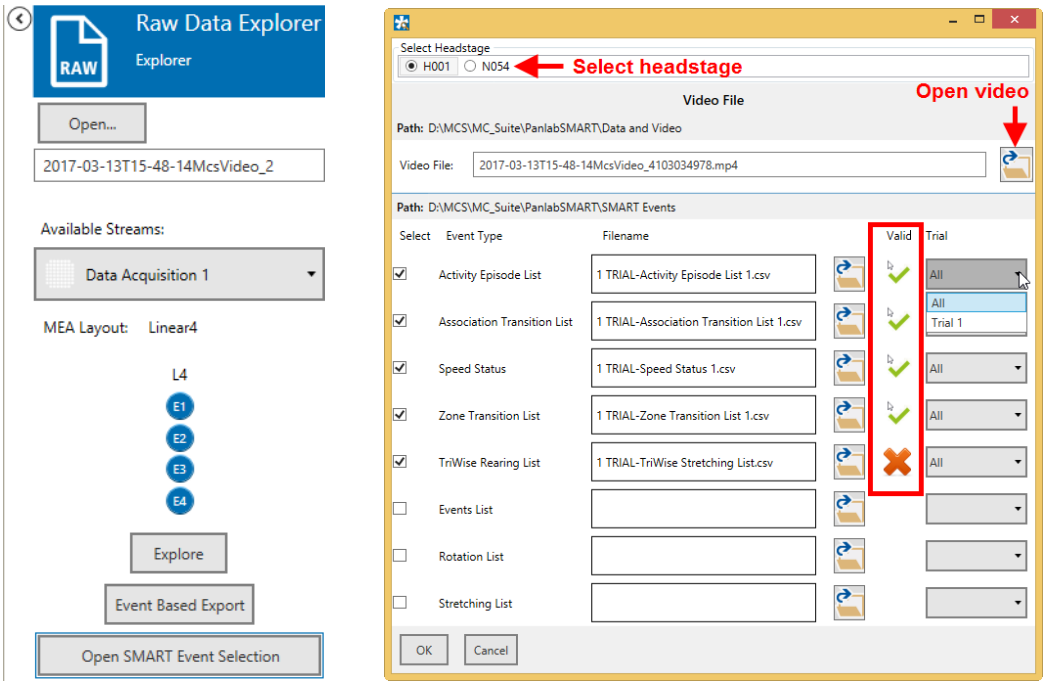
The Panlab SMART event feature is not enabled by default. To enable it, open the **Settings** dialog (see chapter 4.4) and check the Enable Panlab SMART event files check box. Then set the directories where the event and video files are stored.



6.2 Opening the SMART Event Files

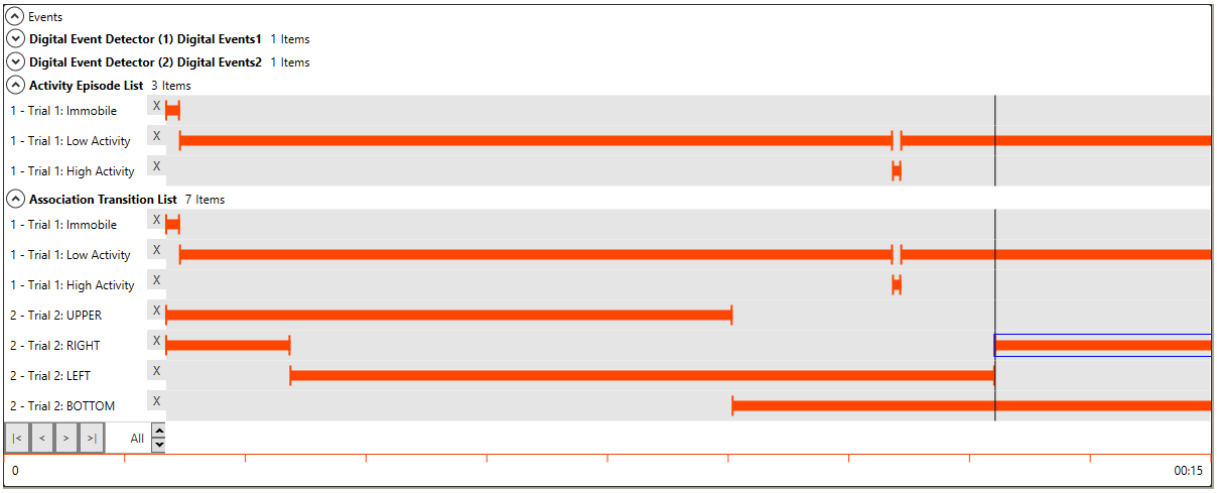
If the SMART events are enabled, and a data file is opened in the Raw Data Explorer or Spike Explorer instrument, the **SMART event dialog** will come up automatically. SMART events are stored in ASCII files (*.csv), and the respective files need to be opened for the different event categories. The **validity** of the files is automatically tested. The trial number can be selected from a drop down list. If more than one headstage was recorded in the datafile in Multi Headstage Mode, the respective **headstage** can be selected by serial number.

The selection of SMART events for each datafile is saved, so it has to be done only once for each datafile, as long as the file paths are not changed. A button to open the SMART event dialog will also come up in the respective Explorer tab.



6.3 Displaying SMART Events

The activated SMART events will show up in the Explorer tab and can be used for navigating the data files like other digital events (see chapter 5.1.1). The video file opens in the Video Player instrument (see chapter 5.6).



7 Index of Application Examples

Below is a list of all application examples mentioned in this manual.

- 5.2.4.1 Extract Spikes from a Raw Data File
- 5.2.4.2 Generate Files with Different Filter Bands from a Raw Data File
- 5.2.4.3 Generate a new File with only a Time Segment of the Original Data
- 5.7.4 How to Extract Sweeps from a Raw Data File
- 5.8.4 How to Analyze EPSPs and Population Spikes from a Hippocampal Slice
- 5.9.4.1 Differentiate Theta Waves and Spike Activity from a Raw Data Signal
- 5.9.4.2 Remove 50 Hz Noise
- 5.10.4.1 Remove Common Artefacts from a Wireless Recording
- 5.10.4.2 Do Differential Measurements between Pairs of Electrodes
- 5.11.4 How to Extract Spikes from a Raw data File
- 5.12.4 How to Analyze Spikes
- 5.13.4.1 How to Analyze Spike Bursts
- 5.13.4.2 Compare Network Synchronization before and after Substance Application
- 5.14.4 How to Analyze Evoked Spikes

8 Support and Troubleshooting

The Multi Channel Suite is a software under development, so bugs may occur more frequently than usual. Also, new software versions are released in short intervals. The software development team of Multi Channel Systems is very grateful for all reported bugs. Due to the modular nature of the software, it is impossible to test all possible configurations for each new release, and any customer feedback is much appreciated to find all problems.

Please report all bugs, feature requests or other issues to Support@multichannelsystems.com. We can only fix things we know about.

In case of problems, please check first whether your **software version** is up to date. The latest release can always be found in the Downloads section of the MCS web site. Maybe the problem has already been solved. If not, reboot the software and power cycle the recording device.

Sometimes bugs are caused by corrupted experiment (*.msa) files, so the next step is to try building a new, identical experiment setup, instead of using an existing one.

If the problem persists, report it together with the **experiment file** (*.msa) which has been used, and possibly a **data file** (*.msrd and respective *.msrs files are needed) to the support e-mail address. An upload option for the files will be provided if need be.

9 Contact Information

Local retailer

Please see the list of official MCS distributors on the MCS web site.

Mailing list

If you have subscribed to the newsletter, you will be automatically informed about new software releases, upcoming events, and other news on the product line. You can subscribe to the newsletter on the contact form of the MCS web site.

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