Multi Channel Experimenter
Manual

Imprint

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1 Welcome to the Multi Channel Experimenter

The Multi Channel Experimenter is the online data acquisition software of the Multi Channel Suite package. The Multi Channel Analyzer is for offline analysis of data generated with the Experimenter, and the Multi Channel DataManager allows to export 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 You Start

2.1 Terms of Use

You are free to use Multi Channel Experimenter 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 Experimenter 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 Experimenter 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 advice prior to install or to use Multi Channel Experimenter. If you do not fulfil all requirements stated below, this may lead to malfunctions or breakage of connected hardware, or even fatal injuries. Always obey 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 Experimenter is only be used for its intended purpose and that it is only used by qualified personnel.

Warning: Multi Channel Experimenter 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 responsibility 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. For operating devices with **USB 3.0 connection**, Windows 10 or 8.1 are strongly recommended, as frequent errors are reported with USB 3.0 under Windows 7.

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. It is possible that at the first use after installation of an update the software offers a **firmware upgrade**. Allow the firmware upgrade 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 Experimenter. 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 Experimenter. Remove all applications from the Autostart folder. Be careful when using a Virus Scanner. These programs are known to disturb Multi Channel Experimenter and even data loss may occur.

Please be sure to install the newest USB driver and/or the driver for main board chip set available.
This is recommended if there are problems with the data acquisition.

When using a MEA2100-120-System or a MEA2100-2x60-System it is recommended to connect a high performance computer with a separate hard disc for program files and data storage. The provided possibility to use up to 240 channels with a sample rate of up to 50 kHz needs high memory capacity. Please remove data and defragment the hard disc regularly to ensure optimal performance.

Warning: If purchased from MCS, the operating system settings of the data acquisition computer were preconfigured by MCS and should not be changed by the user. Changing these settings can lead to program instabilities and data loss.

3.2 Compatible Hardware

The Multi Channel Experimenter will operate only with data acquisition systems from Multi Channel Systems. Currently the MEA2100-, the ME2100-, the W2100-, the Basic Wireless-System, the SE-Wireless and the USB-ME data acquisition systems are supported (USB-ME16/32, USB-ME64/128/256), in all respective variations.

4 General Software Features

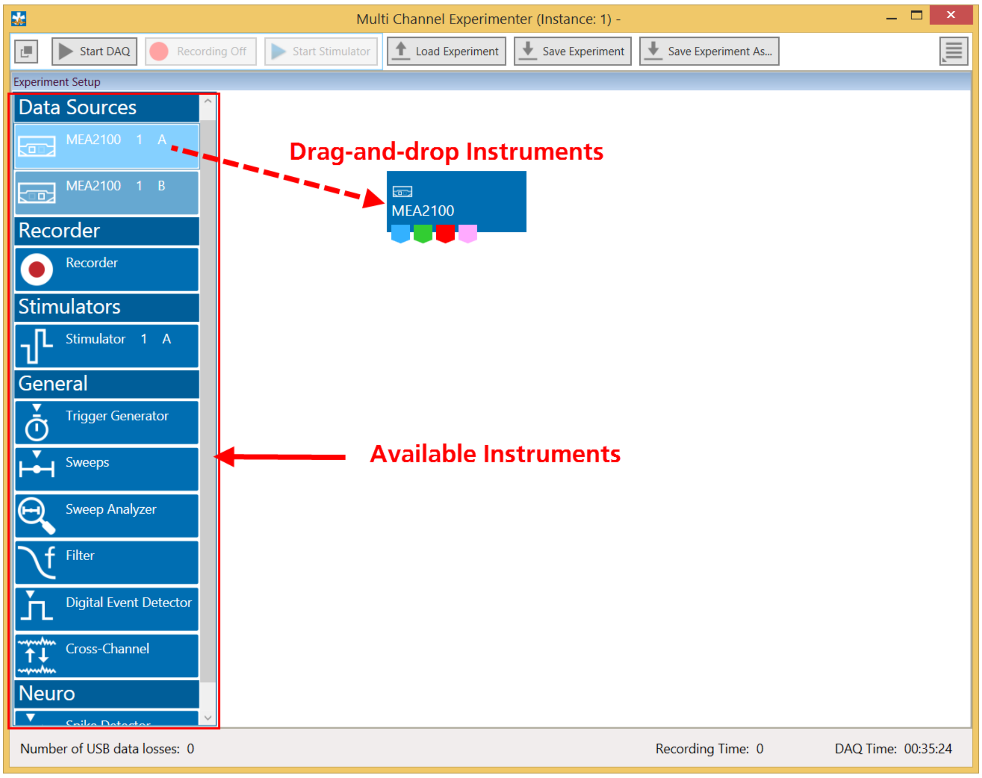
The Multi Channel Suite consists of four parts: The **Multi Channel Experimenter** for online data acquisition, the **Multi Channel Analyzer** for offline data analysis, the **Multi Channel DataManager** for data conversion, and the **Multi Channel VideoControl** for controlling a video camera. Many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

4.1 Functional Principle

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

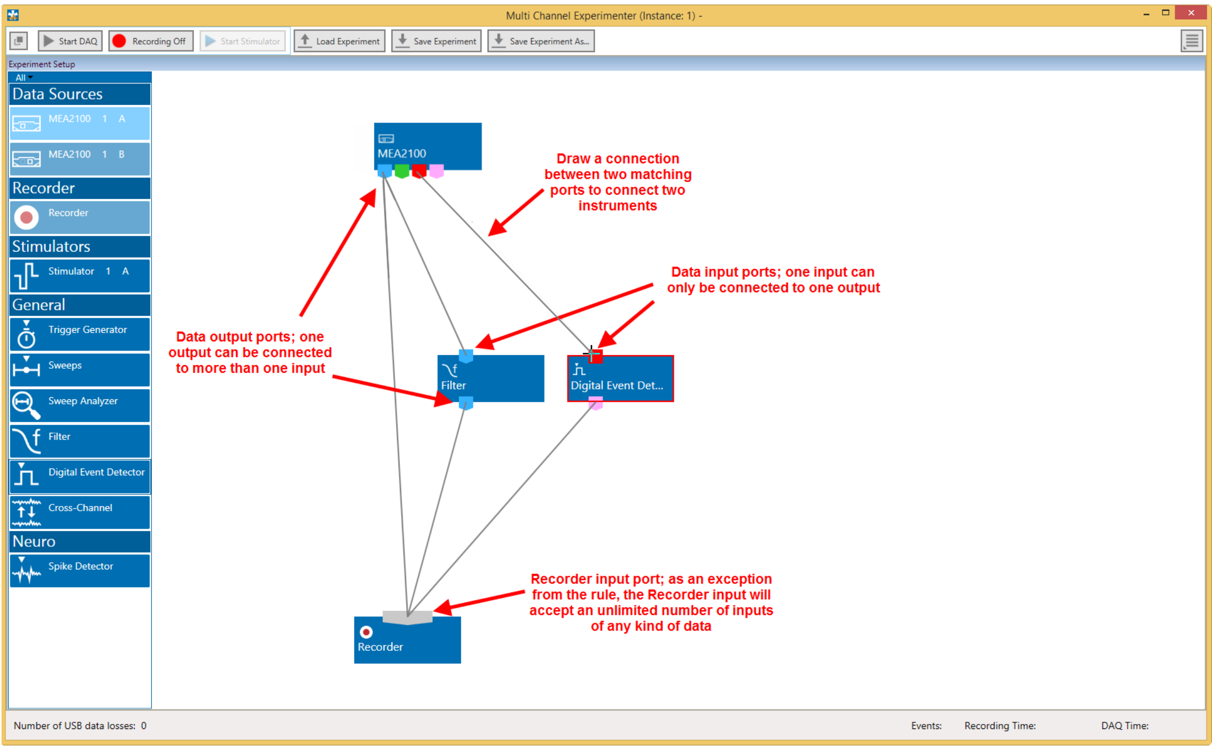
The Multi Channel Experimenter operates with virtual instruments, which can be combined and saved as so called **Experiments** for later use. When opening the software, all available instruments are shown as blue icons on the left hand side of the screen. Each icon can be dragged and dropped in the main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

4.1.1 Designing an Experiment

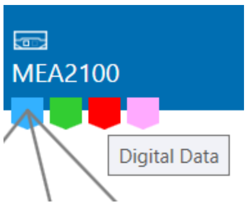


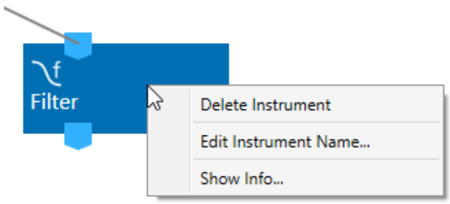
Most instruments have color coded **data ports**. Ports on the upper side represent data input to the instrument, ports on the lower side represent data outputs of the instrument. Only ports with matching colors can be connected by **drawing a connection line** between a data output and one or more data inputs. Data flows along those lines from the data source through the instruments.

An instrument **without connected data inputs** is not functional. Please see movie for illustration.



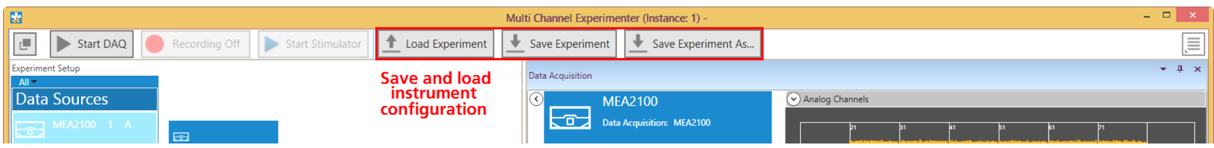
Hovering the mouse pointer above an Output Port will give you a **Tooltip** with the data type the port provides. A right click on a data connection allows to delete the connection. A right click on an instrument brings up a menu which allows to **delete or rename** the instrument.





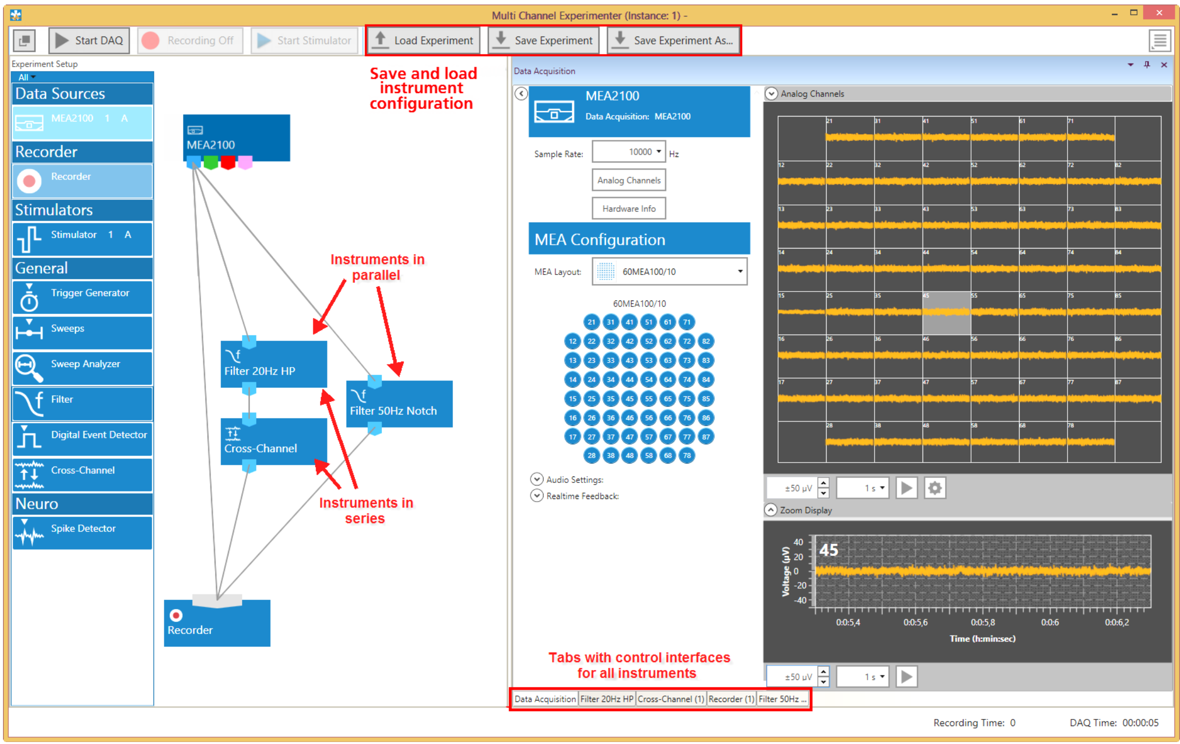
4.1.2 Save and Load an Experiment

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 ***.mse**. 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, the recorder, and the stimulator, which can be selected only once.

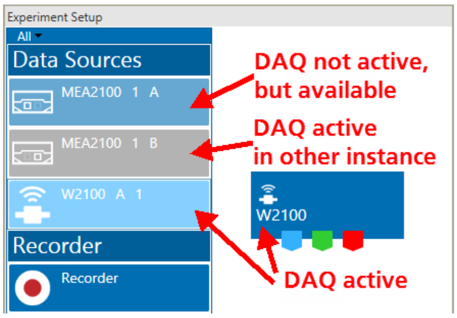


The connecting lines between the instruments represent the flow of data. In the example above, there is a stream of raw data coming from the data source MEA2100 into the recorder directly, and will consequently be recorded in the file. The same raw data stream is processed through a 20 Hz high pass filter, and the filtered data is further processed by the Cross-Channel Tool. The data processed by those two instruments is then connected to the recorder and will generate a second data stream with filtered AND referenced data in the recorded file. A third data stream will be the raw data filtered by a 50 Hz notch filter.

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 Multiple Data Sources, Multiple Software Instances

If **multiple data sources** are connected to the computer, they are indicated as separate icons in the Experimenter. In case of a MEA2100-HS2x60, each individual MEA slot is a separate data source. Only **one data source can be used in one instance** of the Experimenter. **Multiple instances** can be opened simultaneously to control different data sources independently, for example two instances to control the two MEAs of a MEA2100-HS2x60. The active data source is shown in a light blue color. Data sources already occupied by another instance of the software are shown in grey. Not selected but available data sources are darker blue.



4.2 Data Types

The types of data occurring in the Experimenter, 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, the 16 bit digital data stream from the Interface Board.
Pink:	Trigger events.
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.
Light brown:	Gyroscope data, rotation versus time from wireless headstages.
Dark brown:	Accelerometer data, acceleration in x y z direction from wireless headstages.

4.3 File Types

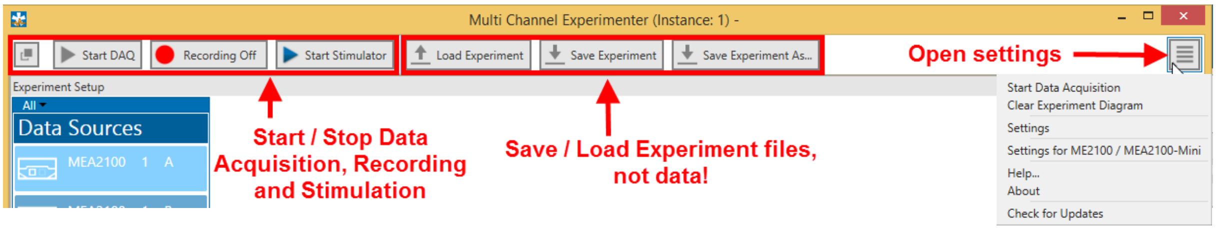
The types of files occurring in the Experimenter are:

- ***.mse:** 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 in the Multi Channel Analyzer.
- ***.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.
- ***.stsd:** Stimulation patterns in the MEA/ME2100 Stimulator instrument can be saved as *.stsd. Earlier Experimenter versions used *.xml.
- ***.nsf:** Stimulation paradigm of the W2100 electrical stimulator.
- ***.osf:** Stimulation paradigm of the W2100 optical stimulator.
- ***.lmp:** A sequence of stimulation electrode patterns in Stimulator List mode, see 5.9.3.9.

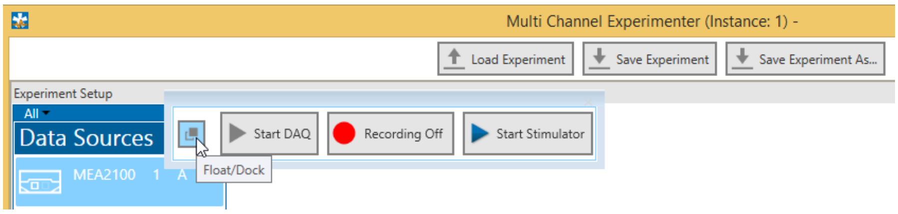
All files generated during a recording have the **same name**, in case of the *.xml files with an extension representing the instrument name ([file name][instrument name].xml).

4.4 Main Menu

The main menu bar contains the major control functions for starting and stopping the **Data Acquisition**, the **Data Recording** and the **Stimulator**, as well as the **Save** or **Load** options for **Experiment files**. Furthermore, there is a button to open the general settings.

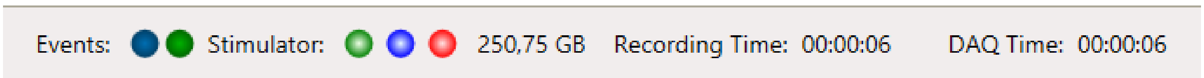


The Start / Stop controls can be **undocked** from the main Menu and be dragged anywhere. They will always stay on top of all other windows.



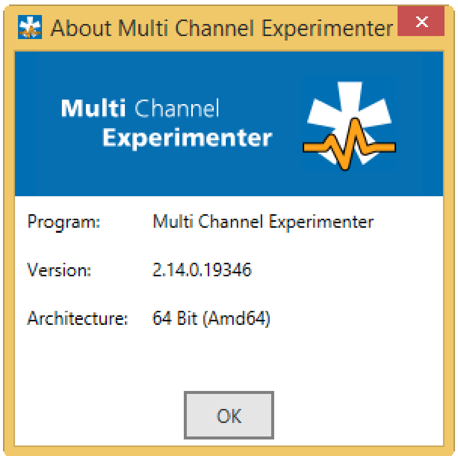
The Start / Stop DAQ button controls the data acquisition, but does not yet start recording data. The Recorder can be in three conditions, **Off, On** (actually recording data) or **Standby** (armed and waiting for start condition). Standby can happen if the Recording is activated, but the DAQ is still off, or if the Recording is set to start on a Trigger event, and the software is waiting for the trigger.

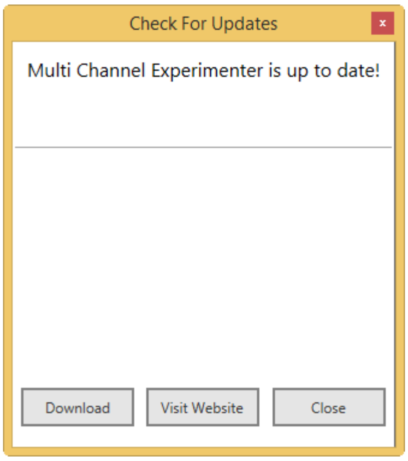
The lower menu bar contains information about the ongoing recording, available disc space and status LEDs for **Trigger events** and the internal **stimulators**, provided that the respective instruments are in use. In the example below, two Digital Event Detectors and all three Stimulator units were used.



4.5 Settings

The settings include the **Start** option again, **Clear** all instruments, the Info page on the software version (About) and the **file path settings**. The **Help** will open the latest version of the manual directly from the MCS web site. This is of course only available if the data acquisition computer is online. **About** will show the currently software version.

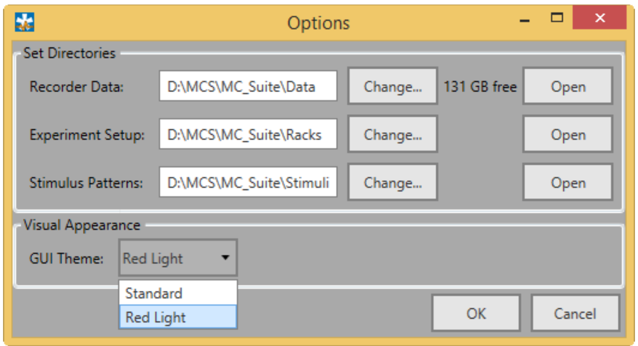




It is recommended to use the **Check for Updates** function on a regular basis. Free upgrades of the Multi Channel Suite are released on a regular basis.

4.5.1 Settings for File Directories and Visual Appearance

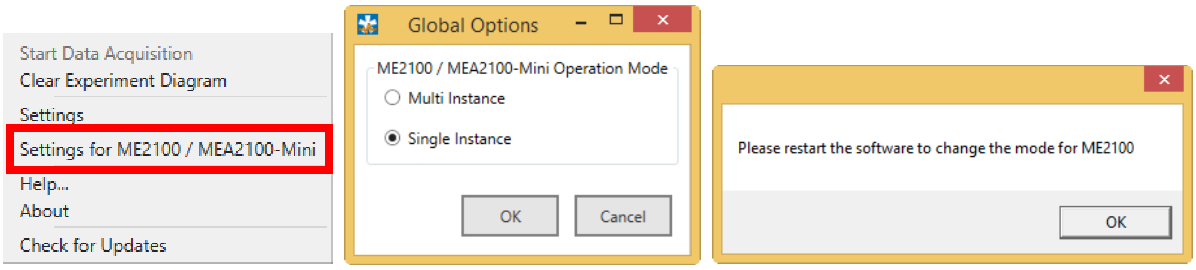
The Multi Channel Experimenter uses a **default file path** for all data, stimulation patterns and Experiment files, which can be changed in this menu.



Furthermore, the **visual appearance** of the complete software can be set to **Standard or Red Light** mode. In Red Light mode, all displays are optimized for minimal brightness and optimized contrast under low light and/or red light conditions. This allows working with light sensitive tissue.

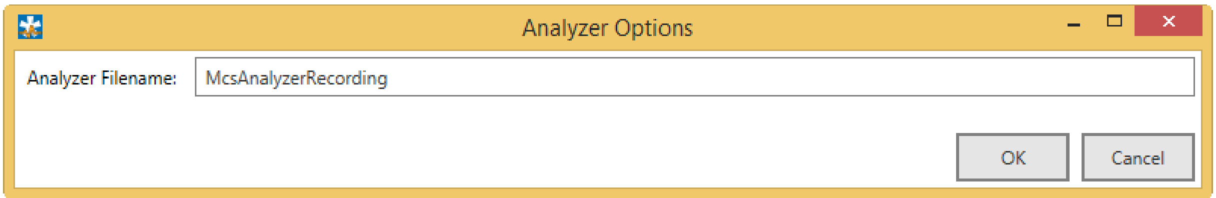
4.5.2 Settings for ME2100 and MEA2100-Mini

For the ME2100 and the MEA2100-Mini Systems, two operation modes are available, **Multi Instance and Single Instance**. In Single Instance Mode, all headstages connected to one SCU are operated from a single Instance of the Experimenter. They start and stop simultaneously. In Multi Instance Mode, each headstage appears as independent data source, and can be operated independently in a separate instance of the Experimenter. More detailed explanations can be found in the description of the respective instruments. This operation mode can be set here. Changing it requires a restart of the software.



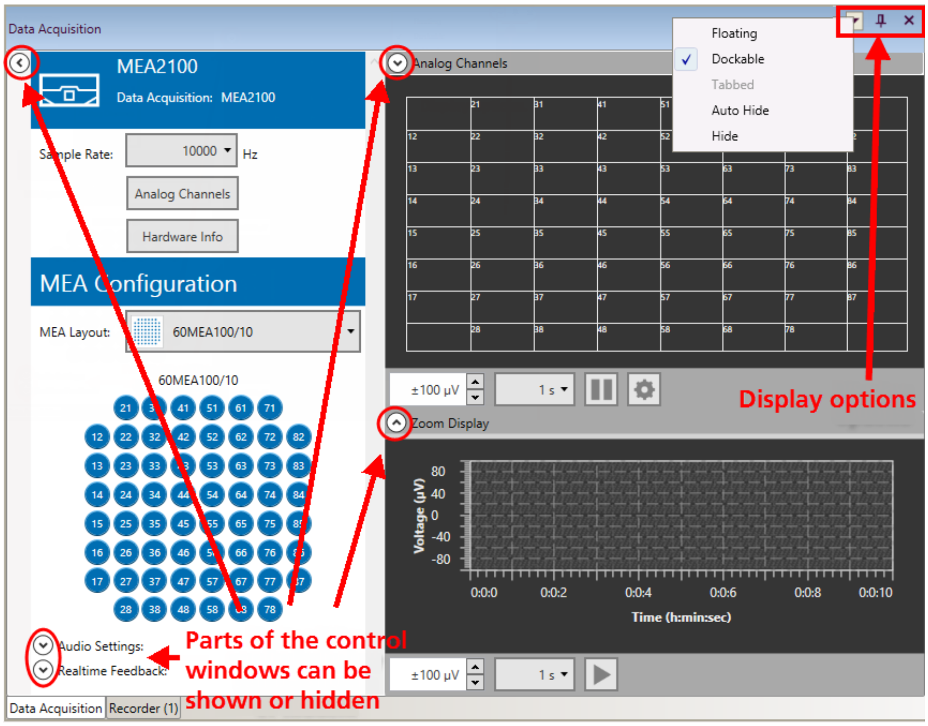
4.5.3 Settings for Analyzer Instruments

Analysis Instruments (Sweep Analyzer, PSTH, Spike Analyzer, Burst Analyzer) allow the **continuous export of analyzed parameters** to ASCII files during recording. In the respective settings, the name for these ASCII files can be selected. In addition to the given name in the settings, there will always be a prefix with **date and time**, and a suffix with the respective **instrument name**, for example “2019-07-22T15-21-51[GivenName]_Burst Analyzer.txt”.

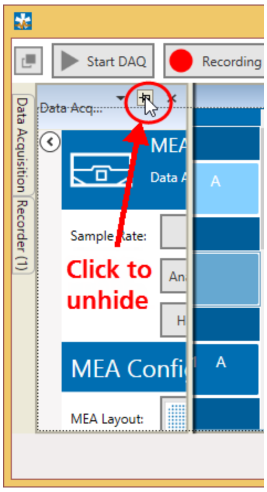


4.6 General Display Features

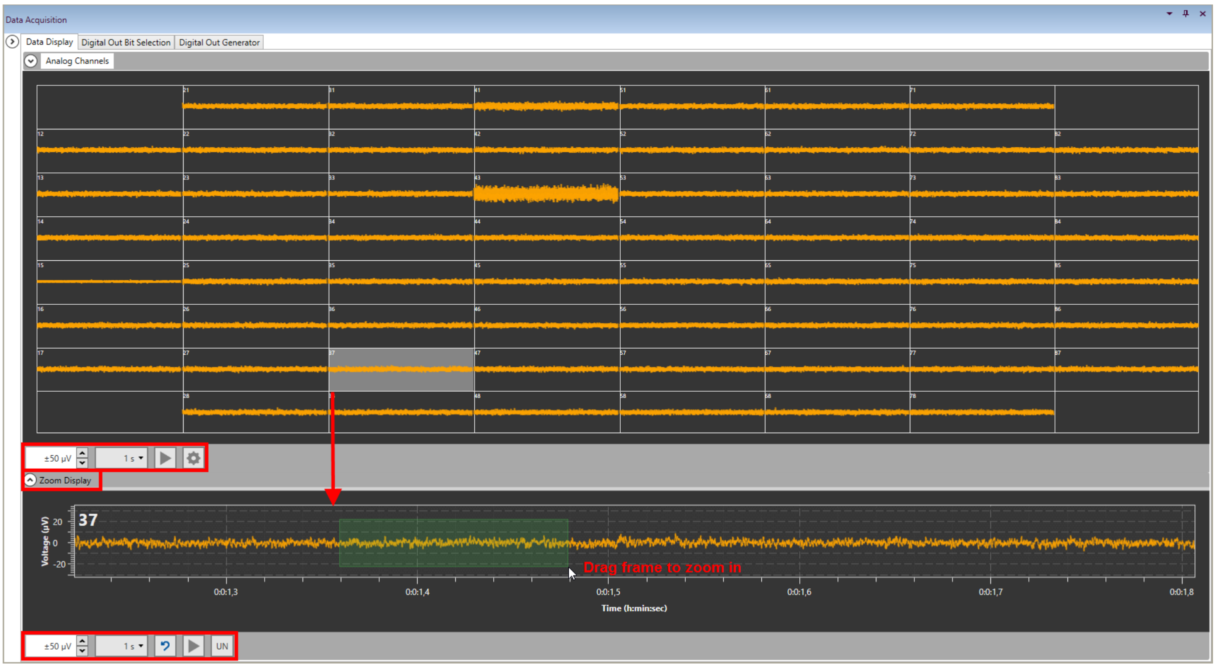
In most control windows, there are additional display or control functions which can be **hidden or shown**, as need be. By default, if more than one control window is open, they are shown as tabs, and only one window is visible at a time. This option is called "**Dockable**".



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.

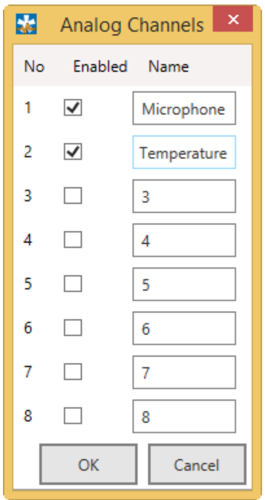


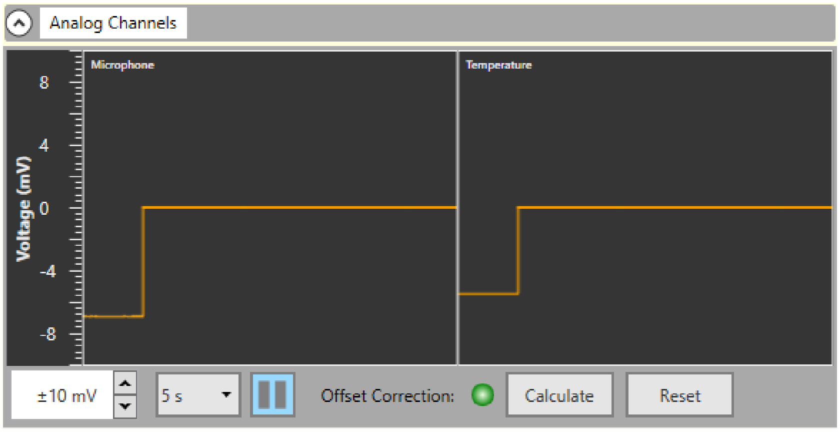
In all control windows, **all channels** of the data acquisition are shown, and one channel can be selected to be shown in the **Zoom Display** below. Both displays can be controlled independently (pause/resume, scaling). All Displays share similar scaling and zoom functions. Under each display the x- and y-axis scaling can be changed. Data displays can be **paused and resumed while the data acquisition is running**, without interfering with the actual data recording.



In a paused display, it's possible to **zoom in by dragging** a frame around the region of interest. A double click will reset the zoom to fit the signal. The return button will return to the scaling currently selected in the drop down menu. After two successive zoom steps, the **UN button** will become active, and allow to go back one zoom step.
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.

For all devices with physical analog inputs, a display for those **Analog Channels** can be toggled. Only analog channels activated in the data source will be shown and can be given random labels. The **Calculate** button will determine the current offset of each channel and compensate it. Active Offset Correction is shown by a green LED. **Reset** will deactivate the Offset Correction.





4.7 Online Export of Analysis Parameters

Analysis Instruments (Sweep Analyzer, PSTH, Spike Analyzer, Burst Analyzer) allow the **continuous export of analyzed parameters** to ASCII files during recording. The file name will consist of a given file name, with date and time as prefix, and the instrument of origin as suffix, see chapter 4.5.3. If enabled in the respective analysis instrument, parameters generated during data acquisition are stored in the same directory as the data, as selected in the Settings (see 4.5.1). This will work while recording, but also if only acquiring data **without recording**.

All parameters generated by a specific instrument are exported. They need to be written into the file as they appear. For example, network burst parameters occur mixed within the other burst parameters, marked with an N instead an electrode label.

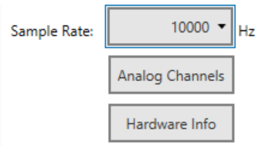
	A	B	C	D	E
1	Multi Channel Experimenter Burst Analyzer ASCII Export				
2					
3	Channel	t	Duration	Count	Frequency
436	E5	58,089	3810	192	50,131
437	E4	58,089	3810	192	50,131
438	E3	56,4845	5414,5	265	48,758
439	E2	58,089	3810	192	50,131
440	E1	58,089	3810	192	50,131
441	E32	57,929	4851,5	310	63,692
442	N	56,5825	203	269	41,41
443	N	56,9075	5873	6729	35,805
444	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

5 Instruments

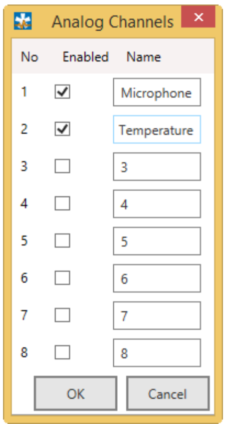
In the following chapters, all instruments of the Multi Channel Experimenter are described individually. Instruments operate as **independent units**. They process the data from the respective input port and generate one or more data streams in the output port(s). Settings have to be done separately 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**.

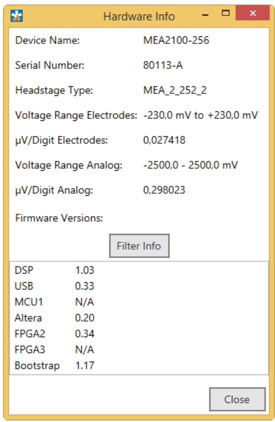
5.1 Data Sources General

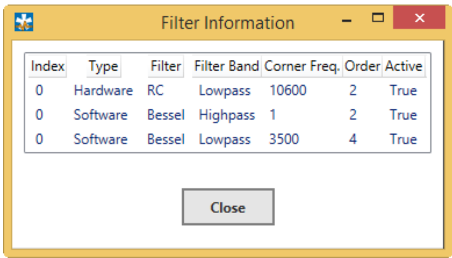
All data sources can be added **only once** to the Experiment. They have only data output ports and no data inputs. All data source instruments show unfiltered raw data for all available channels. The control window of each data source contains the same main control functions:



Only the available sampling rates for the respective device and recording mode turn up in the **Sampling Rate** drop down menu. The additional eight **Analog Input channels** on the Interface Board (green data port) can be individually toggled in a pop up menu. Analog Channel inputs can be given random names. All relevant information about the connected hardware is also available.







The **Filter Info** button is available for all devices using an IFB. It will bring up a menu showing the currently active filters of the device. The 10 kHz low pass filter is fixed in the hardware, and always active. The filters labeled under Software are implemented in the firmware, and may be changed at any given time, using the MCS Filter Config software for all MEA2100 and ME2100 devices, or W2100-System Config for the W2100 wirelsess system.

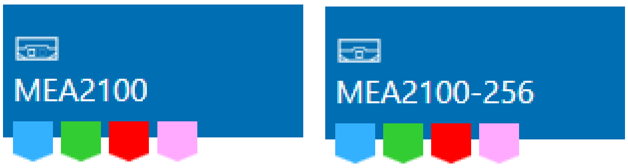
5.2 Data Source: MEA2100-(60/2x60/120/256)-System

5.2.1 Description and Purpose

The MEA2100-System is supported in all available headstage variations. One MEA is always operated independently by one instance of the software, also in case of the MEA2100-2x60 headstage. The icon indicates which headstage and MEA it represents. **1 or 2** indicate the headstage connected to port 1 or 2 of the Interface Board, **A and B** indicate the left or right MEA on a 2x60 headstage. The type of connected headstage (1x32, 2x32, 1x60, 2x60, 1x120 or 1x256) is detected automatically. Once the MEA2100-System is added to the instrument configuration, the MEA2100 Stimulator will also become available as instrument. Please also see movie for illustration.

5.2.2 Data Ports and Export Options

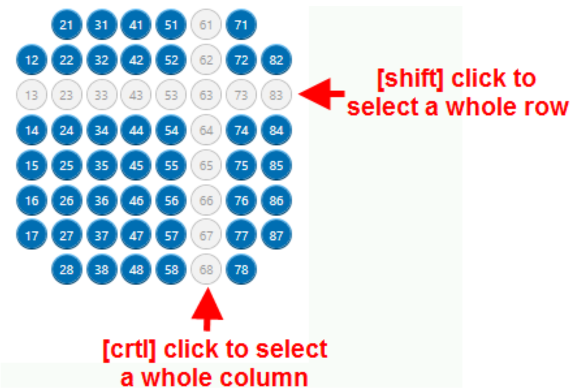
The MEA2100-System has four output ports: Electrode Raw Data (blue), Analog Data (green) Digital Data (red) and Triggers (pink). As with all data sources, there are no input ports. The MEA2100-System can be connected directly with any number of other instruments with blue, red or pink input ports. The green port can only be connected to the Recorder at the moment. There are no export options.

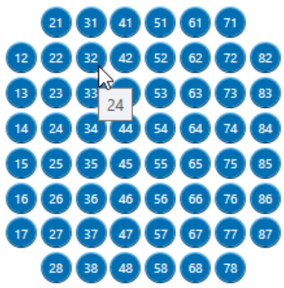


5.2.3 Operation

The MEA2100 data source control window shows **all available data channels**, and optionally also the additional Analog Input channels from the Interface Board. If a standard MEA is used, the respective layout can be selected from a drop down list, to match the display to the actual electrode configuration.

Individual channels can be **toggled** by clicking on them. Deactivated channels will neither be displayed nor recorded. **[shift] click or [crtl] click** on any electrode will toggle the whole row or column. Hover the mouse pointer over an electrode channel for a few seconds to bring up the **hardware channel ID** for that electrode.

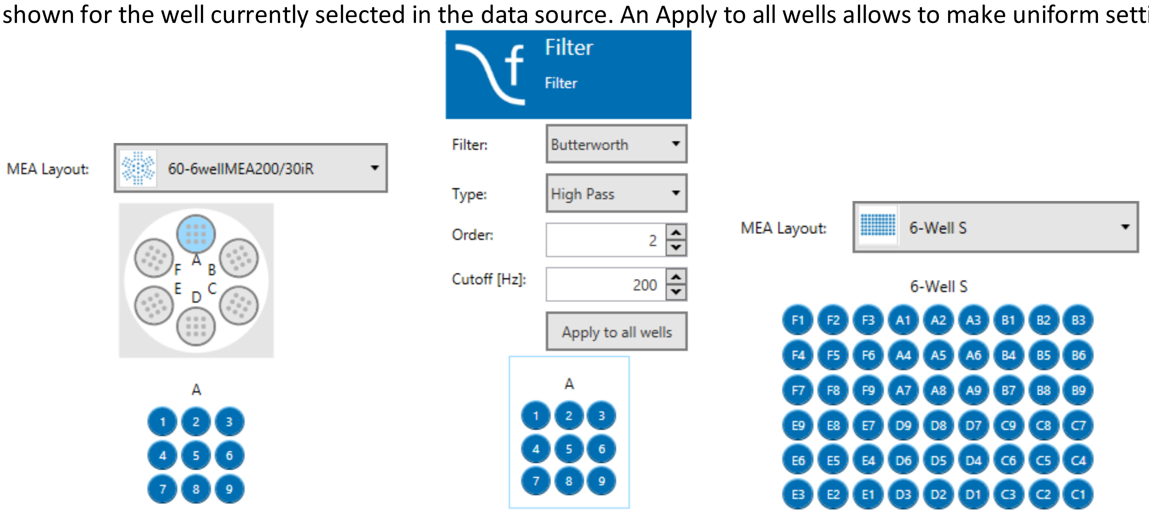




The required **MEA layout**, which will define the display layout for the MEA2100-System data source and all other instruments, can be selected from a drop down list. Based on the automatically detected headstage type, only compatible layouts will show up in the list. If a 60-6wellMEA is selected, channels of only one well are displayed at a time, other wells can be selected by mouse click. This does not affect the recording in any way.

5.2.3.1 Multi Well MEA Layouts

For the 60-6wellMEA200/30iR-Ti, two different layout options are available. The **60-6wellMEA200/30iR** layout shows only one well at a time. You can switch between the wells by clicking at them. Settings for all connected instruments (Filters, Stimulator, Cross Channel Tool…) can be set **independently for each well**. Settings will be shown for the well currently selected in the data source. An Apply to all wells allows to make uniform settings.

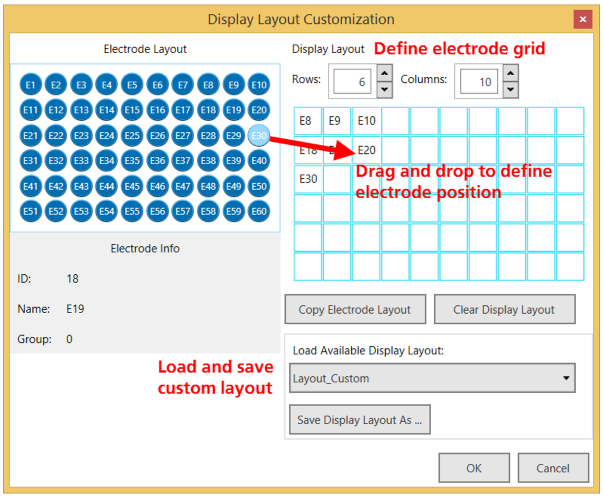


Alternatively, the **6-Well-S layout** shows all electrodes of the 6-Well MEA with the correct electrode label and grouped by wells, but they are all treated as one unit. All settings and stimulation patterns are global for all channels.

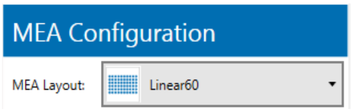
For the **MEA2100-256-System**, two multiwell MEAs are available, the 256-6wellMEA200/30iR-ITO and the 256-9wellMEA300/30iR-ITO. In both cases, depending on the MEA2100-256 contact unit in use, a true multiwell layout or the single well variant is available.

5.2.3.2 Custom Display Layouts

To generate a custom layout for the display, click the Settings icon under the main display. The following menu will allow to generate any desired electrode grid, and then assign channels to any grid position.



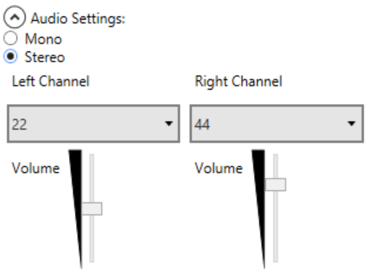
The label of the electrodes is defined by the layout selected from the drop down list. To get the linear hardware channel IDs, select Linear60 as MEA layout from the drop down list.



Currently, the Custom Display Layout is only available for the data source.

5.2.3.3 Audio Settings

To route individual electrode channels to the Stereo Audio Output on the Interface Board, open the Audio Settings.

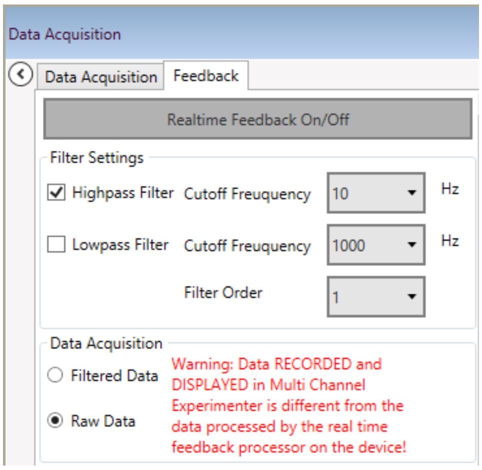


Sound can be either mono or stereo, each electrode channel can be assigned to the two audio channels. Volume control is also independent. The Audio output will generate an audio signal from the raw data of the selected channel(s) in real time.

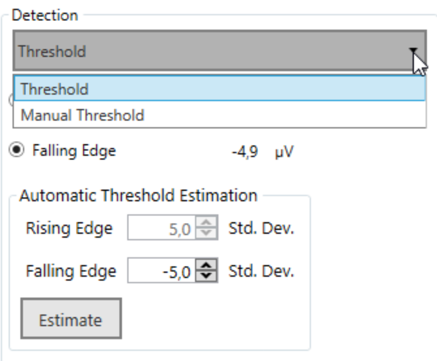
5.2.3.4 Real Time Feedback

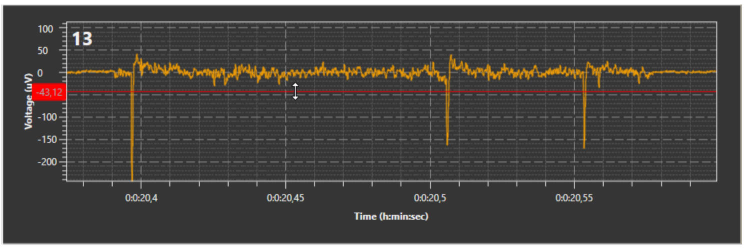
The Real Time Feedback (**RTF**) option can detect spikes and **generate a feedback stimulation with the internal stimulator** with a time delay of only about 1 ms. In addition to starting the internal STG, an **outgoing TTL** can be generated to control an external device by the RTF. The events generated by the RTF are available as triggers on the pink port of the MEA2100 data acquisition. Feedback controls are located on an extra tab in the MEA2100 Data Source. Real Time Feedback is always **only available for the first instance** of the Experimenter.

To achieve this fast feedback, the data is processed on a digital signal processor (DSP) inside the Interface Board before being send to the PC via USB. The DSP can also filter the data before signal detection, therefore the user has the option to send **filtered or unfiltered data** to the PC. A warning is displayed, if filtering is applied, but raw data is recorded.



After filtering, signals are detected based on a detection threshold. The detection threshold can be either positive or negative. Once the RTF is activated, the detection thresholds show up as red lines in the data display. In **Manual Threshold** mode, the threshold for each electrode can be selected manually by typing in a value, or by dragging the red line in the zoom display.

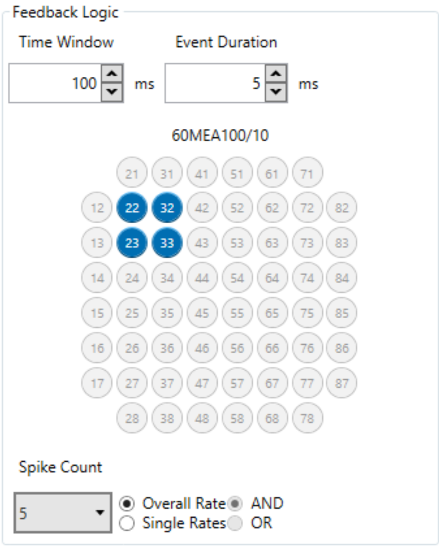




Alternatively, in **Threshold** mode the threshold can be calculated individually for each channel based on the standard deviation of the noise on each channel times a user defined factor. Clicking on **Estimate** will recalculate the standard deviation. Factors between 4 and 6 usually work best, depending on noise and signal size.

Once the data is filtered and events are detected, a user defined **feedback logic** on the DSP determines whether a RTF event is generated. The RTF events can be used as internal Trigger events from the pink port of the MEA2100 Data Source, to generate external TTL pulses via the Digital Out (see 5.2.4.1), or to control the internal Stimulator (see 5.9.3.5).

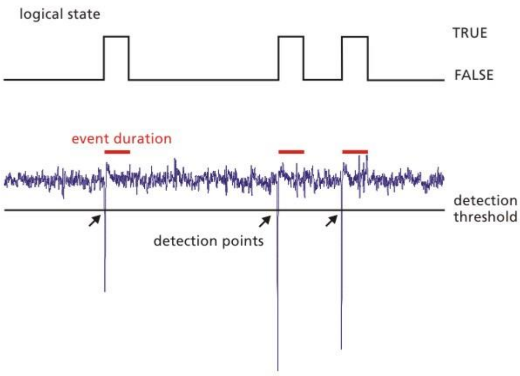
The feedback mechanism operates with **logical states**. The logical state of an electrode, or a combination of electrodes, can be TRUE or FALSE regarding the condition defined by the user. If necessary, the logical states of more than one electrode are combined (see AND and OR function below). An event is generated if the logical states, or the combination of logical states, fulfil the user defined condition.



First, select **one or more channels** from the electrode field which should be monitored for signals. Select a **Time Window** in milliseconds from the numeric updown box. The time window defines the time in which the rates of signals should be counted. For performance reasons, the window is limited to 1000 ms. The time window is not a fixed time bin, but a **moving window**. For example, if the window is set to 1 s, and a rate of 10 Hz is set as condition, the condition is fulfilled as soon as 10 events within a second are detected. Therefore, it may **not** happen that these ten events fall by chance in two separate time bins and are not counted as 10 Hz.

Important: For the purpose of this feature, a Rate is defined as the number of events in the selected time window.

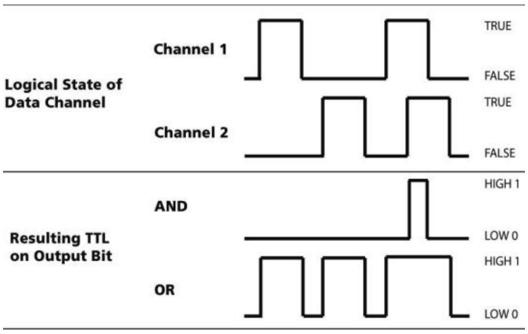
Select the **Event Duration** in milliseconds from the numeric updown box. The Event Duration is the **time after a crossing of the detection threshold** that the detection condition is considered as fulfilled. This duration influences the length of the resulting feedback TTL, if the RTF is used to **control the Digital Out**, and is especially important when the input from several electrodes is combined with the AND and OR function (see below). That means, for the Feedback Logic, the **logical state** of a channel becomes TRUE from the detection point of an event till the end of the event duration. Please see the image below.



If only one channel is used as input, the **duration of the resulting digital TTL pulse** is as long as the logical state is TRUE. If more than one electrode and “Single Rates” is selected, it is necessary to combine the logical states of the different electrodes with the functions **AND or OR**. If AND is selected, a feedback is generated only if **all electrodes** have the logical state TRUE **at the same time**.

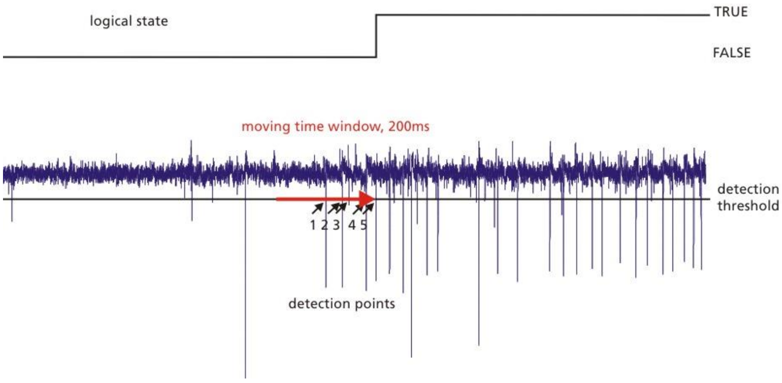
If **OR** is selected, a TTL is generated if **any of the electrodes** has the logical state TRUE. The **length of the TTL** is determined by the **overlap (AND) or addition (OR)** of the TRUE states of the individual electrodes.

Please see the following example:



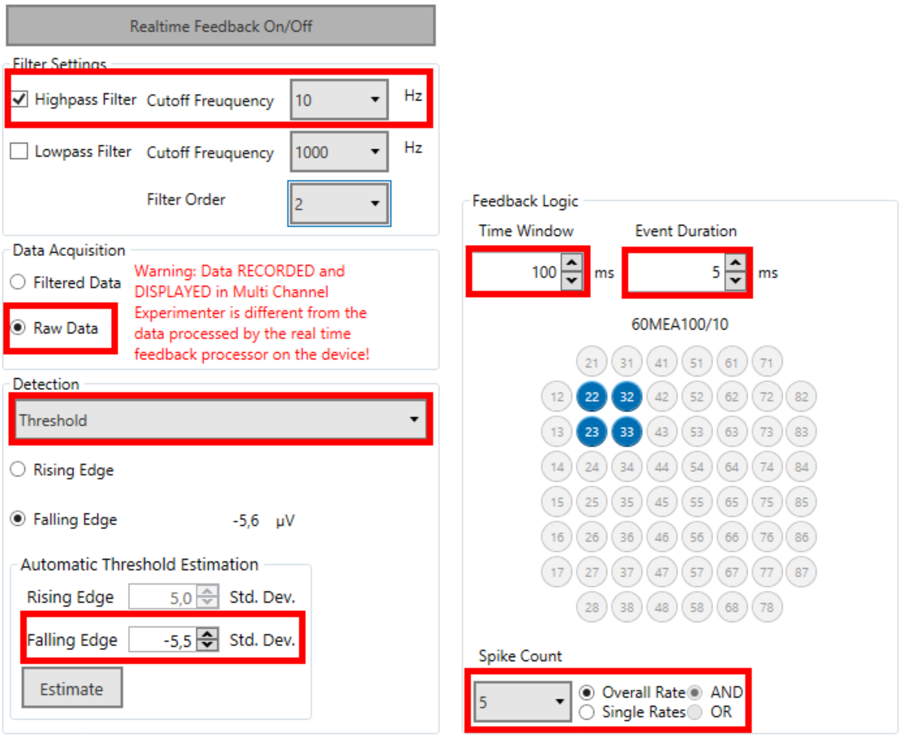
When selecting **Overall Rate** or **one channel only**, the logic option “And” or “Or” is not applicable.

In case of **Rate detection**, the logical state becomes TRUE from the detection point of the **last event that is needed to fulfil the rate condition** till the end of the event duration. If the rate of detected events stays above the rate threshold, the logical state will remain TRUE as long as the rate condition is fulfilled. In the example below, **a window of 200 ms** and a **rate of five events per window** is set as condition. The logical state becomes TRUE upon detection of the fifth spike within 200 ms and stays TRUE because the event rate remains above threshold.



As shown above the **status of the TTL output bit** will remain HIGH as long as the logical state, or the combination of logical states of all selected electrode channels, fulfils the condition defined by the user, including the AND / OR function.

In the example shown below, data is **high pass filtered at 10 Hz** at the DSP before signal detection, and the **raw data is send to the PC** for recording. Four channels are monitored (22, 33, 23 and 33) for spikes, with s detection threshold of 5.5 x SD of the noise. If the spike count is larger than **5 in a 100 ms moving window** in all of the four electrodes combined, the logical state becomes TRUE for 5 ms minimum, or as long as the condition is fulfilled.



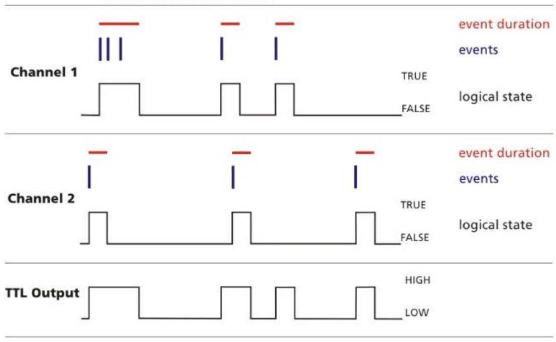
Additional Examples:

The following examples further illustrate the possible combinations of the Feedback Logic. In example one, **single spikes** are detected on two channels combined with the **condition OR**. Hence, the logical state of each channel is TRUE from the detection of an event till the end of the event duration (shown in red). As the two channels are combined with OR, a TTL is generated as long as **any of the two channels**, or both, have the logical state TRUE.

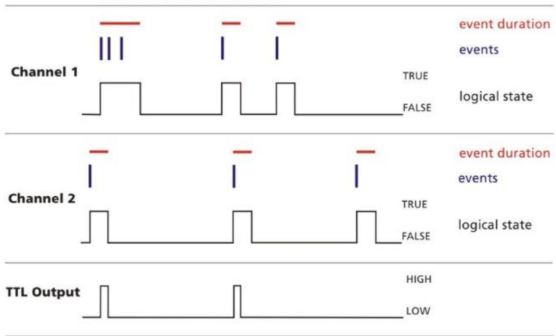
In example two, the same channels are combined with the condition AND. This means, a TTL is generated only if **both channels** have the logical state TRUE. This setting can be used to detect **simultaneous events**. By adjusting the **event duration**, it is possible to define how close together two events have to be to trigger a feedback stimulation.

Important: In extreme cases, if the overlap between the TRUE states of all selected channels is very short (below 20 µs), the generated TTL might be too short to trigger another device.

Example 1: single spike detection, combination with OR

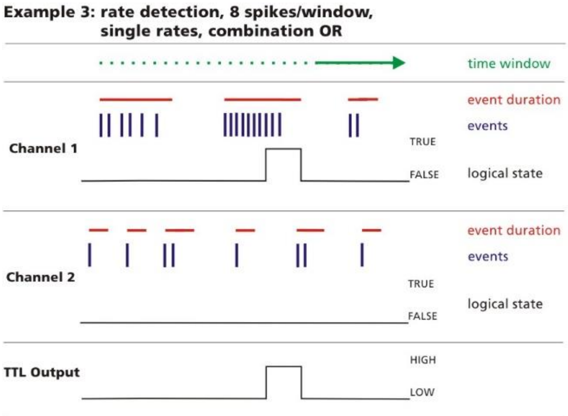


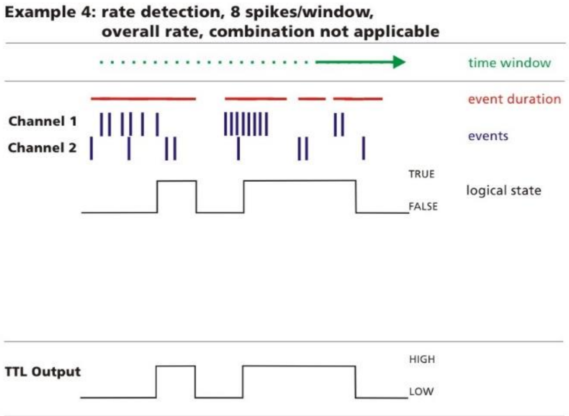
Example 2: single spike detection, combination with AND



In example three, **spike rates** are detected with the condition eight spikes per time window. The two channels are combined with the condition OR. The logical state of channel one becomes TRUE upon **detection of the eighth event** in the time window until the last event that fulfils the condition, **plus the event duration**. In channels two, the event rate is too low. As both channels are combined with OR, the TRUE state of channel one is sufficient to trigger a TTL output.

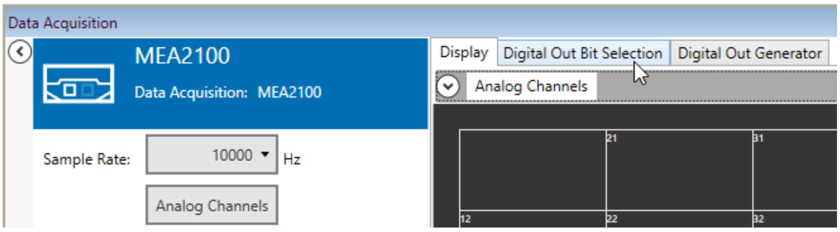
In example four, the **overall event rate** of channel one and two is combined and the condition is again eight events per time window. The addition of both channels results in longer and more TTL outputs. As the activity of both channels is counted together anyway, the combination parameter AND / OR is not applicable.





5.2.4 Digital Out

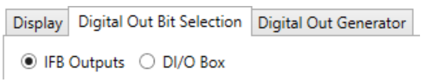
The MEA2100-, ME2100- and W2100-Systems feature an Interface Board with a **16 bit digital output channel** (DigOut). The DigOut channel can generate TTL pulses to **control or synchronize external devices**. The function of the DigOut channel can be controlled in the Data Source instrument. It is possible that several data sources, and hence several instances of the software, share one Interface Board (see chapter 4.1.4). This means all **data sources/software instances have to share** the 16 available DigOut bits. Therefore, one tab in the MEA2100 data source is available to assign the available output bits to the different data sources, and to the available instruments which might use the DigOut.



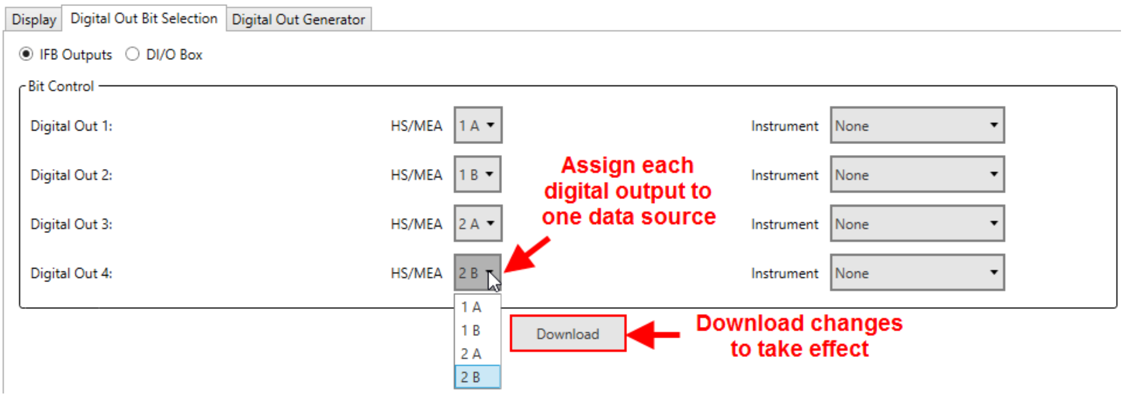
5.2.4.1 Digital Out Bit Selection

The Digital Out Bit Selection tab allows to assign each bit of the Digital Out channel to a certain data source and function. In case of multiple software instances, **only the first instance** in which the Data Source instrument is activated allows to edit the bit assignment. All other instances show the assignment, but only follow passively the settings made in the first instance.

The Digital Out Bit Selection tab is available in two layouts, **IFB Outputs and DI/O Box**. For most applications, four Digital Out bits are enough, so four bits Digital Out 1 to 4, are available as Lemo connectors directly on the Interface Board. If you have this configuration, select IFB outputs, and you will see only the four available outputs.

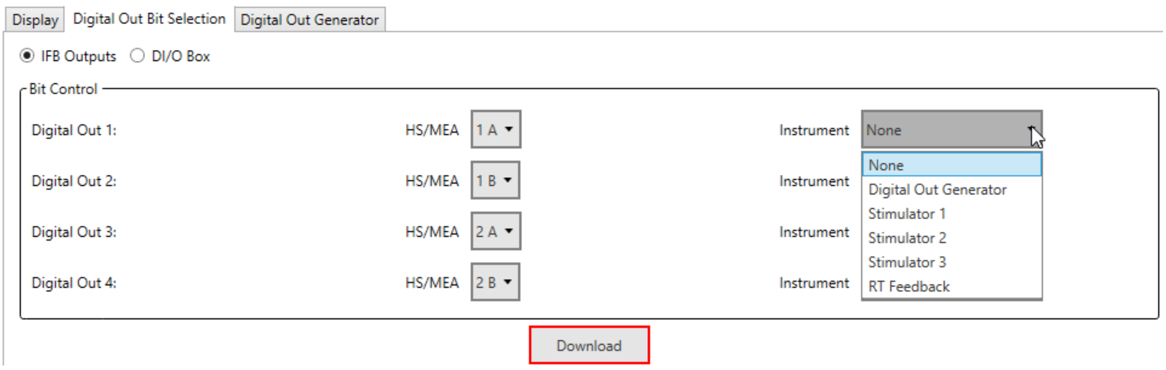


Some applications need all 16 bits. In such cases, a DI/O extension box must be connected to the IFB, which makes all bits available with BNC connectors. If you have such an extension, select DI/O Box to see all available bits. Be aware that the 16 bits on the DI/O are **not in addition** to the four IFB inputs, Digital In 1 - 4 on the IFB are identical to bit 0 - 3 on the DI/O box.



All changes must be downloaded to take effect. The label of a data source is shown on the respective icon.

After assigning the bits to the different data sources, **select the instruments/functions** which should use the respective bit. In a MEA2100-System, three options are available, the Digital Out Generator, the Stimulators and Real Time Feedback. Each bit can be assigned independently to a different function.
Just like the bit assignment to the different data sources, the functions can only be edited in the **first instance.**



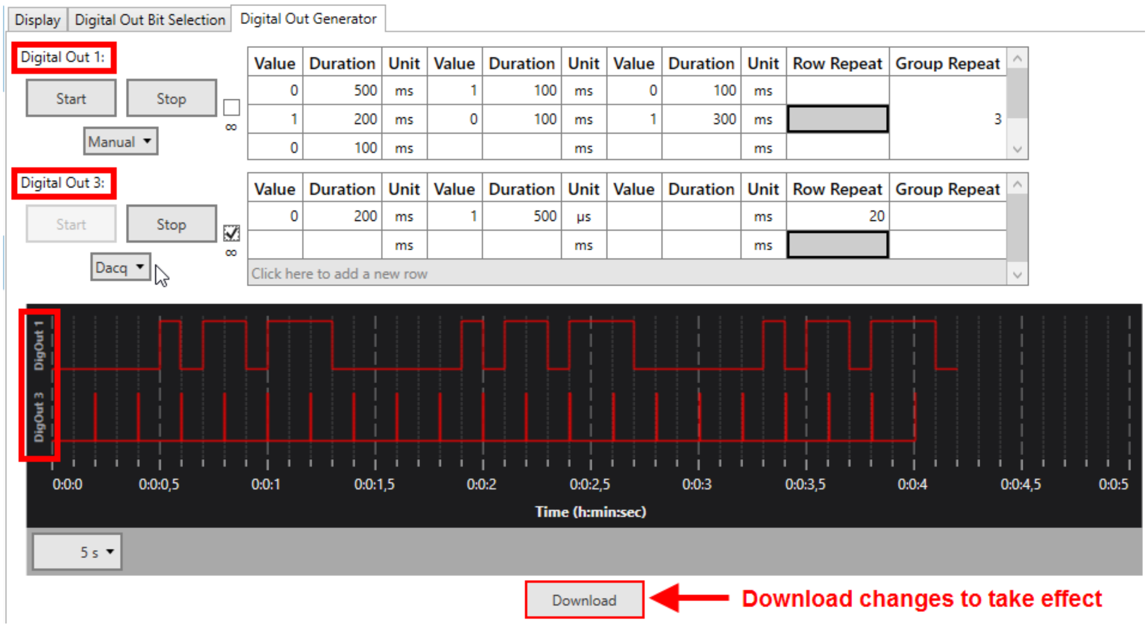
The **Stimulator 1 - 3** option assigns the respective bit to one of the three internal stimulation units. If the Marker option is used (see 5.9.3.8), each Marker can generate a TTL on the Digital Out bit selected here. The **Real Time Feedback** (see 5.2.3.4) can also be used to control the internal stimulators, but also to generate an external TTL pulse on the assigned bit(s).

Finally, the Digital Out Generator can be used to program a selected bit to generate any random pattern of TTL pulses. Again, all changes must be downloaded to take effect.

5.2.4.2 Digital Out Generator

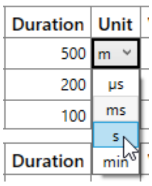
The Digital Out Generator allows to program each bit of the digital out channel to generate a **random pattern of TTL pulses**. The Digital Out Generator tab is only active if at least one digital bit has been assigned to the generator. Only the **assigned bits** will appear in the tab.

Each bit can be programmed with a random pattern of TTLs. Each bit assigned to the Digital Out Generator has its own programming interface and WYSWYG display to show the programmed pattern.

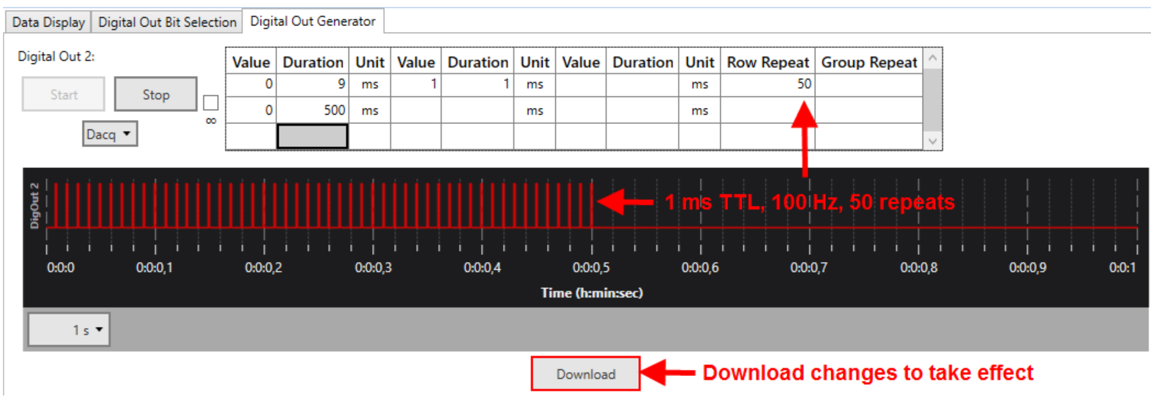


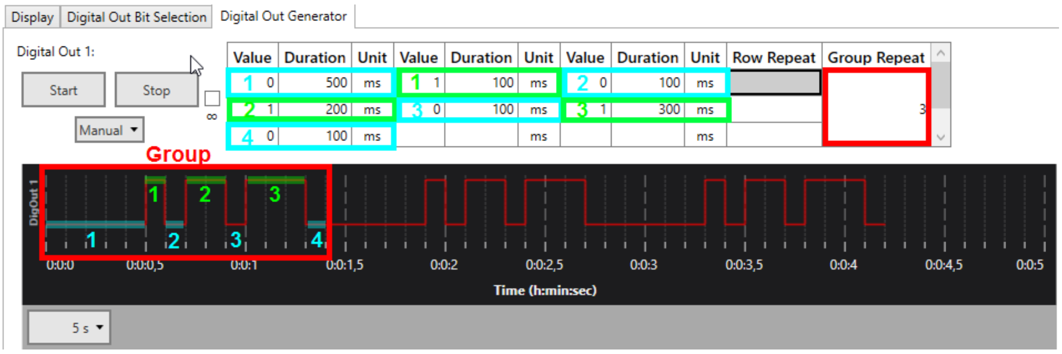
The pattern for each bit can be programmed step by step, with value and duration for each step. Value can **only be 1 (HIGH) or 0 (LOW)**.

Duration can be any time, in **multiples of 20 µs**, which is the time resolution of the Digital Out Generator. The time **unit** can be changed to µs, ms, s or min. Double click on any time unit field and select the desired unit from the drop down menu.

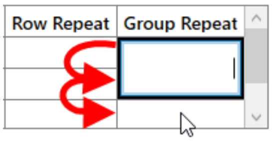


The **Row Repeat** allows repeating the contents of a specific row. If the pattern which should be repeated is longer than one row, it is possible to combine rows to groups, and use the **Group Repeat** function instead.

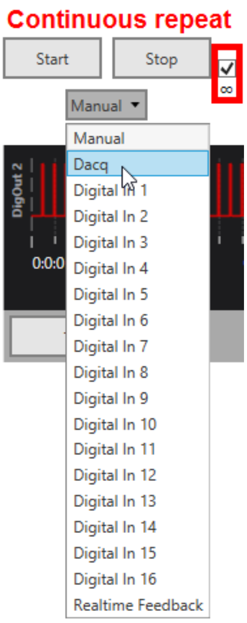




To define a group, hold the **SHIFT and CTRL** key, and click the cells you want to add one by one. You can also remove cells from a group by SHIFT/CTRL click.



The Digital Out Generator can be **started** on different conditions. Stop is always manual. In **Manual** mode, Start and Stop are on manual command. Alternatively, the Generator can be started with the Start of the **data acquisition** (Dacq), or on an incoming TTL on any of the **Digital In** bits. Finally, The **Real Time Feedback** (see 5.2.3.4) can also be used to start the Digital Out Generator.



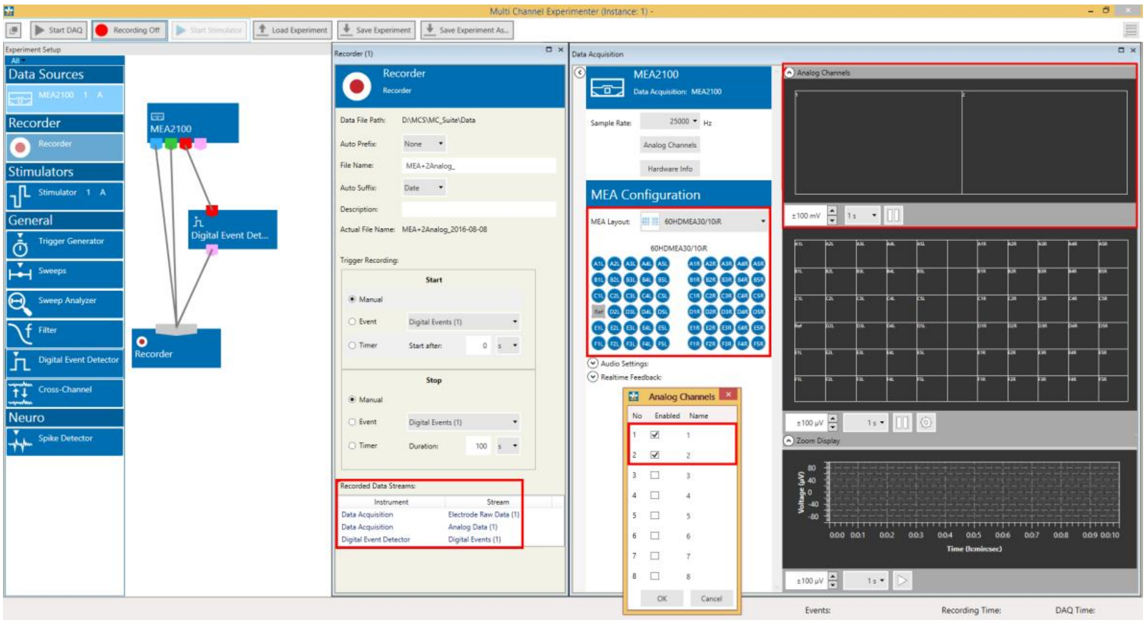
By default, the programmed pattern for each bit will be generated once after each Start command. If the **Continuous** repeat (∞) tick box is active, the pattern will be repeated indefinitely till the data acquisition is stopped, or till a manual Stop command.

The settings and paradigm programmed in the Digital Event Generator are also stored in the Experiment file (see 4.1.2).

5.2.5 Examples: How to ...

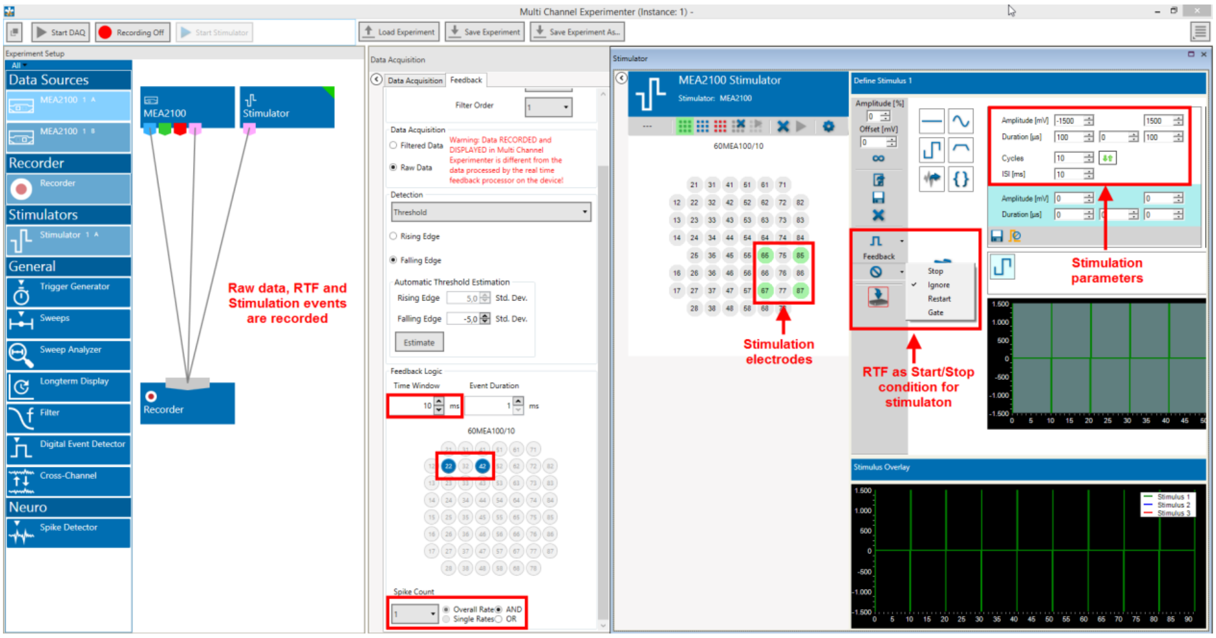
This section highlights a few common uses of the MEA2100-System Data Source and the Digital Out Generator.

5.2.5.1 Record Electrode Raw Data, Analog Data and Trigger Events



In the experiment above, the blue electrode data port and the green analog data port are connected directly to the Recorder. Therefore, both data streams are recorded **continuously**. Analog channels 1 and 2 are activated, and a 60HDMEA layout is selected for the MEA data with 25 kHz sampling rate applied to both. Analog data can come for example from a Patch Clamp amplifier or a temperature sensor. The red digital data port is connected to a Digital Event Detector instrument (please see chapter 5.17, Digital Event Detector), which will generate **Trigger events** based on TTL inputs to the Digital In connectors on the Interface Board. The trigger events will also be recorded, as the pink trigger output port is connected to the Recorder. The digital channel itself will not be recorded, as it is not connected directly to the Recorder.

5.2.5.2 Generate a Closed Loop Stimulation on Simultaneous Spikes on Two Electrodes

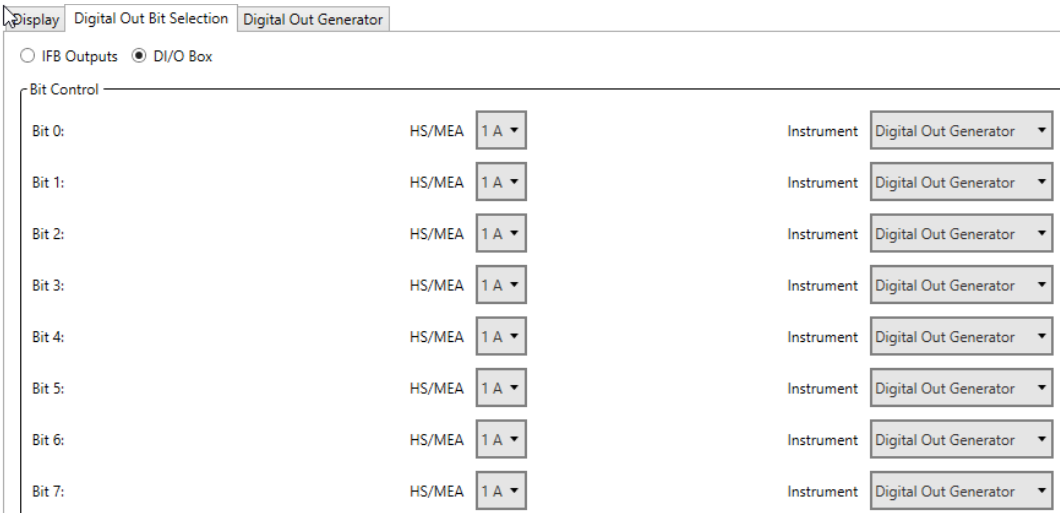


The Real Time Feedback function can for example detect **coincidence of spikes** on two or more electrodes. In the given example, electrodes 22 and 42 are monitored. Data is filtered at 200 Hz high pass to improve spike detection. Even though the RTF works on the filtered data, unfiltered raw data is sent to the computer for recording. Every time spikes occur simultaneously on both electrodes within a time window of 10 ms, the feedback condition is fulfilled. The stimulator instrument is programmed to start on the Feedback, and to deliver a short 100 Hz burst of stimuli to a cluster of four electrodes in the lower right quadrant of the MEA. If the feedback condition is fulfilled again while the Stimulator is still running, this will be ignored. Please see chapter 5.9. Stimulators, MEA2100 Stimulator for details on programming of the Stimulator. Unfiltered raw data and events generated by the RTF and stimulation are recorded.

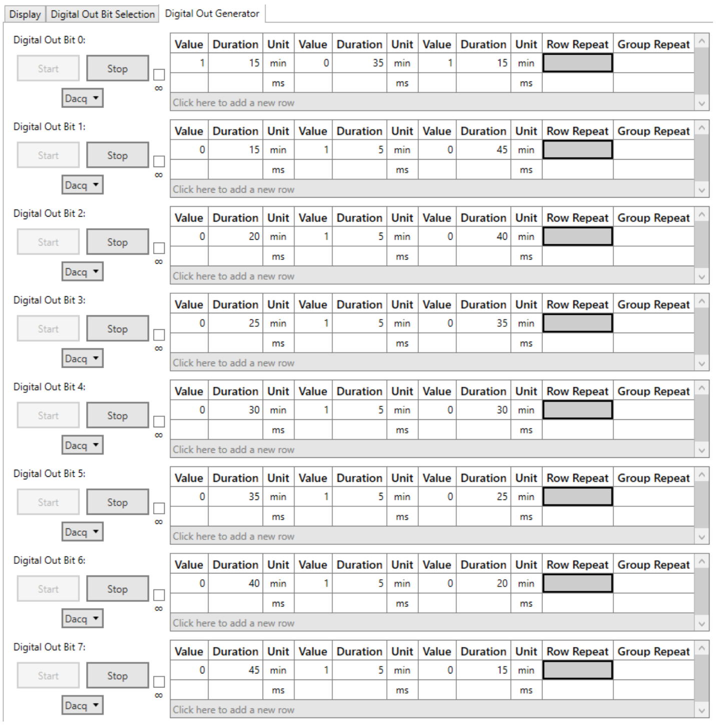
5.2.5.3 Control a Valve Perfusion System with the Digital Out Generator

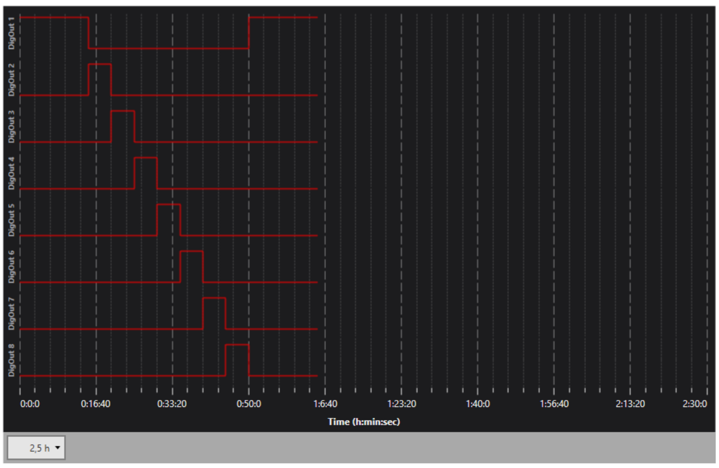
In valve controlled perfusion systems, like the [VC-8 from Warner Instruments](), the valves can often be controlled by external TTL pulses. Usually, a LOW state on the TTL input of each valve means valve closed, while a HIGH state means valve open. Assuming that a DI/O box is connected to the MEA2100 Interface Board, and bits 0 - 7 are connected to the valves 1 - 8, the following setup of the Digital Out Generator would result in valve 1 being open for 15 min (baseline with medium/ACSF), then valves 2 - 8 for five minutes each, and then valve 1 again for 15 min for washout.

First, assign eight bits to the data acquisition you want to use, and select Digital Out Generator as Instrument for all of them. Download the settings.



All Digital Out bits are started simultaneously with the start of the DAQ.





5.3 Data Source: MEA2100-Mini-System

5.3.1 Description and Purpose

The MEA2100-Mini-System is supported in all available headstage variations. One **SCU** (Signal Collector Unit) with all attached headstages is always operated independently by one instance of the software. The icon indicates which SCU it represents. **1 and 2** indicate the SCU connected to port 1 or 2 of the Interface Board. Once the MEA2100-Mini-System is added to the instrument configuration, the MEA2100-Mini Stimulator and the SCU Stimulator will also become available as instruments.

5.3.2 Data Ports and Export Options

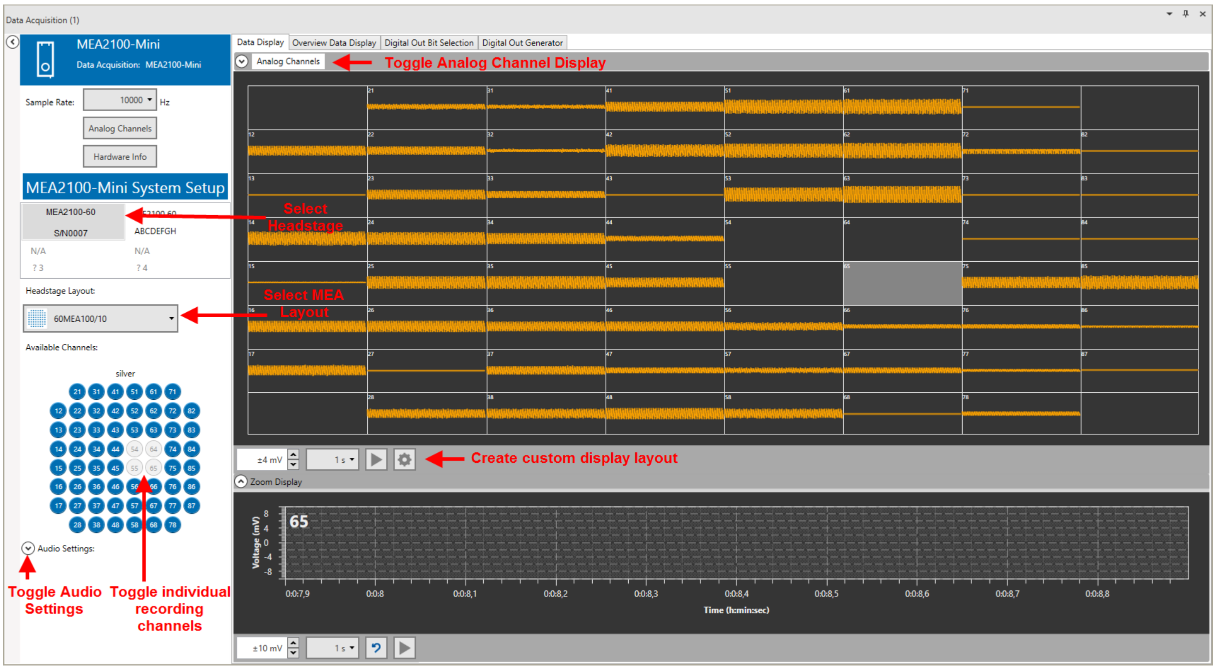
The MEA2100-Mini-System has four output ports: Electrode Raw Data (blue), Analog Data (green) Digital Data (red) and Feedback Events (pink). As with all data sources, there are no input ports. The MEA2100-Mini-System can be connected directly with any number of other instruments with blue or red input ports. The green port can only be connected to the Recorder at the moment. There are no export options.

5.3.3 Single Instance or Multiple Instance Mode

The MEA2100-Mini System can operate up to four headstages on one SCU. These headstages can all be controlled from within a single instance of the Experimenter (Single Instance Mode). Alternatively, each headstage can be treated as an individual device, and can be operated independently by its own instance of the Experimenter. This can be decided by the user in the Settings, see chapter 4.5 for details. The default is Single Instance Mode.

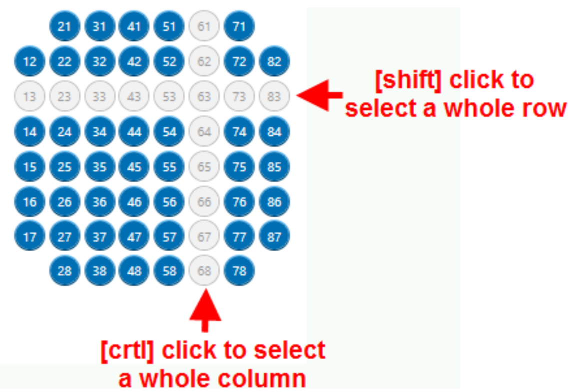
5.3.4 Operation in Single Instance Mode

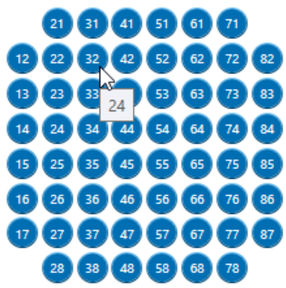
The MEA2100-Mini data source control window shows **all available data channels for all connected headstages**, and optionally also the additional Analog Input channels from the Interface Board. One headstage is visible at a time. All headstages are shown with their serial number. Click on the headstage to see the respective data. This has no influence on recording, all active channels from all active headstages will be recorded, independently from the display.



The MEA layout, settings for the MEA2100-Mini stimulator and other instruments connected to the data source also depend on the **currently selected headstage.** All settings apply only to the selected headstage, which makes **individual changes per headstage** possible, and allows the **independent control of the stimulators** in each headstage. For example, a filter connected to the MEA2100-Mini data source can have different filter settings for each headstage. To adjust them, select the respective headstage and the individual settings will show in the filter tool. The same applies to the MEA2100-Mini stimulator. The stimulation paradigm can be different for each headstage, changes only apply to the stimulator of the currently selected headstage.

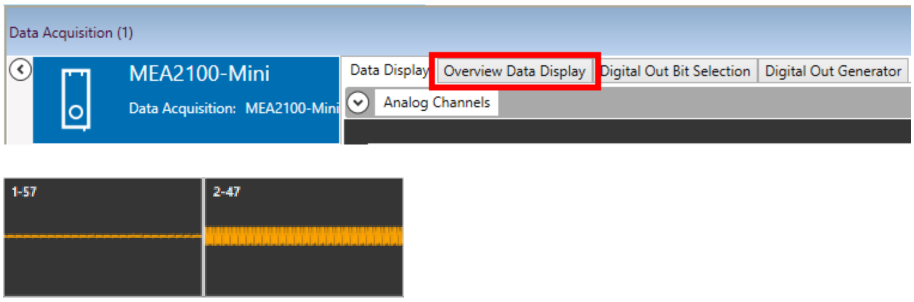
Individual channels can be **toggled** by clicking on them. Deactivated channels will neither be displayed nor recorded. **[shift] click or [crtl] click** on any electrode will toggle the whole row or column. Hover the mouse pointer over an electrode channel for a few seconds to bring up the **hardware channel ID** for that electrode.





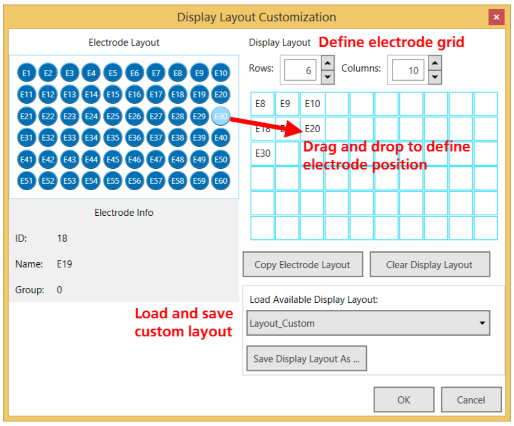
5.3.4.1 Overview Data Display

As soon as more than one headstage is connected, the tab Overview Data Display will appear. On this display, the data of all connected headstages can be seen in parallel. Channels are labeled with 1 - 4 for the headstage and the channel number from the selected layout, respectively.

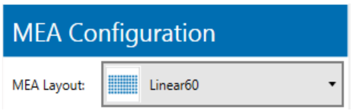


5.3.4.2 Custom Display Layouts

To generate a custom layout for the display, click the Settings icon under the main display. The following menu will allow to generate any desired electrode grid, and then assign channels to any grid position.



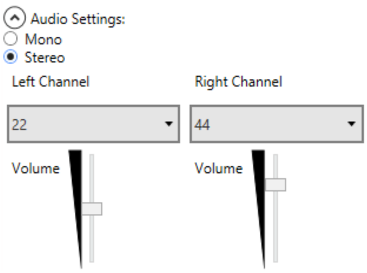
The label of the electrodes is defined by the layout selected from the drop down list. To get the linear hardware channel IDs, select Linear60 as MEA layout from the drop down list.



Currently, the **Custom Display Layout** is only available for the data source. In contrast to the electrode layouts from the drop down list, a custom display layout is **not adopted** by the other instruments.

5.3.4.3 Audio Settings

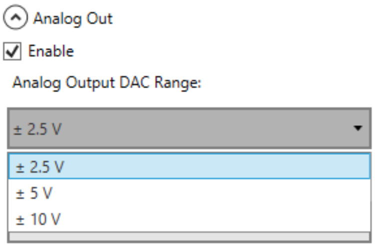
To route individual electrode channels to the Stereo Audio Output on the Interface Board, open the Audio Settings.



Sound can be either mono or stereo, each electrode channel can be assigned to the two audio channels. Volume control is also independent. The Audio output will generate an audio signal from the raw data of the selected channel(s) in real time.

5.3.4.4 Analog Output ADC/DAC Range

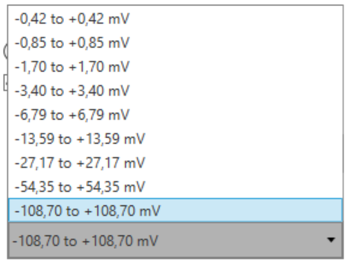
The MCS-SCU has an Analog Out port, and it’s possible to adjust the **Analog Output ADC and DAC Range**. As soon as the Analog Out is enabled, the signal input range of the MEA2100-Mini headstage can be converted to different output ranges on the analog output, depending on the input range of the connected 3rd party data acquisition:



AO DAC Range	Conversion factor	Gain setting
± 2.5 V	0.043	23
± 5 V	0.022	46
± 10 V	0.011	92

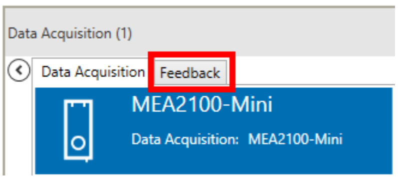
The signal on the AO output **times the conversion factor**, or **divided by the Gain setting**, is the actual signal size.

The **ADC range** setting is necessary as the 24 bit resolution incoming digital data needs to be converted back to an analog signal with only 16 bit resolution. For best results, select the **smallest range** which will cover your expected maximum signal size.



5.3.4.5 Real Time Feedback

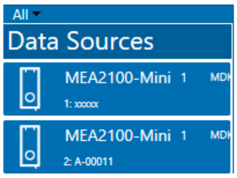
The Real Time Feedback (**RTF**) option can detect spikes and **generate a feedback stimulation with the internal stimulator** with a time delay of only about 1 ms. In addition to starting the internal STG, an **outgoing TTL** can be generated to control an external device by the RTF. The events generated by the RTF are available as triggers on the pink port of the MEA2100-Mini data acquisition. Feedback controls are located on an extra tab in the MEA2100-Mini Data Source. Real Time Feedback is always **only available for the first instance** of the Experimenter.



More details on Real Time Feedback are described in the Data Source: MEA2100 section in chapter 5.2.3.4.

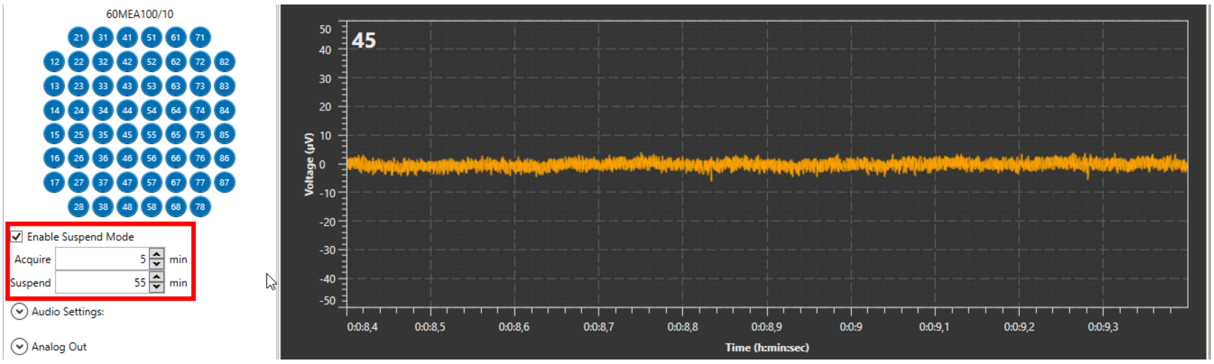
5.3.5 Operation in Multiple Instance Mode

If Multiple Instance Mode is selected in the main Settings menu, each connected MEA2100-Mini headstage will show up as individual Data Source device in the Experimenter and can be operated separate instances of the Experimenter. Otherwise, the operation is identical to Single Instance Mode.



5.3.6 Suspend Mode

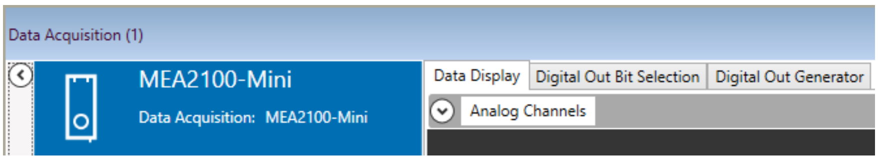
The Suspend Mode function is only available in **Multiple Instance Mode and for the latest generation of SCUs**. It allows to set the MEA2100-Mini to record for a period of time (**Acquire**). After that, the system is automatically powered down for the time set under **Suspend**.



This function allows to reduce the heat emission of the Mini headstage while placed in the incubator, as the electronics are shut down during the Suspend period, and no extra heat is produced.

5.3.7 Digital Out

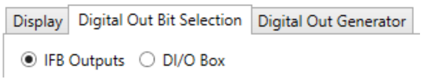
The MEA2100-, ME2100- and W2100-Systems feature an Interface Board with a **16 bit digital output channel** (DigOut). The DigOut channel can generate TTL pulses to **control or synchronize external devices**. The function of the DigOut channel can be controlled in the Data Source instrument. It is possible that several data sources, and hence several instances of the software, share one Interface Board (see chapter 4.1.4). This means all **data sources/software instances have to share** the 16 available DigOut bits. Therefore, one tab in the ME2100 data source is available to assign the available output bits to the different data sources, and to the available instruments which might use the DigOut.



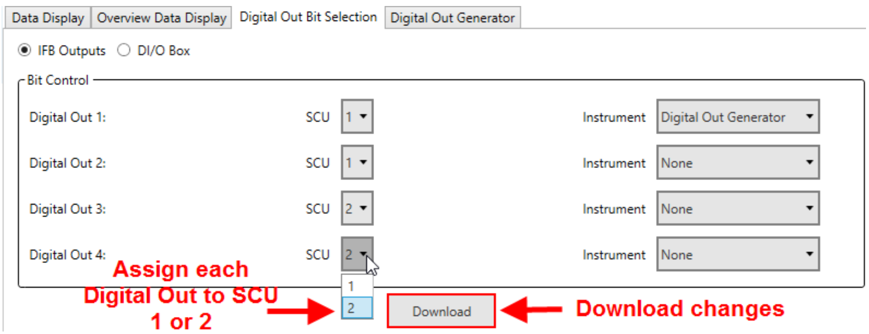
5.3.7.1 Digital Out Bit Selection

The Digital Out Bit Selection tab allows to assign each bit of the Digital Out channel to a certain data source and function. In case of multiple software instances, **only the first instance** in which the Data Source instrument is activated allows to edit the bit assignment. All other instances show the assignment, but only follow passively the settings made in the first instance.

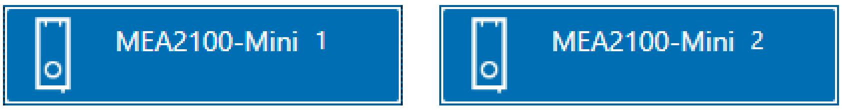
The Digital Out Bit Selection tab is available in two layouts, **IFB Outputs and DI/O Box**. For most applications, four Digital Out bits are enough, so four bits Digital Out 1 to 4, are available as Lemo connectors directly on the Interface Board. If you have this configuration, select IFB outputs, and you will see only the four available outputs.



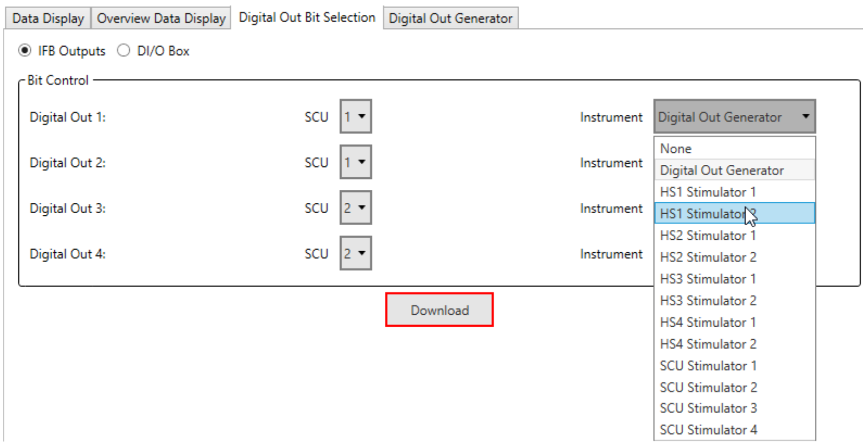
Some applications need all 16 bits. In such cases, a DI/O extension box must be connected to the IFB, which makes all bits available with BNC connectors. If you have such an extension, select DI/O Box to see all available bits. Be aware that the 16 bits on the DI/O are **not in addition** to the four IFB inputs, Digital In 1 - 4 on the IFB are identical to bit 0 - 3 on the DI/O box.



All changes must be downloaded to take effect. The label of a data source is shown on the respective icon. 1 represents the first and 2 the second connected SCU.



After assigning the bits to the different data sources, **select the instruments/functions** which should use the respective bit. In a MEA2100-Mini-System, three options are available, the Digital Out Generator, the headstage stimulators and the SCU stimulators. Each bit can be assigned independently to a different function. Just like the bit assignment to the different data sources, the functions can only be edited in the **first instance.**



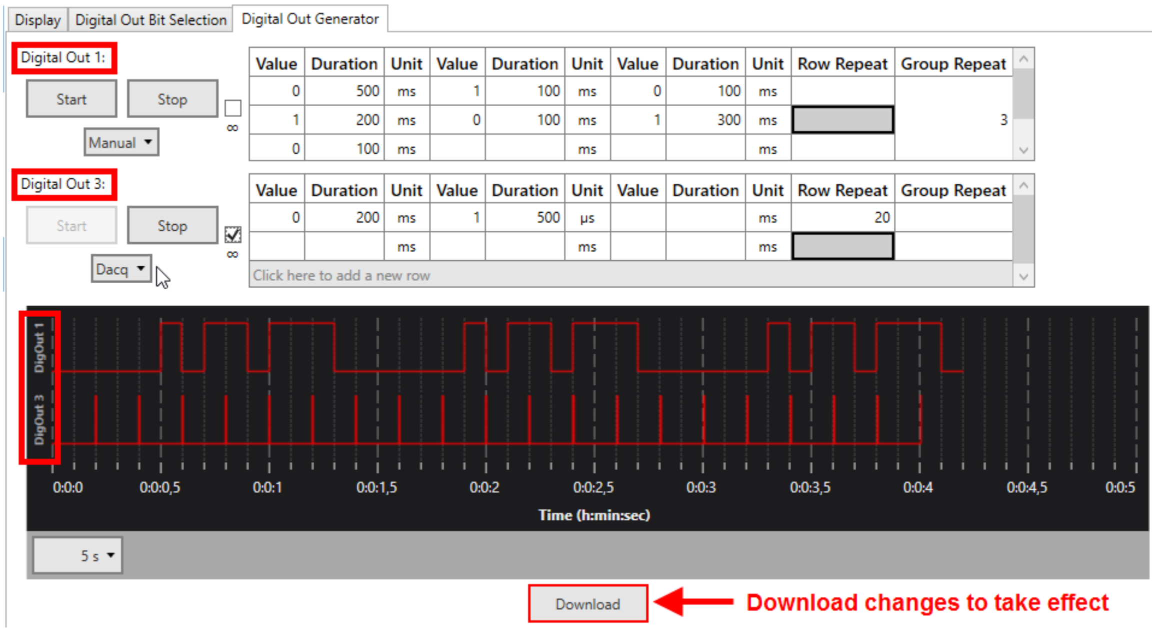
The **HS1 – 4 Stimulator 1 or 2** option assigns the respective DigOut to one of the internal stimulation units of each headstage. If the Marker option is used (see 5.9.3.8), each Marker can generate a TTL on the Digital Out bit selected here. The **SCU Stimulator 1 – 4** refers to the four-channel stimulator integrated into the SCU.

The **Digital Out Generator** can be used to program a selected bit to generate any random pattern of TTL pulses. Again, all changes must be downloaded to take effect.

5.3.7.2 Digital Out Generator

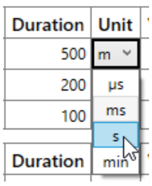
The Digital Out Generator allows to program each bit of the digital out channel to generate a **random pattern of TTL pulses**. The Digital Out Generator tab is only active if at least one digital bit has been assigned to the generator. Only the **assigned bits** will appear in the tab.

Each bit can be programmed with a random pattern of TTLs. Each bit assigned to the Digital Out Generator has its own programming interface and WYSWYG display to show the programmed pattern.

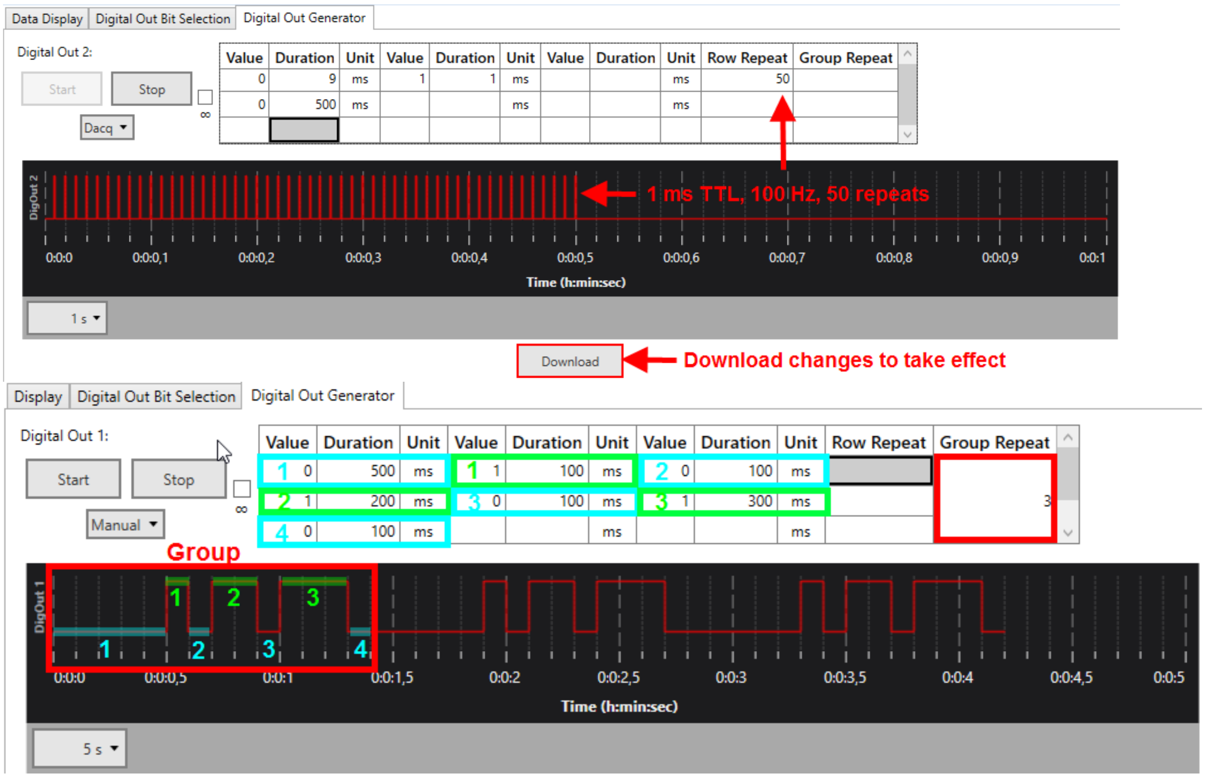


The pattern for each bit can be programmed step by step, with value and duration for each step. Value can **only be 1 (HIGH) or 0 (LOW)**.

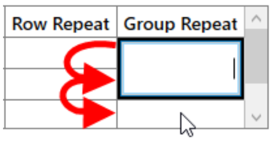
Duration can be any time, in **multiples of 20 µs**, which is the time resolution of the Digital Out Generator. The time **unit** can be changed to µs, ms, s or min. Double click on any time unit field and select the desired unit from the drop down menu.



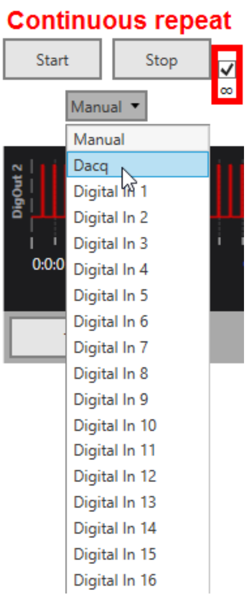
The **Row Repeat** allows repeating the contents of a specific row. If the pattern which should be repeated is longer than one row, it is possible to combine rows to groups, and use the **Group Repeat** function instead.



To define a group, hold the **SHIFT and CTRL** key, and click the cells you want to add one by one. You can also remove cells from a group by SHIFT/CTRL click.



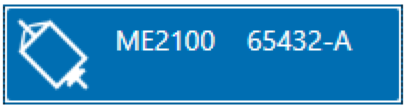
The Digital Out Generator can be **started** on different conditions. Stop is always manual. In **Manual** mode, Start and Stop are on manual command. Alternatively, the Generator can be started with the Start of the **data acquisition** (Dacq), or on an incoming TTL on any of the **Digital In** bits.



By default, the programmed pattern for each bit will be generated once after each Start command. If the **Continuous** repeat (∞) tick box is active, the pattern will be repeated indefinitely till the data acquisition is stopped, or till a manual Stop command.

The settings and paradigm programmed in the Digital Event Generator are also stored in the Experiment file (see 4.1.2).

5.4 Data Source: ME2100-System



5.4.1 Description and Purpose

The ME2100-System is supported in all available headstage variations. One **ME2100-SCU** (Signal Collector Unit) with all attached headstages is always operated independently by one instance of the software. The icon indicates which headstage it represents. **A and B** indicate the SCU connected to port 1 or 2 of the Interface Board. Once the ME2100-System is added to the instrument configuration, the ME2100 Stimulator and the SCU Stimulator will also become available as instruments.

5.4.2 Data Ports and Export Options

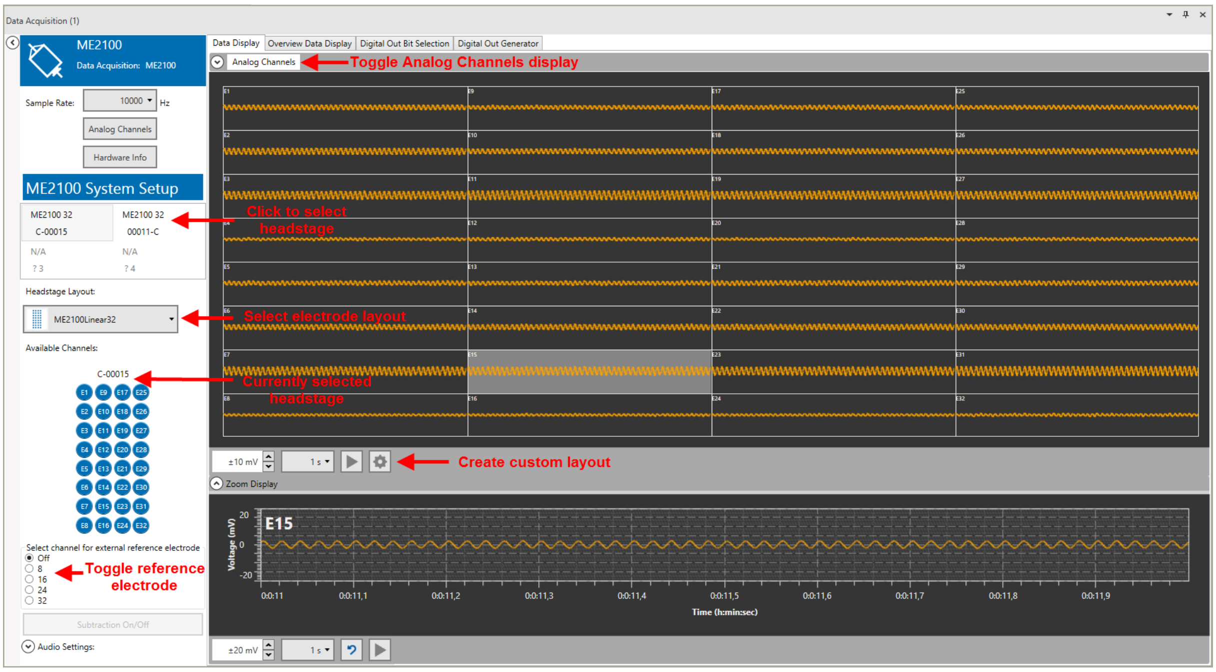
The ME2100-System has four output ports: Electrode Raw Data (blue), Analog Data (green) Digital Data (red) and Feedback Events (pink). As with all data sources, there are no input ports. The ME2100-System can be connected directly with any number of other instruments with blue or red input ports. The green port can only be connected to the Recorder at the moment. There are no export options.

5.4.3 Single Instance or Multiple Instance Mode

The MEA2100-Mini System can operate up to four headstages on one SCU. These headstages can all be controlled from within a single instance of the Experimenter (Single Instance Mode). Alternatively, each headstage can be treated as an individual device, and can be operated independently by its' own instance of the Experimenter. This can be decided by the user in the Settings, see chapter 4.5 for details. The default is Single Instance Mode.

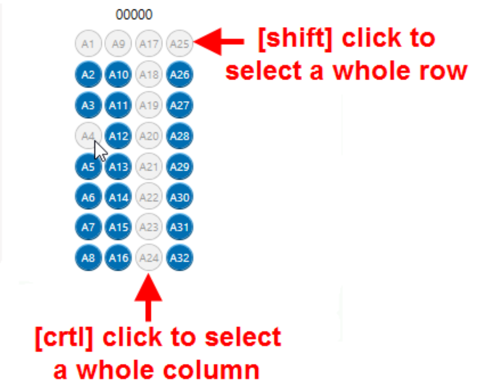
5.4.4 Operation in Single Instance Mode

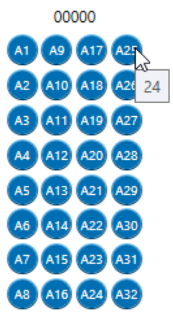
The ME2100 data source control window shows **all available data channels for all connected headstages**, and optionally also the additional Analog Input channels from the Interface Board. One headstage is visible at a time. All headstages are shown with their serial number. Click on the headstage to see the respective data. This has no influence on recording, all active channels from all active headstages will be recorded, independently from the display.



Settings for the ME2100 stimulator and other instruments connected to the ME2100 data source also depend on the **currently selected headstage.** All settings apply only to the selected headstage, which makes **individual changes per headstage** possible, and allows the **independent control of the stimulators** in each headstage. For example, a filter connected to the ME2100 data source can have different filter settings for each headstage. To adjust them, select the respective headstage and the individual settings will show in the filter tool. The same applies to the ME2100 stimulator. The stimulation paradigm can be different for each headstage, changes only apply to the stimulator of the currently selected headstage.

Individual channels can be **toggled** by clicking on them. Deactivated channels will neither be displayed nor recorded. **[shift] click or [crtl] click** on any electrode will toggle the whole row or column. Hover the mouse pointer over an electrode channel for a few seconds to bring up the **hardware channel ID** for that electrode.

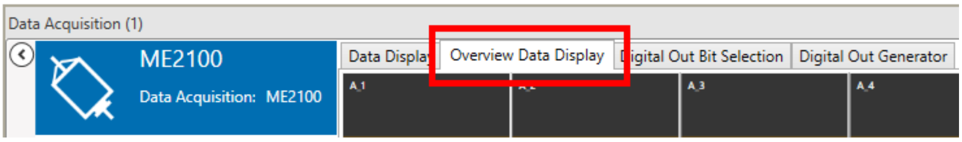




By default, all channels are shown in linear layout. To adjust the display layout to the electrode layout, it is possible to create a custom layout.

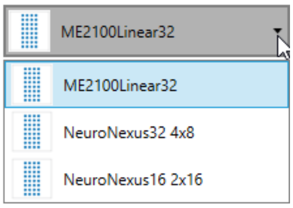
5.4.4.1 Overview Data Display

As soon as more than one headstage is connected, the tab Overview Data Display will appear. On this display, the data of all connected headstages can be seen in parallel. Channels are labeled with A - D for the headstage and the linear channel number.

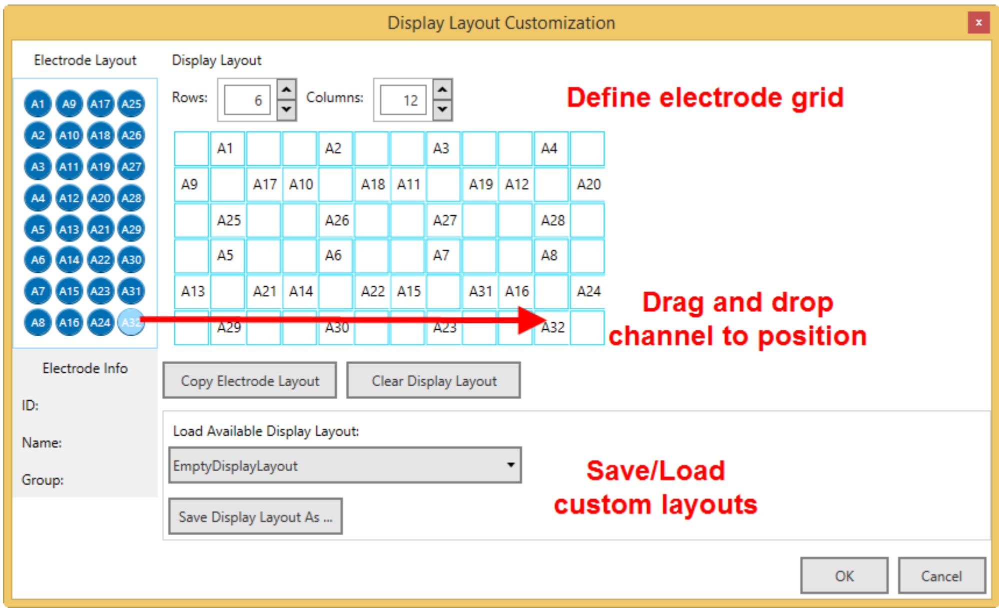


5.4.4.2 Electrode Layouts and Display Layouts

The channel layout of some commonly used electrodes is available from a drop-down list. This **electrode layout** will automatically also be used in **all other instruments**. If you need a layout implemented into the list, please write to [MCS support](), and provide the datasheet of the electrode.



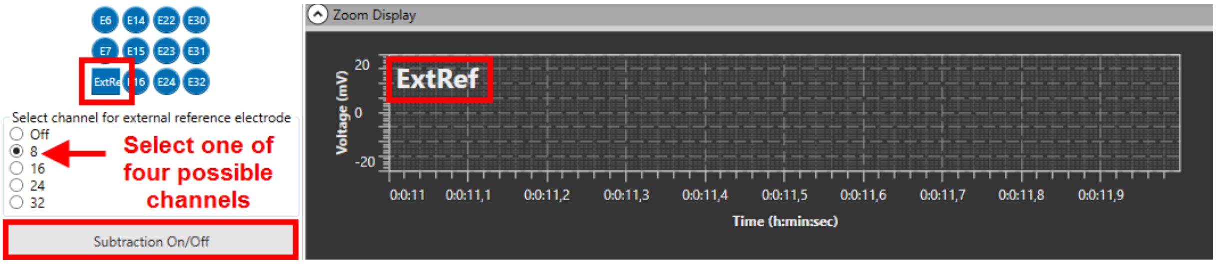
To generate a custom layout for the display, click the Settings icon under the main display. The following menu will allow to generate any desired electrode grid, and then assign channels to any grid position.



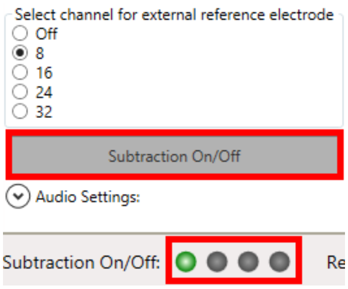
Currently, the **Custom Display Layout** is only available for the data source. In contrast to the electrode layouts from the drop down list, a custom display layout is **not adopted** by the other instruments.

5.4.4.3 Reference Electrode

The ME2100 Headstages have a **reference input**, which can be connected to an external reference electrode. By default, the reference input is not used. To use the reference, one recording channel must be sacrificed. Recording channels 8, 16, 24 or 32 can be used to be **replaced by the reference input**. If activated, the respective data display shows the data from the reference input instead the selected channel, but no subtraction happens yet. This allows monitoring the **signal on the reference input**.

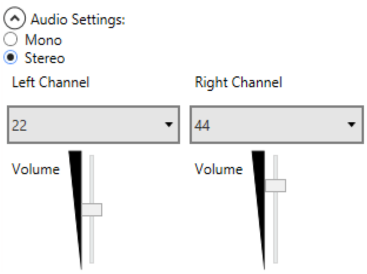


If the subtraction is activated, the Ref input signal gets subtracted from all other channels. This can be set individually for each headstage and is indicated by a virtual LED on the lower main bar.



5.4.4.4 Audio Settings

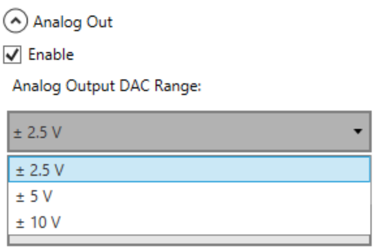
To route individual electrode channels to the Stereo Audio Output on the Interface Board, open the Audio Settings.



Sound can be either mono or stereo, each electrode channel can be assigned to the two audio channels. Volume control is also independent. The Audio output will generate an audio signal from the raw data of the selected channel(s) in real time.

5.4.4.5 Analog Output ADC/DAC Range

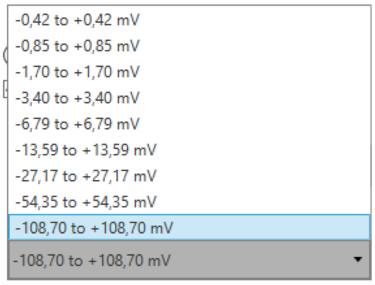
The MCS-SCU has an Analog Out port, and it's possible to adjust the **Analog Output ADC and DAC Range**. As soon as the Analog Out is enabled, the signal input range of the ME2100 can be converted to different output ranges on the analog output, depending on the input range of the connected 3rd party data acquisition:



AO DAC Range	Conversion factor ME2100-HS / µPA	Gain setting ME2100-HS / µPA
± 2.5 V	0.092 / 0.1	11 / 10
± 5 V	0.046 / 0.05	22 / 20
± 10 V	0.023 / 0.025	43 / 40

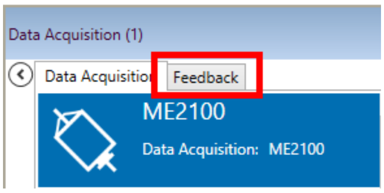
The signal on the AO output **times the conversion factor**, or **divided by the Gain setting**, is the actual signal size.

The **ADC range** setting is necessary as the 24 bit resolution incoming digital data needs to be converted back to an analog signal with only 16 bit resolution. For optimal results, select the **smallest range** which will cover your expected maximum signal size.



5.4.4.6 Real Time Feedback

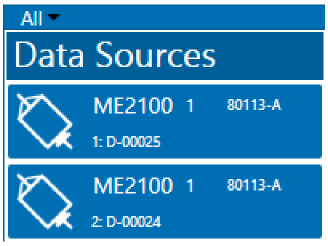
The Real Time Feedback (**RTF**) option can detect spikes and **generate a feedback stimulation with the internal stimulator** with a time delay of only about 1 ms. In addition to starting the internal STG, an **outgoing TTL** can be generated to control an external device by the RTF. The events generated by the RTF are available as triggers on the pink port of the ME2100 data acquisition. Feedback controls are located on an extra tab in the ME2100 Data Source. Real Time Feedback is always **only available for the first instance** of the Experimenter.



More details on Real Time Feedback are described in the Data Source: MEA2100 section in chapter 5.2.3.4.

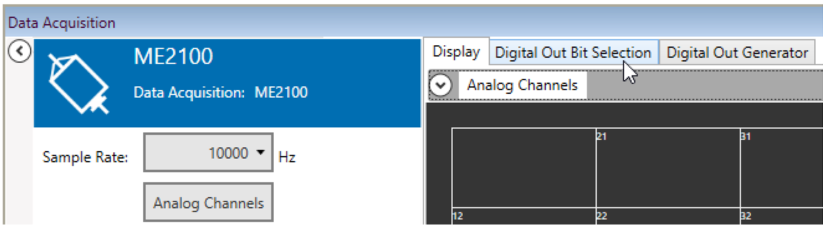
5.4.5 Operation in Multiple Instance Mode

If Multiple Instance Mode is selected in the main Settings menu, each connected ME2100 headstage will show up as individual Data Source device in the Experimenter, and can be operated separate instances of the Experimenter. Otherwise, the operation is identical to Single Instance Mode.



5.4.6 Digital Out

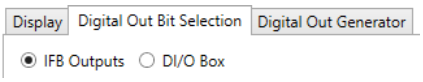
The MEA2100-, ME2100- and W2100-Systems feature an Interface Board with a **16 bit digital output channel** (DigOut). The DigOut channel can generate TTL pulses to **control or synchronize external devices**. The function of the DigOut channel can be controlled in the Data Source instrument. It is possible that several data sources, and hence several instances of the software, share one Interface Board (see chapter 4.1.4). This means all **data sources/software instances have to share** the 16 available DigOut bits. Therefore, one tab in the ME2100 data source is available to assign the available output bits to the different data sources, and to the available instruments which might use the DigOut.



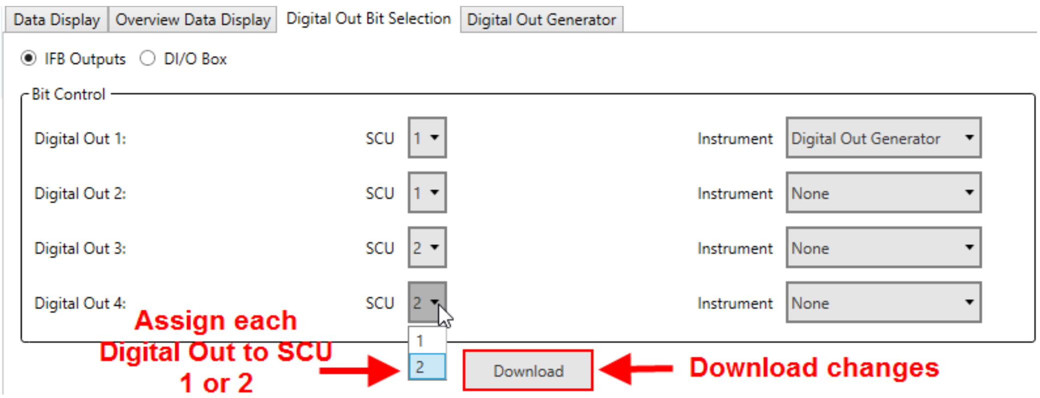
5.4.6.1 Digital Out Bit Selection

The Digital Out Bit Selection tab allows to assign each bit of the Digital Out channel to a certain data source and function. In case of multiple software instances, **only the first instance** in which the Data Source instrument is activated allows to edit the bit assignment. All other instances show the assignment, but only follow passively the settings made in the first instance.

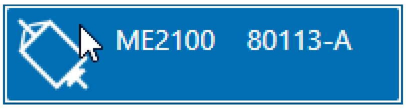
The Digital Out Bit Selection tab is available in two layouts, **IFB Outputs and DI/O Box**. For most applications, four Digital Out bits are enough, so four bits Digital Out 1 to 4, are available as Lemo connectors directly on the Interface Board. If you have this configuration, select IFB outputs, and you will see only the four available outputs.

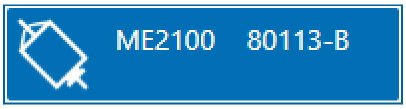


Some applications need all 16 bits. In such cases, a DI/O extension box must be connected to the IFB, which makes all bits available with BNC connectors. If you have such an extension, select DI/O Box to see all available bits. Be aware that the 16 bits on the DI/O are **not in addition** to the four IFB inputs, Digital In 1 - 4 on the IFB are identical to bit 0 - 3 on the DI/O box.

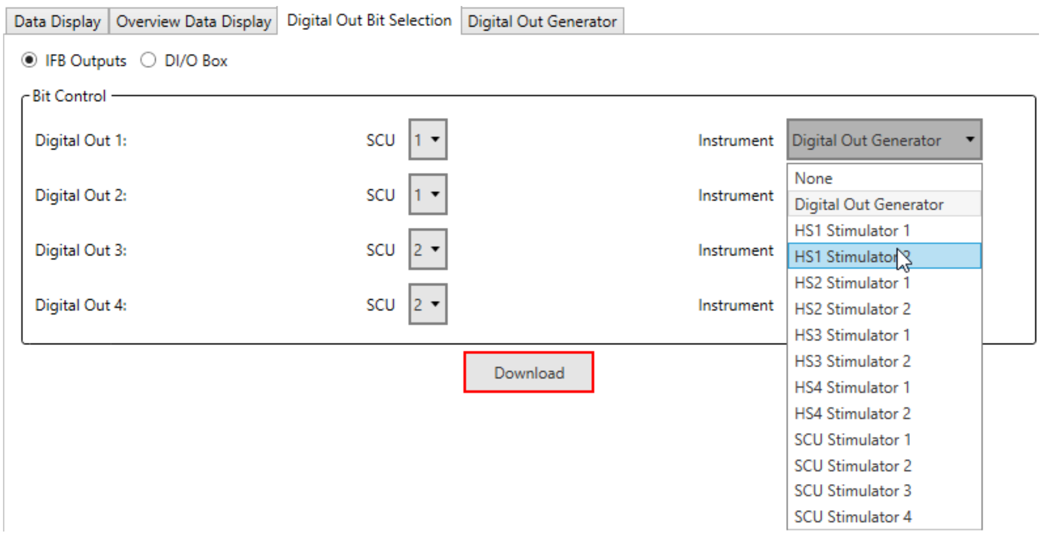


All changes must be downloaded to take effect. The label of a data source is shown on the respective icon. A represents the first and B the second connected SCU.





After assigning the bits to the different data sources, **select the instruments/functions** which should use the respective bit. In a MEA2100-Mini-System, three options are available, the Digital Out Generator, the headstage stimulators and the SCU stimulators. Each bit can be assigned independently to a different function. Just like the bit assignment to the different data sources, the functions can only be edited in the **first instance.**

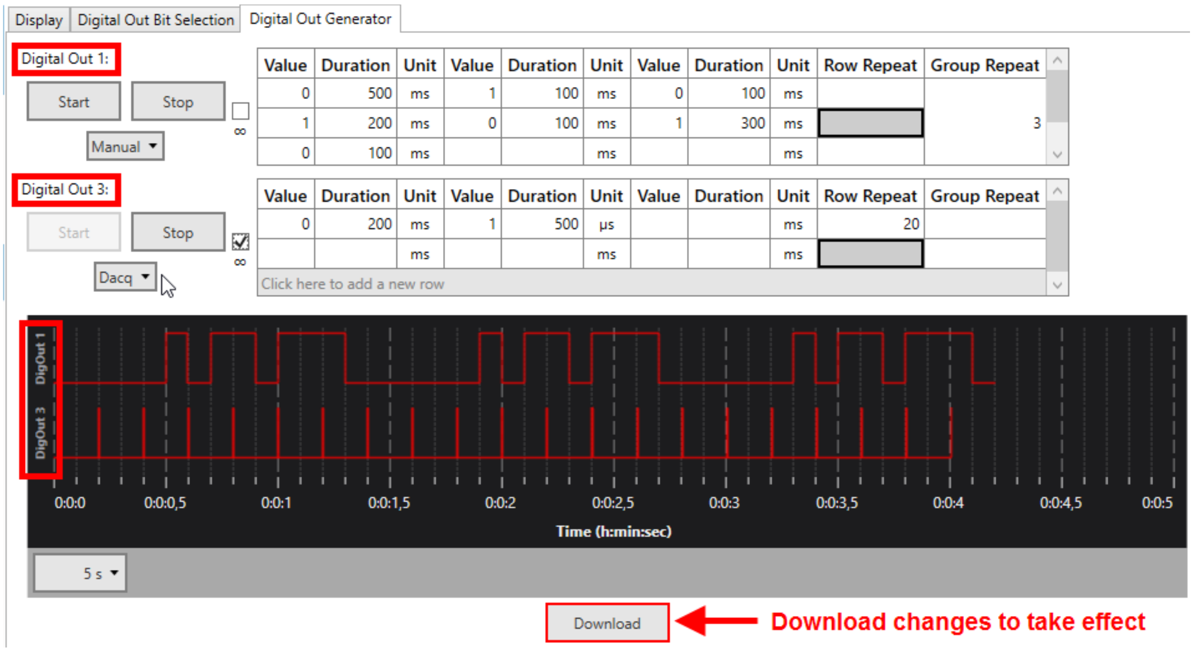


The **HS1 – 4 Stimulator 1 or 2** option assigns the respective DigOut to one of the internal stimulation units of each headstage. If the Marker option is used (see 5.9.3.8), each Marker can generate a TTL on the Digital Out bit selected here. The **SCU Stimulator 1 – 4** refers to the four-channel stimulator integrated into the SCU. The **Digital Out Generator** can be used to program a selected bit to generate any random pattern of TTL pulses. Again, all changes must be downloaded to take effect.

5.4.6.2 Digital Out Generator

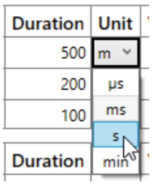
The Digital Out Generator allows to program each bit of the digital out channel to generate a **random pattern of TTL pulses**. The Digital Out Generator tab is only active if at least one digital bit has been assigned to the generator. Only the **assigned bits** will appear in the tab.

Each bit can be programmed with a random pattern of TTLs. Each bit assigned to the Digital Out Generator has its own programming interface and WYSWYG display to show the programmed pattern.

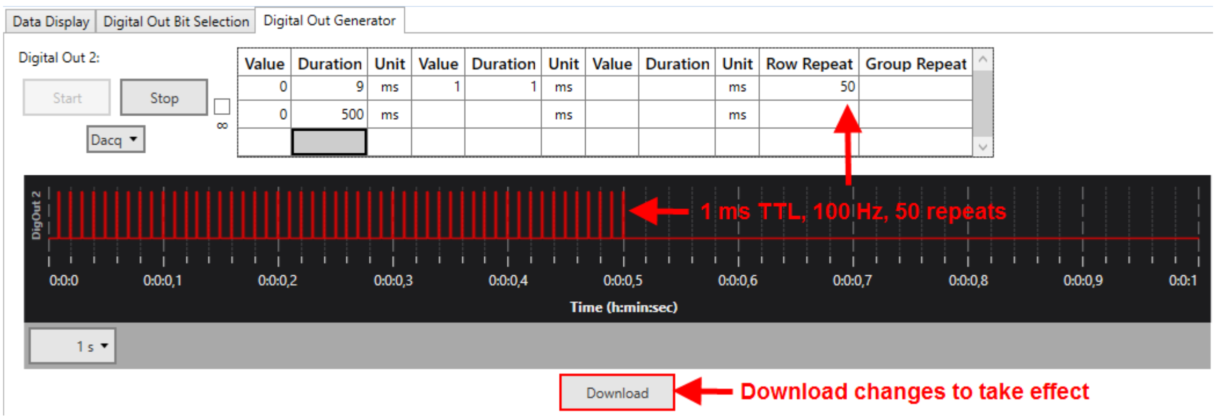


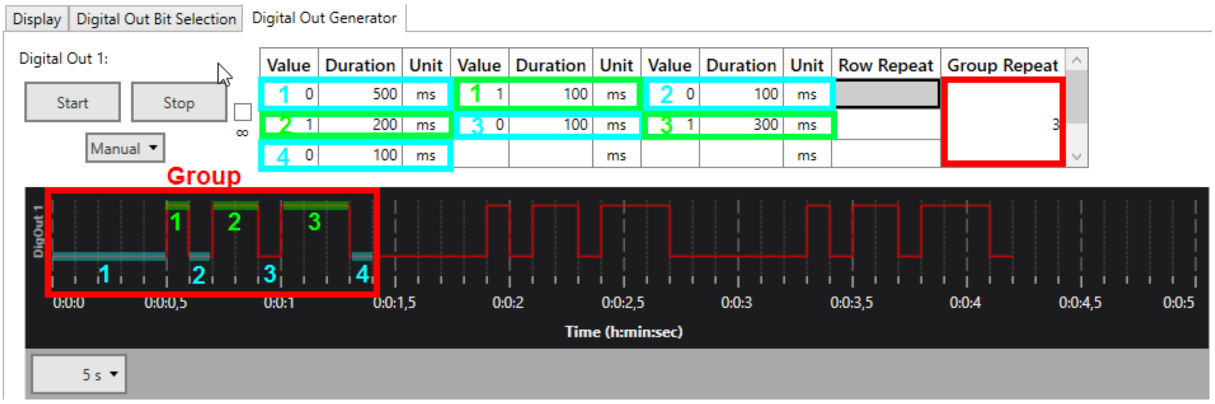
The pattern for each bit can be programmed step by step, with value and duration for each step.
Value can **only be 1 (HIGH) or 0 (LOW)**.

Duration can be any time, in **multiples of 20 µs**, which is the time resolution of the Digital Out Generator. The time **unit** can be changed to µs, ms, s or min. Double click on any time unit field and select the desired unit from the drop down menu.

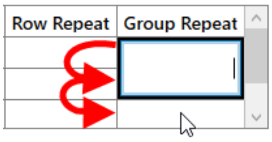


The **Row Repeat** allows repeating the contents of a specific row. If the pattern which should be repeated is longer than one row, it is possible to combine rows to groups, and use the **Group Repeat** function instead.

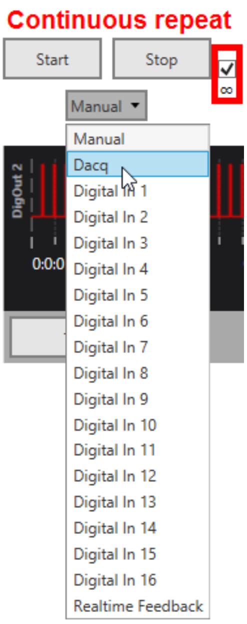




To define a group, hold the **SHIFT and CTRL** key, and click the cells you want to add one by one. You can also remove cells from a group by SHIFT/CTRL click.



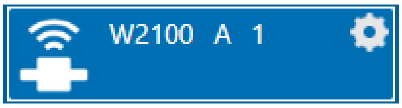
The Digital Out Generator can be **started** on different conditions. Stop is always manual. In **Manual** mode, Start and Stop are on manual command. Alternatively, the Generator can be started with the Start of the **data acquisition** (Dacq), or on an incoming TTL on any of the **Digital In** bits. Finally, The **Real Time Feedback** (see 5.2.3.4) can also be used to start the Digital Out Generator.



By default, the programmed pattern for each bit will be generated once after each Start command. If the **Continuous** repeat (∞) tick box is active, the pattern will be repeated indefinitely till the data acquisition is stopped, or till a manual Stop command.

The settings and paradigm programmed in the Digital Event Generator are also stored in the Experiment file (see 4.1.2).

5.5 Data Source: W2100-System

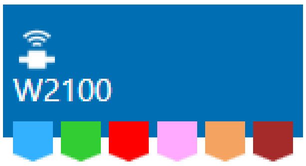


5.5.1 Description and Purpose

The W2100-System is the new generation of MCS wireless recording systems. One receiver is always operated independently by one instance of the software. Multiple headstages connected to one receiver are operated by the same instance. **1 or 2** indicate the receiver connected to port 1 or 2 of the Interface Board. Once the W2100-System is added to the instrument configuration, the W2100 Stimulator will also become available as instrument. Please also see movie for illustration.

5.5.2 Data Ports and Export Options

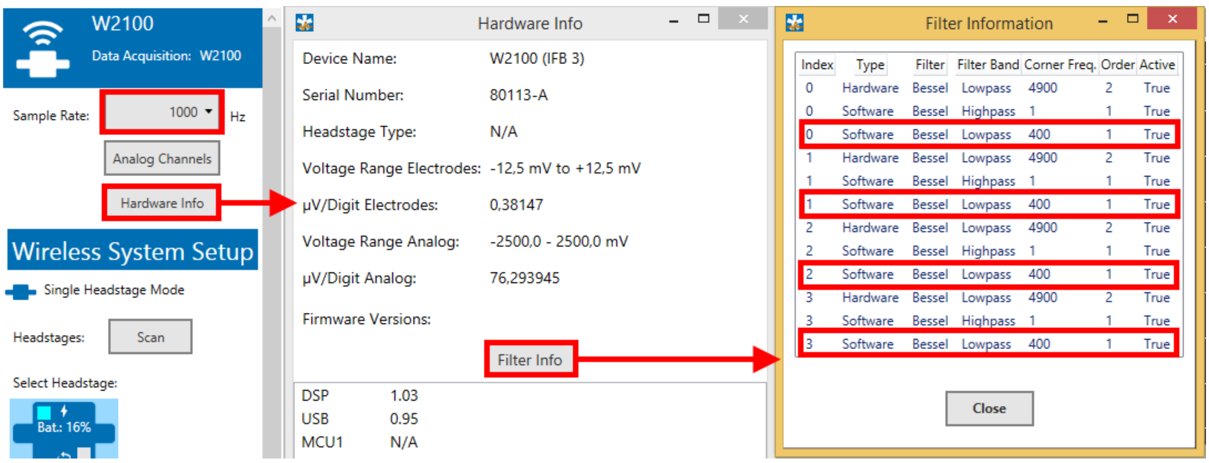
The W2100-System has six output ports. Electrode Raw Data (blue), Analog Data (green), Digital Data (red) and Real Time Feedback events (pink) are present and active in all headstages. Headstages of the latest generation also have Gyroscope Data (light brown) and Accelerometer Data (dark brown). The brown ports are always visible but **contain no data in older headstages**. As with all data sources, there are no input ports. The W2100-System can be connected directly with any number of other instruments with blue or red input ports. The green and brown ports can only be connected to the Recorder at the moment. There are no export options.



5.5.3 Operation

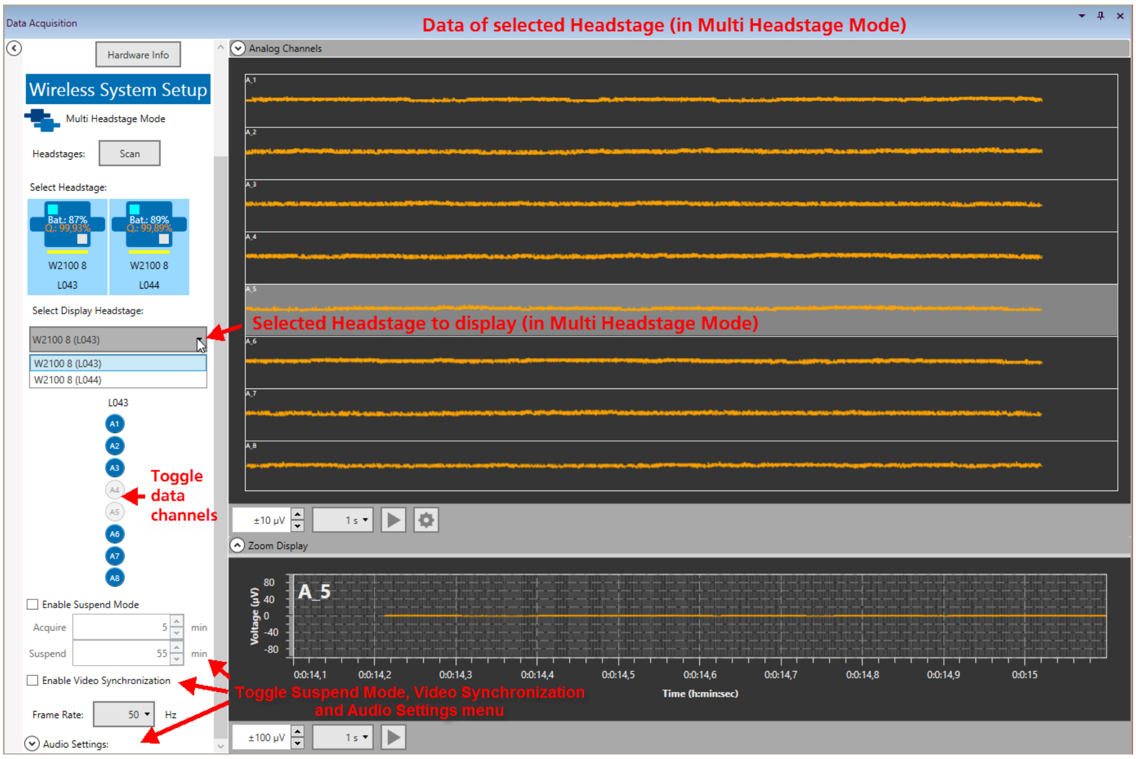
The W2100-System data source control window shows **all available headstages.** All channels of all active headstages can be displayed, and optionally also the additional Analog Input channels from the Interface Board.

To avoid aliasing issues with **low sampling rates**, the W2100 System will apply automatic adaptive **Low Pass filtering**, if sampling rates below 10 kHz are used. The active filters can be seen in the hardware info tab. Filters for all potential headstages are shown, whether or not they are currently present.



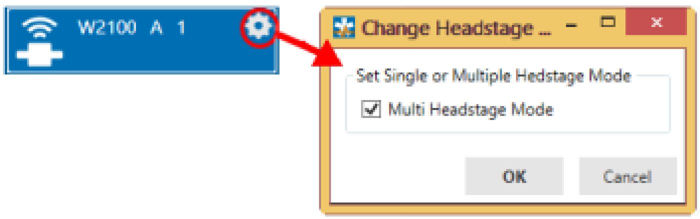
The **Scan** function will screen for available headstages in range and display them as icons. The additional functions, Audio, Suspend Mode and Video Synchronization can be toggled.

In Multi Headstage Mode, a drop down menu with all active headstages allows to select the active headstage to be displayed. This has no influence on recording, all active channels from all active headstages will be recorded, independently from the display.



5.5.3.1 Single and Multi Headstage Mode

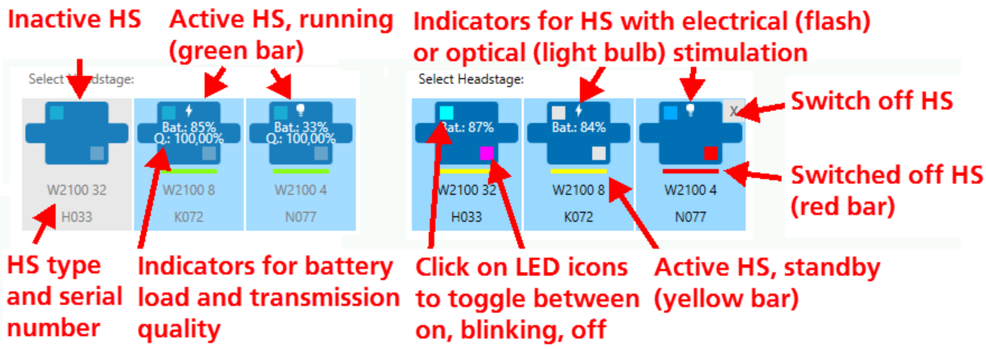
When hovering the mouse pointer above the W2100-System instrument, a **Settings icon** appears. This allows to select Single or Multi Headstage Mode before actually activating the W2100-System data source by dragging it to the main window.



In Single Headstage Mode, only **one headstage** per receiver can be used at a time, at full sampling rate. In Multiple Headstage Mode, up to **four headstages** can be used per receiver simultaneously, but at reduced sampling rate. The Sampling Rate drop down menu will adjust the selectable sampling rates automatically. Different types of headstages can be combined in Multi Headstage Mode. A **separate file** will be generated for each headstage. The file name is identical, as defined in the Recorder tool, with the headstage serial number at the end ([file name][HS serial #]).

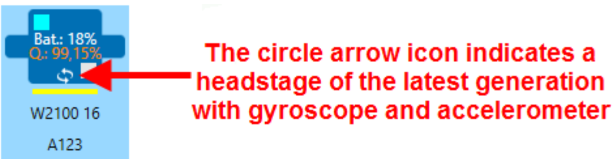
5.5.3.2 Select Headstage

After scanning, all available headstages (HS) are displayed as icons. To select a headstage for recording, simply click on it. The HS icons contain status information about each headstage. Grey outlined headstages are inactive (not selected), selected headstages are outlined in blue. Each HS icon displays type and serial number, and battery status (if active). A bar indicates whether the HS is actively acquiring data (green), in standby (yellow) or switched off (red). Lost contact to the receiver is indicated by a purple bar.



Headstages with electrical or optical stimulation capability show a **flash or light bulb icon**, respectively. The two LEDs (red and blue) on the HS have also individual indicators on the HS icon. Clicking on the indicator will switch each LED between on, blinking and off. The indicator colors are:

	Off	**ON**	**Blinking**
Red LED	grey	red	purple
Blue LED	grey	blue	cyan



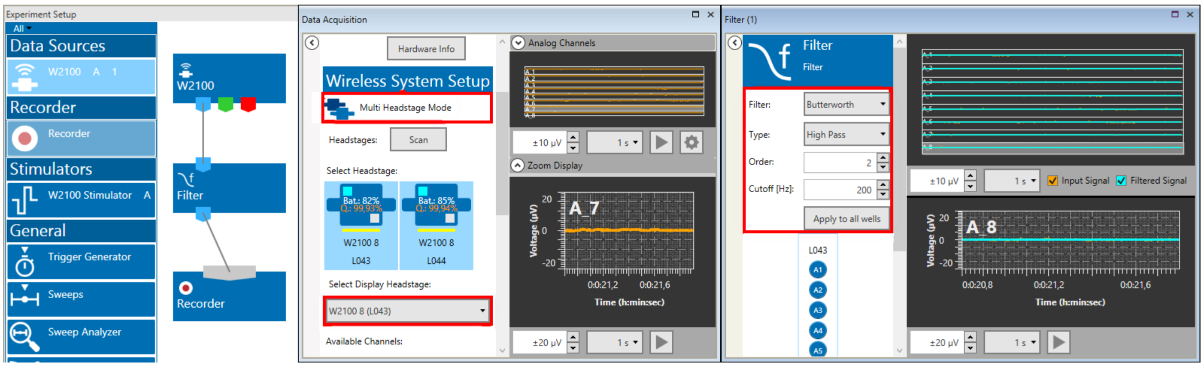
5.5.3.3 Available Channels

In Single Headstage Mode, all channels of the selected headstage are displayed in linear fashion. In Multi Headstage Mode, the headstage to be displayed can be selected from a drop down list by serial number. Individual channels can be toggled by clicking on them. Deactivated channels will neither be displayed nor recorded.

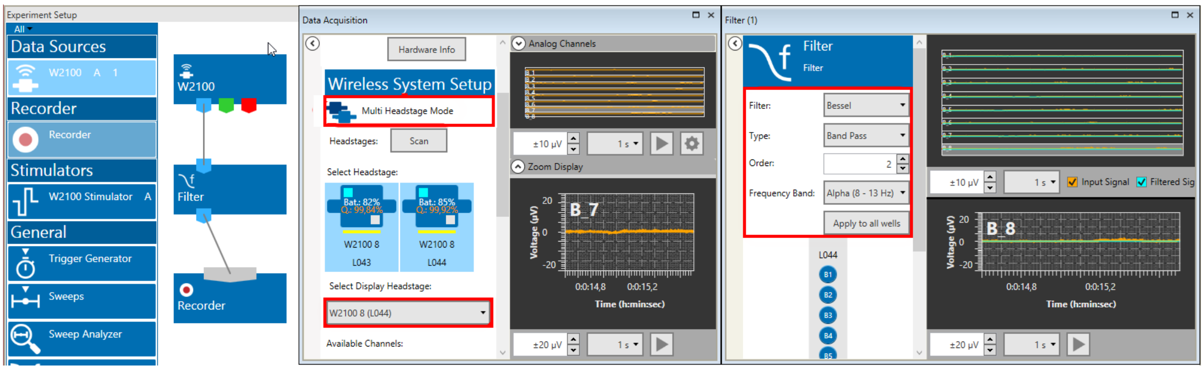
5.5.3.4 Individual Instrument Settings in Multiple Headstage Mode

In Multiple Headstage Mode, up to four Headstages can be active simultaneously. Only one of those Headstages is displayed at a time, as selected from a drop down menu by serial number. Settings of instruments connected to the W2100-System data source, like filters or spike detectors, can be set individually for each active headstage. Settings in all connected instruments can be different for each headstage, and will change automatically if the displayed headstage in the Data Acquisition instrument is changed.

First headstage selected



Second headstage selected

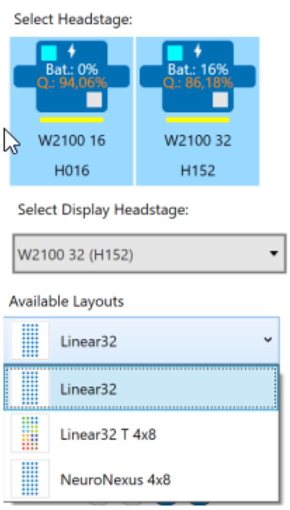


5.5.3.5 Overview Data Display in Multi Headstage Mode

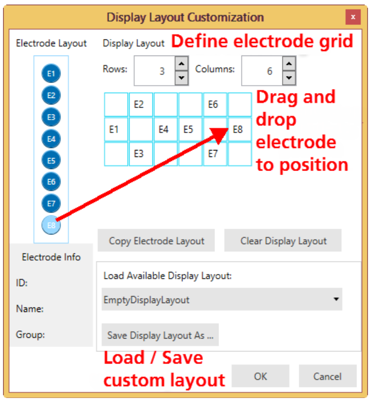
In Multi Headstage Mode, the tab Overview Data Display will appear. On this display, the data of all selected headstages can be seen in parallel. Channels are labeled with A-D for the headstage and the linear channel number.

5.5.3.6 Electrode Layouts

For all headstages, a generic linear layout is available. Some specific electrode layouts can be selected via a drop down menu. To have a layout implemented in the software, please contact [MCS support](#).

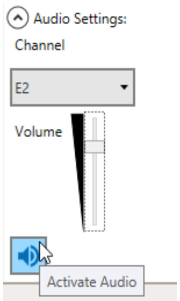


To generate a custom layout for the data source display only, click the **Settings icon** under the main display. The following menu will allow to generate any desired electrode grid, and then assign channels to any grid position. The label of the electrodes is the **linear channel ID** from the headstage connector. Currently, the Custom Display Layout is only available for the data source.



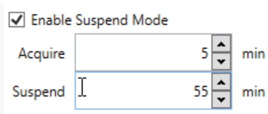
5.5.3.7 Audio Settings

To route any electrode channel to the Audio Output on the Interface Board, open the Audio Settings and select a channel from the drop down list. Volume control is also available. Once activated, the signal from the selected channel is audible via speakers or headphones connected to the **Audio Out** on the Interface Board.



5.5.3.8 Suspend Mode

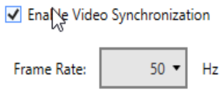
Suspend Mode allows repeated episodic recordings with **low power consumption**. Usually, the headstages are in stand by while selected and ready for recording. In standby, they react quickly on start commands, but still use relatively much battery power. In suspend mode, the HS is switched off automatically for a predefined **Suspend** period in between recordings, to safe battery power. Then it gets reactivated, and does a recording for as long as defined in the **Acquire** window. This will be repeated till stopped manually. The reactivation takes 1 - 2 seconds, so the timing of the Acquire and Suspend times is only accurate on a second scale.



If the suspended mode is combined with **electrical or optical stimulation**, the stimulation will also be interrupted during the Suspend time, and continue automatically in the next Acquire phase.

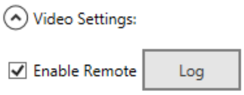
5.5.3.9 Video Synchronization

Activate the Video Synchronization if you want to record a video together with the electrophysiological data with the **Multi Channel VideoControl** software. The selected **frame rate** must be identical to the frame rate of the video acquisition, which is selected in the VideoControl software.



This needs to be done **manually**, if Multi Channel Experimenter and VideoControl software are not connected. If **Enable Remote** function is active, the Experimenter and the VideoControl software communicate via a pipe connection. In that case, the Frame Rate control in the Experimenter is inactive and the Frame rate selected in the VideoControl software will **automatically** be adjusted. Please see manual of Multi Channel VideoControl for details.

This works if both programs run on the same PC, but also if they run on **different PCs** which are in the **same network.** To enable a connection, both programs must be running on the respective PC(s).

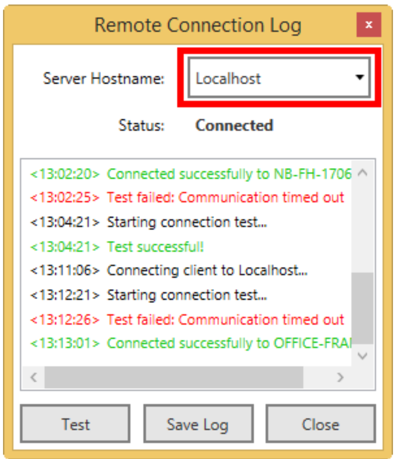


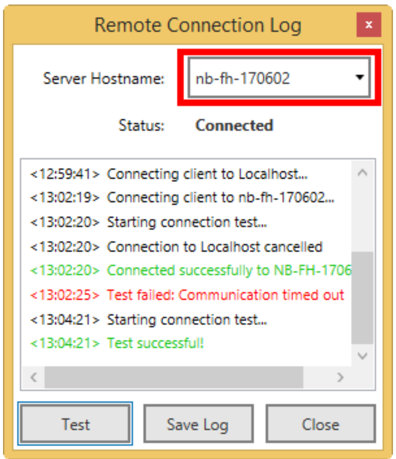
By default, the Multi Channel Experimenter software will attempt to connect to a VideoControl instance running on the same PC (**Localhost**).

To connect to a different **PC in the network**, press the "Log" button in the Video Settings of the W2100 Data Source of the Experimenter and select a different Hostname from the drop down list or enter a new one.

The hostname of a PC is the computer name shown in the **Windows Settings / System.**

If the connection is established, press "Test" for a quick check. The hostnames with successful connections are stored by the Experimenter software in its *.ini file and the last connected one will be used in the next session automatically.



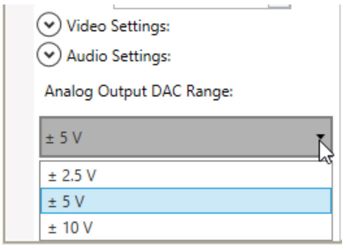


If the connection is established, **VideoControl remotely controls** the Video **Frame Rate** setting, the **base file name** in the Recorder (see chapter 5.12.3) and whether Video Sync is enabled in the Multi Channel Experimenter. The Experimenter sends the ID stored in the W2100 data file via this connection to VideoControl, so that the same ID can be stored in the VideoControl movie file. This ID identifies video files and recordings belonging together. Make sure the appropriate bits of the Digital Out channel are assigned to the Video Sync function (see chapter 5.5.5.1).

Known problems with pipe connections in Windows:
The windows firewall might interfere with the pipe connection. The connection uses UDP ports 137 and 139 and TCP port 445 for communication, so if these are blocked a connection to a remote PC might not work. More detailed info about pipe connections can be found here.

5.5.3.10 Analog Output DAC Range

Receivers with the AO (Analog Out) option have the **Analog Output DAC Range** as additional setting. The ±12.4 mV input range of the W2100 headstage can be converted to different output ranges on the analog output, depending on the input range of the connected 3rd party data acquisition:

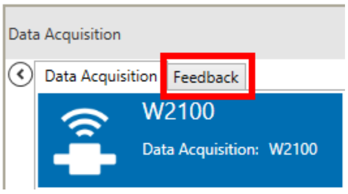


AO DAC Range	Conversion factor	Gain setting
± 2.5 V	0.00496	201
± 5 V	0.00248	403
± 10 V	0.00124	806

The signal on the AO output **times the conversion factor**, or **divided by the Gain setting**, is the actual signal size.

5.5.3.11 Real Time Feedback

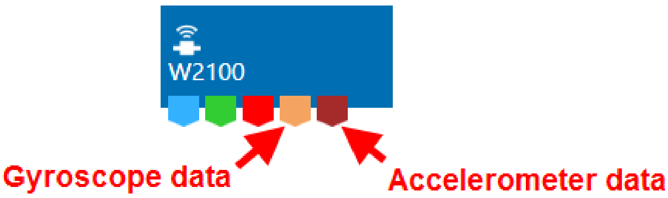
The Real Time Feedback (**RTF**) option can detect spikes and **generate a feedback stimulation with the internal stimulator** with a time delay of only about 10 ms. The delay is longer for the W2100 than for the tethered devices (MEA2100, ME2100) as information needs to be transferred wirelessly between the headstage and the IFB. In addition to starting the internal STG, an **outgoing TTL** can be generated to control an external device by the RTF. The events generated by the RTF are available as triggers on the pink port of the MEA2100-Mini data acquisition. Feedback controls are located on an extra tab in the MEA2100-Mini Data Source. Real Time Feedback is always **only available for the first instance** of the Experimenter and in **Single Instance Mode**.



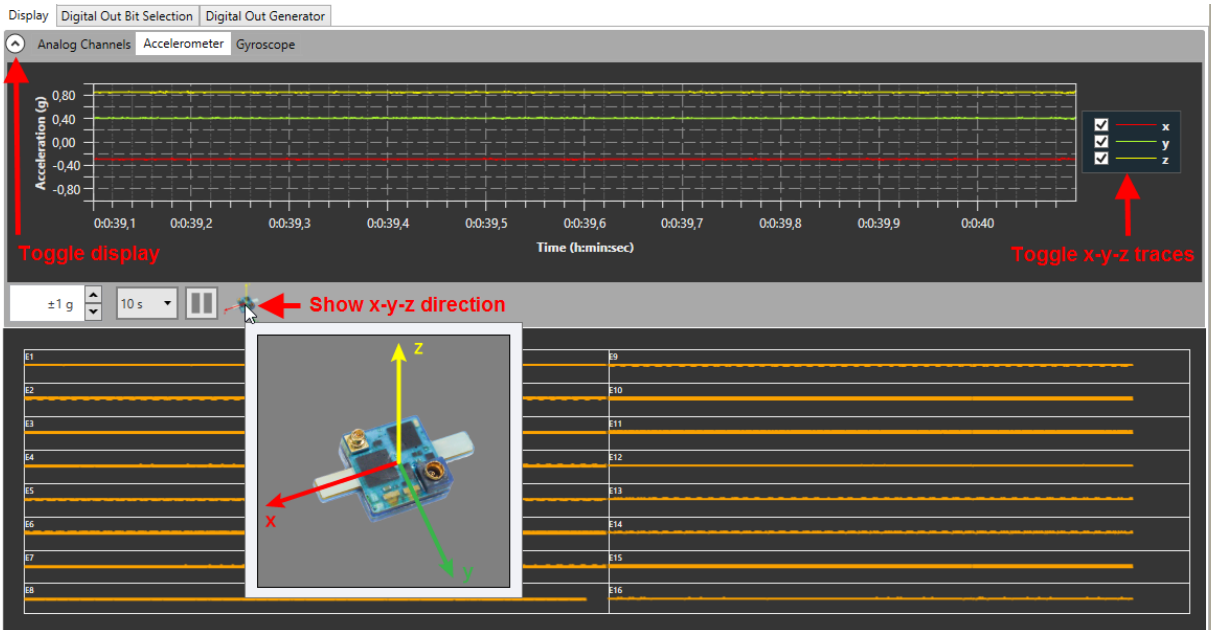
More details on Real Time Feedback are described in the Data Source: MEA2100 section in chapter 5.2.3.4.

5.5.4 Gyroscope and Accelerometer Data

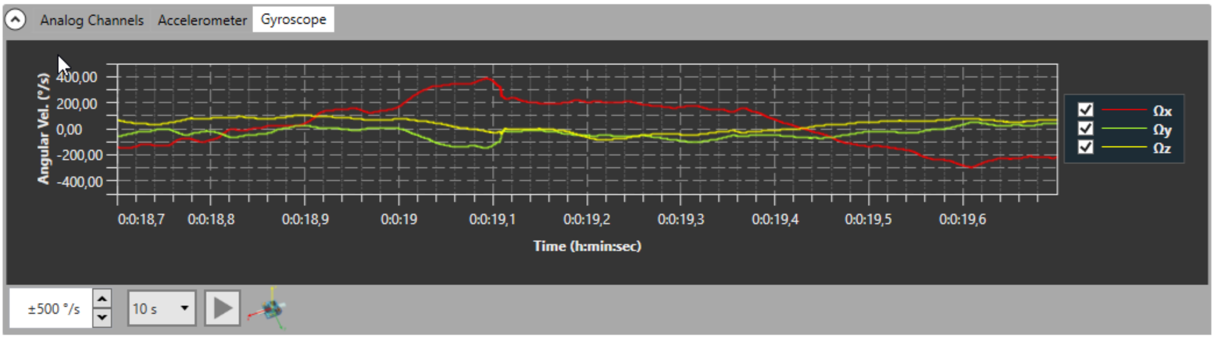
Most Headstages (all but the W210-HS4 and W2100-HS4-opto) of the **latest generation** are equipped with sensors for rotation and acceleration, each in x-y-z-direction. Currently, this data can only be recorded and exported by the Data Manger. These headstages show a circle arrow icon, and the W2100 data source ports in light brown and dark brown are usable.



If such a headstage is selected, toggle the **display for auxiliary analog channels**, which now contains also tabs for the Accelerometer and Gyroscope data. All three special directions are shown as individual traces, and can also be toggled individually. Acceleration is shown in g (gravity acceleration, 9.81 m/s^2), rotation in angular degrees per second (°/s).

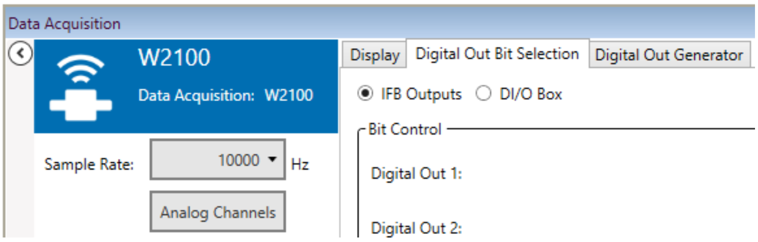


Be aware that due to **gravity**, there is a constant acceleration of 1 g in downward direction. That means, if the headstage is held still and perfectly horizontal, the Acceleration z value is 1, and x and y are 0. In all other positions, earth gravity is distributed between all three spatial directions. Any kind of movement causes fluctuations in acceleration and rotation.



5.5.5 Digital Out

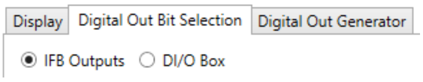
The MEA2100- and W2100-Systems feature an Interface Board with a **16 bit digital output channel** (DigOut). The DigOut channel can generate TTL pulses to **control or synchronize external devices**. The function of the DigOut channel can be controlled in the Data Source instrument. It's possible that several data sources, and hence several instances of the software, share one Interface Board (see chapter 4.1.4). This means all **data sources/software instances have to share** the 16 available DigOut bits. Therefore, one tab in the W2100 data source is available to assign the available output bits to the different data sources, and to the available instruments which might use the DigOut.



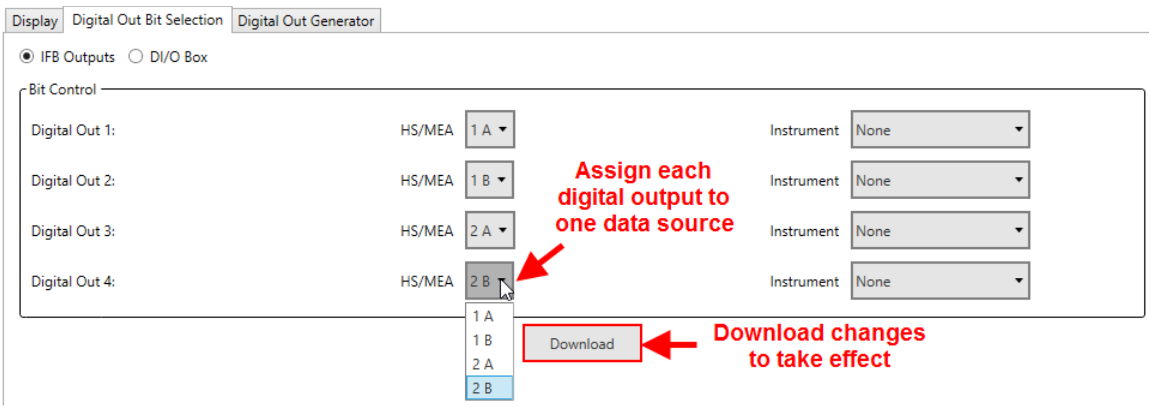
5.5.5.1 Digital Out Bit Selection

The Digital Out Bit Selection tab allows to assign each bit of the DigOut channel to a certain data source and function. In case of multiple software instances, **only the first instance** in which the Data Source instrument is activated allows to edit the bit assignment. All other instances show the assignment, but only follow passively the settings made in the first instance.

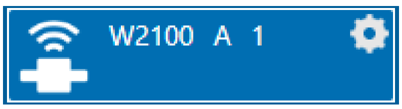
The Digital Out Bit Selection tab is available in two layouts, **IFB Outputs and DI/O Box**. For most applications, four Digital Out bits are enough, so four bits Digital Out 1 to 4, are available as Lemo connectors directly on the Interface Board. If you have this configuration, select IFB outputs, and you will see only the four available outputs.

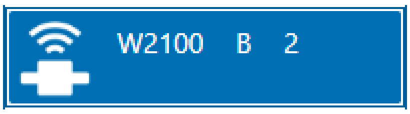


Some applications need all 16 bits. In such cases, a DI/O extension box must be connected to the IFB, which makes all bits available with BNC connectors. If you have such an extension, select DI/O Box to see all available bits. Be aware that the 16 bits on the DI/O are **not in addition** to the four IFB inputs, Digital In 1 - 4 on the IFB are identical to bit 0 - 3 on the DI/O box.

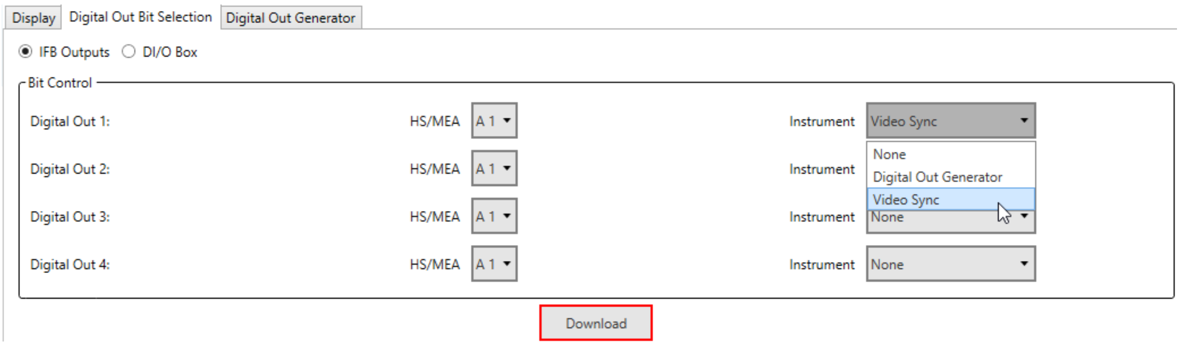


All changes must be downloaded to take effect. The label of a data source is shown on the respective icon.





After assigning the bits to the different data sources, **select the instruments/functions** which should use the respective bit. In a W2100-System, three options are available, the Digital Out Generator, the Stimulators and Real Time Feedback. Each bit can be assigned independently to a different function. Just like the bit assignment to the different data sources, the functions can only be edited in the **first instance.**



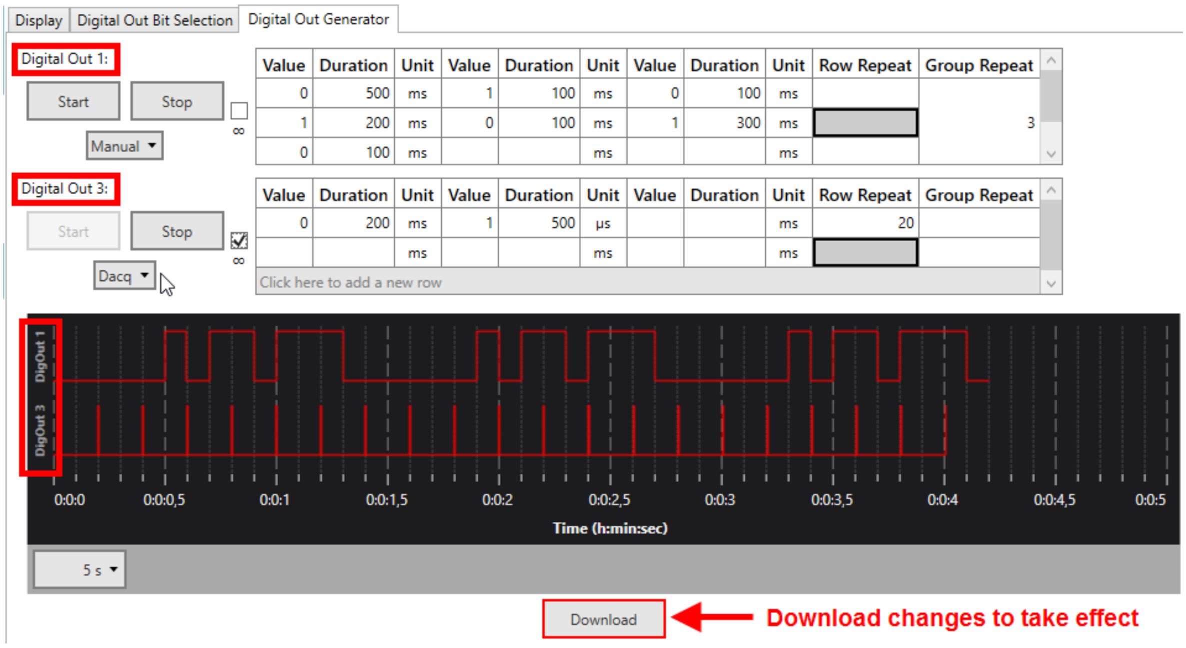
In the W2100-System, the digital out can be used in combination with the W2100 Video System to record a video file synchronized with the electrophysiological data (see chapter 5.5.3.9). Up to four cameras can be operated from one IFB. Assign one bit per camera to the Video Sync.

Finally, the Digital Out Generator can be used to program a selected bit to generate any random pattern of TTL pulses. Again, all changes must be downloaded to take effect.

5.5.5.2 Digital Out Generator

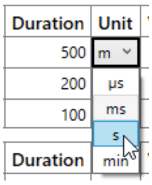
The Digital Out Generator allows to program each bit of the digital out channel to generate a **random pattern of TTL pulses**. The Digital Out Generator tab is only active if at least one digital bit has been assigned to the generator. Only the **assigned bits** will appear in the tab.

Each bit can be programmed with a random pattern of TTLs. Each bit assigned to the Digital Out Generator has its own programming interface and WYSWYG display to show the programmed pattern.

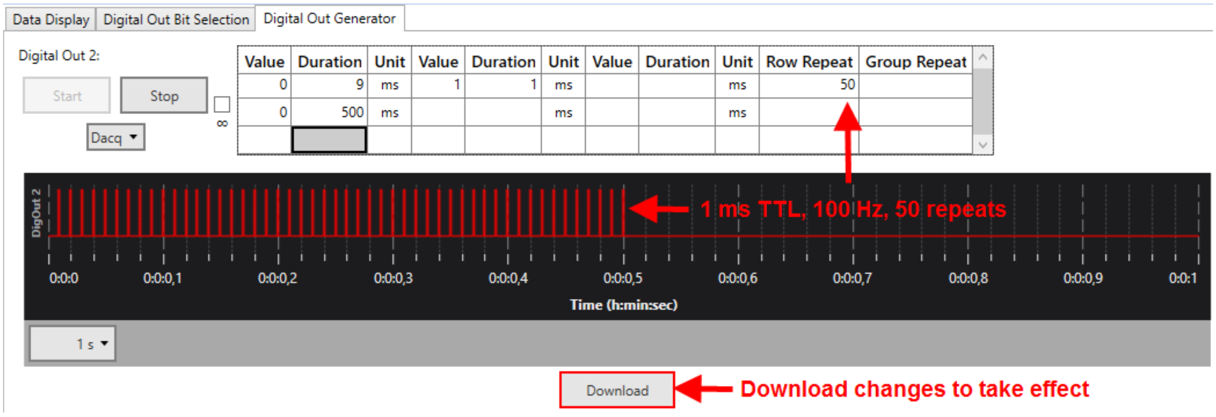


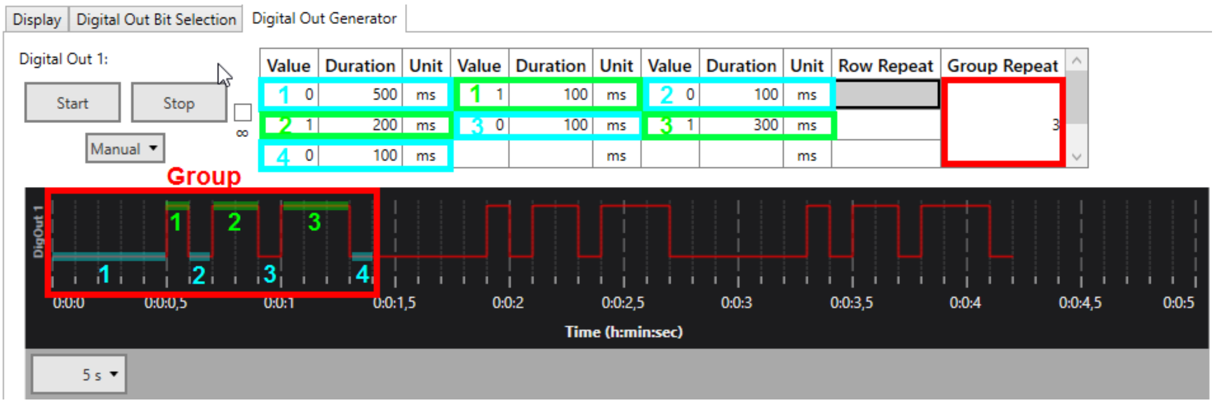
The pattern for each bit can be programmed step by step, with value and duration for each step. Value can **only be 1 (HIGH) or 0 (LOW)**.

Duration can be any time, in **multiples of 20 µs**, which is the time resolution of the Digital Out Generator. The time **unit** can be changed to µs, ms, s or min. Double click on any time unit field and select the desired unit from the drop down menu.

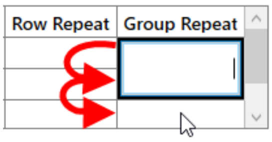


The **Row Repeat** allows repeating the contents of a specific row. If the pattern which should be repeated is longer than one row, it is possible to combine rows to groups, and use the **Group Repeat** function instead.

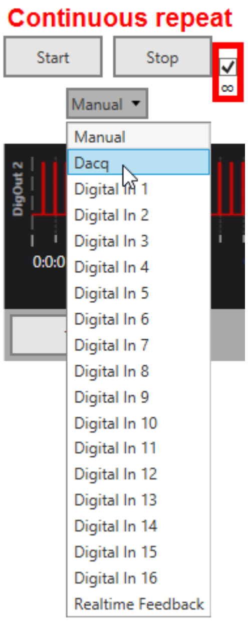




To define a group, hold the **SHIFT and CTRL** key, and click the cells you want to add one by one. You can also remove cells from a group by SHIFT/CTRL click.



The Digital Out Generator can be **started** on different conditions. Stop is always manual. In **Manual** mode, Start and Stop are on manual command. Alternatively, the Generator can be started with the Start of the **data acquisition** (Dacq), or on an incoming TTL on any of the **Digital In** bits. Finally, The **Real Time Feedback** (see 5.2.3.4) can also be used to start the Digital Out Generator.



By default, the programmed pattern for each bit will be generated once after each Start command. If the **Continuous** repeat (∞) tick box is active, the pattern will be repeated indefinitely till the data acquisition is stopped, or till a manual Stop command.

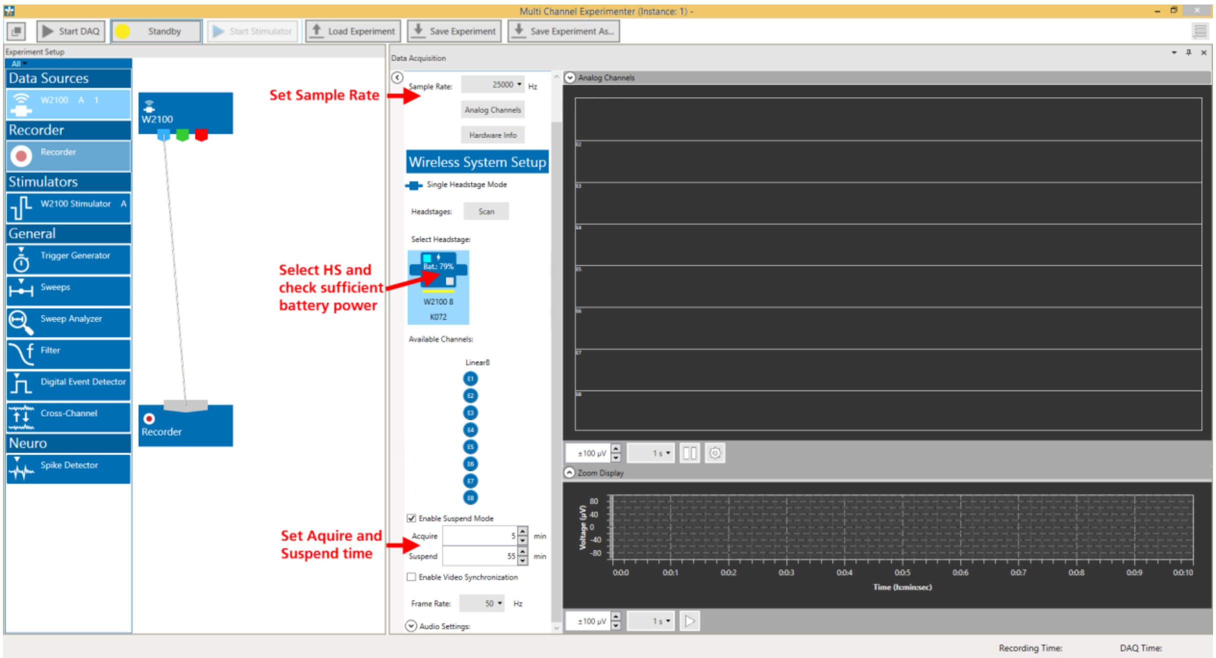
The settings and paradigm programmed in the Digital Event Generator are also stored in the Experiment file (see 4.1.2).

5.5.6 Examples: How to …

This section highlights a few common uses of the W2100-System Data Source.

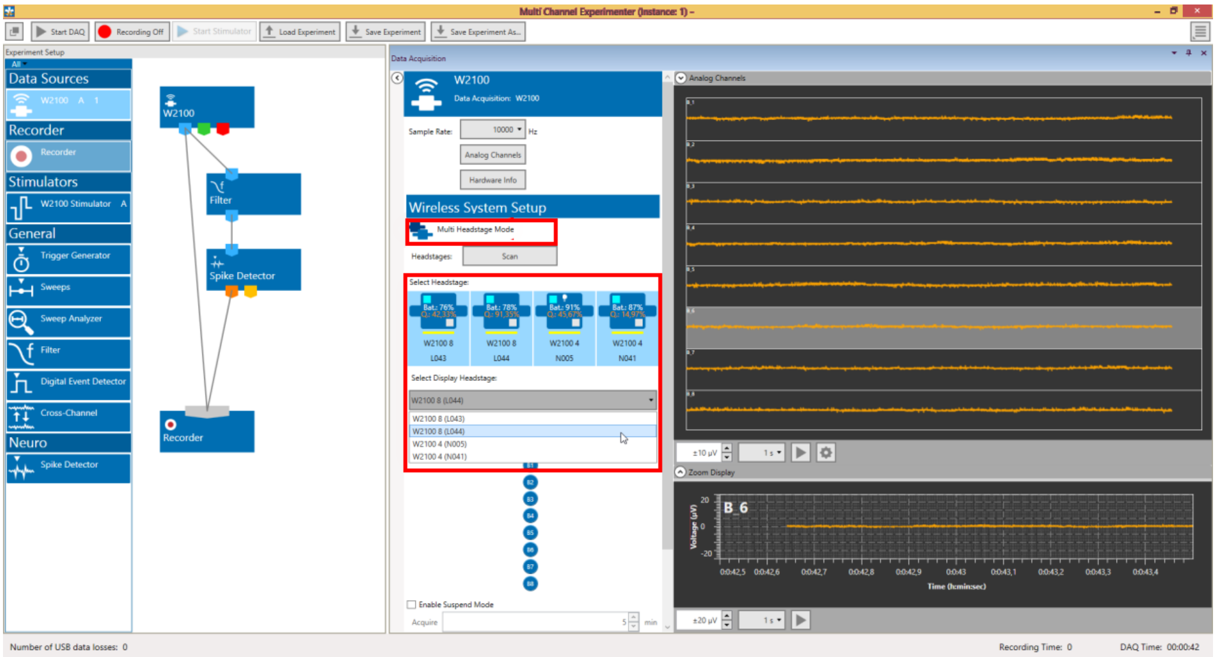
5.5.6.1 Record Five Minutes per Hour Overnight Automatically

To do repeated recordings on a minutes or hours time scale, it is recommended to use the Suspend Mode to save battery power. Connect the W2100-System data source to the recorder and open the control window of the W2100-System. Enable suspend mode, and set the Suspend and Acquire times, as well as the sampling rate. Set the data acquisition to Standby. Once the DAQ is started, the W2100-System will record for 5 min, and then shut down the HS for 55 min. This will repeat till manually stopped.



5.5.6.2 Record Four Headstages in Parallel

Before dragging the W2100-System Headstage to the main window, select Multi Headstage Mode with the Settings Icon (please see chapter 0, Single and Multi Headstage Mode). Scan, and select all four headstages for recording. The HS with the highest channel count will determine the **maximum sampling rate**. Set recording to Standby and start the DAQ manually. A separate file will be generated for each headstage. The file name is identical, as defined in the Recorder tool, with the headstage serial number at the end ([file name][HS serial #]). In this example, unfiltered raw data and spike cutouts will be recorded for all four headstages.

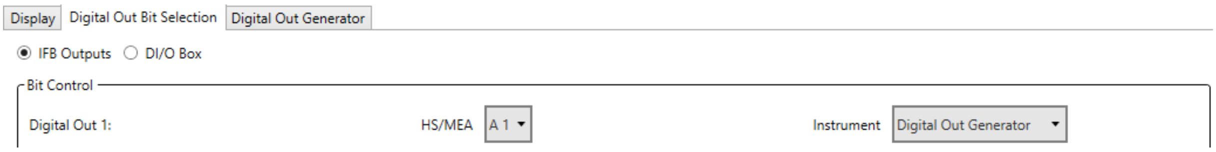


Only one headstage is displayed at a time, the **displayed headstage** can be selected from a drop down menu. **Settings** for the filter and spike detector, like filter cut off and spike detection levels, **can be selected individually** for each headstage, even though there is only one filter and spike detector instrument. Any changes from the default settings will be applied to the displayed headstage only. Change the displayed headstage in the W2100-System data source, and the settings in the subsequent instruments will change accordingly.

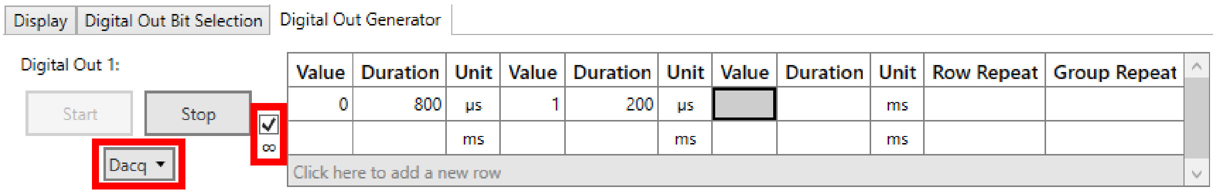
5.5.6.3 Generate a Synchronization Pulse for an External Device

If more than one data acquisition device is used in the same experiment, synchronization of data is always an issue, as each device runs on its own clock, and time lines will drift apart over time. In this example, the W2100-System will generate a constant stream of TTL pulses via one Digital Out bit at 1 kHz, which can be connected to an external device. This TTLs can later be used to synchronize the W2100 data with the external data, and the TTLs are synced to the internal clock of the W2100-System.

Assign one Digital Out bit to the Digital Out Generator.



Next, program the Digital Out Generator to start on the start of the data acquisition, and repeat the programmed pulse continuously. Program a TTL pattern with 800 µs LOW an 200 µs HIGH. Depending on the sampling rate of the external device, it might be necessary to have a longer HIGH phase to make sure the TTL is detected. Generally, the faster the sampling rate, the shorter the HIGH phase and the faster the frequency of the synchronization signal is recommended. For slower acquisition systems, a slower frequency with longer HIGH phases should be used.



5.6 Data Source: Basic Wireless-System

5.6.1 Description and Purpose

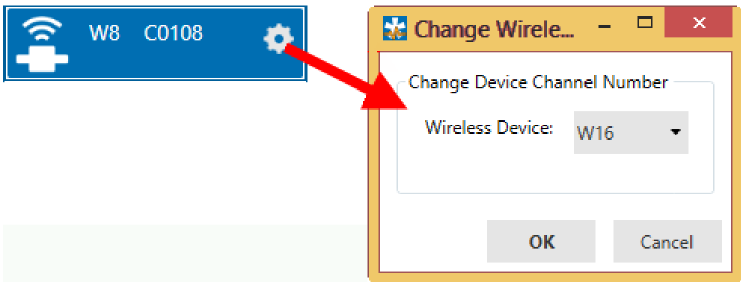
The Basic Wireless-System is the first generation of MCS wireless recording systems. One system is always operated independently by one instance of the software. Multiple headstages can be operated by one system, but only one headstage can be active at a time. The icon shows the serial number of the recording system, and the type of headstage which is currently selected (W8 in the example above).

5.6.2 Data Ports and Export Options

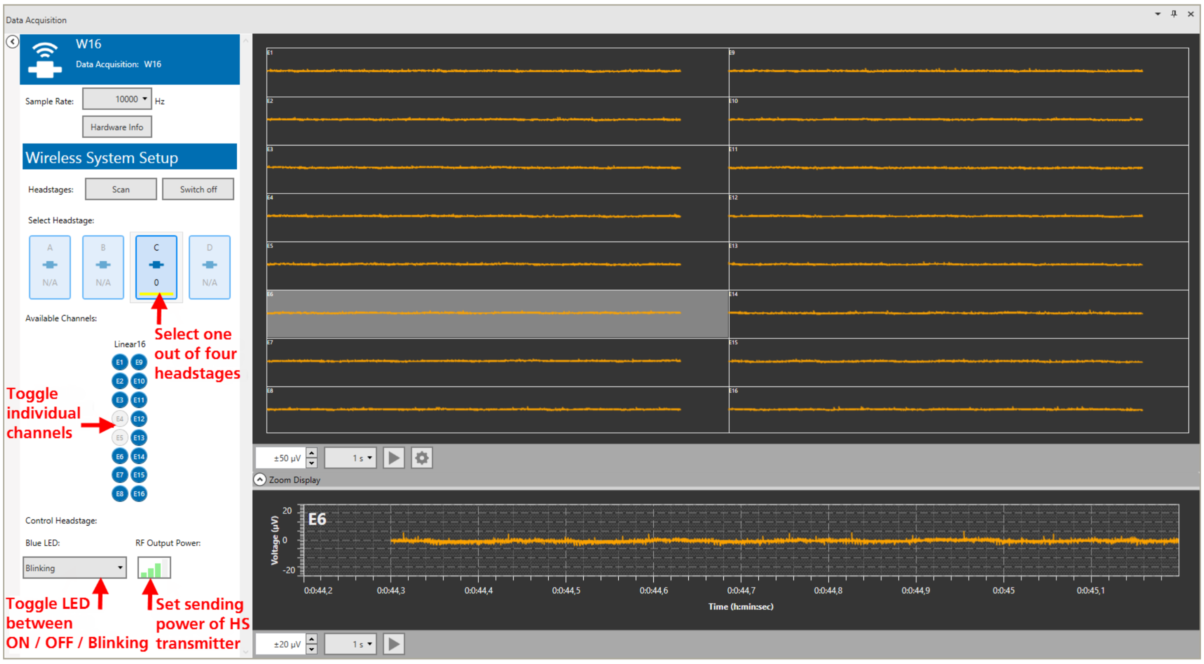
The Basic Wireless-System has two output ports: Electrode Raw Data (blue) and Digital Data (red). As with all data sources, there are no input ports. The Basic Wireless-System can be connected directly with any number of other instruments with blue or red input ports. There are no export options.

5.6.3 Operation

The Basic Wireless-System data source needs to be set to **one type of headstage** (W4, W8, W16 or W32) first. When hovering the mouse pointer above the Basic Wireless instrument, a Settings icon appears. This allows to select the headstage type **before** actually activating the Basic Wireless-System data source by dragging in to the main window.



The **Scan** function will screen for available headstages in range and display up to four of them as icons (A to D). One headstage can be selected for recording at a time. In the control window, all channels of the active headstage will be displayed. Grey headstages are inactive (not selected), the selected headstage are outlined in blue. Each headstage icon displays a serial number. A bar indicates whether the headstage is actively acquiring data (green), in standby (yellow) or switched off (red). Switched Off headstages need to be turned on again by power cycling or with an IR remote control. The status of the LED on the headstage and the output power of the transmitters can also be adjusted. Individual channels can be deactivated, these channels will neither be displayed nor recorded. Deactivation of channels can increase the available sampling rate.



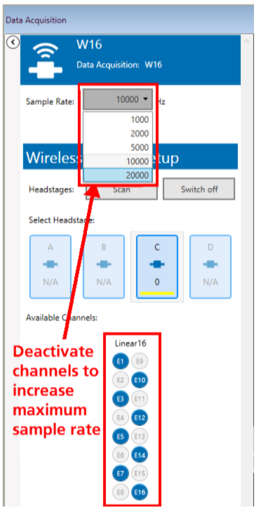
Data Acquisition
W16
Data Acquisition: W16
Sample Rate:
10000
Hz
Hardware Info
Wireless System Setup
Headstages:
Scan
Switch off
Select Headstage:
Available Channels:
Linear16
Control Headstage:
Blue LED:
RF Output Power:
Blinking
Select one out of four headstages
Toggle individual channels
Toggle LED between ON / OFF / Blinking
Set sending power of HS transmitter
Zoom Display
E6
Voltage (µV)
Time (h:min:sec)

5.6.4 Examples: How to ...

This section highlights a few common uses of the Basic Wireless-System Data Source.

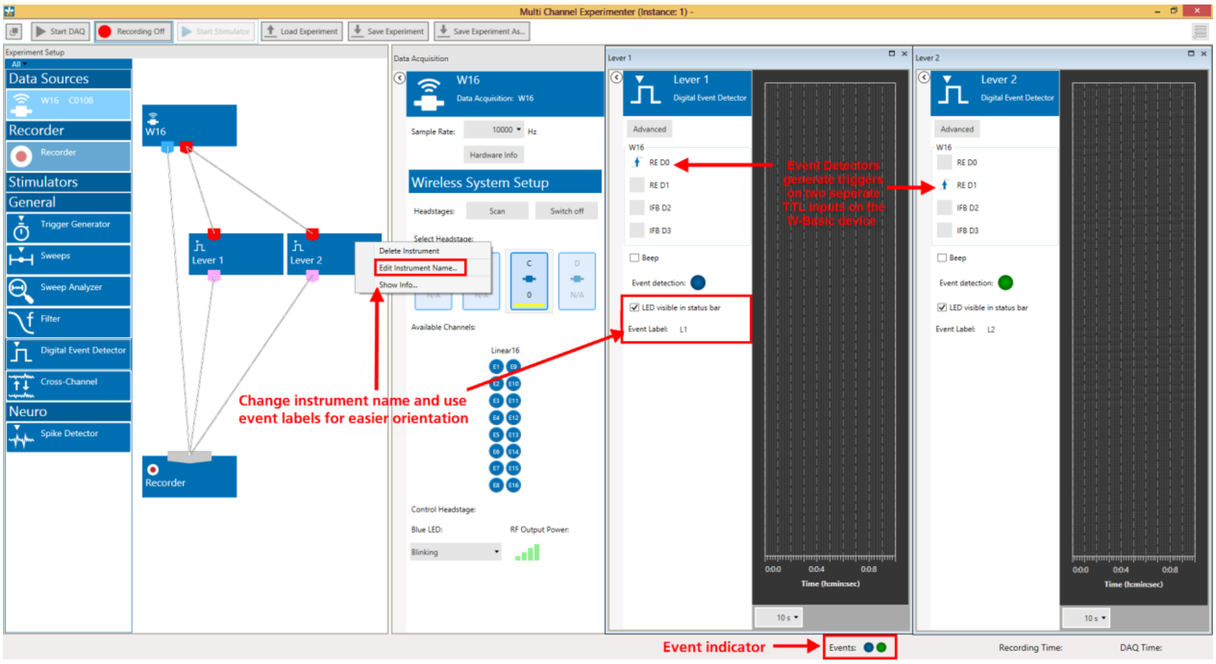
5.6.4.1 Record with Increased Sampling Rate by Sacrificing Recording Channels

The maximum sampling rate is mostly determined by the number of channels which are actually recorded, not the number of channels available on the headstage. In this example, the maximum sampling rate of a W16 is usually 10 kHz. By deactivating half of the channels, this can be increased to 20 kHz. The available options in the sampling rate drop down menu will adjust automatically based on the number of recorded channels. Reducing channels will also **decrease power consumption**.



5.6.4.2 Record Electrode Data and Time Stamps for Two Different Behavioral Tasks

In some behavioral experiments, animals are trained to perform certain tasks, for example pressing a lever. To analyze the brain activity in relation to the performed task, it is necessary to have a **time stamp** in the file. In this experiment, the animal has the choice between two different levers. Using any one lever will generate a TTL pulse, which is applied to one of two digital inputs on the Basic Wireless-System device. The TTL is detected by two Digital Event Detectors, and will show up as time stamp in the data file.



Events and instruments can be given **custom labels**, to make orientation easier. These labels will also be saved in the data file. Raw Data and both Trigger data streams are connected to the Recorder, and will therefore be recorded.

5.7 Data Source: SE-Wireless-System

5.7.1 Description and Purpose

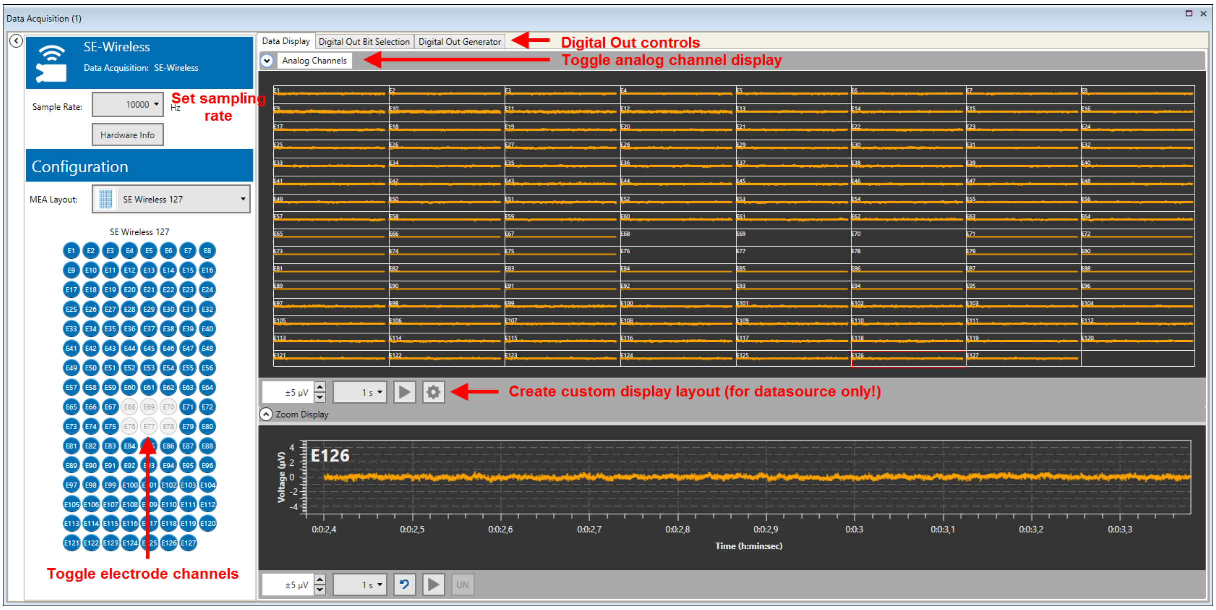
The SE-Wireless-System is supported in all available channel count variations. One receiver is always operated independently by one instance of the software. The type of connected receiver (5, 15, 31, 63 or 127 channels) is detected automatically.

5.7.2 Data Ports and Export Options

The SE-Wireless-System has three output ports: Electrode Raw Data (blue), Analog Data (green) and Digital Data (red). As with all data sources, there are no input ports. The SE-Wireless data source can be connected directly with any number of other instruments with blue or red input ports. The green port can only be connected to the Recorder at the moment. There are no export options.

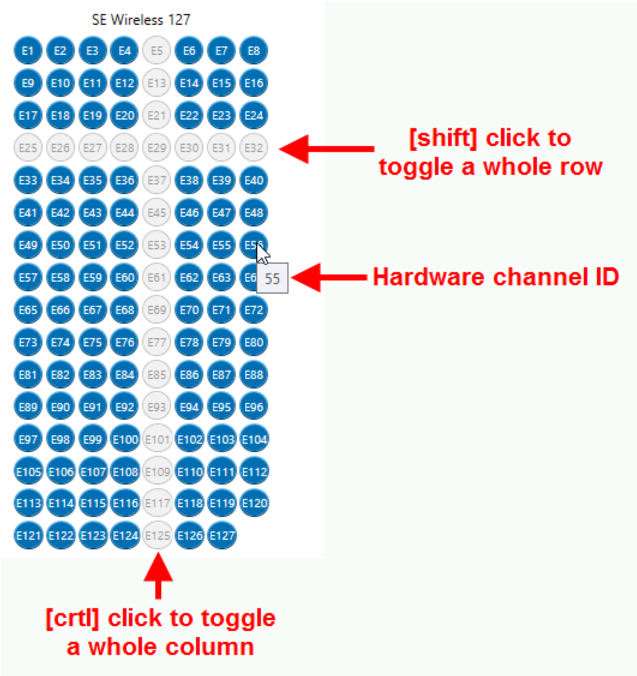
5.7.3 Operation

The SE-Wireless data source control window shows **all available data channels**, and optionally also the additional Analog Input channels from the Interface Board. The numbering of the channels reflects the channel numbers on the TBSI headstage documentation.

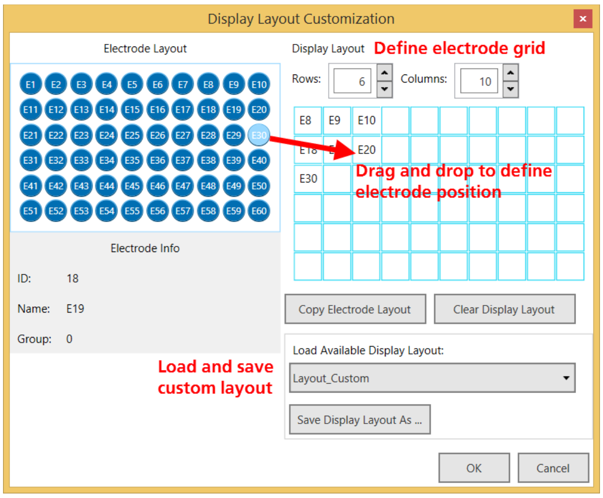


Warning: TBSI headstages have a low pass filter of 7 kHz. Sampling rates below 20 kHz may lead to aliasing effects and should be avoided.

Individual channels can be **toggled** by clicking on them. Deactivated channels will neither be displayed nor recorded. **[shift] click or [crtl] click** on any electrode will toggle the whole row or column. Hover the mouse pointer over an electrode channel for a few seconds to bring up the **hardware channel ID** for that electrode.

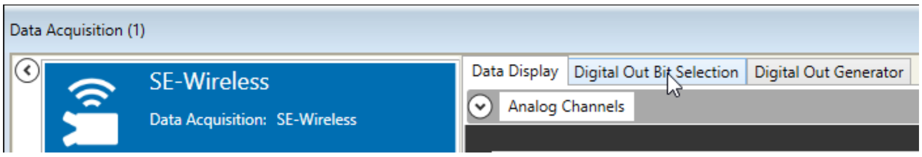


To generate a **custom layout** for the display, click the Settings icon under the main display. The following menu will allow to generate any desired electrode grid, and then assign channels to any grid position. Currently, the Custom Display Layout is **only available for the data source**.



5.7.4 Digital Out

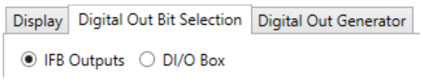
The SE-Wireless-System features an Interface Board with a **16 bit digital output channel** (DigOut). The DigOut channel can generate TTL pulses to **control or synchronize external devices**. The function of the DigOut channel can be controlled in the Data Source instrument. It is possible that several data sources, and hence several instances of the software, share one Interface Board (see chapter 4.1.4).
This means all **data sources/software instances have to share** the 16 available DigOut bits. Therefore, one tab in the MEA2100 data source is available to assign the available output bits to the different data sources, and to the available instruments which might use the DigOut.



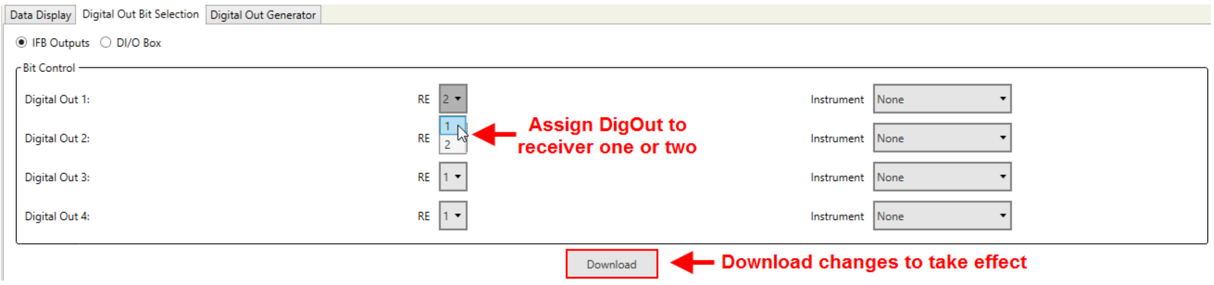
5.7.4.1 Digital Out Bit Selection

The Digital Out Bit Selection tab allows to assign each bit of the Digital Out channel to a certain data source and function. In case of multiple software instances, **only the first instance** in which the Data Source instrument is activated allows to edit the bit assignment. All other instances show the assignment, but only follow passively the settings made in the first instance.

The Digital Out Bit Selection tab is available in two layouts, **IFB Outputs and DI/O Box**. For most applications, four Digital Out bits are enough, so four bits Digital Out 1 to 4, are available as Lemo connectors directly on the Interface Board. If you have this configuration, select IFB outputs, and you will see only the four available outputs.

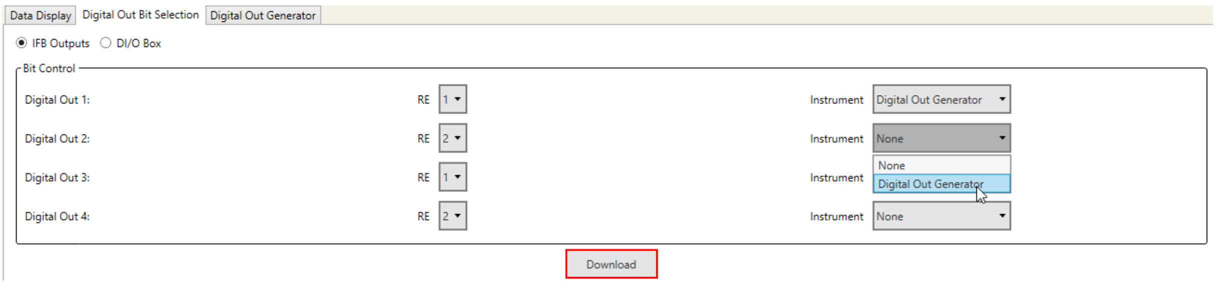


Some applications need all 16 bits. In such cases, a [DI/O extension box]() must be connected to the IFB, which makes all bits available with BNC connectors. If you have such an extension, select DI/O Box to see all available bits. Be aware that the 16 bits on the DI/O are **not in addition** to the four IFB inputs, Digital In 1 - 4 on the IFB are identical to bit 0 - 3 on the DI/O box.



Two receivers can be connected to one Interface Board. Assign each Dig Out you want to use to receiver one or two. All changes must be downloaded to take effect.

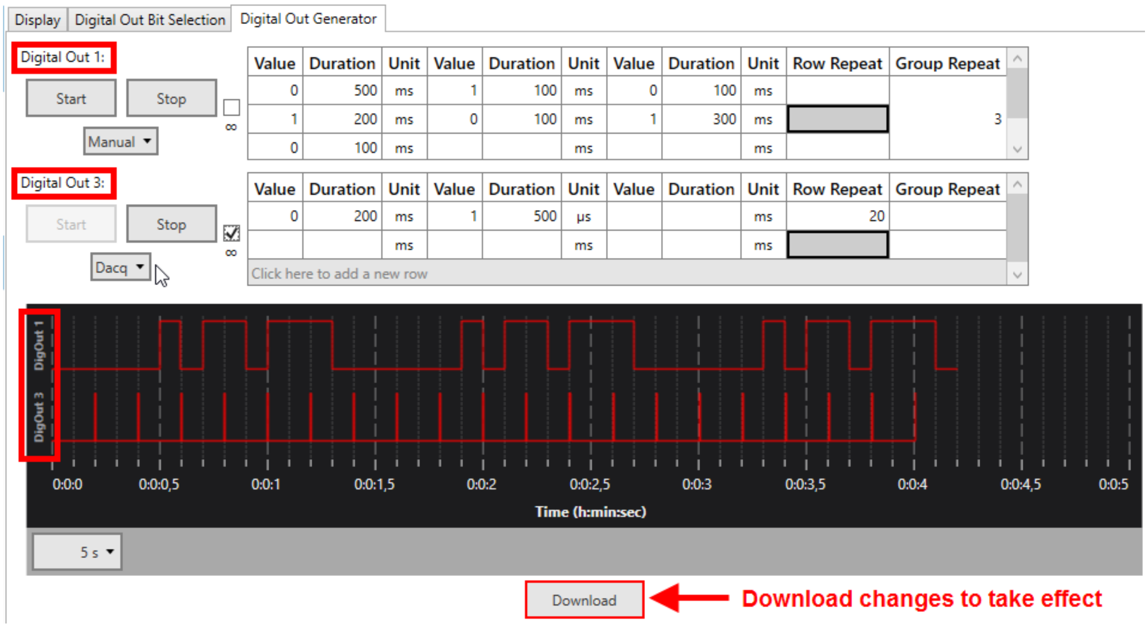
After assigning the bits to the different data sources, **select the instruments/functions** which should use the respective bit. In a SE-Wireless-System, only the Digital Out Generator can use the DigOut. The Digital Out Generator can be used to program a selected bit to generate any random pattern of TTL pulses. Just like the bit assignment to the different data sources, the functions can only be edited in the **first instance**, and all changes must be downloaded.



5.7.4.2 Digital Out Generator

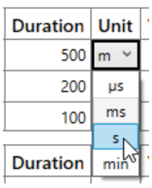
The Digital Out Generator allows to program each bit of the digital out channel to generate a **random pattern of TTL pulses**. The Digital Out Generator tab is only active if at least one digital bit has been assigned to the generator. Only the **assigned bits** will appear in the tab.

Each bit can be programmed with a random pattern of TTLs. Each bit assigned to the Digital Out Generator has its own programming interface and WYSWYG display to show the programmed pattern.

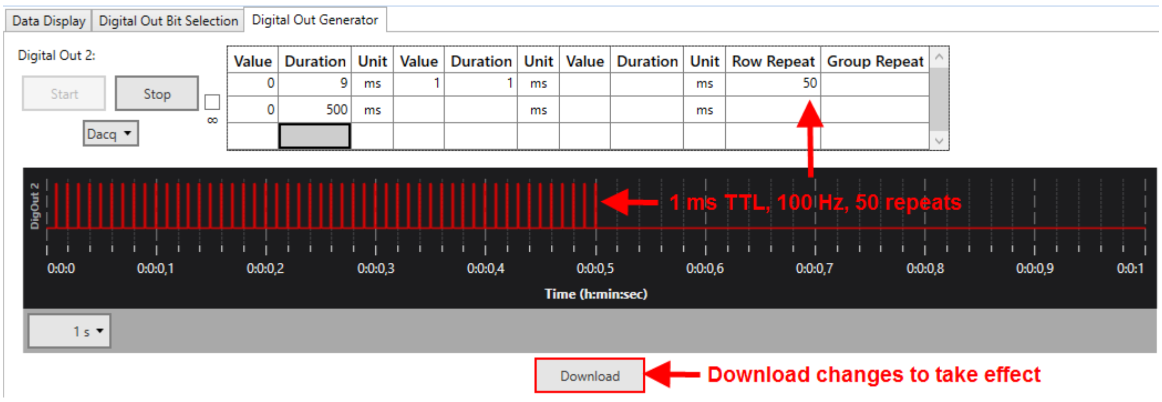


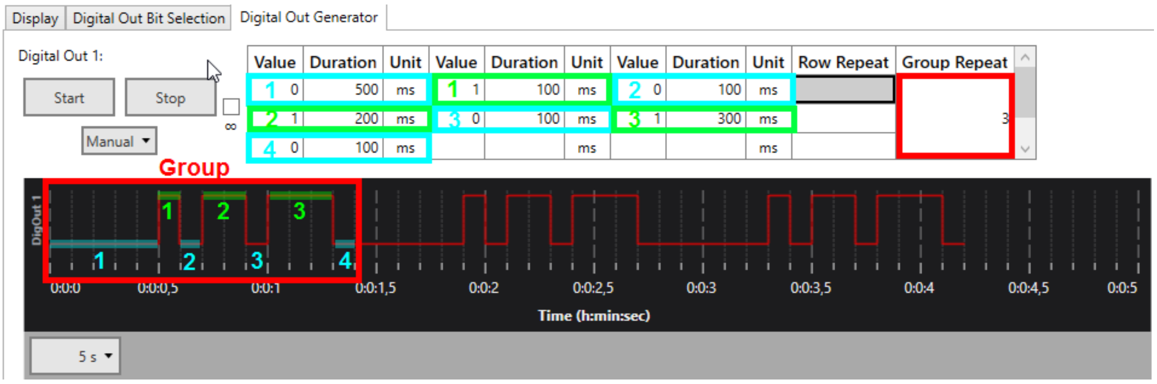
The pattern for each bit can be programmed step by step, with value and duration for each step.
Value can **only be 1 (HIGH) or 0 (LOW)**.

Duration can be any time, in **multiples of 20 µs**, which is the time resolution of the Digital Out Generator. The time **unit** can be changed to µs, ms, s or min. Double click on any time unit field and select the desired unit from the drop down menu.

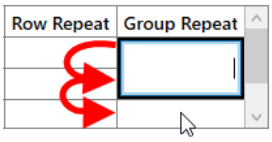


The **Row Repeat** allows repeating the contents of a specific row. If the pattern which should be repeated is longer than one row, it is possible to combine rows to groups, and use the **Group Repeat** function instead.

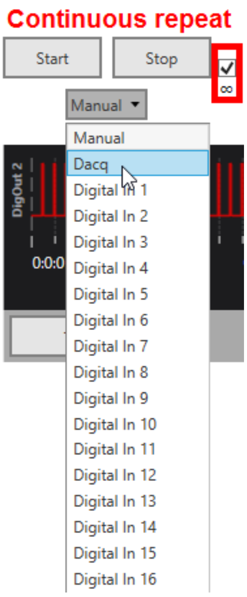




To define a group, hold the **SHIFT and CTRL** key, and click the cells you want to add one by one. You can also remove cells from a group by SHIFT/CTRL click.



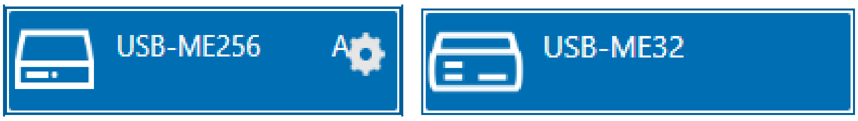
The Digital Out Generator can be **started** on different conditions. Stop is always manual. In **Manual** mode, Start and Stop are on manual command. Alternatively, the Generator can be started with the Start of the **data acquisition** (Dacq), or on an incoming TTL on any of the **Digital In** bits.



By default, the programmed pattern for each bit will be generated once after each Start command. If the **Continuous** repeat (∞) tick box is active, the pattern will be repeated indefinitely till the data acquisition is stopped, or till a manual Stop command.

The settings and paradigm programmed in the Digital Event Generator are also stored in the Experiment file (see 4.1.2).

5.8 Data Source: USB-ME-System



5.8.1 Description and Purpose

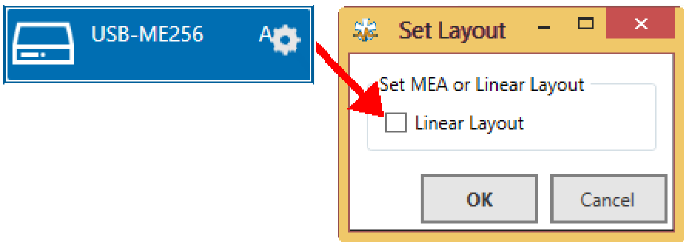
The USB-ME-System data acquisition systems come in three versions, with 64, 128 or 256 channels. Each 64 channels have an individual physical input connector on the device, labeled with A, B, C and D. Most often, one MEA1060 amplifier or one ME recording system is attached to each input. Consequently, the Multi Channel Experimenter handles each input as **separate data source**, which must be opened in separate instances of the software. The Multi Channel Experimenter can't control the MEA1060 amplifiers or the STG stimulators of the 2000, 3000 and 4000 series. MEA-Select and MC_Stimulus II must still be used for this purpose. The compact USB-ME16 and USB-ME32 *in vivo* systems are also supported.

5.8.2 Data Ports and Export Options

The USB-ME-System Data Source has three output ports: Electrode Raw Data (blue), Analog Data (green) and Digital Data (red). The USB-ME16 and USB-ME32 systems have no Analog Data port. As with all data sources, there are no input ports. The USB-ME-System can be connected directly with any number of other instruments with blue or red input ports. The green port can only be connected to the Recorder at the moment. There are no export options.

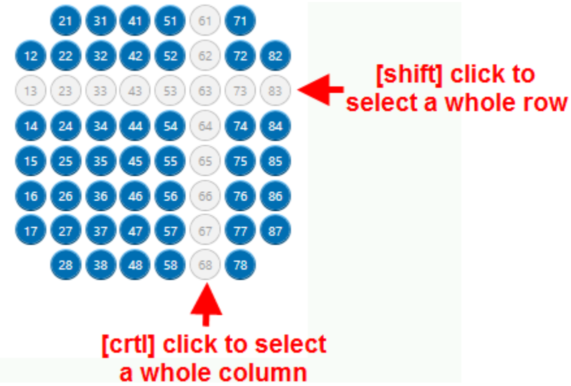
5.8.3 Operation

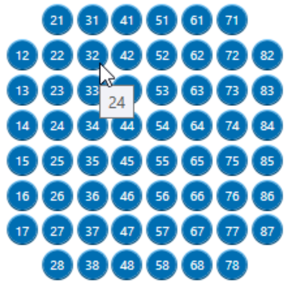
The USB-ME-System data acquisition can operate MEA1060 and ME amplifiers (FA and PGA). If you use a MEA1060 with a standard MEA layout, you can use a MEA layout from a drop down list. If you want to use the linear channel layout and create a custom layout, set the device channel layout to **Linear Layout** before dragging the instrument icon to the main window. The USB-ME16- and USB-ME32-Systems support only 16 or 32 channel linear layout. When hovering the mouse pointer above the USB-ME-System instrument, a Settings icon appears. This allows to select Linear Layout or MEA Layout.

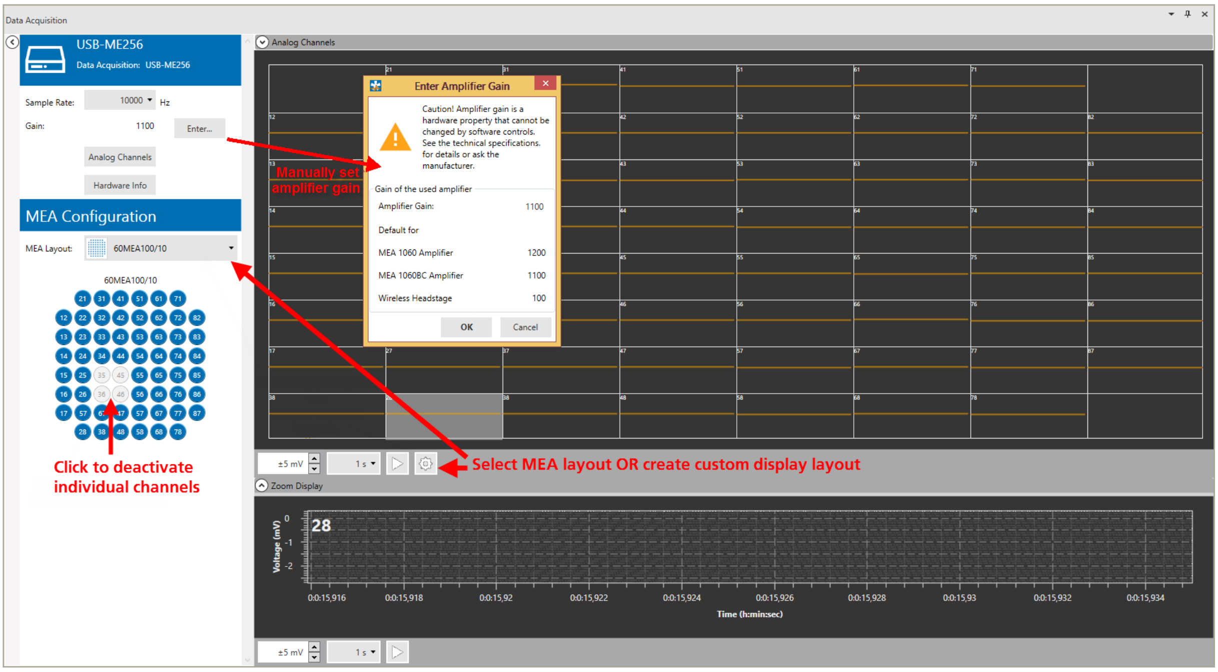


The USB-ME-System data source control window shows **all available data channels**, and optionally also the additional Analog Input channels. If a standard MEA is used, the respective layout can be selected from a drop down list, to match the display to the actual electrode configuration.

Individual channels can be **toggled** by clicking on them. Deactivated channels will neither be displayed nor recorded. **[shift] click or [crtl] click** on any electrode will toggle the whole row or column. Hover the mouse pointer over an electrode channel for a few seconds to bring up the **hardware channel ID** for that electrode.







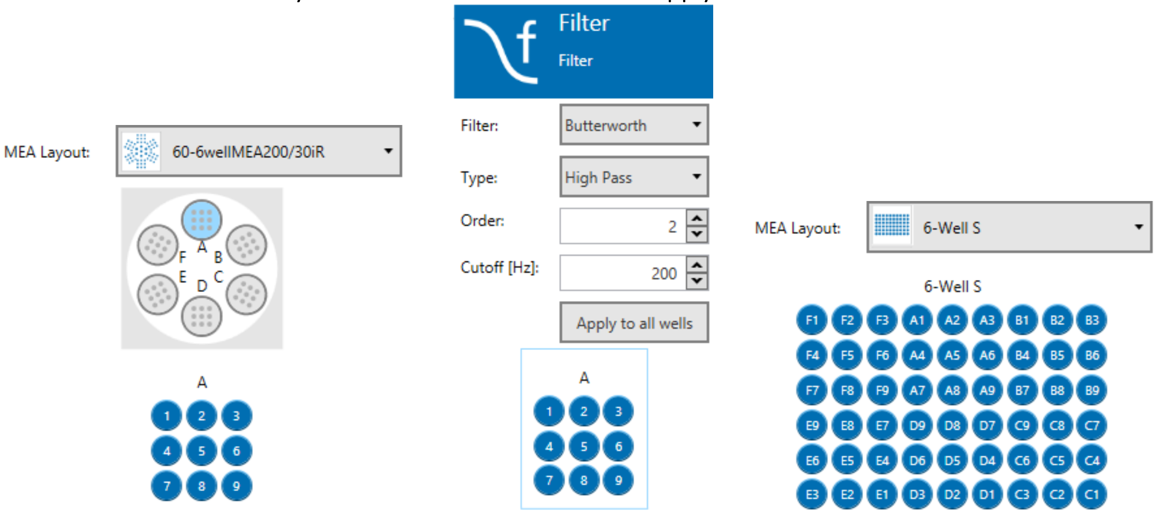
The required **MEA layout**, which will define the display layout for the USB-ME-System data source and all other instruments, can be selected from a drop down list. If a 60-6well MEA is selected, channels of only one well are displayed at a time, other wells can be selected by mouse click. This does not affect the recording in any way.

5.8.3.1 Gain Setting

In contrast to the later MCS recording systems, the analog MEA1060 and FA and PGA amplifiers cannot provide gain and filter setting information to the software. Therefore, the gain for the amplifier connected to the USB-ME-System data acquisition must be selected manually. Click the **Enter** button to open the Amplifier Gain menu. The gain values for the most common amplifiers are listed.

5.8.3.2 Multiwell MEA Layouts

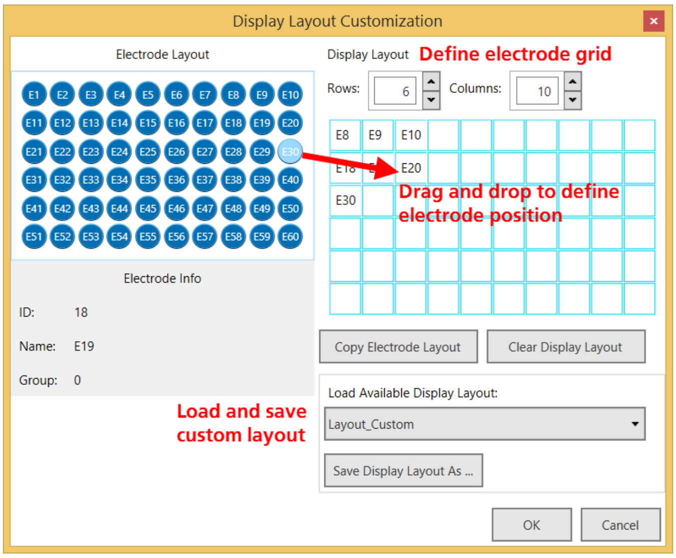
For the 60-6wellMEA200/30iR-Ti, two different layout options are available. The **60-6wellMEA200/30iR** layout shows only one well at a time. You can switch between the wells by clicking at them. Settings for all connected instruments (Filters, Stimulator, Cross Channel Tool) can be set **independently for each well**. Settings will be shown for the well currently selected in the data source. An Apply to all wells allows to make uniform settings.



Alternatively, the **6-Well-S layout** shows all electrodes of the 6-Well MEA with the correct electrode label and grouped by wells, but they are all treated as one unit. All settings and stimulation are global for all channels.

5.8.3.3 Custom Layouts

To generate a custom layout for the display, click the Settings icon under the main display. The following menu will allow to generate any desired electrode grid, and then assign channels to any grid position.

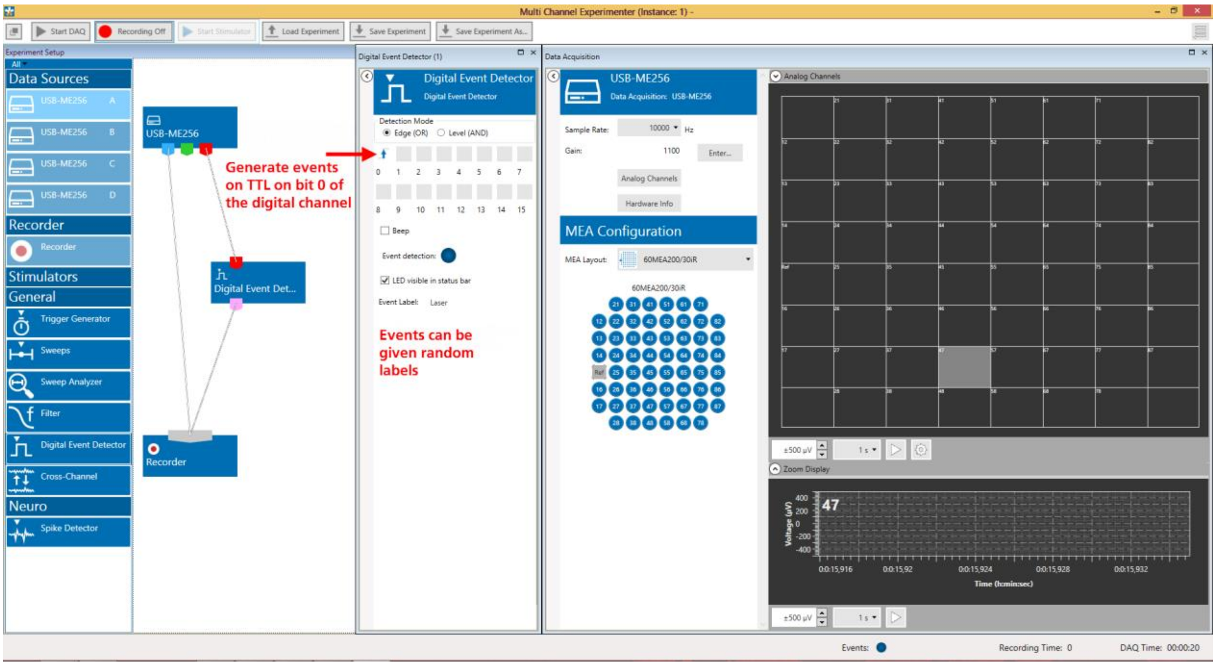


The label of the electrodes is defined by the layout selected from the drop down list. To get the linear hardware channel IDs, select Linear Layout in the Set Layout menu (see above). Currently, the Custom Display Layout is only available for the data source.

5.8.4 Examples: How to …

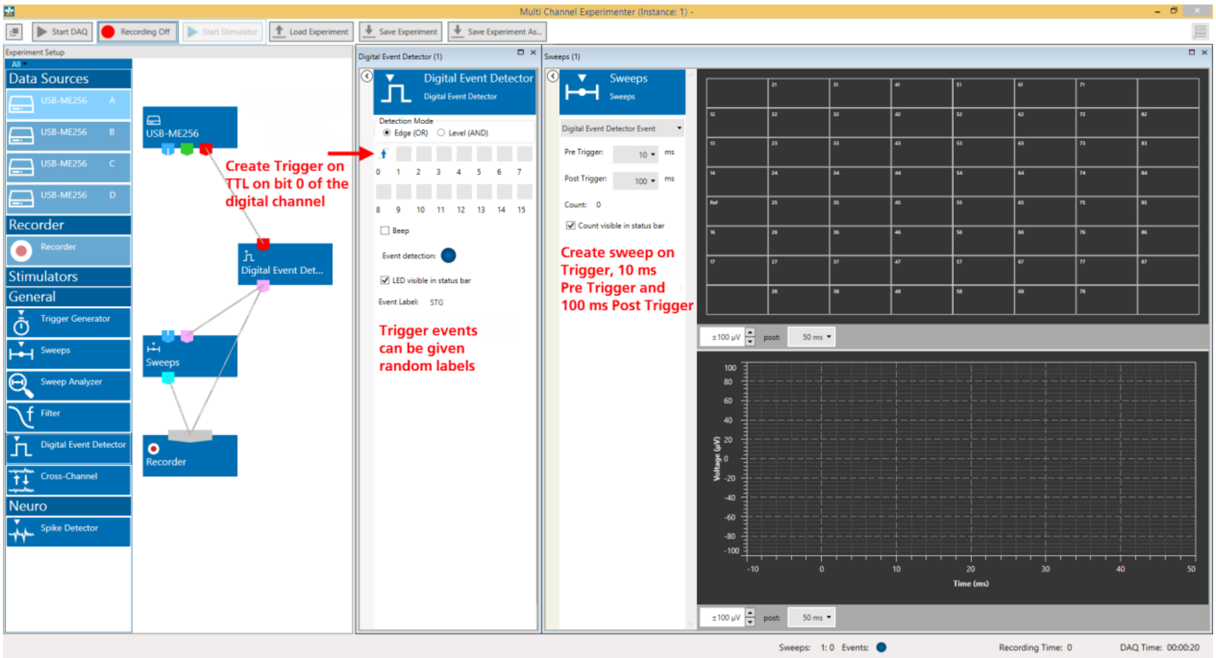
This section highlights a few common uses of the USB-ME-System Data Source.

5.8.4.1 Record Electrode Raw Data and Trigger Events



In the experiment above, the blue electrode data port is connected directly to the Recorder. Therefore, the electrode data recorded continuously. A 60MEA200/30iR layout is selected for the MEA data with 10 kHz sampling rate applied. The red digital data port is connected to a Digital Event Detector instrument (please see chapter 5.17, Digital Event Detector), which will generate Trigger events based on TTL inputs to the Digital In connectors on USB-ME-System. The trigger events will also be recorded, as the pink trigger output port is connected to the Recorder. The digital channel itself will not be recorded, as it is not connected directly to the Recorder.

5.8.4.2 Record Sweeps Triggered by an External Stimulator



External Stimulators can be programmed to generate TTL pulses together with every stimulation pulse. To record segments of data, so called **sweeps**, around each stimulation, connect the TTL output of the stimulator to a **digital input** of the USB-ME-System data acquisition. In the present example, the TTL is applied to bit 0 of the digital channel. The Digital Event Detector generates a trigger event on each rising flank of a TTL on the respective bit. The Sweeps tool uses the raw electrode data from the Data Source and the event to generate sweeps of -10 ms to 100 ms around each stimulation. Only the events and the sweeps are recorded, not the continuous raw data.

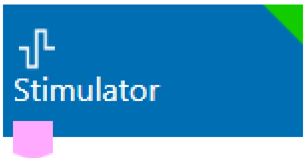
5.9 Stimulators: Stimulator of the MEA2100- and ME2100-System

5.9.1 Description and Purpose

The MEA2100 and ME2100 Stimulator instrument controls the three internal stimulation units of the MEA/ME2100-System. The Stimulator instrument becomes available only after a MEA/ME2100-System data source has been activated (dragged into the main window). Each instance of the experimenter with a MEA/ME2100-System has an independent Stimulator. Please note, the **MEA2100-60, MEA2100-2x60 and MEA2100-120** headstages have **three stimulator units** per MEA. The **MEA2100-256** and the **MEA2100-Mini** headstages have **only two stimulator** units. The **ME2100** has two stimulator units for each attached headstage.

5.9.2 Data Ports and Export Options

The Stimulator has a pink port to generate trigger events. There are no export options.

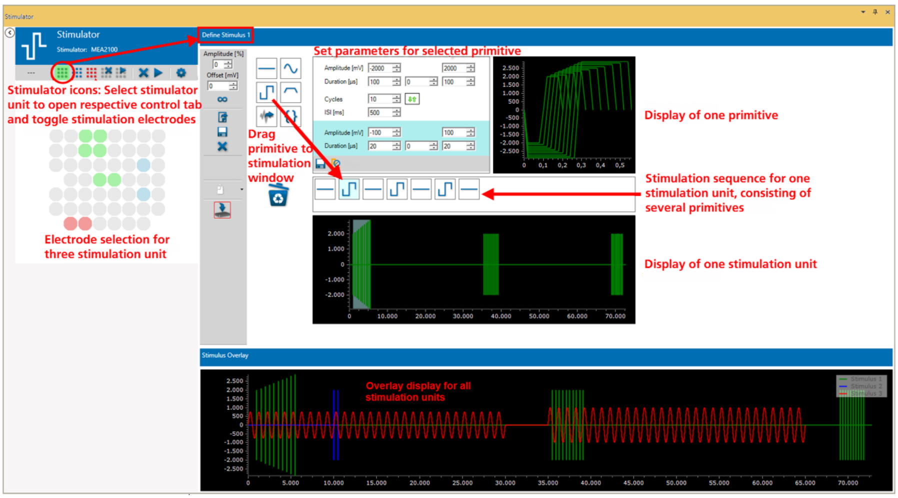


5.9.3 Operation

The operation of the stimulators of the MEA2100- and ME2100-Systems are largely identical, hence, they are described together in this chapter. The differences are shown in the first two subchapters.

5.9.3.1 Operation MEA2100

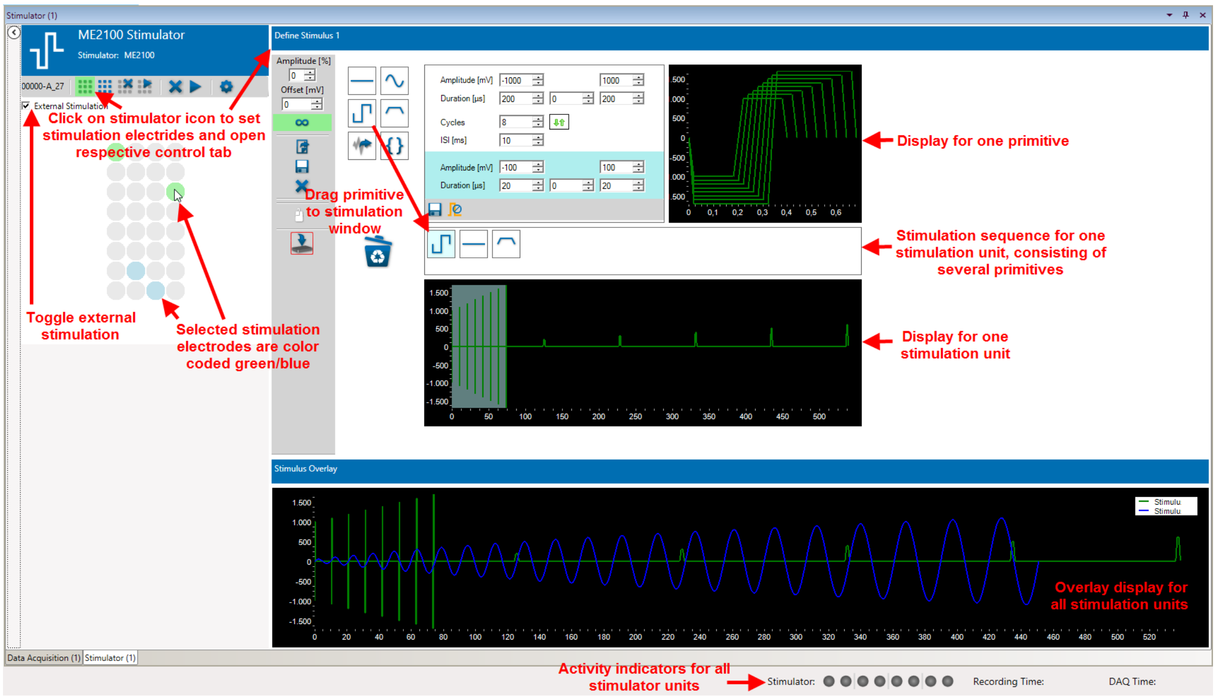
The three units of the MEA2100 stimulator are color coded in **Stimulator 1** green, **Stimulator 2** blue **and Stimulator 3** red. The MEA2100-256 and the MEA2100-Mini only have the first two, green and blue. Each stimulator has an independent control tab. The programming of a stimulation pattern is achieved by combining predefined waveforms, so called **primitives**. Click on a Stimulator unit icon to open the respective control tab.



5.9.3.2 Operation ME2100

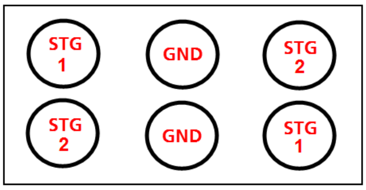
The two units of the ME2100 stimulator are color coded in **Stimulator 1** green, **Stimulator 2** blue. Each stimulator has an independent control tab. Each connected headstage has two independent stimulator units. All control functions apply to the stimulator of the headstage currently selected in the ME2100 data source (see also chapter 5.4.4).

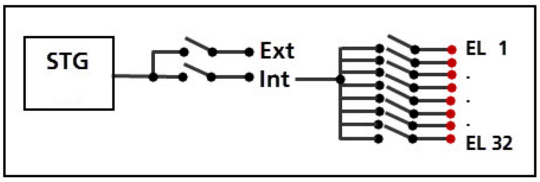
The programming of a stimulation pattern is achieved by combining predefined waveforms, so called **primitives**. Click on a Stimulator unit icon to open the respective control tab. The stimulator control window will display the electrode layout selected in the data source. Click on any electrode to toggle it as stimulation electrode for the selected Stimulator unit. One electrode can only be assigned to one Stimulator unit at a time.



The "**External Stimulation**" feature is unique to the ME2100 stimulator. If activated, the programmed stimulation patterns will be applied to the selected channels of the recording electrode attached to the respective headstage **and** to the external stimulation output on the side of the ME2100 headstage. If you want to use the external output only, deselect all recording electrodes. The external stimulation output allows the connection of one or two external stimulation electrodes. The six-pin socket is **protected against polarity reversal,** as the connector can be plugged in either way. The pins for STG 1 and 2 and GND are physically connected internally.

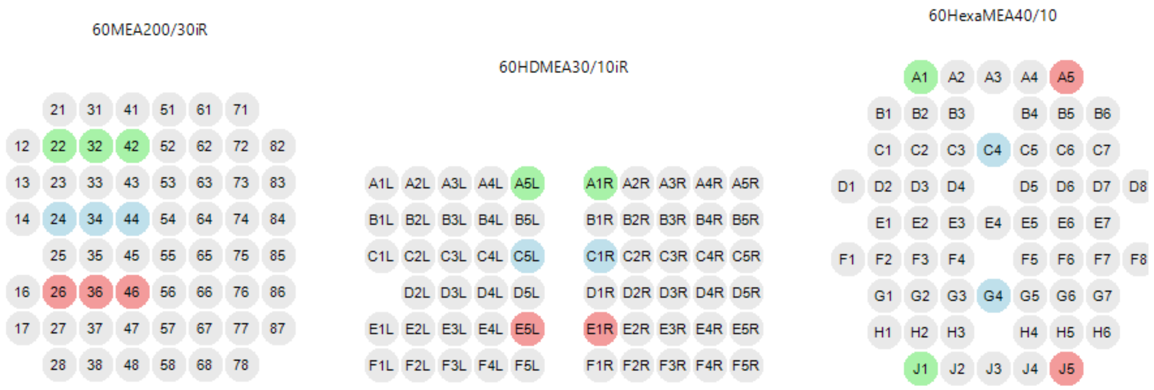
Please see the pin layout of the connector and the connection scheme below.





5.9.3.3 Selection of Stimulation Electrodes

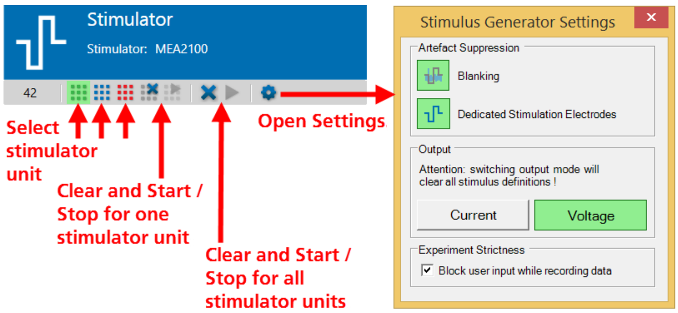
The stimulator control window will display the electrode layout selected in the data source. Click on any electrode to toggle it as stimulation electrode for the selected Stimulator unit. **One electrode** can only be assigned to **one stimulator** unit at a time.



Attention: In voltage-controlled stimulation, any number of electrodes can be selected for each stimulator. If more than one electrode per stimulator is selected in current controlled stimulation, the applied current would be divided between all selected electrodes according to their impedance in an unpredictable way. Therefore, in current mode only one stimulation electrode per stimulator unit is allowed.

5.9.3.4 Start / Stop / Settings

Stimulator units can be started and stopped **independently or simultaneously**. Similarly, stimulation electrodes can be cleared for individual units or altogether. In the Settings menu, it's possible to switch between **current or voltage** controlled stimulation.

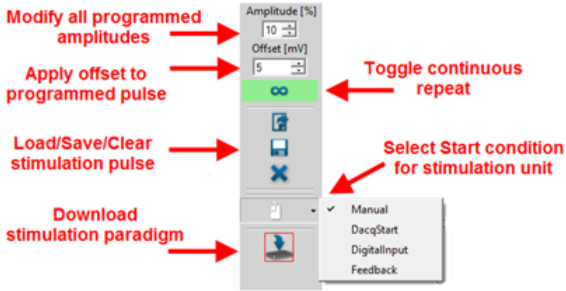


The **Artefact Suppression** functions are also toggled here. **Blanking** disconnects all electrodes for a short period of time during the stimulation pulse, to reduce stimulation artefacts. **Dedicated Stimulation Electrodes** will permanently connect the selected stimulation electrodes to the respective stimulator units, instead of switching back and forth between recording and stimulation.

This will decrease artefacts even more, but the stimulation electrodes are lost for recoding. With this function activated, the stimulation electrodes will show an **increased noise level** and no signals whatsoever. Finally, under **Experiment Strictness** the user can decide whether changes in the stimulation paradigm should be possible while recording data.

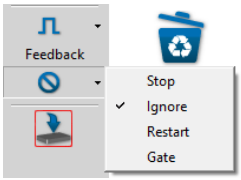
5.9.3.5 Stimulator Control Functions

Each Stimulator unit has independent controls to select a start condition, load/save stimulation patterns and so on. It is possible to modify the programmed **amplitude** of all pulses in a pulse pattern by a certain percentage with a single up down window. Likewise, it is possible to apply a constant **offset** to the complete stimulation paradigm.



Stimulation pulses can be applied once, as programmed, or repeated till manually stopped, if the **Loop Stimulus** function is active. Once programmed, stimulation paradigms can be saved and reloaded. Stimulation paradigms are saved as *.xml files. Each Stimulation unit can also generate **external Tigger** events, in addition to the internal triggers which are generated with each individual stimulation pulse in any case. In the data source, digital Out bits can be assigned to each stimulator instrument, you have to select a different bit for each of the three stimulation units (see 5.2.4.1, Digital Out Bit Selection). The TTLs will be generated on the Digital Out connectors on the Interface Board or the DI/O box, respectively.

The **Start condition** for each Stimulation unit can be selected from a drop down menu. Start can be either on manual command, with start of the Data Acquisition, on an external TTL on the Digital Inputs on the Interface Board, or controlled by the Real Time Feedback (please see chapter 5.2.3.4, Real Time Feedback). If Feedback or Digital Input are selected, additional options appear to select the Digital Input bit, and to set how to handle repeated start commands.



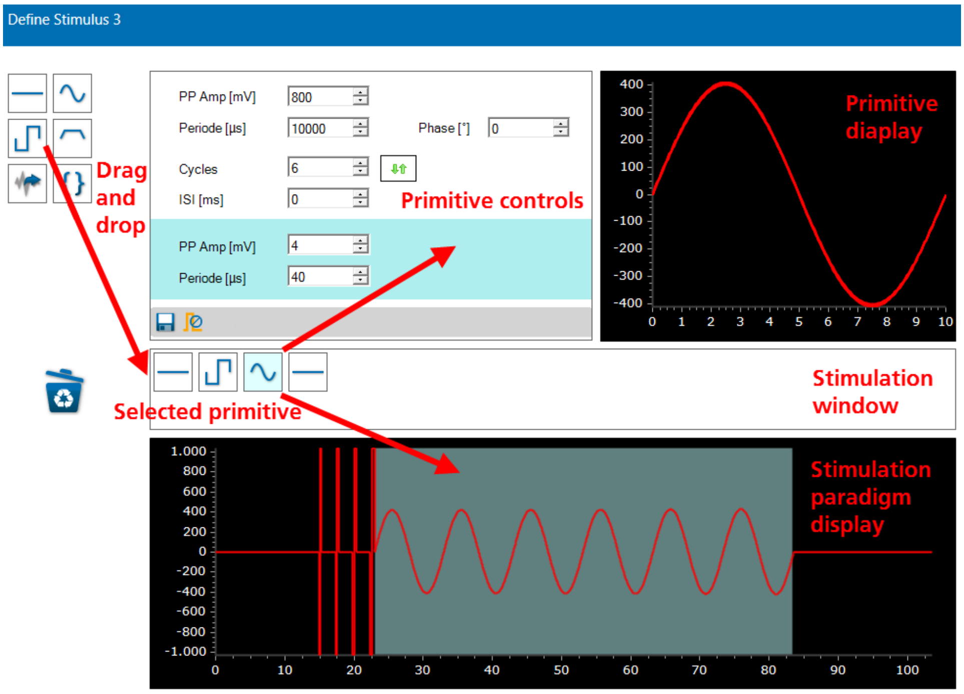
A repeated Start trigger, either external or from the RTF, while a stimulation is running can either:

- stop the current stimulation.
- be ignored.
- restart the stimulation paradigm.
- be used as a Gate. Gate means the Stimulation will be applied as long as the Start Trigger condition is fulfilled. This can either be as long as an external TTL is HIGH, or as long as the RTF condition is fulfilled, and the resulting trigger event continues.

All changes in the stimulation paradigm must be **downloaded** to the MEA/ME2100-System device. If the Download icon is grey, no changes have been made since the last download.

5.9.3.6 Defining a Stimulation Paradigm

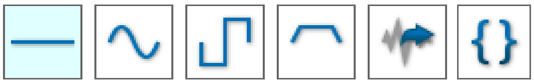
A stimulation paradigm can be assembled from predefined waveforms, so called **Primitives**. The primitives can simply be **dragged** to the stimulation window. Please also see movie for illustration. To delete a primitive, drag it to the **trash bin**. Click on any primitive in the stimulation window to open the respective controls. There is one display for the selected primitive, and one for the complete stimulation paradigm. The selected primitive is highlighted in the Stimulation paradigm display and the Stimulation window, and the respective controls are available.



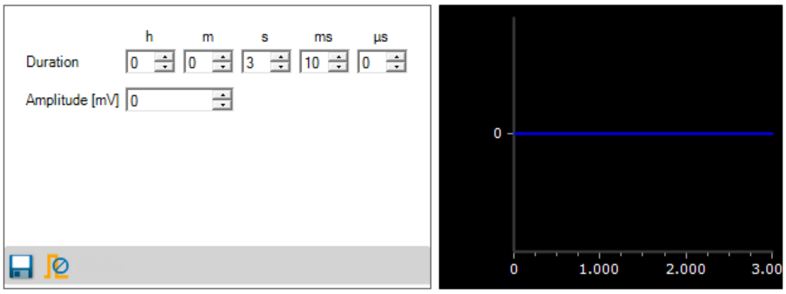
5.9.3.7 Primitives

The following primitives are available:

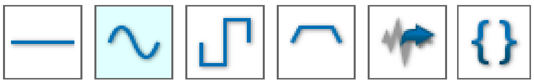
Flat Line



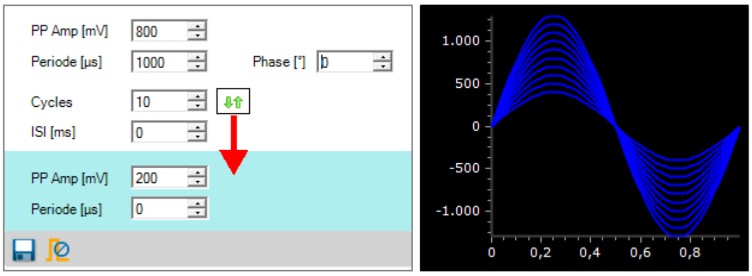
A flat line usually defines a break between two stimulation pulses. The controls allow to set a duration and an offset with an amplitude in mV.



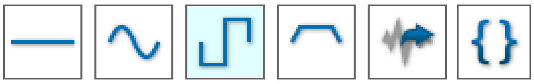
Sine Wave



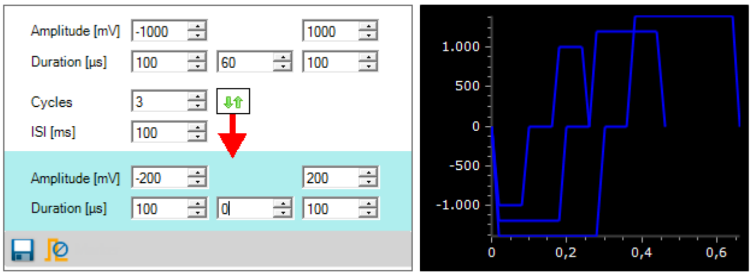
A sine wave stimulation can be defined by amplitude and period. A phase shift of 90, 180, 270 or 360° can also be selected, as well as a number of **cycles (repeats)** with an inter stimulus interval (ISI). If the number of cycles is >1, the advanced settings become available. Advanced settings allow to change the pulse parameters with each cycle. In the example below, there will be ten consecutive sine waves without ISI, each wave will have an amplitude 200 mV larger than the one before.



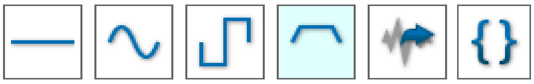
Rectangular Pulse



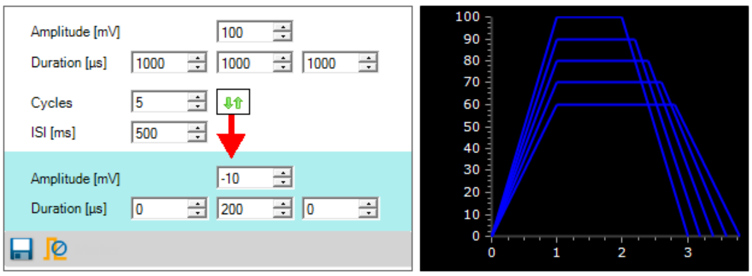
Rectangular pulses are the most commonly used stimulation pattern on MEAs. It's recommended to use biphasic, **charge balanced** pulses with the **negative phase first**. A rectangular pulse can be defined by amplitude and duration, independently for both phases. A break between the phases can also be selected, as well as a number of **cycles (repeats)** with an inter stimulus interval (ISI). If the number of cycles is >1, the advanced settings become available. Advanced settings allow to change the pulse parameters with each cycle. In the example below, there will be three consecutive pulses with 100 ms ISI, each pulse will have an amplitude 200 mV larger, and a duration 100 µs longer than the one before.



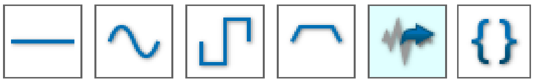
Ramp



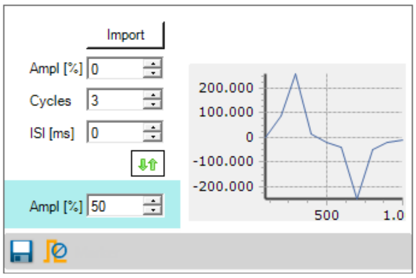
A ramp can be defined by amplitude rising and falling flank, and plateau, as well as a number of **cycles (repeats)** with an inter stimulus interval (ISI). If the number of cycles is >1, the advanced settings become available. Advanced settings allow to change the pulse parameters with each cycle. In the example below, there will be five consecutive ramps with 100 ms ISI, each ramp will have an amplitude 10 mV smaller, and a plateau 200 µs longer than the one before.

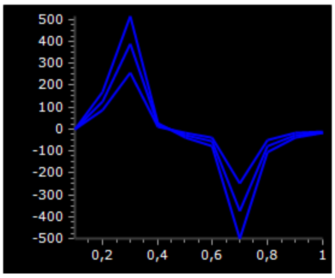


Custom Waveform



To generate a custom waveform, it's possible to **import an ASCII file**, and modify it in terms of amplitude. If more than one cycle is selected, amplitude steps are also possible. In the example below, there will be three consecutive repeats of the imported waveform with no ISI, each signal will have an amplitude 50 % larger than the one before.



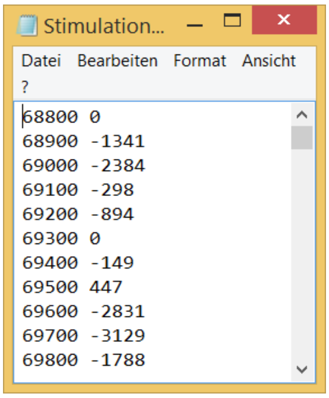


To use, for example, a biological signal as stimulation pulse, the signal needs to be converted to ASCII with a certain format. After that, the ASCII file can be imported to the primitive by clicking the **Import** button. The following format is mandatory for the ASCII file:

Voltage Mode
Time stamp Voltage
Time stamp Voltage
Time stamp Voltage
...

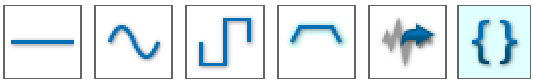
OR

Current Mode
Time stamp Current
Time stamp Current
Time stamp Current
...

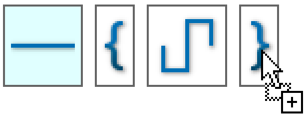


The unit for the **time stamp is µs** and for the **voltage/current value µV or nA**. The units are not part of the file. Only integers are accepted and commas, tabulators or spaces to separate the time stamp and the voltage / current value. Remove all possible headers and use a new line for each integer pair.

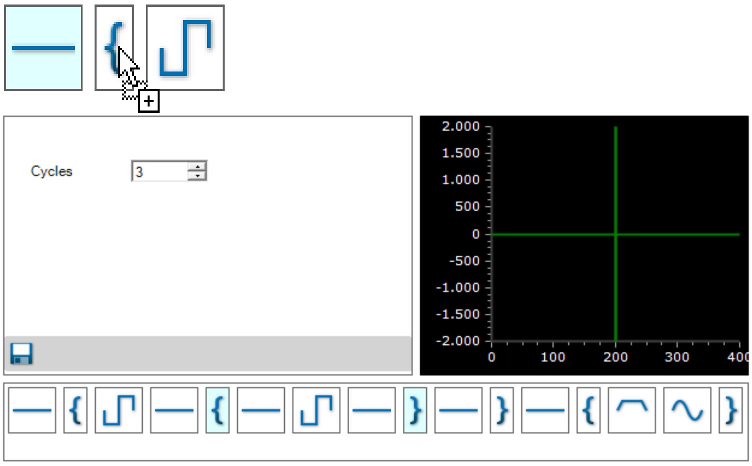
Brackets



Brackets are not a waveform, but allow to introduce repeats to parts of the complete stimulation paradigm. If you drag the bracket icon into the paradigm, it will appear as a separated pair of brackets. If you drop a primitive on the **second** bracket, it will appear **inside** the brackets.



If you drop the primitive on the **first** bracket, it will appear **before** the brackets.



Pairs of brackets can be used **in series**, one after the other, or also as **brackets within brackets**. The sequence will be executed by the same rules as a mathematical operation. Click on each pair of brackets to select the number of cycles for the complete sequence of primitives within the brackets.

Primitive Defaults

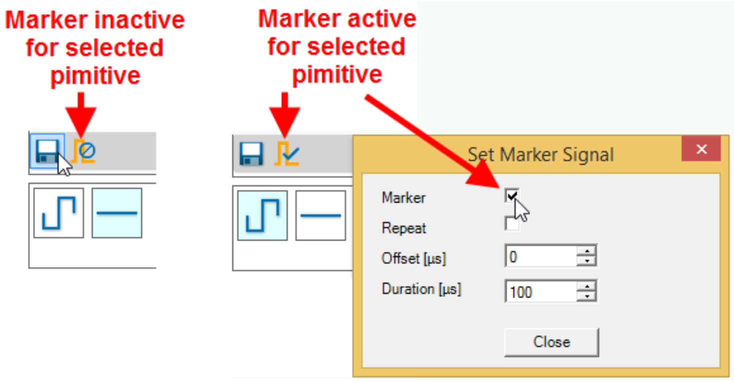
Each primitive has certain default values. However, for each primitive, the **Set primitive as default** function is available.

This function will **temporary** set the currently selected parameters as default for a certain primitive type. This can be useful if a specific pulse is to be used repetitively in a pulse paradigm. For example, if a rectangular pulse with +/-3 V / 200 µs is required repetitively, drag the rectangular pulse primitive to the stimulation window as usual. It will open with the standard default values. Change the settings as needed, and click Set primitive as default. The next rectangular pulse primitive dragged to the stimulation window will have the selected parameters as default.

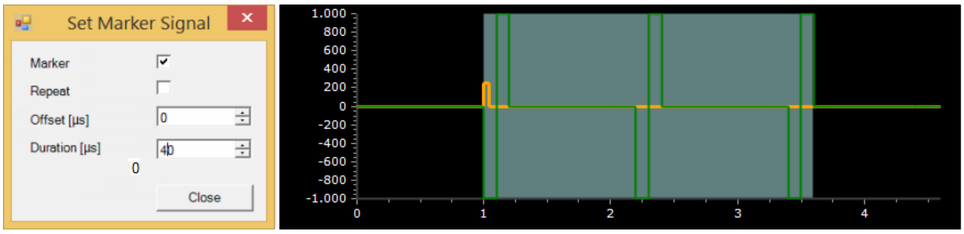
5.9.3.8 Marker and Digital Outputs

Each stimulator will automatically generate triggers for start and stop of each individual pulse on its trigger output (pink port). In addition, it is possible to add a **Marker** to the stimulation paradigm, which can be activated or deactivated individually for each primitive.

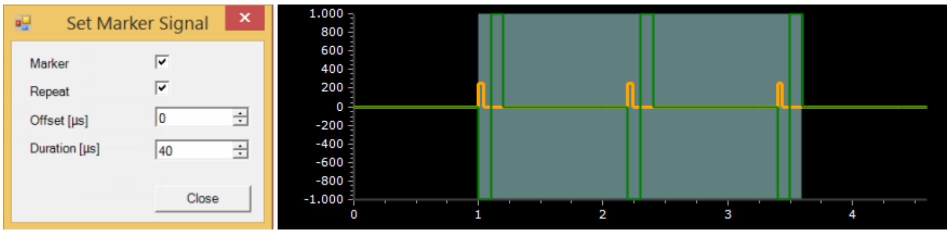
The Marker can be used for internal triggering, or to generate **external TTL pulses** from the Digital Out ports on the Interface Board. Select the **DigOut port bit** for any stimulator unit in the data source, you have to select a different bit for each of the three stimulation units (see 5.2.4.1, Digital Out Bit Selection). Please note, **bit 0 refers to the Lemo connector Digital Out 1** on the IFB, bit 1 to Digital Out 2 and so on. Bits 4 and up are only accessible if the DI/O extension is used. Select a primitive, by default the Marker is inactive. Click on the Marker icon to open the **Set Marker Signal** menu and activate the Marker tick box. The icon will change to indicate the active Marker.



The Marker by default is generated at the beginning of each primitive. The **duration** of the Marker and a positive **offset** in relation to the beginning of the primitive can be set. The Marker is shown in yellow.



If the **Repeat** tick box is selected, the Marker will be generated not only at the beginning of the primitive, but at the beginning of each pulse within the primitive. Duration and Offset settings apply to all markers.

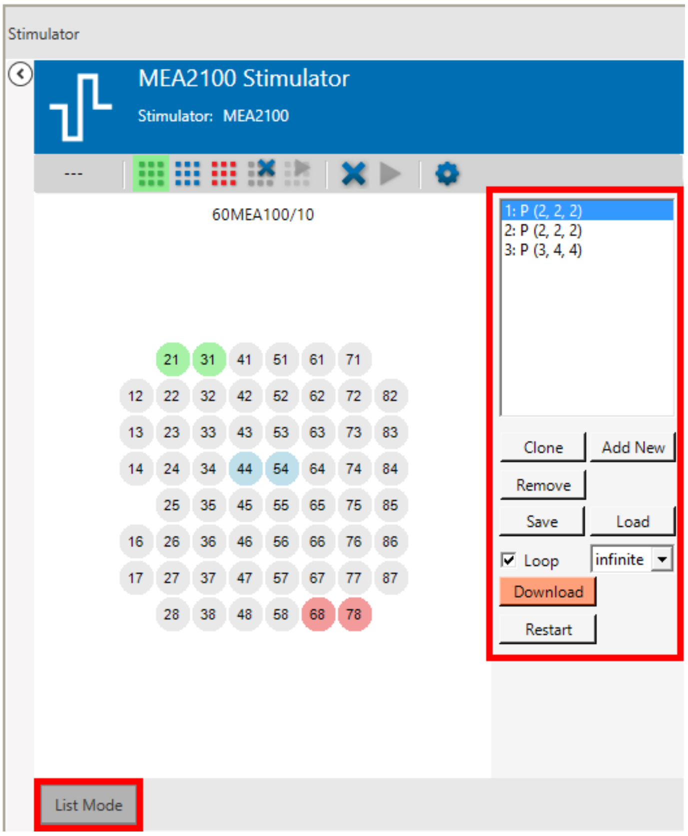


5.9.3.9 Stimulator List Mode

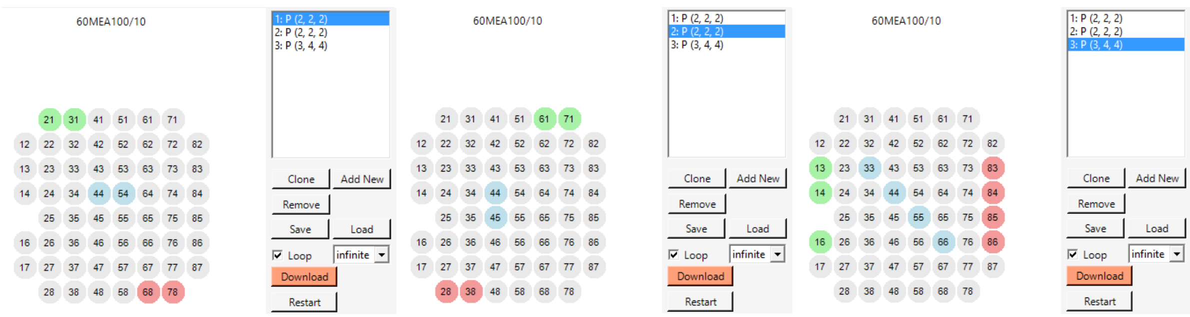
Stimulator List Mode (SLM) allows the **automated application** of a list of predefined stimulation electrode patterns. The patterns are applied one after another after the start signal. The pattern list may be repeatedly applied depending on the Loop setting. The same stimulation pulse is applied to each of the patterns.

Attention: List Mode is currently only available for MEA2100-60/2x60/120/256 headstages. It only works for single well MEA layouts.

The List Mode can be toggled via the **List Mode button** in the Stimulator control window. If enabled, a panel is made visible to the right of the electrode layout.



Each entry in the list defines one pattern and can contain a single or multiple selected stimulation electrodes, for one or more stimulators (green, blue, red). The list shows entries in numbered order with the number of selected electrodes for each stimulator in parenthesis. When an entry in the list is selected the electrode pattern for all stimulators is shown. Due to restrictions of the internal memory, the **number of list entries is limited to 256**.



The functions of the Stimulator List Mode interface are as follows:

- **Clone:** Add an entry in the list with the same pattern as the currently selected.
- **Add New:** Add a new entry to the end of the list with an empty electrode pattern.
- **Remove:** Removes the currently selected entry from the list.
- **Save/Load:** Saves the pattern list to a file or load from a file (*.lmp). Only pattern files with the same MEA layout can be loaded. Loading overwrites the current list entries.
- **Loop:** Determines the switching to the next pattern in the list.
    - turned off: only one pattern is applied, until the next trigger occurs
    - 1: all the patterns in the list are applied once, one after the other
    - 2,3: all the patterns in the list are applied one after the other, then the list is repeated two or three times, respectively
    - infinite: the pattern list is applied until the stimulation is stopped
- **Download:** After each change in the GUI the patterns and loop settings must be downloaded in order to be activated on the hardware.
- **Restart:** Resets the current pattern to the first entry in the list.

If SLM is active and an electrode pattern is downloaded, the first pattern of the list is applied when the selected start trigger occurs. The **start trigger** is defined in the stimulus pattern of the **first (green) stimuluator unit** (Manual, DacqStart, Digital Input, or Feedback). For the other stimulator units, the start trigger selection is disabled in List Mode. If a trigger occurs while the stimulus pulse is still running, it is ignored. A start trigger will always **start all three stimulator units** simultaneously. If more than one stimulator unit is used, the duration of the longest programmed stimulation pattern determines the end of the stimulation and the switch to the electrode pattern

If **Loop** is turned off, the stimulator is halted after the stimulation pattern has ended until the next start trigger occurs. The next electrode pattern in the list is then preselected and gets applied by the next trigger. If Loop is turned on, the patterns in the list are automatically applied one after each other (see above). There must be at least one electrode selected, otherwise the switching to the next pattern doesn’t occur. If the stimulator is stopped manually, then no switch to the next electrode pattern occurs.

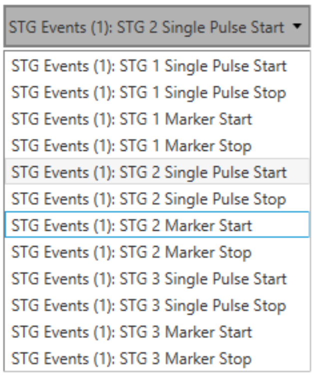
The handling of the stimulus pattern is not changed by the List Mode, with one exception: a stimulation pattern with **inifinite loop** is incompatible with the List Mode function. A pattern with infinite duration would prohibit the switching of the electrode patterns.

5.9.4 Trigger Output

The stimulator of the MEA2100- and ME2100-System has a pink data port for trigger events. These events will be generated automatically, for **each stimulator unit of each headstage** there are four events:

- Single Pulse Start
- Single Pulse Stop
- Marker Start
- Marker Stop

This means there will be on trigger at the beginning, and one at the end of each pulse, and also at the beginning and end of each marker. These trigger events can be recorded, and used to control other instruments, like the **Sweeps** tool or the **Recorder**.

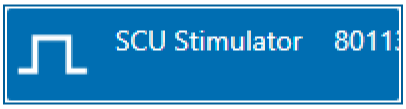


5.9.5 Example: How to Apply a Stimulation Automatically to all Electrodes Row by Row

With the Stimulator List Mode function, it is possible to scan through all electrodes of an MEA automatically. To scan through an MEA in lines with a single stimulator, first program the required stimulation pattern with a proper pause between the individual pulses for the first stimulator unit (green). Make sure *not* to activate the infinite loop function for the stimulator. Then open the List Mode interface and select the first row of electrodes for stimulation in the first list entry. Then click “Add new” to create a new entry. Select the second row of electrodes. Repeat this till you have eight list entries with one row each. Set the “Loop” in the List Mode interface to infinite, and the start condition in the green Stimulator unit to Manual. Download the settings.

As soon as the stimulation is manually started, the programmed pulse will be applied to the first row of electrodes. After the pulse is over, including the pause, the pattern switches to the next row and continues. Stimulation will be applied till manually stopped.

5.10 Stimulators: SCU Stimulator

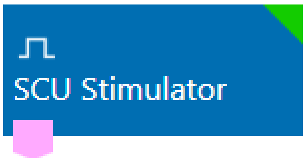


5.10.1 Description and Purpose

The SCU Stimulator instrument controls the **Opto Stim** output on the ME2100 and MEA2100-Mini SCU. The Stimulator instrument becomes available only after a data source has been activated (dragged into the main window). Each instance of the experimenter controlling one SCU has an independent SCU Stimulator.

5.10.2 Data Ports and Export Options

The SCU Stimulator has a Trigger data port, which produces Trigger events marking start and stop of the stimulator and individual pulses. There are no export options.

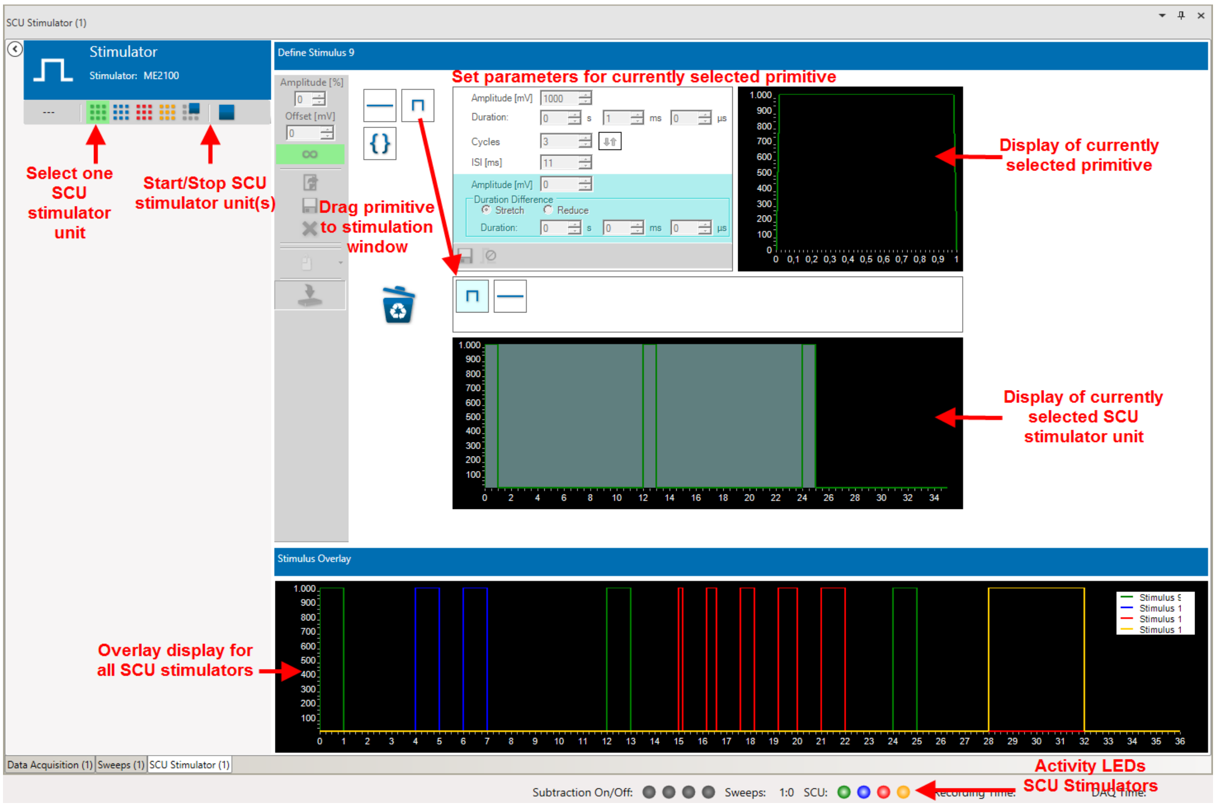


5.10.3 Operation

The operation of the SCU stimulator is similar to the MEA2100- and ME2100-System stimulator. Four stimulator units are available for each SCU.

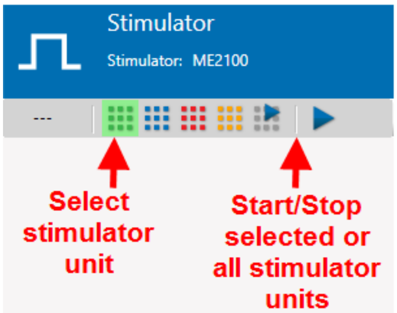
The four units of the SCU stimulator are color coded in **Stimulator 9** green**, Stimulator 10** blue**, Stimulator 11** **red** and **Stimulator 12** orange. The numbering starts with nine, as potentially four ME2100 or four MEA2100-Mini headstages with two stimulators each could be connected to each SCU, occupying Stimulator numbers 1 to 8.

Each stimulator has an independent control tab. The programming of a stimulation pattern is achieved by combining predefined waveforms, so called **primitives**. Click on a Stimulator unit icon to open the respective control tab.



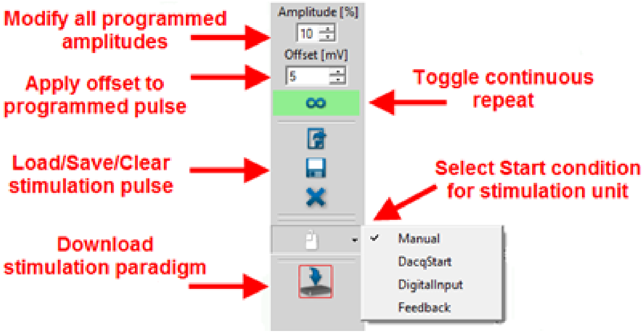
5.10.3.1 Start / Stop

Stimulator units can be started and stopped **independently or simultaneously**. Changes in the stimulation pattern are downloaded automatically if needed.



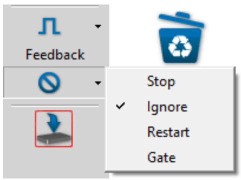
5.10.3.2 Stimulator Control Functions

Each Stimulator unit has independent controls to select a start condition, load/save stimulation patterns and so on. It is possible to modify the programmed **amplitude** of all pulses in a pulse pattern by a certain percentage with a single up down window. Likewise, it is possible to apply a constant **offset** to the complete stimulation paradigm.



Stimulation pulses can be applied once, as programmed, or repeated till manually stopped, if the **Loop Stimulus** function is active. Once programmed, stimulation paradigms can be saved and reloaded. Stimulation paradigms are saved as *.xml files. Each Stimulation unit can also generate **external Tigger** events, in addition to the internal triggers which are generated with each individual stimulation pulse in any case. In the data source, digital Out bits can be assigned to each stimulator instrument, you have to select a different bit for each of the four stimulation units (see 5.4.6.1 Digital Out Bit Selection). The TTLs will be generated on the Digital Out connectors on the Interface Board or the DI/O box, respectively.

The **Start condition** for each Stimulation unit can be selected from a drop down menu. Start can be either on manual command, with start of the Data Acquisition, on an external TTL on the Digital Inputs on the Interface Board, or controlled by the Real Time Feedback (please see chapter 5.2.3.4, Real Time Feedback). If Feedback or Digital Input are selected, additional options appear to select the Digital Input bit, and to set how to handle repeated start commands.



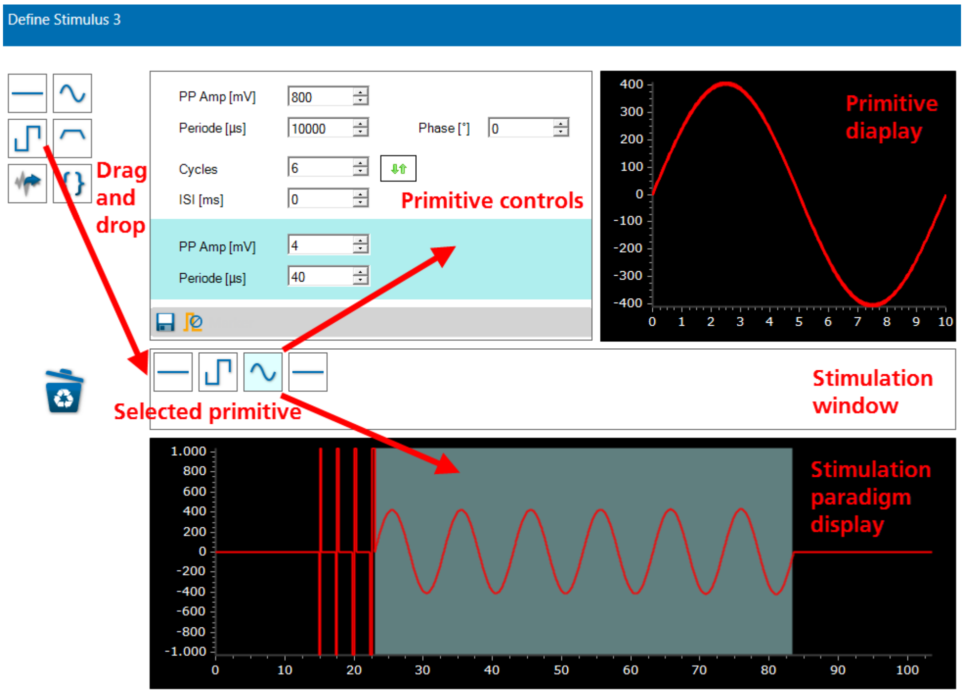
A repeated Start trigger, either external or from the RTF, while a stimulation is running can either:

- stop the current stimulation.
- be ignored.
- restart the stimulation paradigm.
- be used as a Gate. Gate means the Stimulation will be applied as long as the Start Trigger condition is fulfilled. This can either be as long as an external TTL is HIGH, or as long as the RTF condition is fulfilled, and the resulting trigger event continues.

All changes in the stimulation paradigm must be **downloaded** to the MEA/ME2100-System device. If the Download icon is grey, no changes have been made since the last download.

5.10.3.3 Defining a Stimulation Paradigm

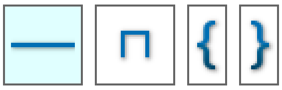
A stimulation paradigm can be assembled from predefined waveforms, so called **Primitives**. The primitives can simply be **dragged** to the stimulation window. Please also see movie for illustration. To delete a primitive, drag it to the **trash bin**. Click on any primitive in the stimulation window to open the respective controls. There is one display for the selected primitive, and one for the complete stimulation paradigm. The selected primitive is highlighted in the Stimulation paradigm display and the Stimulation window, and the respective controls are available.



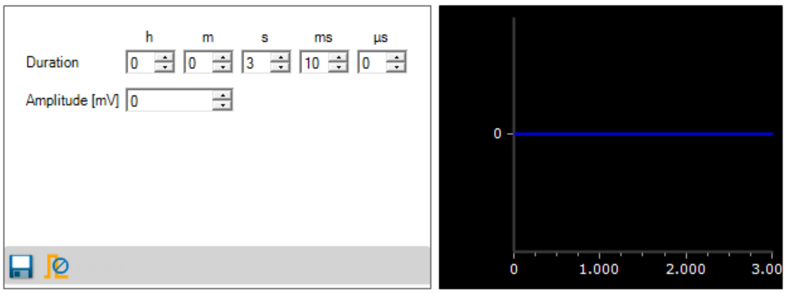
5.10.3.4 Primitives

The following primitives are available:

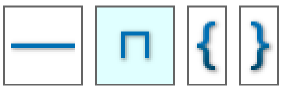
Flat Line



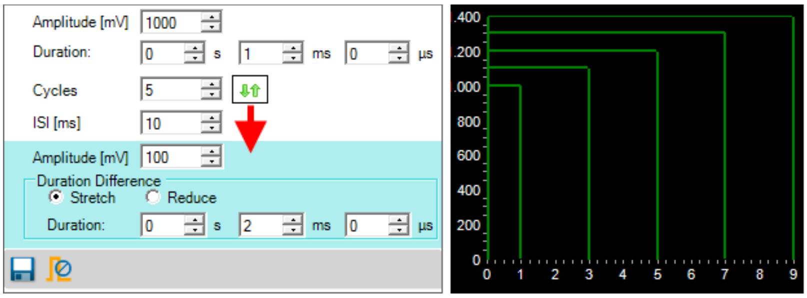
A flat line usually defines a break between two stimulation pulses. The controls allow to set a duration and an offset with an amplitude in mV.



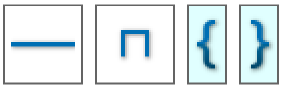
Rectangular Pulse



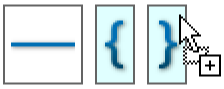
Positive, rectangular pulses can be used to control external light sources. A rectangular pulse can be defined by amplitude and duration. A break between the phases can also be selected, as well as a number of **cycles (repeats)** with an inter stimulus interval (ISI). If the number of cycles is >1, the advanced settings become available. Advanced settings allow to change the pulse parameters with each cycle. In the example below, there will be ten consecutive pulses with 10 ms ISI, each pulse will have an amplitude 100 mV larger, and a duration 2 ms longer than the one before.



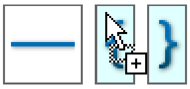
Brackets



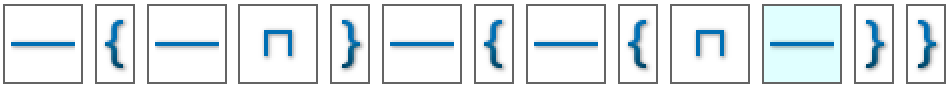
Brackets are not a waveform, but allow to introduce repeats to parts of the complete stimulation paradigm. If you drag the bracket icon into the paradigm, it will appear as a separated pair of brackets.
If you drop a primitive on the **second** bracket, it will appear **inside** the brackets.



If you drop the primitive on the **first** bracket, it will appear **before** the brackets.



Pairs of brackets can be used **in series**, one after the other, or also as **brackets within brackets**.
The sequence will be executed by the same rules as a mathematical operation. Click on each pair of brackets to select the number of cycles for the complete sequence of primitives within the brackets.



Primitive Defaults

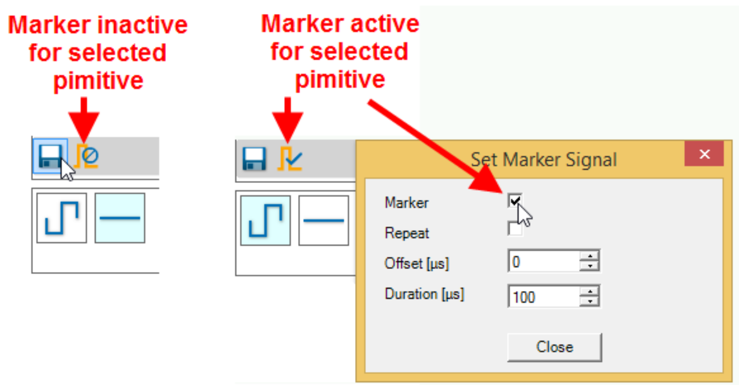
Each primitive has certain default values. However, for each primitive, the **Set primitive as default** function is available.

This function will **temporary** set the currently selected parameters as default for a certain primitive type. This can be useful if a specific pulse is to be used repetitively in a pulse paradigm. For example, if a rectangular pulse with +/-3 V / 200 µs is required repetitively, drag the rectangular pulse primitive to
the stimulation window as usual. It will open with the standard default values. Change the settings as needed, and click Set primitive as default. The next rectangular pulse primitive dragged to the stimulation window will have the selected parameters as default.

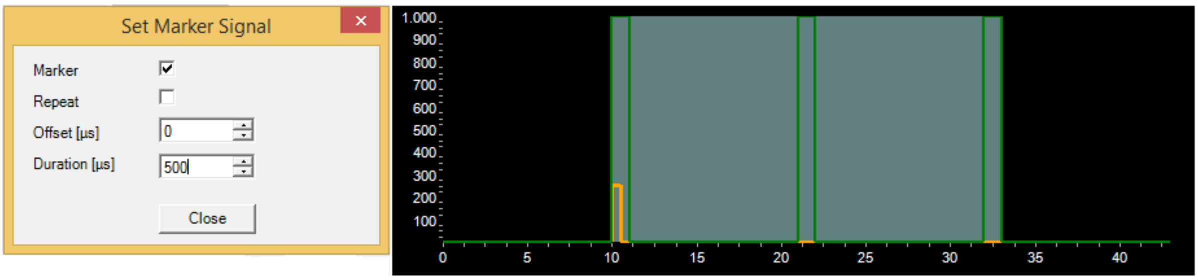
5.10.3.5 Marker and Digital Outputs

Each stimulator will automatically generate triggers for start and stop of each individual pulse on its trigger output (pink port). In addition, it is possible to add a **Marker** to the stimulation paradigm, which can be activated or deactivated individually for each primitive.

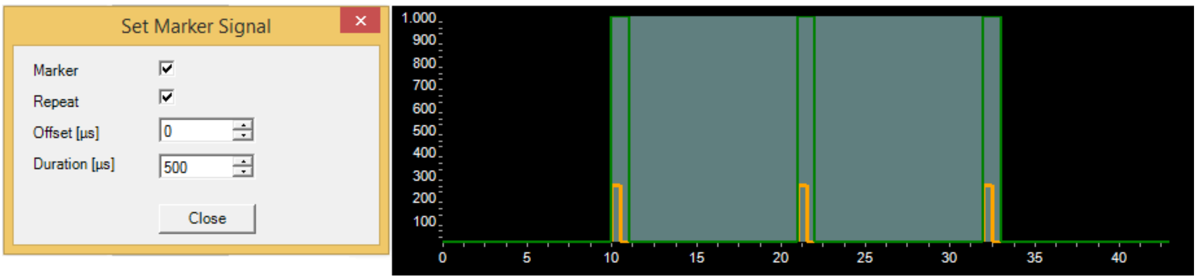
The Marker can be used for internal triggering, or to generate **external TTL pulses** from the Digital Out ports on the Interface Board. Select the **DigOut port bit** for any stimulator unit in the data source, you have to select a different bit for each of the three stimulation units (see 5.2.4.1, Digital Out Bit Selection). Please note, **bit 0 refers to the Lemo connector Digital Out 1** on the IFB, bit 1 to Digital Out 2 and so on. Bits 4 and up are only accessible if the DI/O extension is used. Select a primitive, by default the Marker is inactive. Click on the Marker icon to open the **Set Marker Signal** menu and activate the Marker tick box. The icon will change to indicate the active Marker.



The Marker by default is generated at the beginning of each primitive. The **duration** of the Marker and a positive **offset** in relation to the beginning of the primitive can be set. The Marker is shown in yellow.



If the **Repeat** tick box is selected, the Marker will be generated not only at the beginning of the primitive, but at the beginning of each pulse within the primitive. Duration and Offset settings apply to all markers.

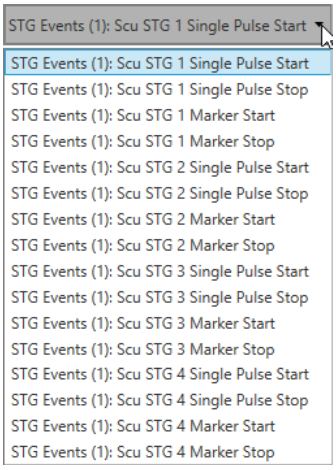


5.10.4 Trigger Output

The SCU stimulator has a pink data port for trigger events. These events will be generated automatically, for **each stimulator unit** there are four events:

- Single Pulse Start
- Single Pulse Stop
- Marker Start
- Marker Stop

This means there will be one trigger at the beginning, and one at the end of each pulse, and also at the beginning and end of each marker. These trigger events can be recorded, and used to control other instruments, like the **Sweeps** tool or the **Recorder**.



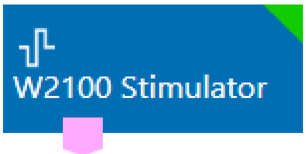
5.11 Stimulators: Stimulator of the W2100-System

5.11.1 Description and Purpose

The W2100-System Stimulator instrument controls the **optical or electrical stimulation** outputs of W2100-System headstages with stimulation capabilities. The Stimulator instrument becomes available only after a W2100-System data source has been activated (dragged into the main window). Each instance of the experimenter with a W2100-System has an independent Stimulator.

5.11.2 Data Ports and Export Options

The Stimulator has a Trigger data ports, which produces Trigger events marking start and stop of the stimulator and individual pulses. There are no export options.

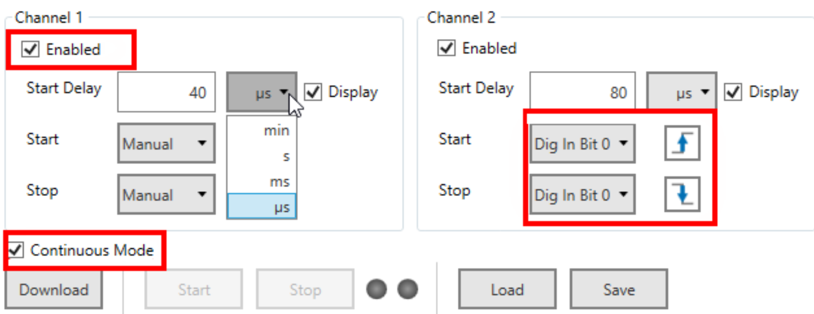


5.11.3 Operating Multiple Stimulation Headstages

If more than one headstage with stimulation capabilities, either optical or electrical, is selected in **Multi Headstage Mode**, the Stimulator tool will display multiple tabs with the respective serial numbers. It is possible to control each headstage independently.

5.11.4 Start and Stop

The Start and Stop options are identical for optical and electrical stimulation. Each stimulation channel can be **enabled** with a tick box. Only enabled channels are active.



A **start delay** can be set individually for both channels. This allows a time offset between the two channels, but also in relation to the start commend. The start command can be Manual, or a TTL pulse on any selected bit of the **Digital In** Channel. A **manual** start command always affects both stimulation channels, while both channels can be started and stopped on separate TTLs. Either the rising or the falling flank of the TTL can be used as Start/Stop signal. This allows for example the use of a TTL as a **gate trigger** for the stimulation channels, as shown above for Channel 2. An incoming start signal while the stimulator is still running will be **ignored**. In **Continuous Mode**, the programmed pulse is repeated till manually stopped.

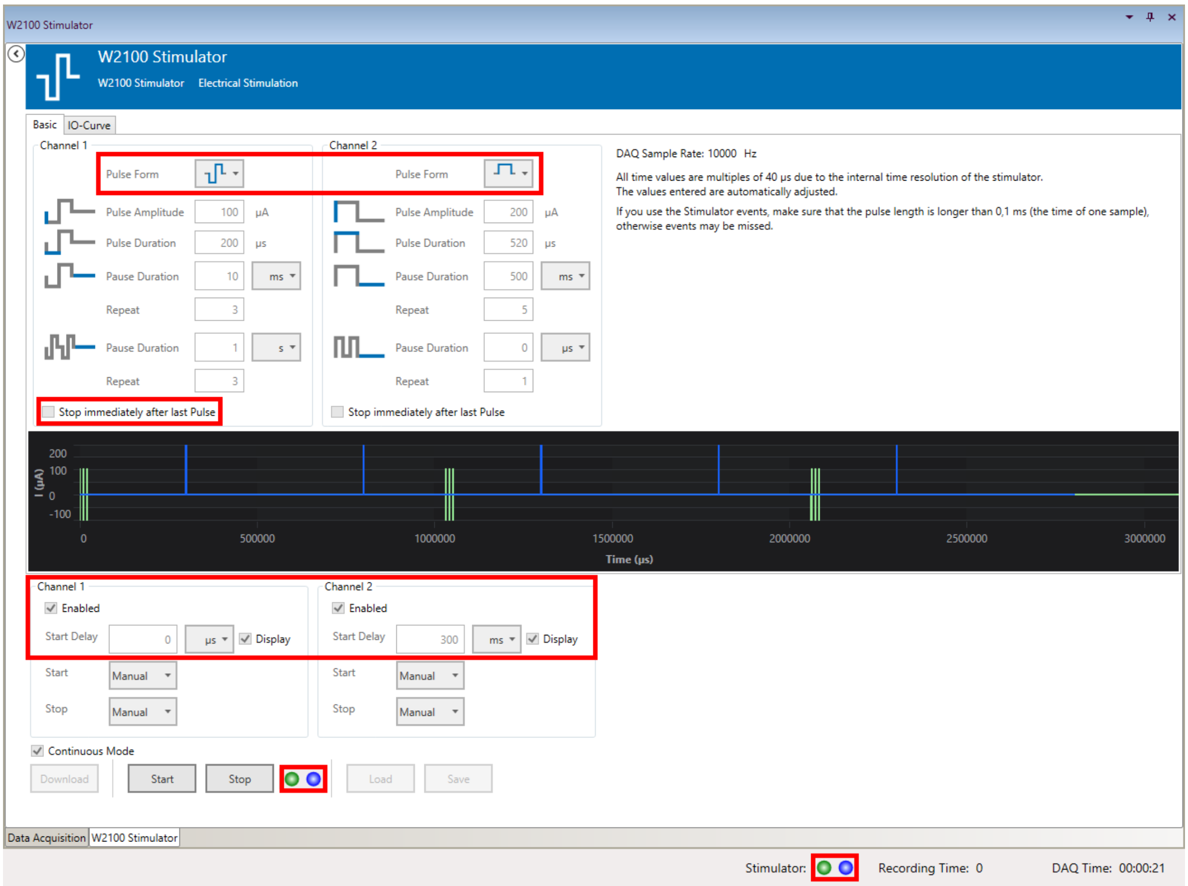
A stimulation paradigm needs to be **downloaded** before it can be used. Start/Stop is only available while the data acquisition is running, while Download is only available while it's stopped. Two **virtual LEDs** indicate the activity of the two stimulation channels. Stopping the data acquisition will also stop the stimulator.

5.11.5 Operation: Electrical Stimulation

If a headstage with electrical stimulation capabilities is selected, the stimulator control window will display two tabs, **Basic and IO-Curve**.

5.11.5.1 Basic

In the Basic tab, the stimulation paradigm for the two available stimulation channels can be programmed. Only rectangular current pulses are available. The **Pulse Form** can be selected individually for each channel from a drop down menu (pos/neg, neg/pos, only positive, only negative). If identical but inverted pulses without time offset are programmed for both stimulation channels, this results in a **bipolar stimulation**.



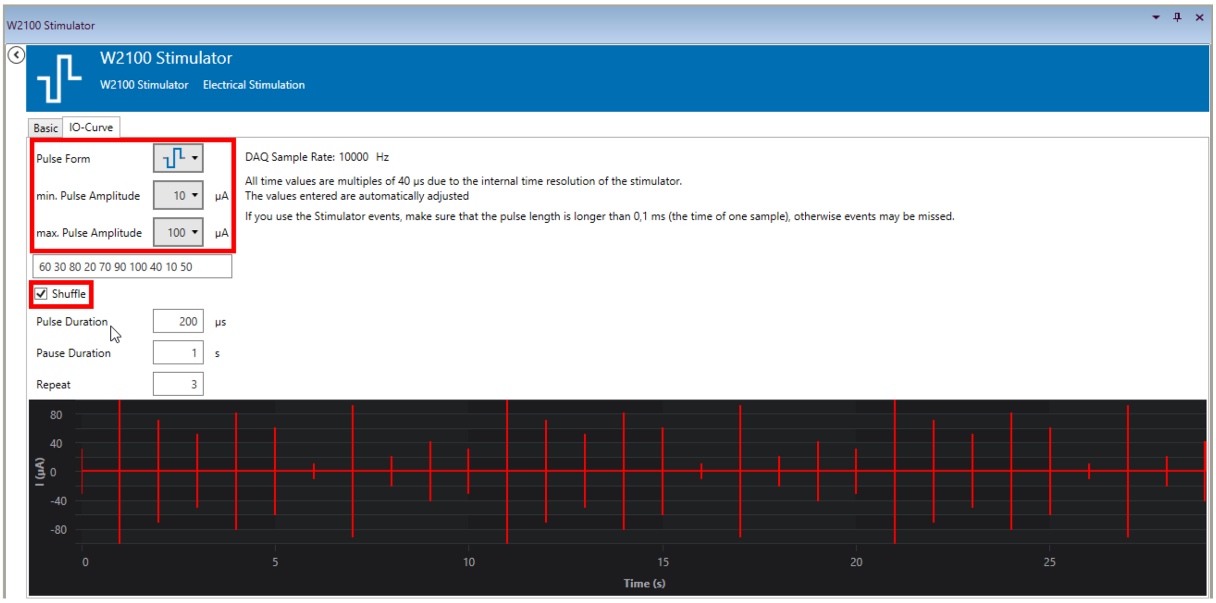
Amplitude, duration and inter pulse interval can be selected individually for each channel. Furthermore, a pulse train can be repeated a number of times, with a pause in between. Once programmed, a pulse paradigm can be **Saved/Loaded** as *.nsf file.

The programmed stimulation paradigm for both channels is shown in overlay in the stimulation display, **green** for Channel 1 and **blue** for Channel 2. It's possible to skip the inter pulse interval and pause between trains at the end of the stimulus paradigm and stop immediately by activating the **Stop immediately after last pulse** tick box. Each stimulation channel can be selected to be **enabled** or **disabled** with a second tick box. In **Continuous Mode**, the programmed pulse is repeated till manually stopped. A **start delay** can be set individually for both channels. This allows a time offset between the two channels, but also in relation to the start command.

A stimulation paradigm needs to be **downloaded** before it can be used. Start/Stop is only available while the data acquisition is running, while Download is only available while it's stopped. The green/blue **virtual LEDs** in the Stimulator window and in the lower Main Menu indicate ongoing stimulation on stimulation channels one and two, respectively.

5.11.5.2 IO-Curve

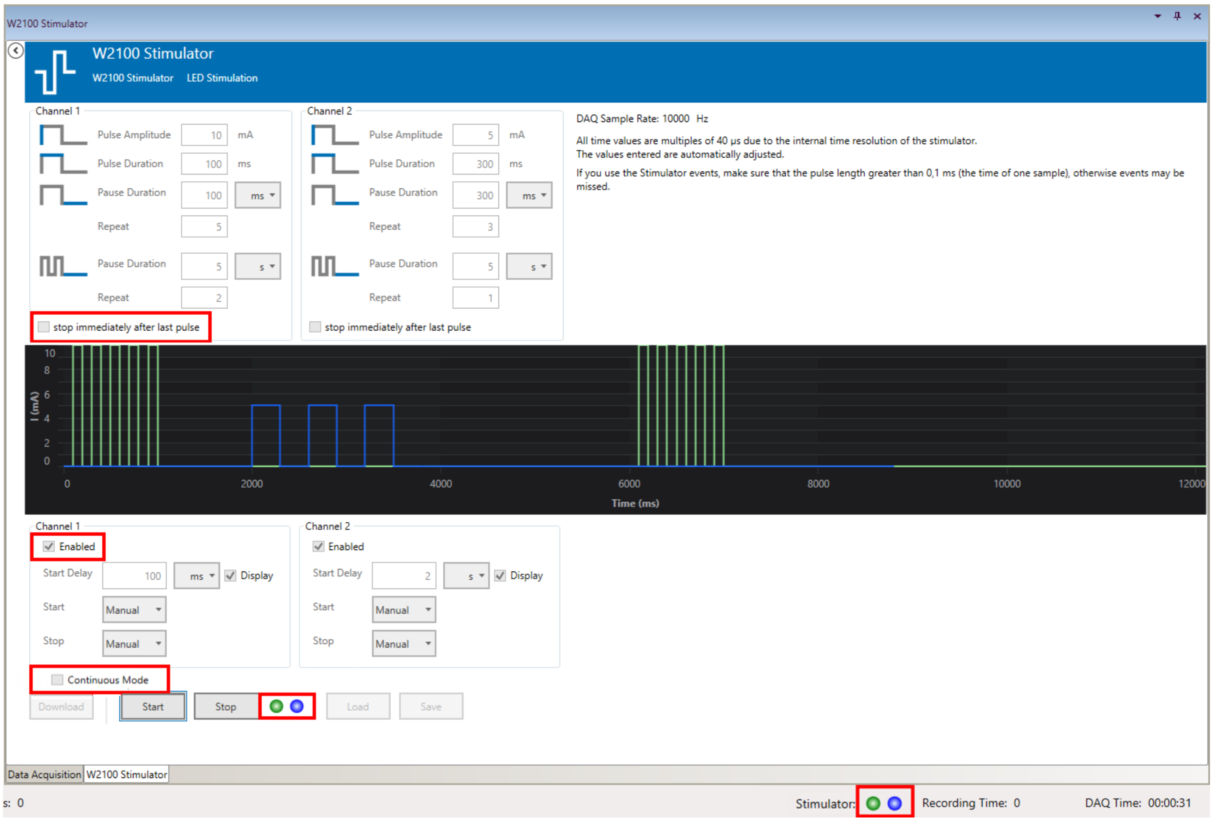
The IO-Curve is displayed only, but is applied to all **enabled** channels with the user defined **Start Delay**. For the IO-Curve, the highest and lowest value can be selected, and the software automatically calculates ten steps in between. These ten steps can be applied once, or repeatedly. The **Pulse Form** for the I/O-Curve can be selected from a drop down menu (pos/neg, neg/pos, only positive, only negative). Different stimulation amplitudes will be applied either linear, from lowest to highest value, or **shuffled**. Start, Stop and Repeat option are identical to the Basic mode.



Again, an IO-Curve needs to be **downloaded** before it can be used. Start/Stop is only available while the data acquisition is running, while Download is only available while it's stopped. The green/blue **virtual LEDs** in the Stimulator window and in the lower Main Menu indicate ongoing stimulation on stimulation channels one and two, respectively.

5.11.6 Operation: Optical Stimulation

If a headstage with optical stimulation capabilities is selected, the stimulator control window will display only one tab.

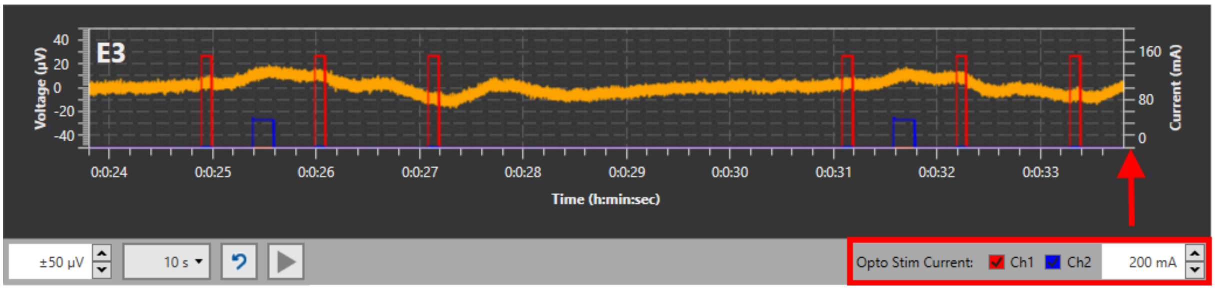


In this tab, the stimulation paradigm for the two available optical stimulation channels can be programmed. Only rectangular positive current pulses are available. If more than one headstage with stimulation capabilities is selected in Multi Headstage Mode, it is possible to select a headstage by serial number. Amplitude, duration and inter pulse interval can be selected individually for each channel. Furthermore, a pulse train can be repeated a number of times, with a pause in between. Once programmed, a pulse paradigm can be **Saved / Loaded** as *.osf file.

The programmed stimulation paradigm for both channels is shown in overlay in the stimulation display, **green** for Channel 1 and **blue** for Channel 2. It's possible to skip the inter pulse interval and pause between trains at the end of the stimulus paradigm and stop immediately by activating the **Stop immediately after last pulse** tick box. Each stimulation channel can be selected to be **enabled** or **disabled** with a second tick box. In **Continuous Mode**, the programmed pulse is repeated till manually stopped. A **start delay** can be set individually for both channels. This allows a time offset between the two channels, but also in relation to the start command.

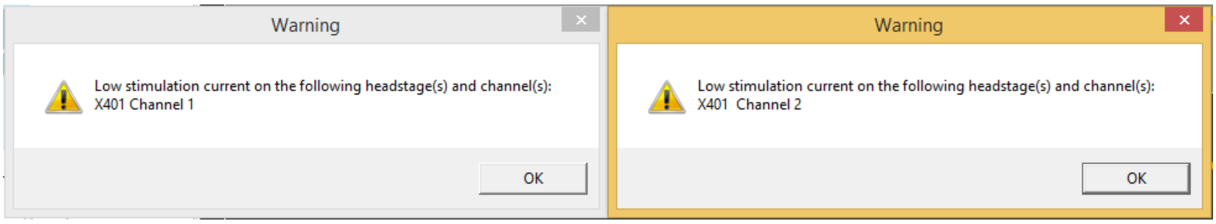
A stimulation paradigm needs to be **downloaded** before it can be used. Start and Stop is only available while the data acquisition is running, while Download is only available while it's stopped. The green or blue **virtual LEDs** in the Stimulator window and in the lower Main Menu indicate ongoing stimulation on stimulation channels one and two, respectively.

Stimulation Current Display
The maximum current which can be set to drive the connected LED is 1000 mA. However, due to a limited capacity of the attached battery, or a decreasing battery charge, it is possible that the actual current is **lower than the set current**. This usually is only a problem for high currents. The W2100-Opto-Headstages measure the actual current used during optical stimulation. It can be displayed in the independently for both optical stimulation channels in the **Zoom Display of the W2100 Data Source**.



The selected data channel and the current for both stimulation channels are displayed in the same graph, with individual y-axis. Display for both channels can be toggled.

If the actual current is more than 10% lower the set current, a **popup window with a warning** is shown.



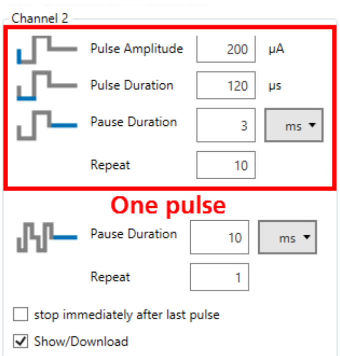
The same warning will not be shown again till the W2100 Data Source has been stopped and restarted. The actual stimulation current is also recorded in the datafile.

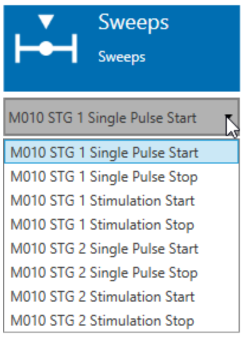
5.11.7 Trigger Output

The stimulator of the W2100-System has a pink data port for trigger events. These events will be generated automatically, for **each enabled stimulation channel of each active headstage** there are four events:

- Stimulation Start
- Stimulation Stop
- Single Pulse Start
- Single Pulse Stop

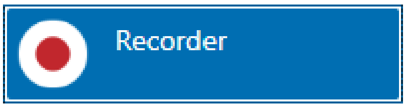
Stimulation in this case is the whole stimulation paradigm, including all repeats, also in continuous mode. There will be on trigger at the very beginning, and one at the end of a stimulation. **Single Pulse** in this case means a pulse train, with all repeats.





There will be on trigger at the beginning, and one at the end of each pulse. The Trigger events can be recorded, and used to control other instruments, like the **Sweeps** tool.

5.12 Recorder



5.12.1 Description and Purpose

The Recorder is essential to record acquired data. Only one recorder can be used in an experiment. All data streams which are directly connected to the Recorder will be recorded. Data from instruments not connected to the Recorder will be lost. Please also see movie for illustration.

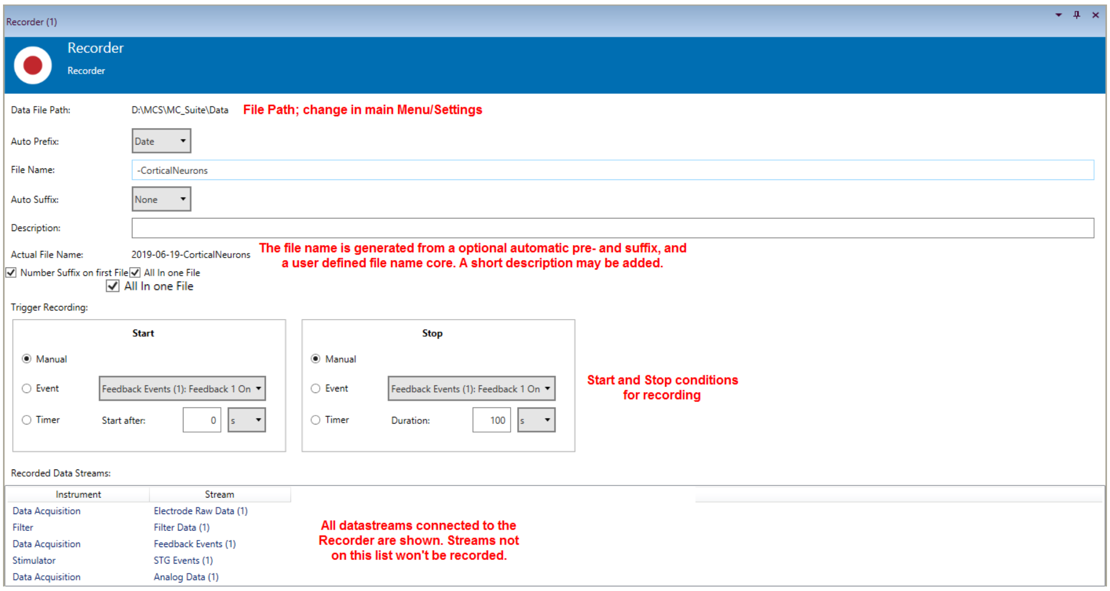
5.12.2 Data Ports and Export Options

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.

5.12.3 Operation

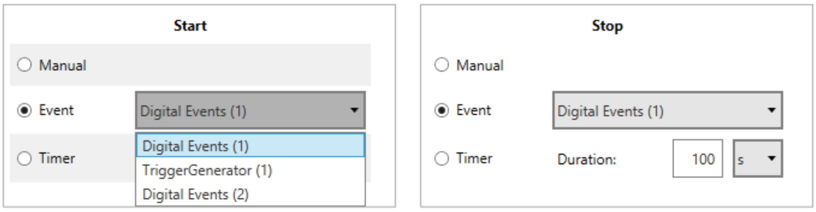
The Recorder is **mandatory to record data**. The simplest experiment would consist of the Data Source connected to the Recorder. The default **file path** is set in the Main Window settings. A **file name** is generated from a random file core, and an optional pre and/or postfix. If the same file name 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.

The “**All in one File**” tick box is relevant for W2100 in Multi Headstage Mode, ME2100 and MEA2100-Mini in Single Instance Mode or Multiwell MEAs. If “All in one File” is selected, data from all headstages/wells is stored in a single data file. Otherwise, one *.msrd file per headstage/well is generated.



The Start and Stop condition for recording can be a **Manual** command, an **Event** or a **Timer**. Data can only be recorded if the **Data Acquisition is running**. Manual Start or Stop commands are given with the Recording button in the main window:

If the Start condition is Manual, and the DAQ is running, the recoding will start immediately once the Recording button is pressed. If the DAQ is stopped, the Recorder will go to Standby till the DAQ is started
Likewise, if the recorder should be started on an Event, the Recording needs to be set to **Standby manually**. It will remain so till the Start Event starts recording. If the start condition is Timer, the recording will start the selected time after giving a manual start command.



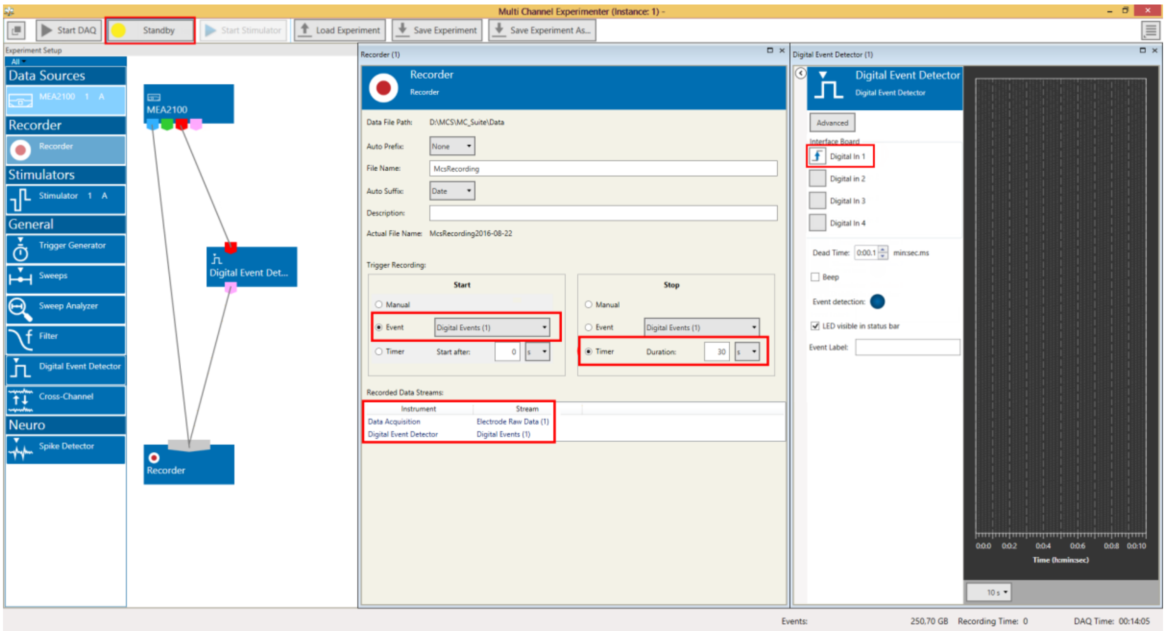
Events can be generated by the Digital Event Detector or the Trigger Generator, for example. Both instruments can be present multiple times in an experiment and generate multiple options for start and stop commands.

Stopping the recorder manually can have two effects: if the Start condition is also Manual, the Recorder will be Off. If the start condition is an Event, it will go to Standby, and wait for the next start command. Likewise, if a recording is stopped by an Event or a Timer, recording will be Off or in Standby afterwards, depending on what the Start condition is.

5.12.4 Examples: How to …

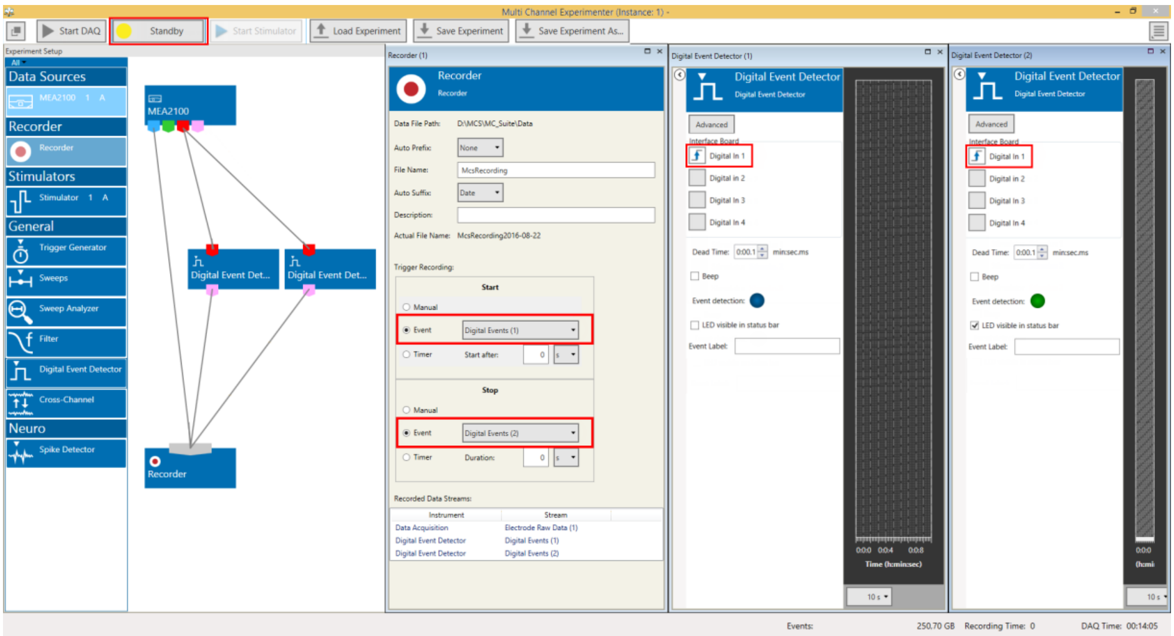
This section highlights a few common uses of the Recorder.

5.12.4.1 Record Segments of Electrode Raw Data after each external TTL



In the experiment above, the blue electrode data port and a pink Trigger port are connected directly to the Recorder. The red digital data port is connected to a Digital Event Detector instrument (please see chapter 5.17, Digital Event Detector), which will generate **Trigger events** based on TTL inputs to the **Digital In connector 1** on the Interface Board. The trigger events will also be recorded, as the pink trigger output port is connected to the Recorder. The digital channel itself will not be recorded, as it is not connected directly to the Recorder. The recording is set to **Standby**. Each TTL applied to the Digital In 1 of the IFB will generate a Start command for recording. The system will record for 30 s, as the Stop command is **Timer/30 s**. After 30 s, the Recording go will go back to Standby and wait for the next Start command. This will continue till manually interrupted.

5.12.4.2 Use a Gate Trigger to Control Recording



In the experiment above, the blue electrode data port and two pink Trigger ports are connected directly to the Recorder. The red digital data port is connected to two Digital Event Detector instruments (see chapter 5.17, Digital Event Detector), which will generate **Trigger events** based on TTL inputs to the **Digital In connector 1** on the Interface Board. The trigger events will also be recorded, as the pink trigger output ports are connected to the Recorder. The digital channel itself will not be recorded, as it is not connected directly to the Recorder. The recording is set to **Standby**.

Each TTL applied to the Digital In 1 of the IFB will generate a Start command for recording on the rising flank of the TTL (Digital Event Detector 1), and a Stop command on the falling flank of the same TTL (Digital Event Detector 2). As an effect, data will be recorded as long as the TTL on the Digital In 1 of the Interface Board is HIGH. This will continue till manually interrupted.

5.13 Trigger Generator

5.13.1 Description and Purpose

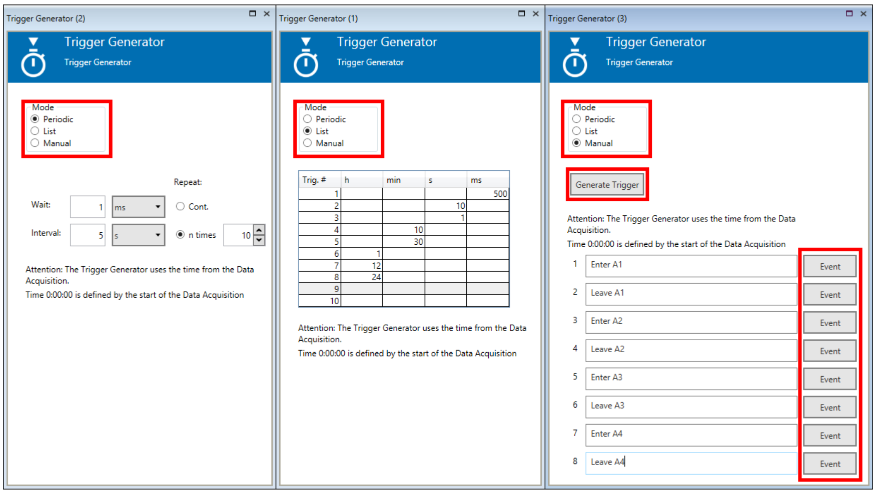
The Trigger Generator can generate trigger events **independently from an external input**. This can be controlled by a timer, or by a manual input. The generated events can be used to control all other instruments with a trigger input. More than one Trigger Generator can be used in one experiment, and more than one instrument can be connected to each Trigger Generator.

5.13.2 Data Ports and Export Options

The Trigger Generator has a blue Electrode Raw Data input and a pink Trigger output. The Electrode Raw Data input is necessary to get a **timing signal** from the Data Source. Without that input, the instrument is not functional. There are no direct export options.

5.13.3 Operation

The Trigger Generator has three different options to generate events, **Periodic**, **List** based and **Manual**.

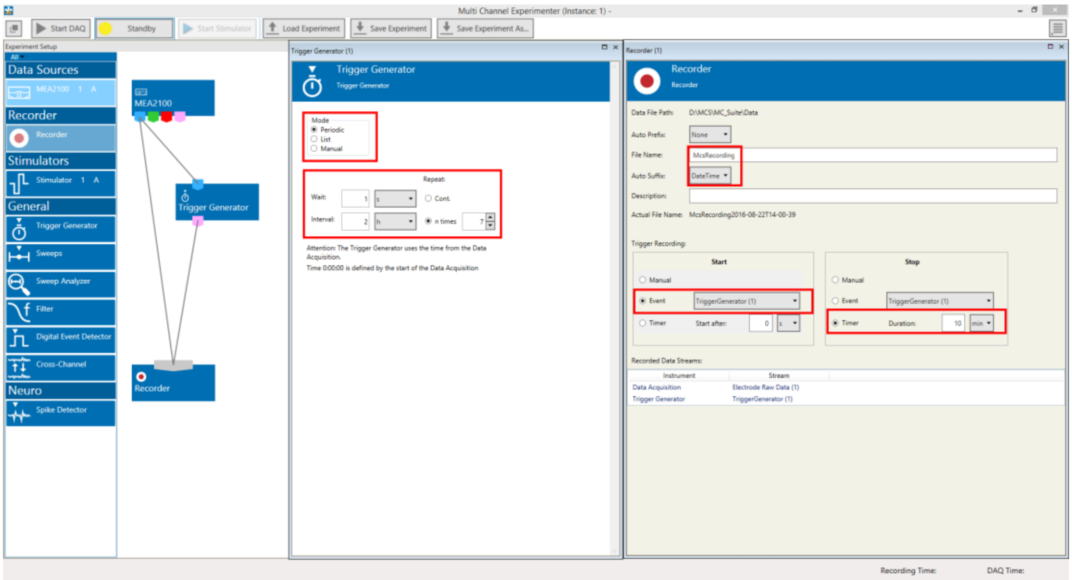


The **time base** is always the **start of the Data Acquisition**. A periodic list of events can be generated continuously, or a predefined number of times, with or without a delay in relation to the DAQ start. It's also possible to generate a random list of time stamps at which a trigger should be generated.
At the example above, events are generated at increasing intervals between 500 ms and 24 h after start of the DAQ. The third option is to generate events manually on mouse click. One unlabeled trigger is available (Generate Trigger). Alternatively, eight independent manual buttons are available to generate events, which will show up with the respective label when opening the data file in the Multi Channel Analyzer.

5.13.4 Examples: How to ...

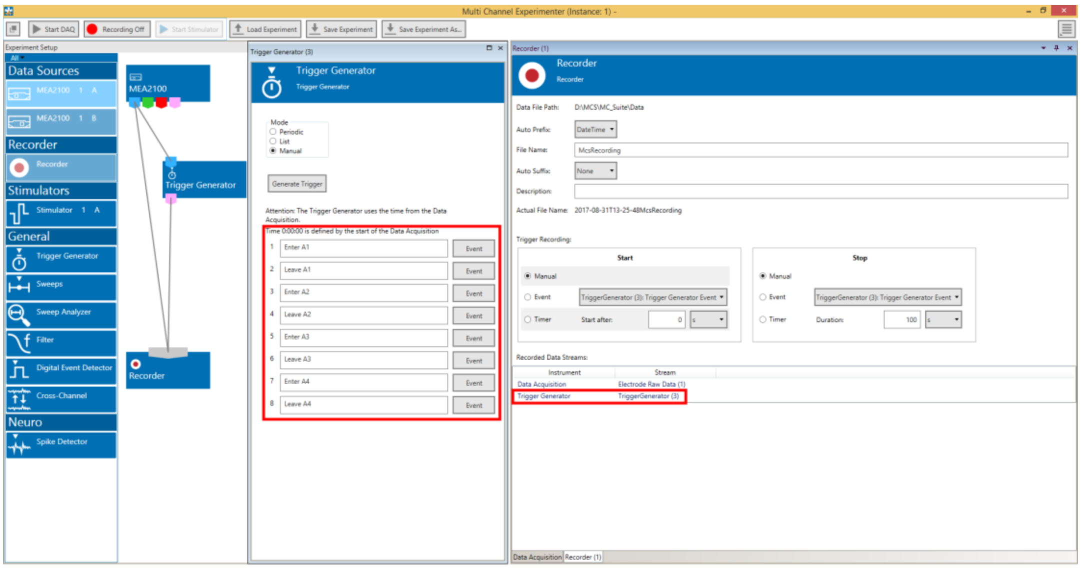
This section highlights a few common uses of the Trigger Generator.

5.13.4.1 Record Segments of Electrode Raw Data automatically overnight



In the experiment above, the blue electrode data port and a pink Trigger port are connected directly to the Recorder. The Electrode Raw Data port is also connected to a Trigger Generator instrument. Events will be generated every 2 hours, seven times in total (14 h). Every event will trigger a 10 min recording period. File names will contain the time of the recording.

5.13.4.2 Set Manual Marker Events during Recording



In the experiment above, the blue electrode data port and a pink Trigger port are connected directly to the Recorder. The Electrode Raw Data port is also connected to a Trigger Generator instrument. Recording of continuous data is controlled manually. Eight labeled events will be generated on every manual mouse click on the respective button during recording. These events can mark certain time stamps in the file and can be used in the Multi Channel Analyzer to navigate the file.

5.14 Sweeps

5.14.1 Description and Purpose

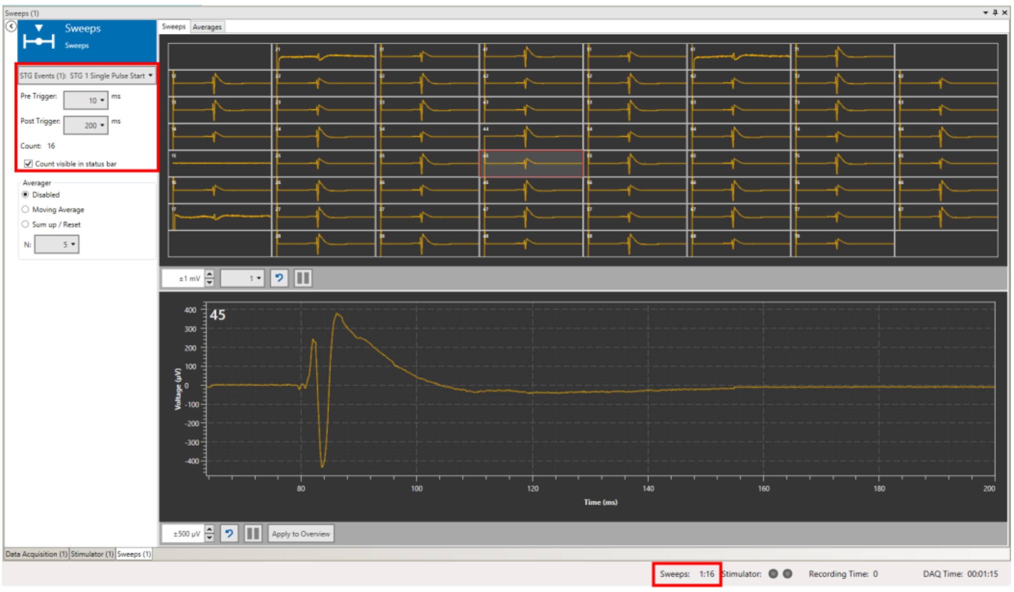
The Sweeps instrument is supposed to record **short segments of data (sweeps)** around a trigger event. More than one Sweeps instrument can be used in one experiment and more than one instrument can be connected to each Sweeps tool. The Sweeps tool is most often used to record the response to an **electrical or optical stimulation**, where the trigger marks the time stamp of stimulation. Please also see [movie] for illustration.

5.14.2 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 outputs are a cyan Sweeps (individual segments) and a purple Averages (averages of multiple segments) data stream, which can be connected to a **Sweep Analyzer and the Recorder**. There are no direct export options.

5.14.3 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.

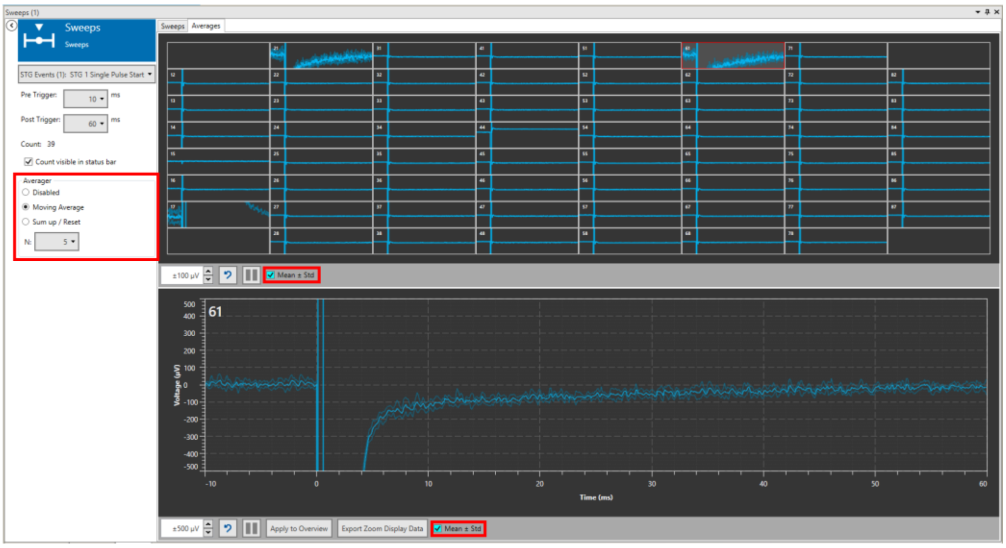


The time base (time 0) is always the selected trigger event. The number of sweeps generated since start of the data acquisition is counted, optionally also in the status bar.

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.



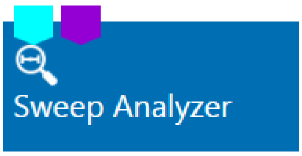
5.15 Sweep Analyzer

5.15.1 Description and Purpose

The Sweep Analyzer instrument can analyze sweep data and averages generated by the Sweeps tool. 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.

5.15.2 Data Ports and Export Options

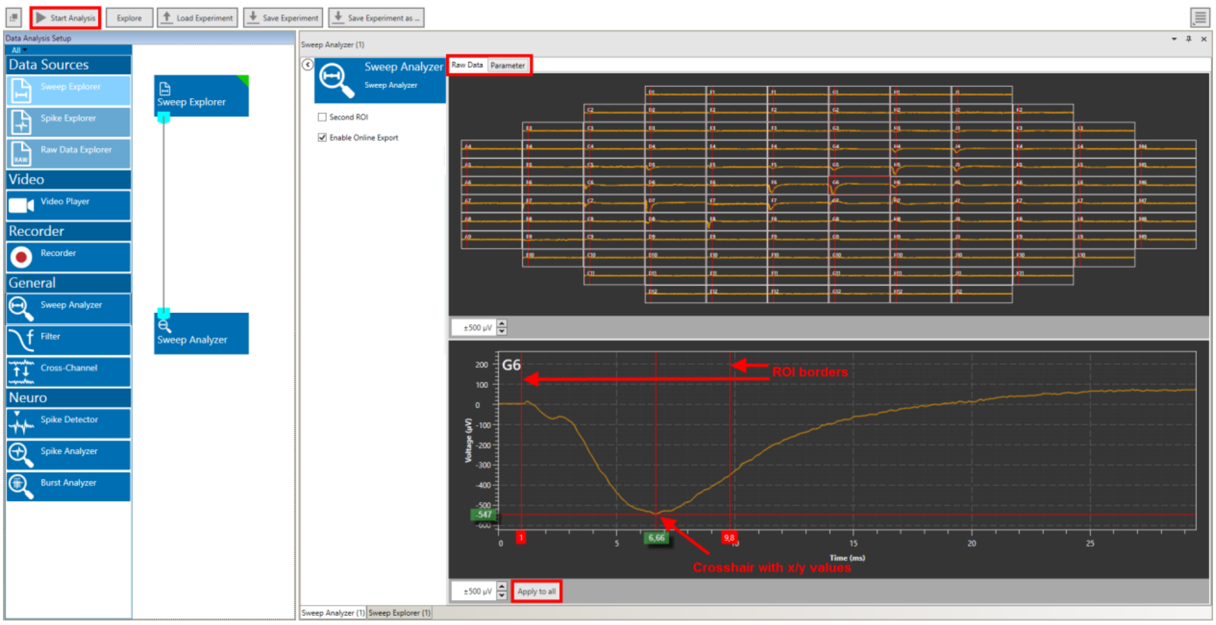
The Sweep Analyzer has a cyan sweep data input, a purple averages input and no outputs. Analysis parameters can be exported as ASCII data, see 4.7. Only **one of the two inputs** can be used at a time.



5.15.3 Operation

5.15.3.1 ROI Definition

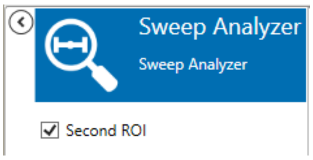
The Sweep Analyzer has two tabs, Raw Data and Parameter. The **Raw Data** tab shows the sweeps send from the Sweep Explorer with the **Explore** function and allows to define a **region of interest (ROI)** for the analysis. To define a ROI, click on any channel. The selected channel will show up on the 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.



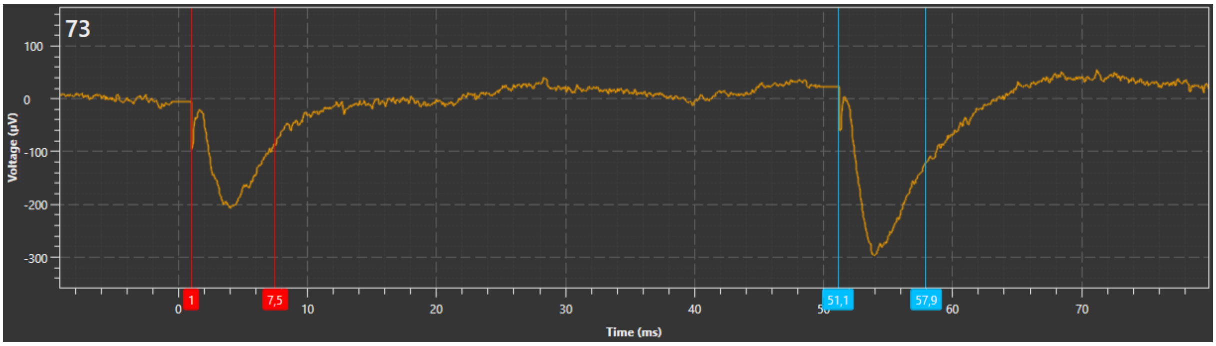
A cross hair is available, which can show x and y values of any specific point in the plot. Once the ROIs are set for all relevant channels, the **Start Analysis** function can start the processing of all sweeps in a file.

5.15.3.2 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.



5.15.3.3 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.

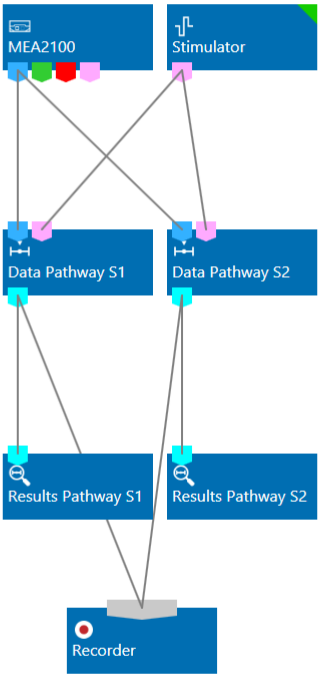
The parameters are defined as follows:

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.
Ratio	For each parameter, the ratio parameter of ROI 2 divided by the parameter of ROI 1

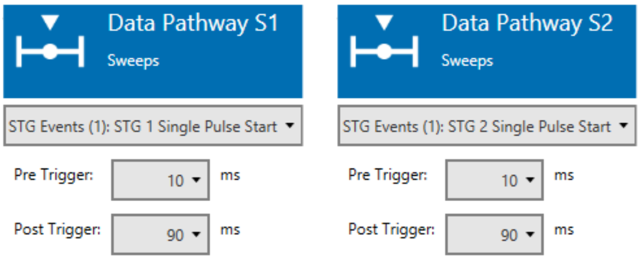
All analyzed parameters can be exported as on-line as ASCII data, if the **Enable Online Export** function is active, see 4.7.

5.15.4 Example: How to do an LTP Experiment with two Stimulation Pathways

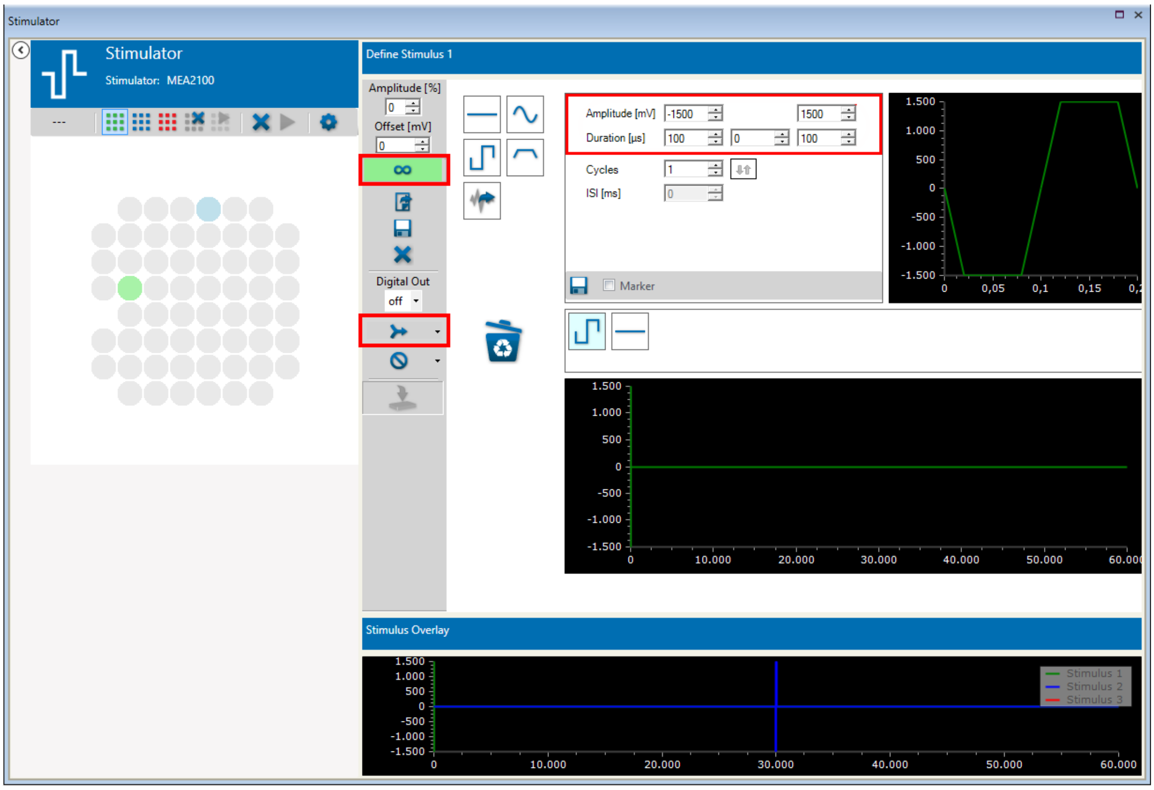
This example illustrates the most common use of the Sweeps and Sweep Analyzer instrument, the recording and analysis of evoked activity. A combination of several instruments, the Stimulator, the Sweeps instrument and Sweep Analyzer is used. The electrical stimulation applied to a hippocampal slice by two individual stimulators are marked by individual trigger events, generated by two internal stimulator units. The evoked responses to stimulation via both stimulator units are detected in independent Sweeps and instruments, both are recorded as independent data streams. Sweep Analyzer instruments are used to analyze the evoked responses on-line.

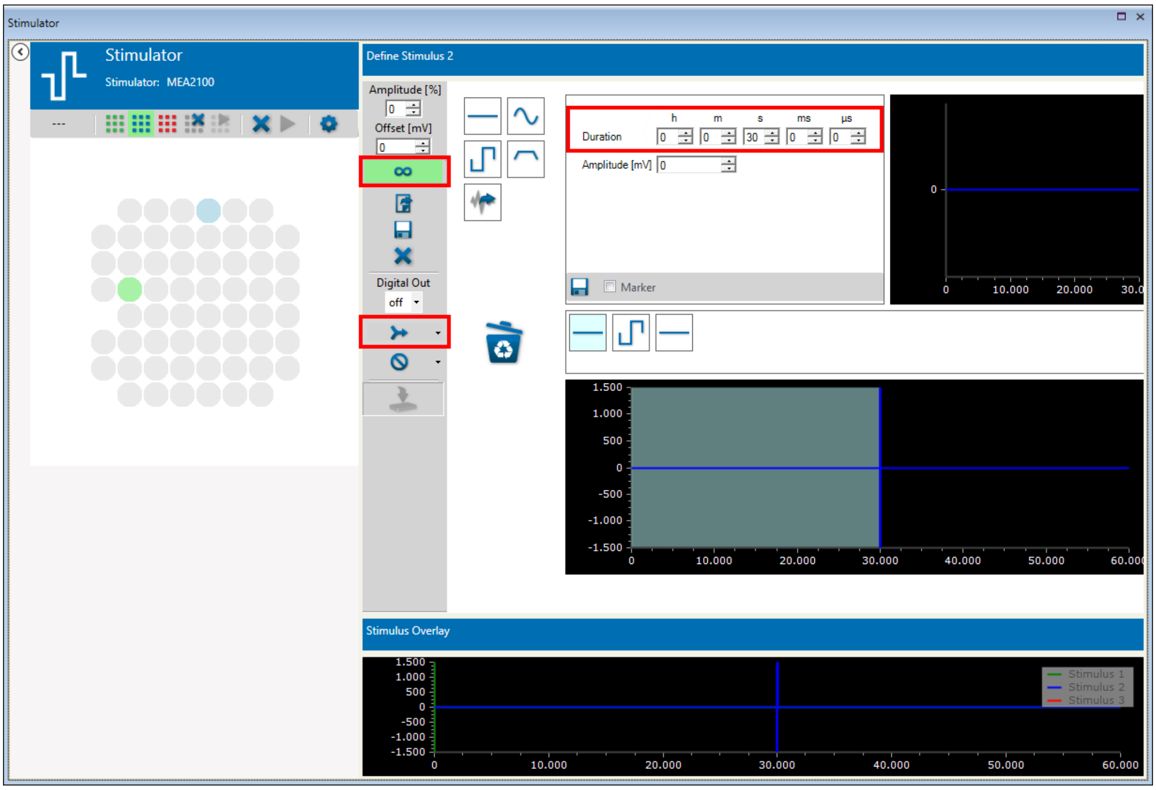


Stimulation pathways S1 and S2 will be operated by the Stimulator units one and two. For each pathway, raw data is running from the Data Source to the Sweeps tool, and the resulting sweeps are recorded and analyzed by a Sweep Analyzer. Sweeps of 100 ms duration are triggered by each pulse start from Stimulator one and two, respectively. The two Sweeps data streams are recorded manually.



The stimulators are both programmed to start simultaneously with DAQ start, and then deliver alternately voltage pulse with 1500 mV amplitude every minute, till manually stopped. This would be a common **baseline stimulation** paradigm for a LTP experiment.





5.16 Filter

5.16.1 Description and Purpose

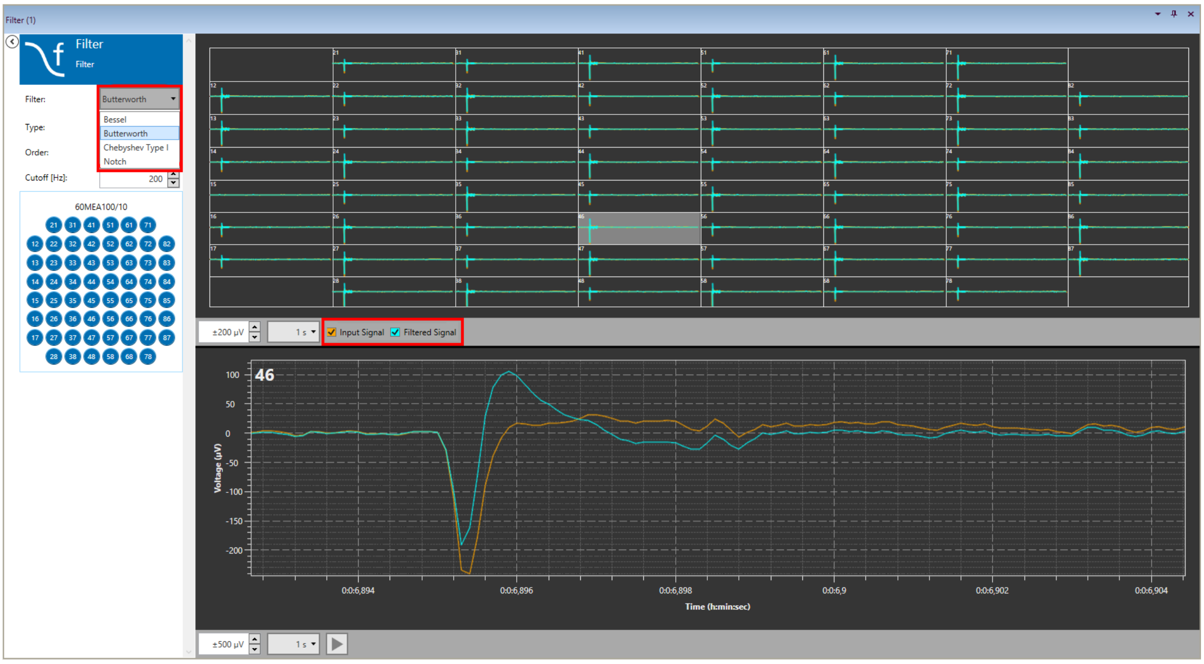
The Filter can apply time based filters with different characteristics to the raw data. More than one Filter can be used in one experiment, the filters can be used in series or in parallel. Please also see [movie]() for illustration.

5.16.2 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 Data Source, or from another instrument with a blue output, like a Cross-Channel Tool or another Filter. Likewise, the Filter Data output is compatible with any other blue input port. There are no direct export options.

5.16.3 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.



5.16.3.1 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.

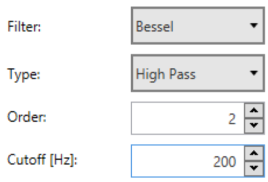
The **Butterworth** filter is designed to have a frequency response which is as flat as mathematically possible in the passband.

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.

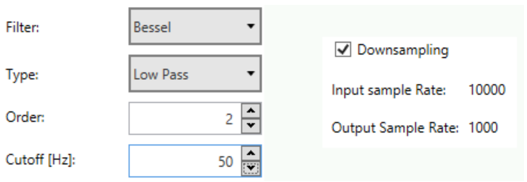
5.16.3.2 High Pass

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.



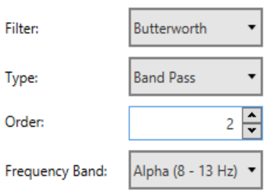
5.16.3.3 Low Pass

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. Additionally, **Downsampling** is available. If Downsampling is active, the sampling rate for the downsampled data will adjust automatically, based on the Cut Off frequency. The outgoing Filtered Data stream will then have the **lower sampling rate**.



5.16.3.4 Band Pass

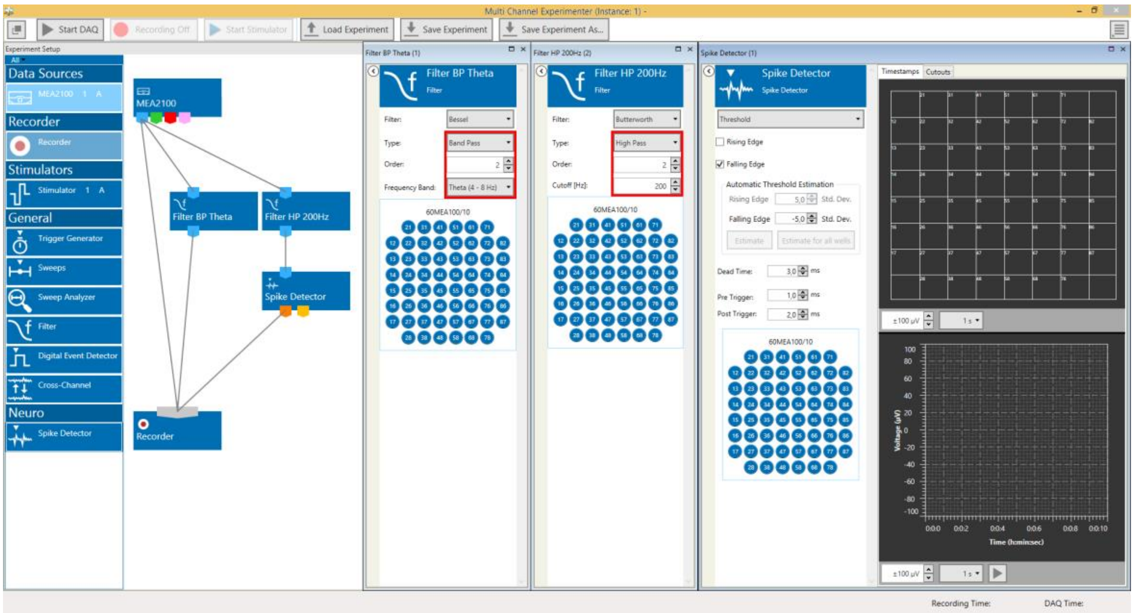
Band Pass filters remove all frequencies outside the selected frequency band. Fixed Band Pass options are available. Select Alpha, Beta, Gamma and Theta band from the drop down menu.



5.16.4 Examples: How to …

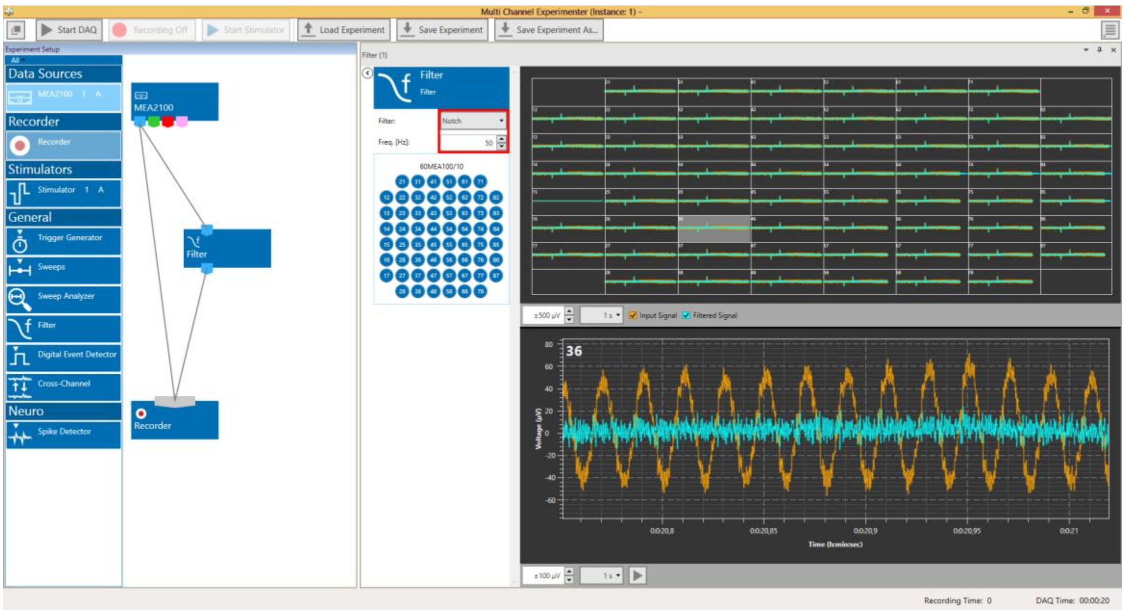
This section highlights a few common uses of the Filter.

5.16.4.1 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 (please see chapter 5.20, Spike Detector), and the detected spikes are also recorded. In addition, the raw, unfiltered data is also recorded. Recording of the spikes and filtered data is not strictly necessary, as filtering and spike detection could also be done offline in the Multi Channel Analyzer as long as the raw data stream has been recorded.

5.16.4.2 Remove 50 Hz Noise



If problems with 50 Hz noise occur, the best option is to find the source of the noise. However, if everything else fails, a Notch filter can be used to remove the noise. Periodic noise is very often caused by other electronic instruments. Europe usually has an AC frequency of 50 Hz, while in the US it is 60 Hz. Adjust the filter accordingly.

5.17 Digital Event Detector

5.17.1 Description and Purpose

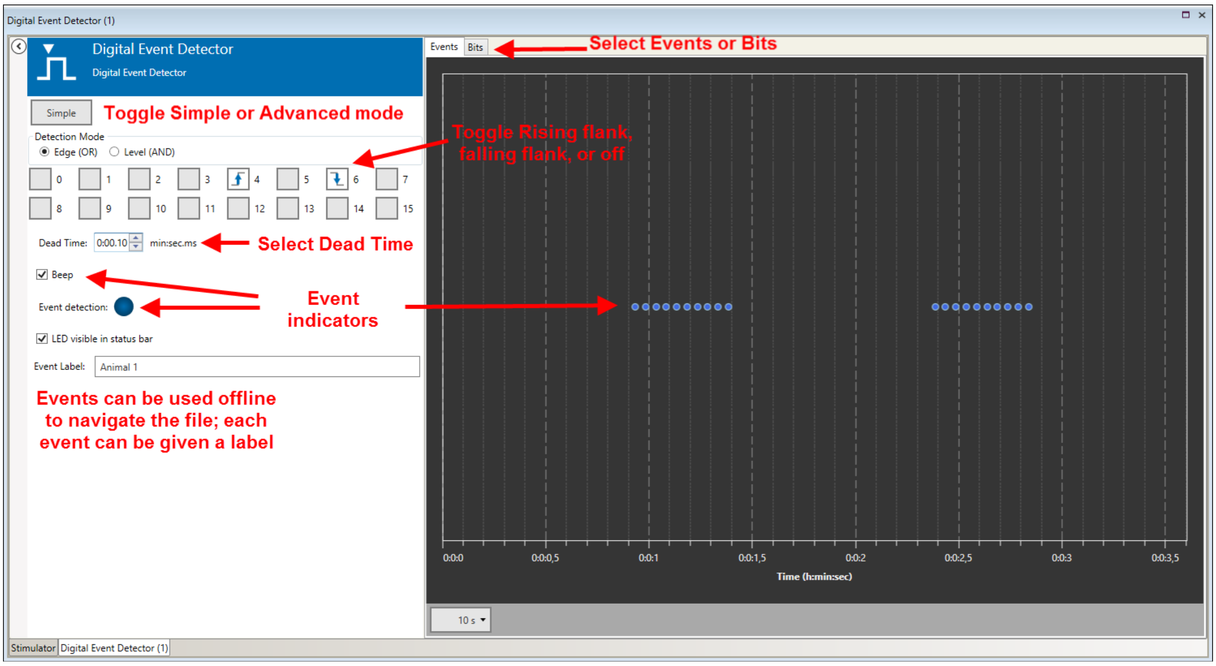
The Digital Event Detector can generate trigger events upon external inputs, or upon stimulation signals of the internal Stimulator. The generated events can be used to control all other instruments with a trigger input. More than one Digital Event Detector can be used in one experiment, and one than one instrument can be connected to each Digital Event Detector. Please also see movie for illustration.

5.17.2 Data Ports and Export Options

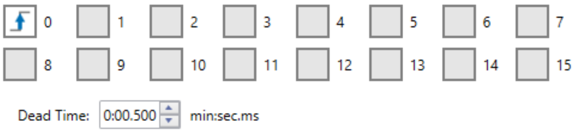
The Digital Event Detector has a red Digital Data input and a pink Trigger output. The Digital Data stream from the Data Acquisition has 16 bits, each of which can be used to generate trigger events. Without that input, the instrument is not functional. There are no direct export options.

5.17.3 Operation

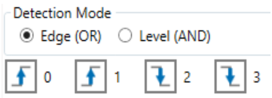
The Digital Event Detector monitors the 16 incoming bits of the digital channel. Trigger events can be generated on the **rising or falling flank** of an external TTL or an internal sideband signal from the MEA2100-System Stimulator. Generated events are indicated in a display, and as optical and optionally also as an acoustical signal.



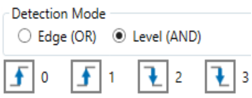
Each event can be given a random **label**, which will show up in the Analyzer and helps with navigating the file. A **Dead Time** can be selected, the dead time is the time after each event, during which any another TRUE condition to evoke an event will be ignored. For example, if the condition to evoke an event is the rising flank of a TTL on bit 0. Three TTLs come with 100 ms interval, and this train is repeated every second. The Dead Time is set to 500 ms (see below). An event will be generated only for the first TTL of each train, as the next two are within the Dead Time after the first.



Several bits can be combined with an **AND** or **OR** condition. The default is OR, which means that if any of selected bits detects a rising or falling flank, an event is generated. In the condition AND, all selected bits must fulfill the selected condition simultaneously to generate an event.



Event if bit 0 or 1 detects a rising flank **or** bit 2 or 3 detects a falling flank.



Event only if bit 0 **and** 1 is HIGH (1) **and** bit 2 and 3 are LOW (0) simultaneously.

On different data acquisition devices, the digital bits can be addressed in different ways. For all devices, the **Advanced** mode is available, which simply lists the available 16 bits from 0 to 15. For the Basic Wireless-System, the MEA2100- and the W2100-System, there is also the **Simple** mode, which lists the bits with the respective function/connector on that instrument.

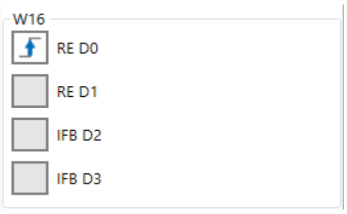
5.17.3.1 Advanced Mode

In Advanced mode, all 16 bits are listed from 0 to 15. They can be used as described above. On most devices, four bits are accessible via Lemo connectors. To gain physical access to all bits, it's necessary to connect a DIO extension box with 32 BNC connectors, 16 bits for Digital Out and 16 bits Digital In.

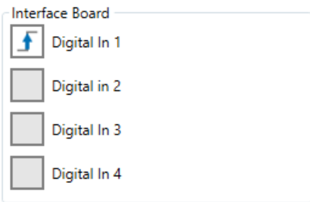
5.17.3.2 Simple Mode

The interface of the Simple mode looks different, depending on which data acquisition is in used. For the **USB-ME-System** data acquisitions the Simple mode is not available. For the **Basic Wireless-System**, the four bits are shown as available on the Receiver (RE) and the Interface Board (IFB).

On the **MEA2100, ME2100 and W2100**-Systems, the Digital In and Out bits are located on the Interface Board. Bits 0 to 3 are assigned to the four Digital In connectors on the Interface Board.



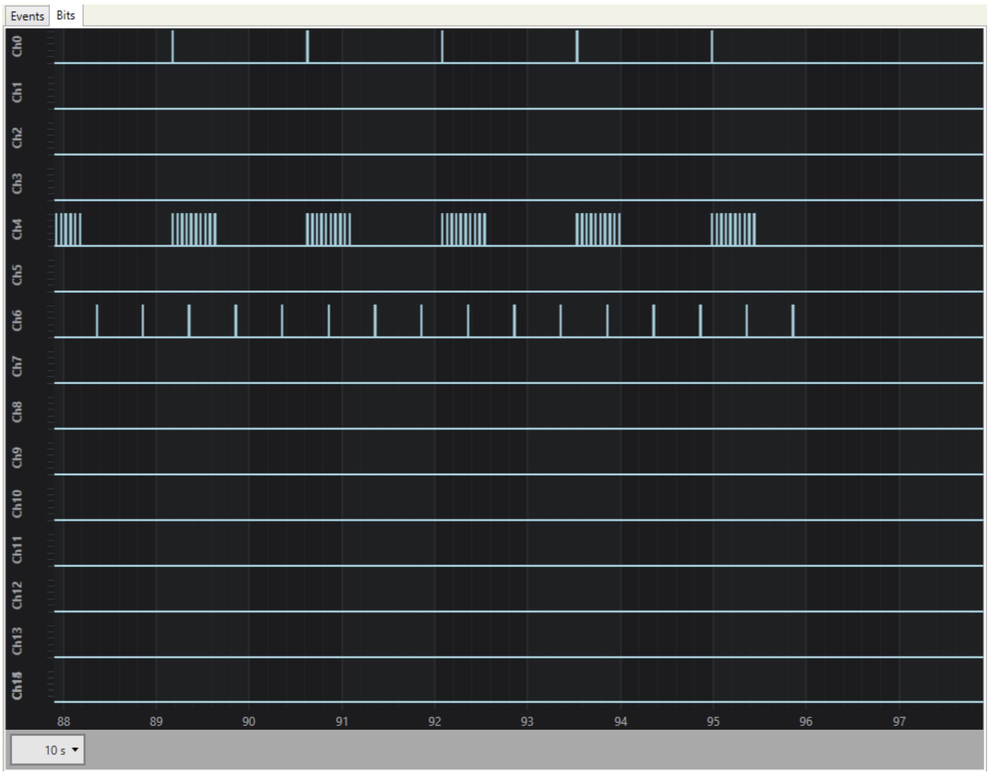
Basic W-System System



MEA2100, ME and W2100 System

5.17.4 Events and Bits tab

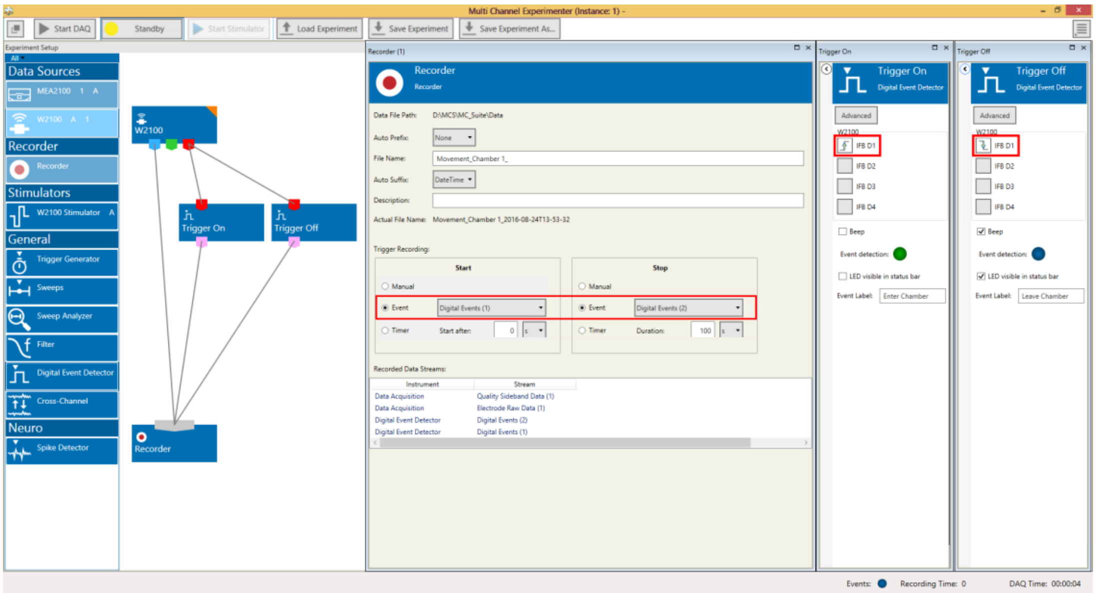
The Digital Event Detector has an **Events** and a **Bits** tab. The Events tab shows only detected events as markers (see 5.17.3). The **Bits** tab shows the status HIGH or LOW of all 16 Digital Input bits (0 to 15). A HIGH on a specific bit is not automatically an event (Trigger).



5.17.5 Examples: How to …

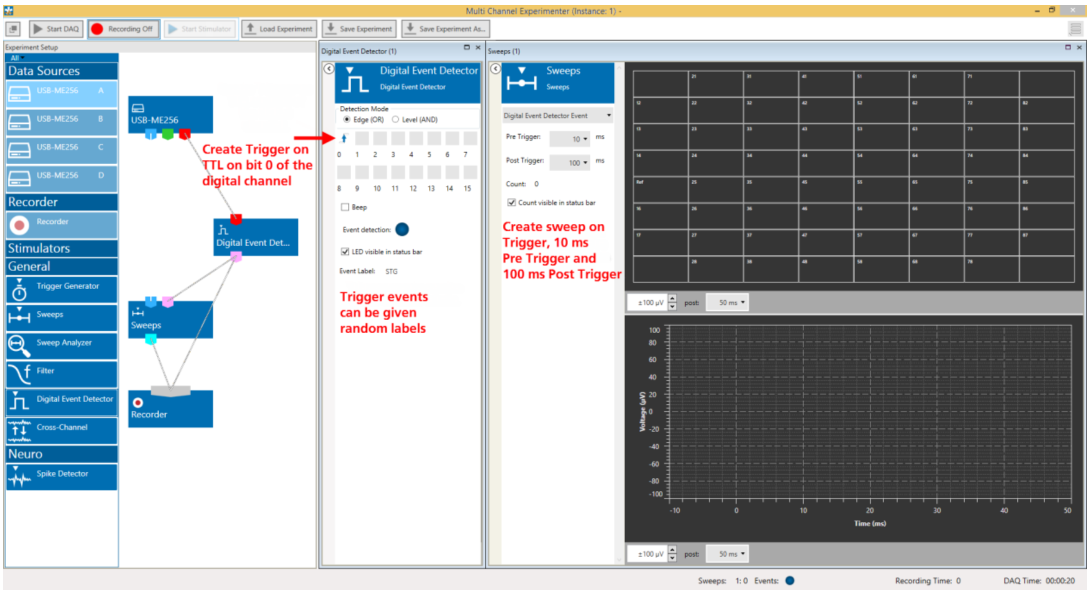
This section highlights a few common uses of the Digital Event Detector. Several examples have already been shown in the earlier sections.

5.17.5.1 Record Automatically with a W2100 System as long as a Sensor Detects Movement



In the example above, a motion sensor detects movement in a certain chamber, and generates a gate trigger which remains high as long as there is movement. The gate trigger is connected to the Digital In 1 on the Interface Board of the W2100-System. An event is generated on the rising flank of the TTL, and a second event on the falling flank of the same TTL. The first event starts the recorder, the second one stops it, and recording goes back to Standby. As a consequence, data is only recorded if the animal is in the chamber, which saves battery power.

5.17.5.2 Record Sweeps Triggered by an External Stimulator



External Stimulators can be programmed to generate TTL pulses together with every stimulation pulse. To record segments of data, so called **sweeps**, around each stimulation, connect the TTL output of the stimulator to a **digital input** of the USB-ME data acquisition. In the present example, the TTL is applied to bit 0 of the digital channel. The Digital Event Detector generates a trigger event on each rising flank of a TTL on the respective bit. The Sweeps tool uses the raw electrode data from the Data Source and the event to generate sweeps of -10 ms to 100 ms around each stimulation. Only the events and the sweeps are recorded, not the continuous raw data.

5.18 Cross-Channel

5.18.1 Description and Purpose

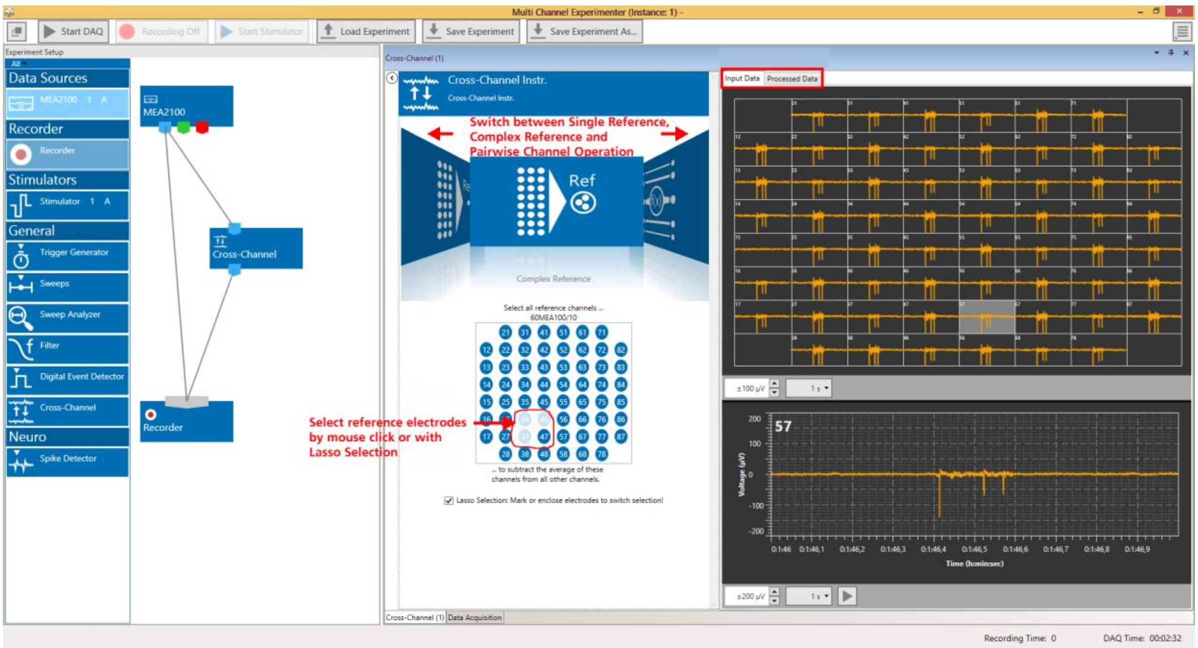
The Cross-Channel instrument can do calculations **between data channels**, for example to remove common noise. More than one Cross-Channel can be used in one experiment, and more than one instrument can be connected to a Cross-Channel data output.

5.18.2 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 Data Source, 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.

5.18.3 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.



5.18.3.1 Simple Reference

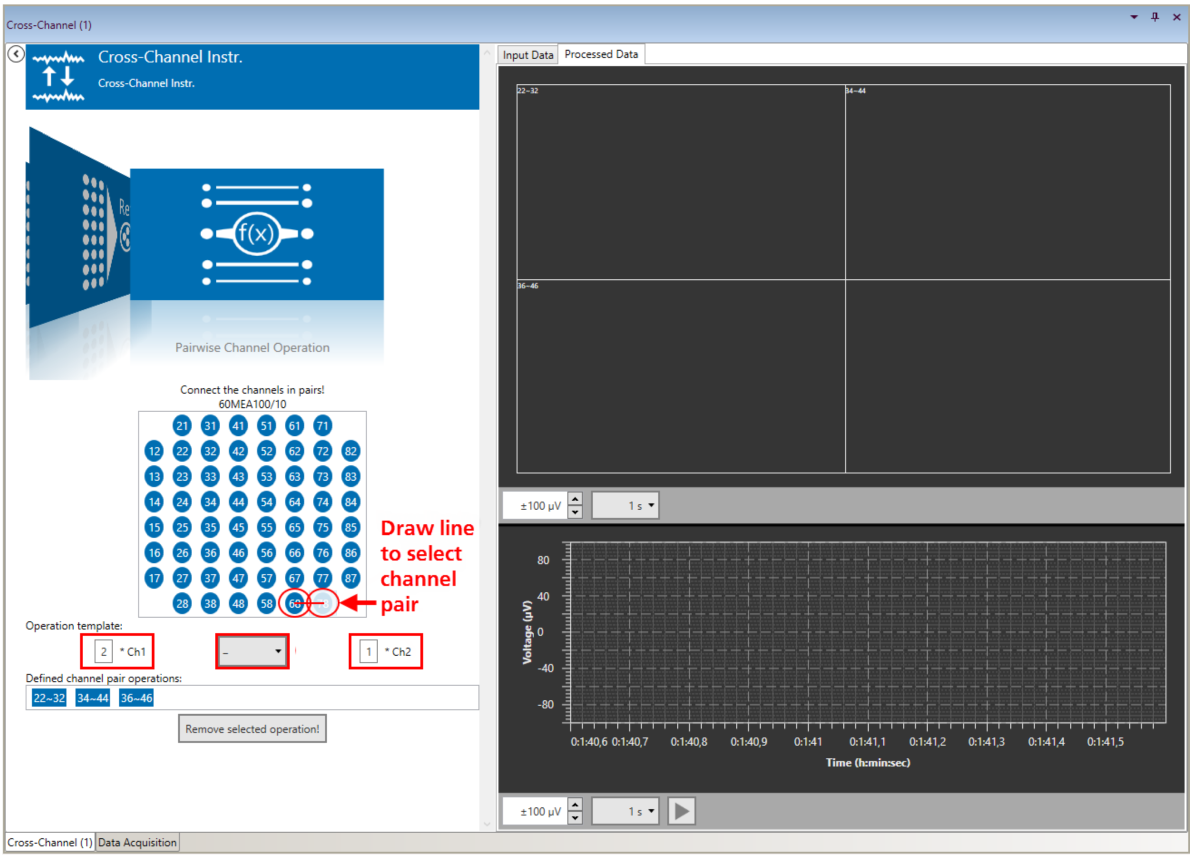
In Simple Reference mode, the signal **one selected reference** channel is subtracted from all others. The number of output channels in the Cross-Channel Data output is identical to the number of input channels.

5.18.3.2 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.

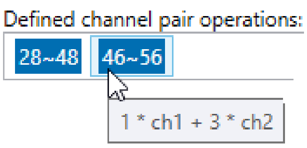
5.18.3.3 Pairwise Channel Operation

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.

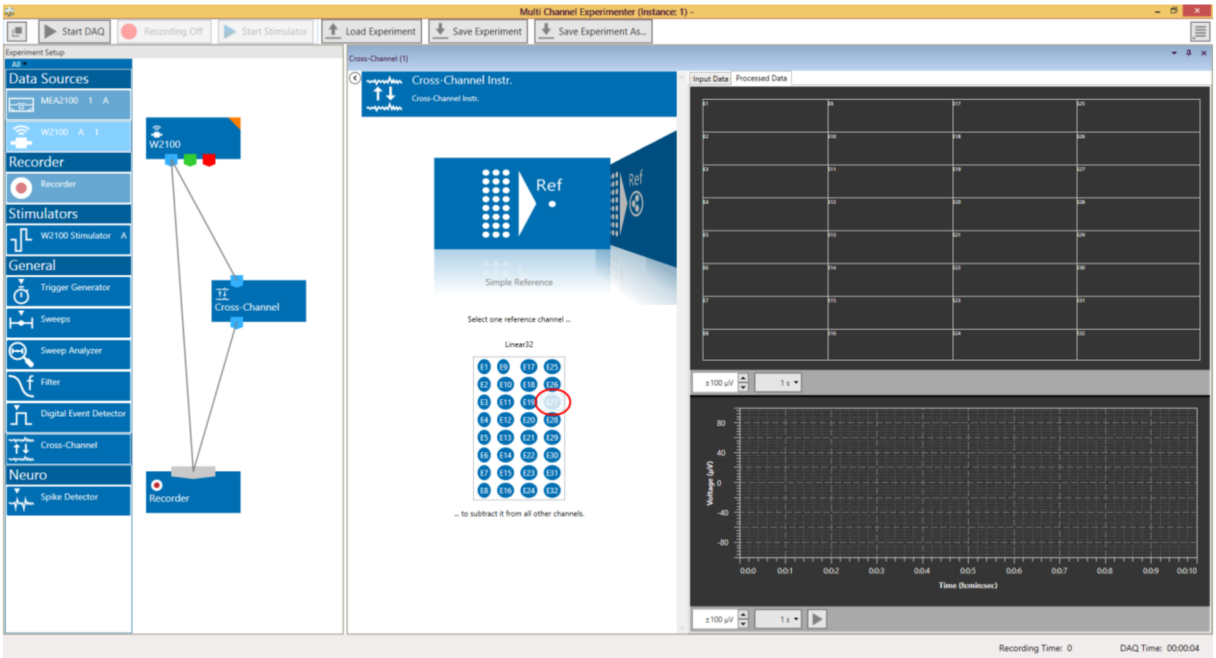
The operation can be set **individually for each pair of electrodes**. The operation for each pair can be seen when in a tooltip. First select the operation, then connect the electrode pair.



5.18.4 Examples: How to …

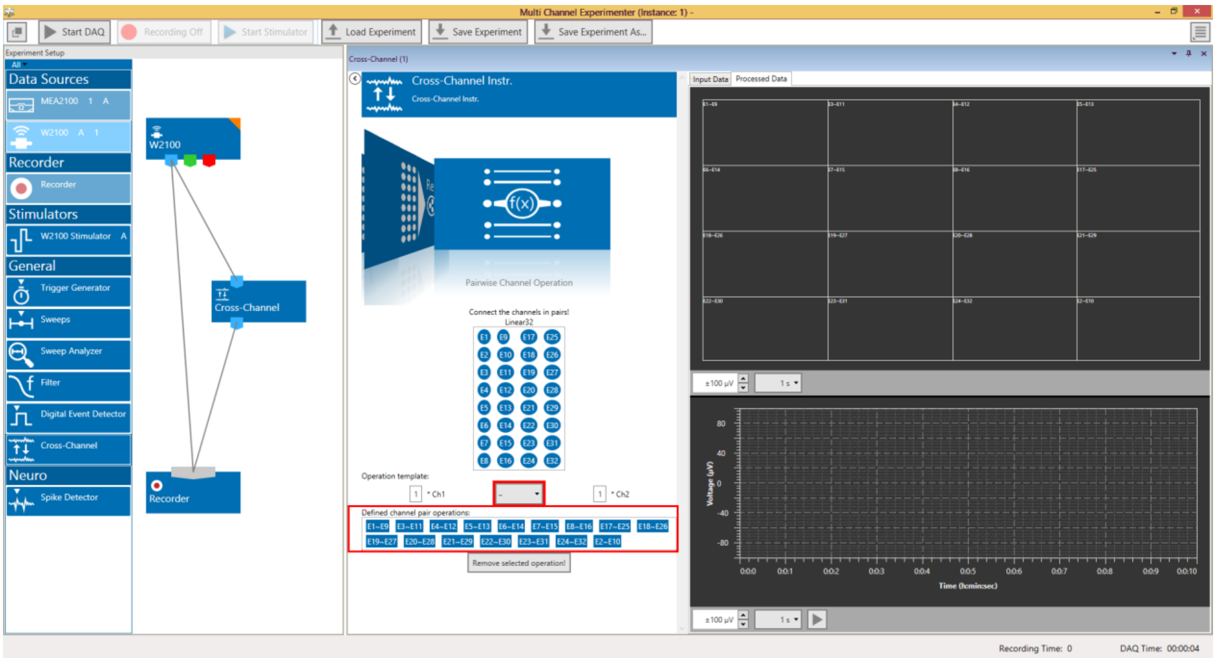
This section highlights a few common uses of the Cross-Channel Tool.

5.18.4.1 Remove Common Artefacts from a Wireless Recording



In the example above, a single electrode, which shows common artefacts as all other channels (breathing, heartbeat), but no actual signals is selected as Simple Reference. In the processed data, the common noise is removed. It's always advisable to record the raw data, too.

5.18.4.2 Do Differential Measurements between Pairs of Electrodes



In the example above, 16 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.

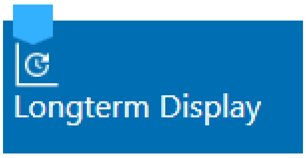
5.19 Longterm Display

5.19.1 Description and Purpose

The Longterm Display allows to display continuous data for extended periods of time, up to **72 hours**. The complete course of even an extended experiment can be displayed for all channels.

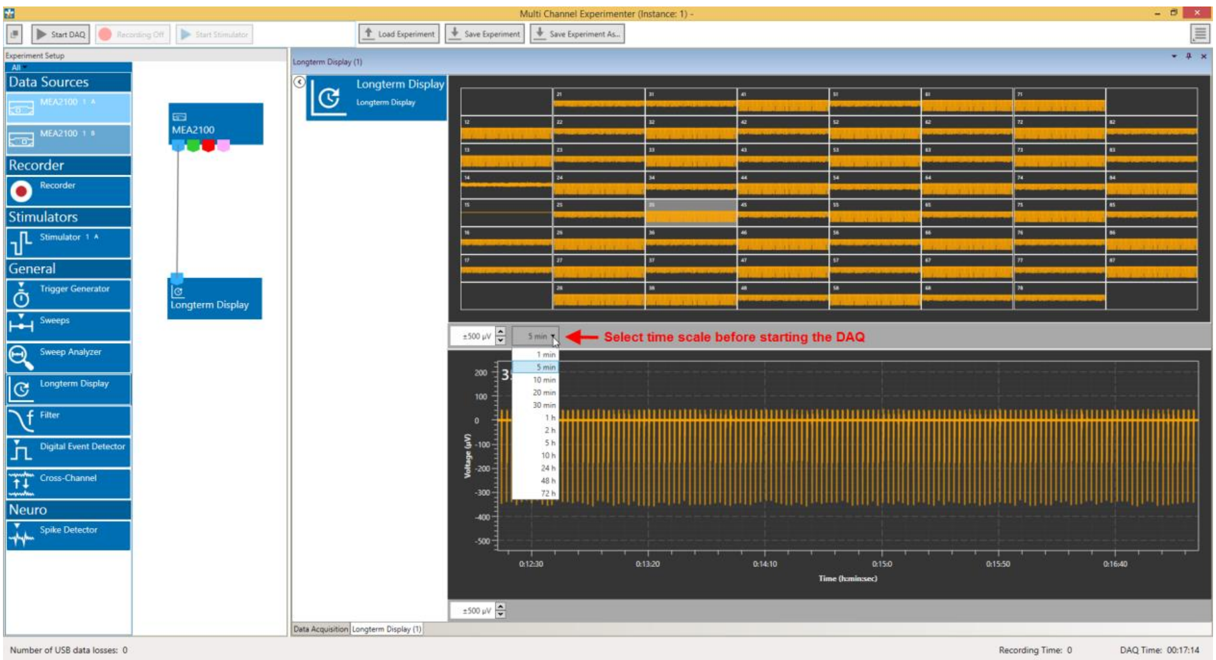
5.19.2 Data Ports and Export Options

The Longterm Display Instrument has a blue Electrode Raw Data input only. The input data can be directly from the Data Source, or from another instrument with a blue output, like a Filter. There are no direct export options.



5.19.3 Operation

The operation of the Longterm Display is very simple, the only important thing is to select the time scale **before starting the data acquisition**. Once the DAQ is running, the scaling for the time axis is fixed.
To allow such long-time data display, not all raw data is stored in the cache of the PC, just the extrema and as much data points to fill a full screen display of a single channel. Therefore, no zoom options in x direction are available. Only the y-axis can be changed.



5.20 Spike Detector

5.20.1 Description and Purpose

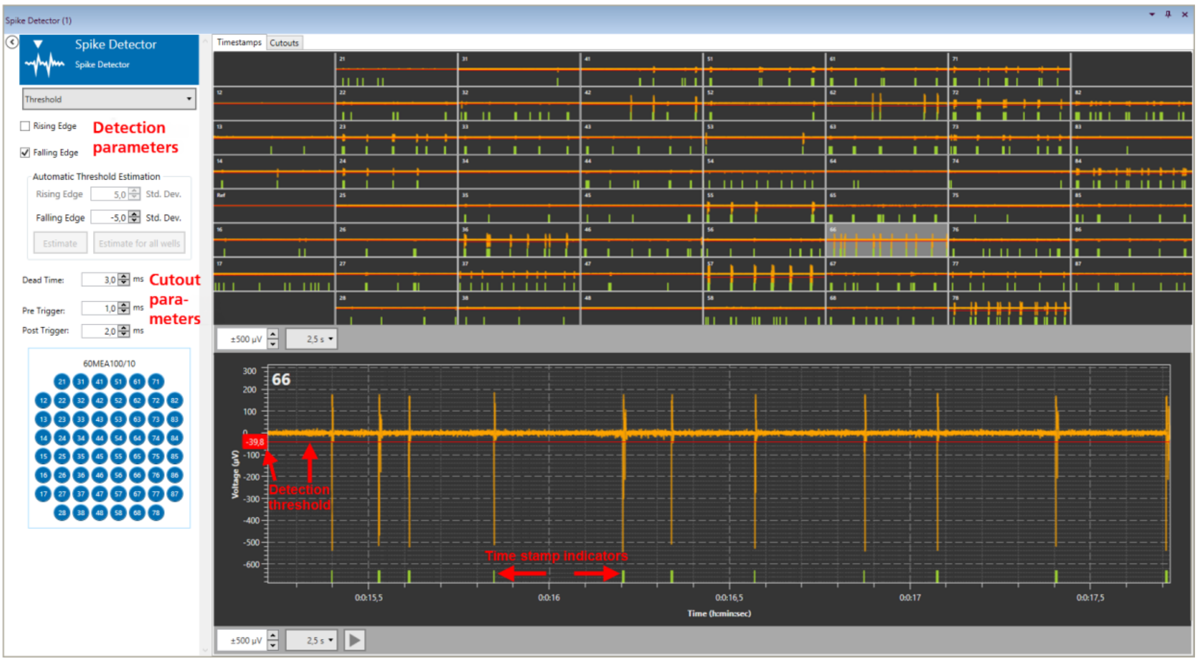
The Spike Detector can extract spikes from a raw data stream by different methods, and generate time stamps and cutouts with spike waveforms. Please also see movie for illustration.

5.20.2 Data Ports and Export Options

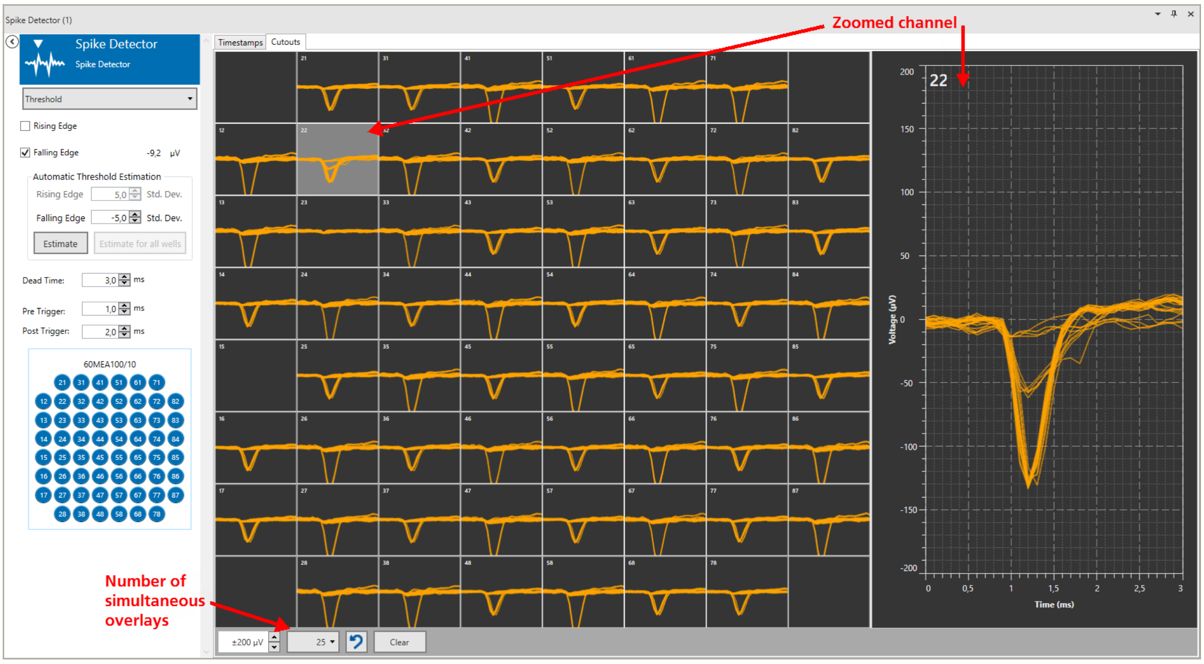
The Spike Detector has a blue Electrode Raw Data input and orange Spike Data and yellow Spike Time Stamps output. The input data can be directly from the Data Source, or from another instrument with a blue output, like a Filter or a Cross-Channel instrument. There are no direct export options.

5.20.3 Operation

The Spike Detector has a tab which shows the input data and indicates the **time stamps**, and one which shows the detected **spike cutouts** in an overlay plot. 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.

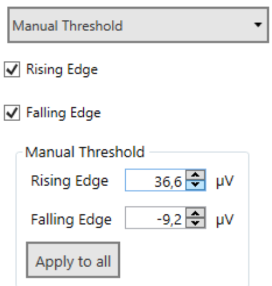


The spike cutouts are displayed in an overlay plot in the Cutouts tab. The number of simultaneously shown cutouts can be adjusted. If that number is reached, the earliest detected cutout will be erased and replaced by a new one.



5.20.3.1 Detection by Manual Threshold

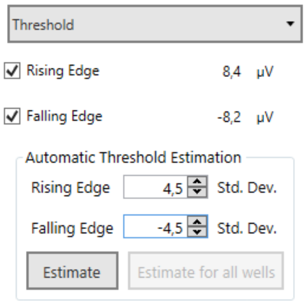
If Manual Threshold is selected from the drop down menu, the user needs to either set the detection threshold manually for all channels, or apply an identical threshold to all. Threshold values can either be changed with the updown box, or the threshold can be dragged with the mouse in the Zoom window.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



5.20.3.2 Detection by Threshold

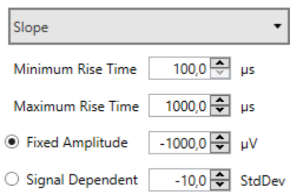
If Threshold is selected from the drop down menu, the thresholds are calculated individually for each channel by multiplying a user defined factor with the standard deviation (SD) of the noise of each specific channel. The SD is calculated from 500 ms of data every time the “**Estimate”** button is pressed.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

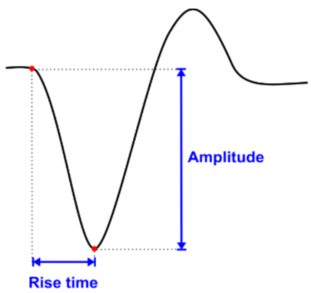
The Threshold detection takes into account small differences in noise level between the channels, and is usually superior to a manually selected threshold value The SD is strongly depending on the 500 ms of data used for calculation. If a lot of spiking activity occurs within these 500 ms, the SD will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



5.20.3.3 Detection by Slope

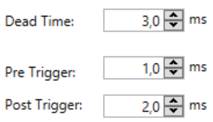
Spikes can also be detected using a combination of minimum and maximum **slope**, and a minimum **amplitude**. This method is sometimes more effective in detecting small signals close to the noise, without risking too many false detections caused by random peaks. Amplitudes can be fixed, or dependent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





5.20.3.4 Spike Cutouts

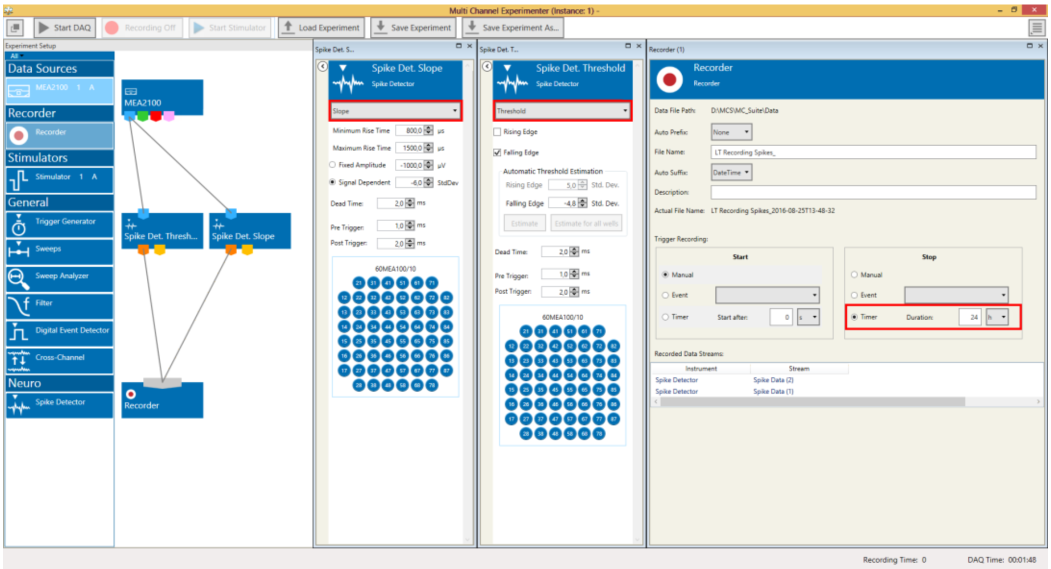
The Spike Data stream consist of cutouts, short segments of data around each detection time stamp which contain the spike waveform. The **length of this cutout** in relation to the detection time stamp (Trigger) can be adjusted. The Dead Time is the time after each detection time stamp after which a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



5.20.4 Examples: How to ...

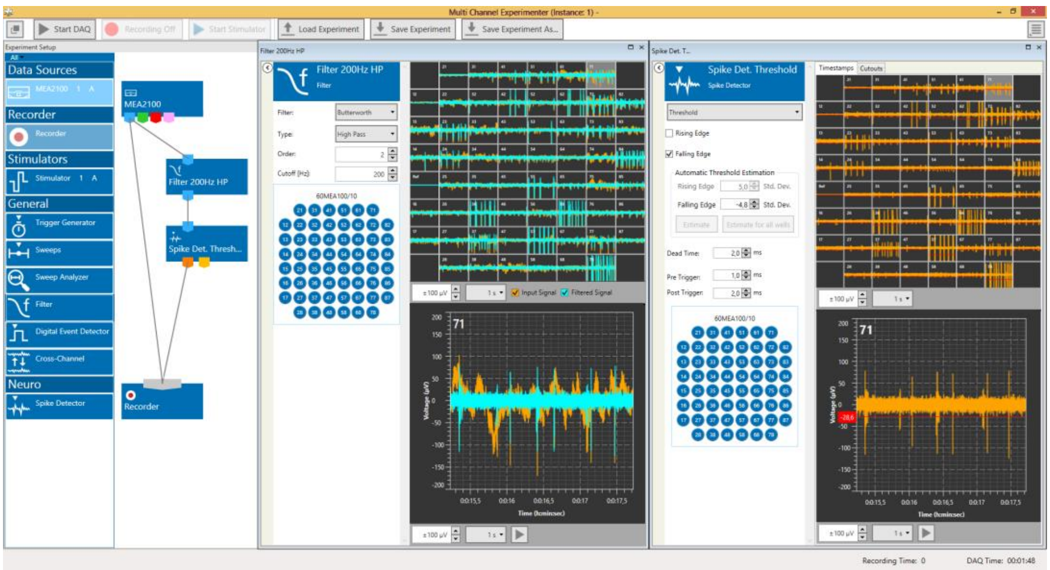
This section highlights a few common uses of the Spike Detector.

5.20.4.1 Do Long Term Recordings with Spike Data only



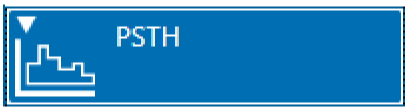
The sole purpose of the Spike Detector is to extract spikes from a raw data signal. The instrument works identical **online** (Experimenter) or **offline** (Analyzer). So, it is not strictly necessary to record the time stamps and/or cutouts, as long as the raw data stream is recorded. The exception can be very long recordings, where raw data files would simply become too large. The obvious downside is that everything which escapes the detection for some reason is permanently lost. In the example above, two Spike Detectors with **Threshold** and **Slope** detection method are used in parallel. Both Spike Cutouts are recorded, but not the raw data. Recording is started manually and stopped by a timer after 24 h.

5.20.4.2 Extract Spikes from a Noisy Signal



Usually it's helps to improve spike detection if the data is filtered before the spike detection. High pass filters with cutoff frequencies between 100 and 200 Hz are used. If the signal contains low frequency components, the baseline **drifts through the detection threshold** and leads to false detections. As always, it is recommended to record the raw data stream also.

5.21 PSTH



5.21.1 Description and Purpose

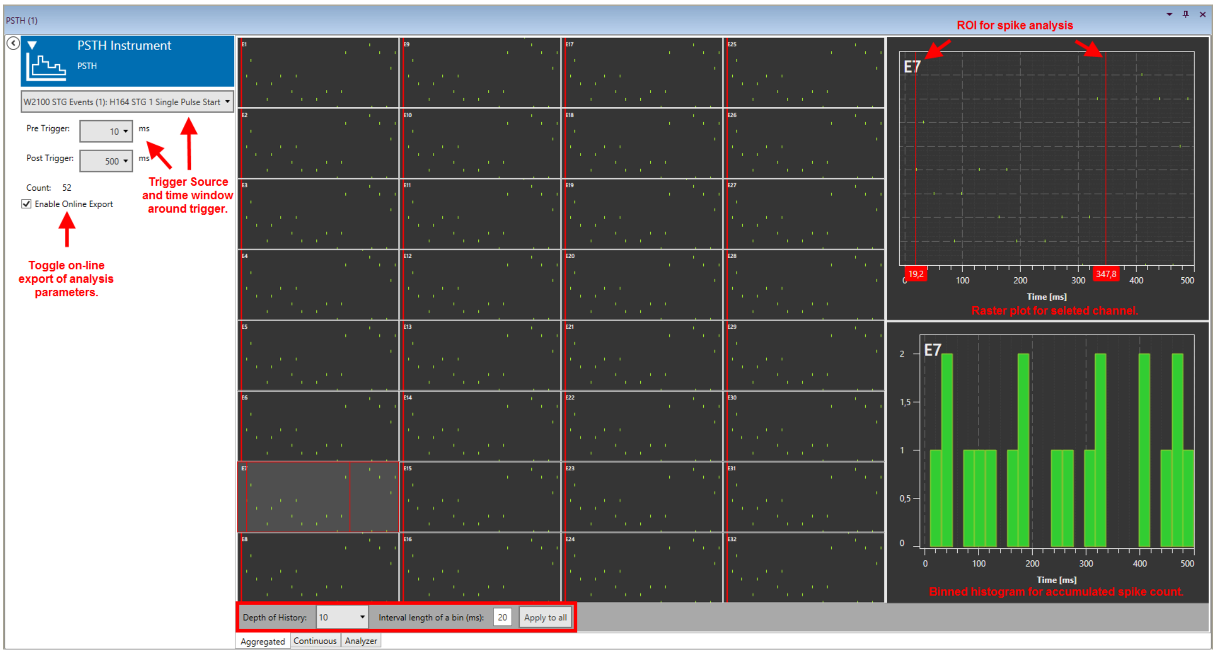
The PSTH (Peri Stimulus Time Histogram) tool can display spike time stamps arranged in relation to a Trigger input, usually a stimulation timepoint.

5.21.2 Data Ports and Export Options

The PSTH has a yellow Spike Time stamp and a pink Trigger input. There are no outputs. Analysis parameters can be exported as ASCII data, see 4.7.

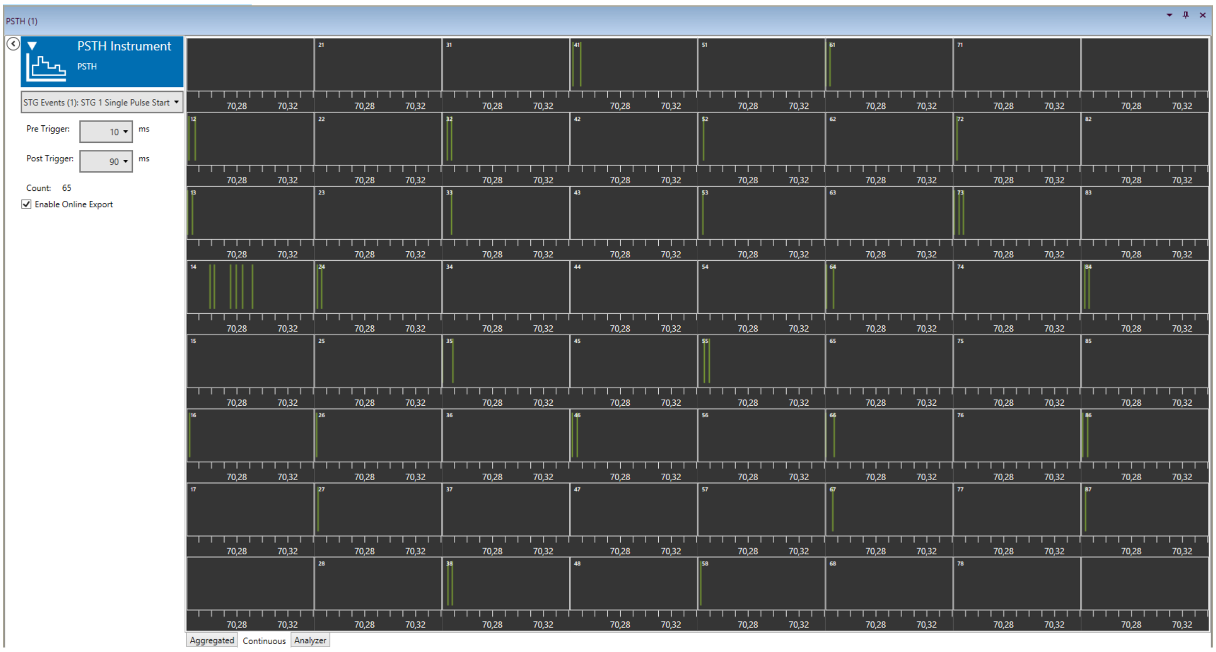
5.21.3 Operation

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**.

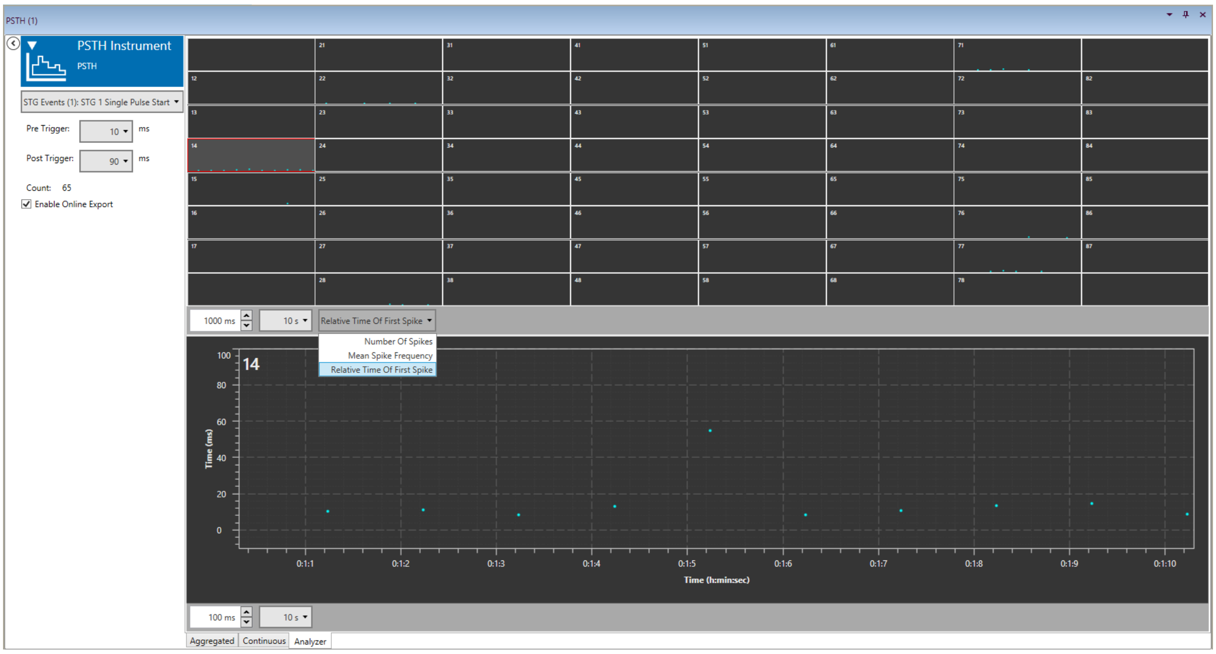


In the zoomed raster plot, a **Region Of Interest (ROI)** can be set by dragging the red lines. The same ROI can be **applied to all** channels, or the ROIs can be set individually channel by channel. Within the ROI, the number, average frequency, and relative time of first spike is analyzed and plotted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



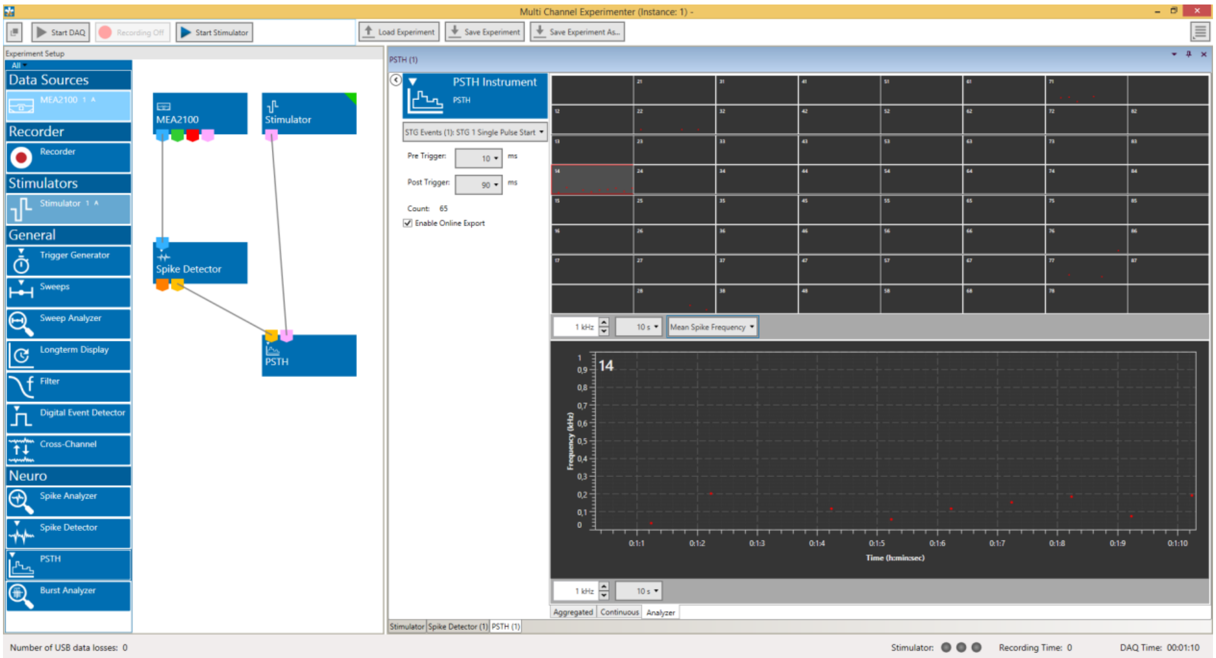
The **Analyzer** tab shows the analyzed results for all spikes within the ROI. Three parameters are shown, the **Number of Spikes** in each ROI, the **Mean Spike Frequency** (if there is more than one spike in the ROI), and the **Relative Time of First Spike**, in relation to the trigger.



All results can be exported as ASCII data continuously, for all channels, into a single ASCII file, if the **Enable Online Export** function is selected, see chapter 4.7.

5.21.4 Example: How to Analyze Evoked Spikes

To be able to analyze evoked spikes, a spike data stream and trigger events from a stimulator are needed. The spikes 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.

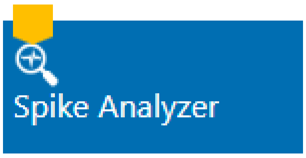
5.22 Spike Analyzer

5.22.1 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.

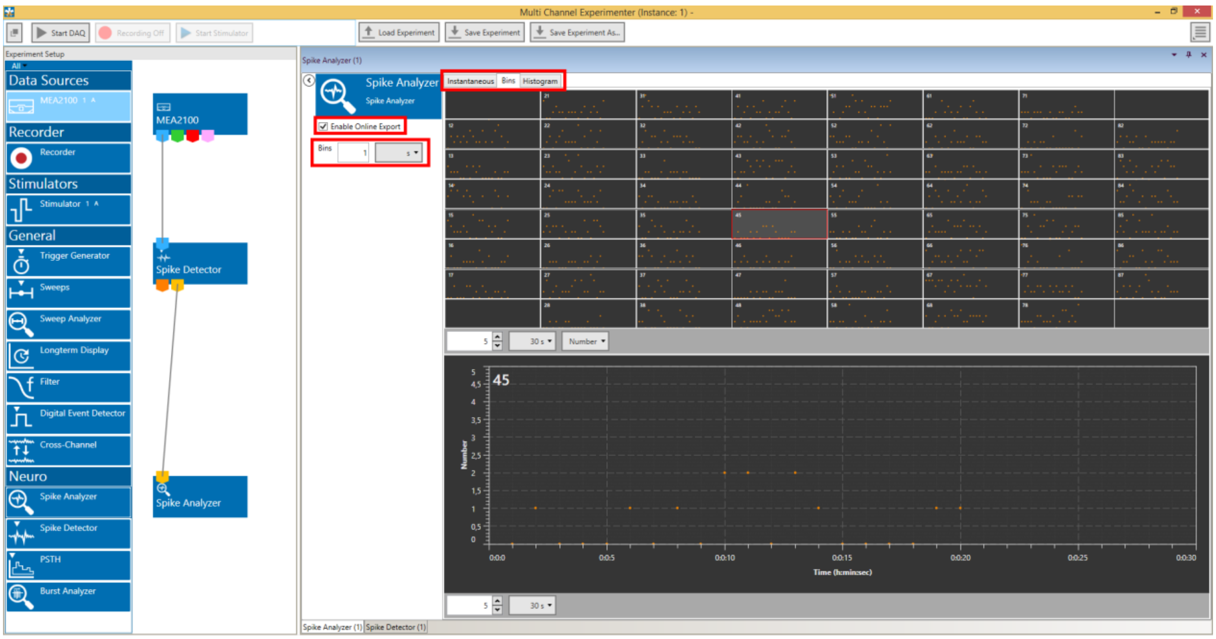
5.22.2 Data Ports and Export Options

The Spike Analyzer has a yellow Spike Time Stamps input only. The input data must be from the Spike Detector. Analysis results can be exported in ASCII format.



5.22.3 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 online as ASCII data with the Enable Online Export function, see chapter 4.7.

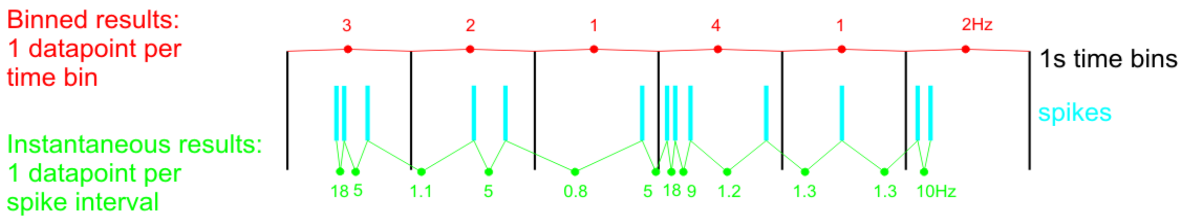


5.22.3.1 Instantaneous Versus Binned Analysis

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

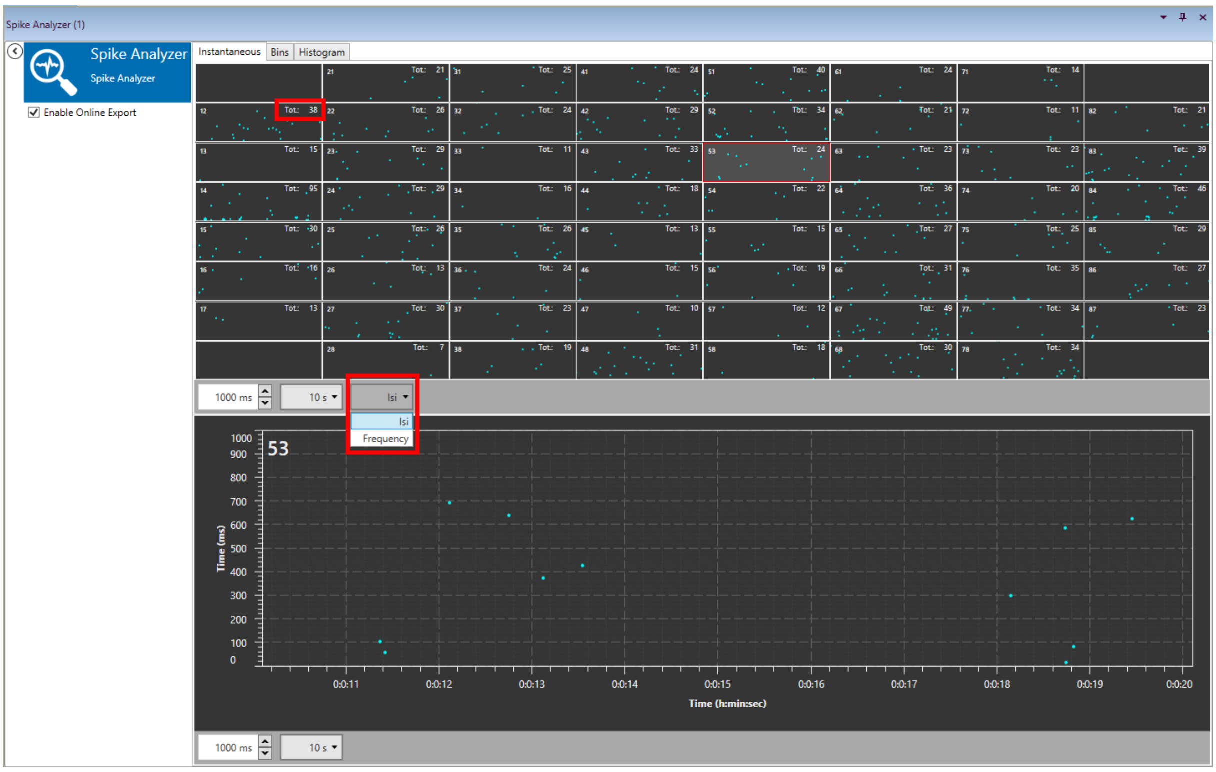
Bin wise analysis allows averaging of spike activity over longer time periods.



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.

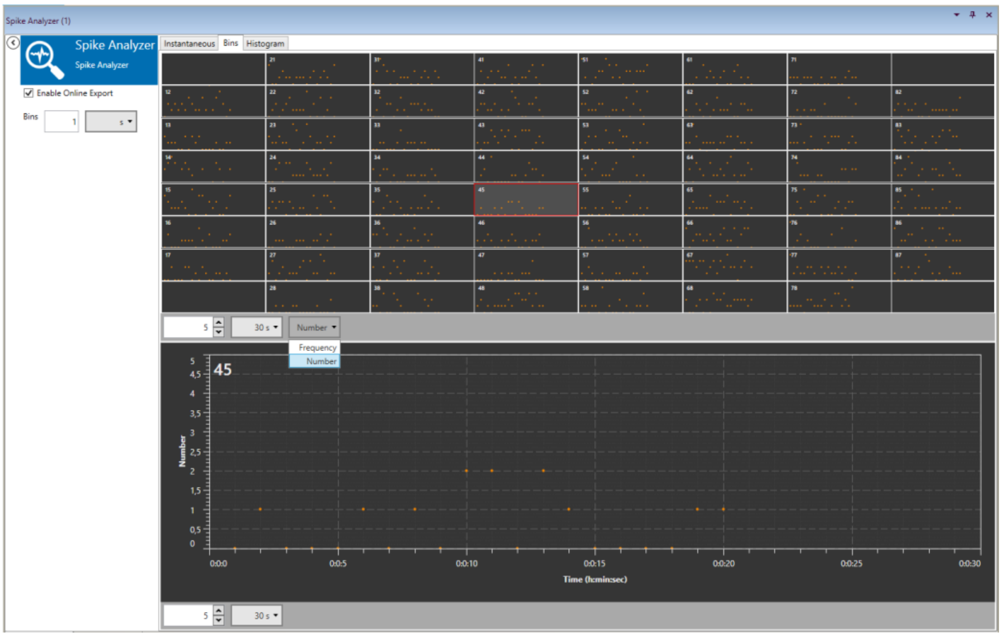
5.22.3.2 Instantaneous

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.



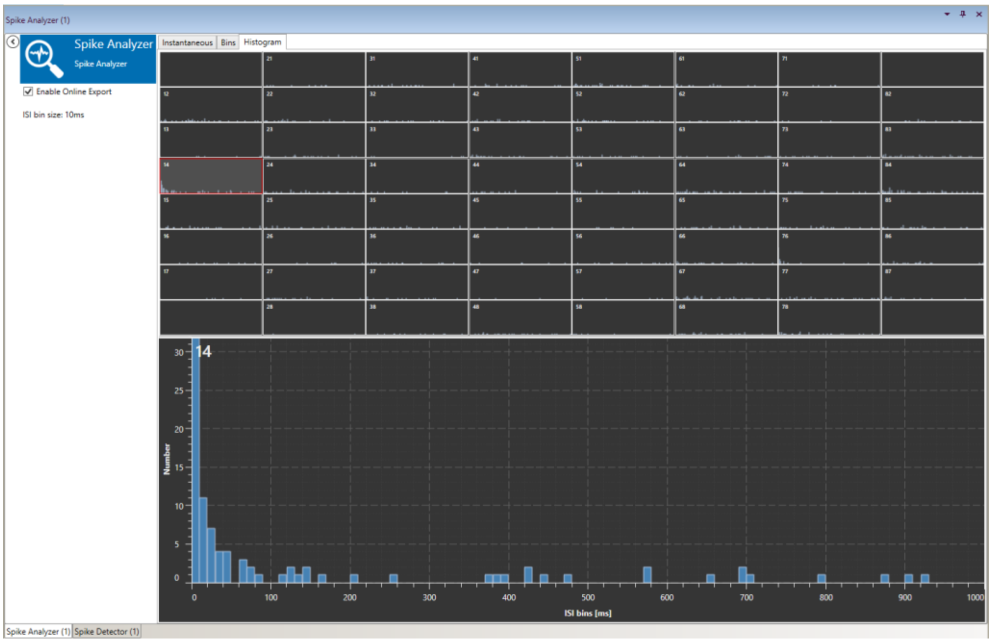
5.22.3.3 Bins

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.



5.22.3.4 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 Histogram is not updated continuously, but will be calculated once the data acquisition is stopped. In the example below, the results for the zoomed channel 14 mean that an ISI of 0 - 10 ms occurred on channel 14 around 32 times in the complete file, an ISI of 11 - 20 ms occurred around 11 times, 21 - 30 ms around 7 times, and so on.



5.23 Spike Sorter

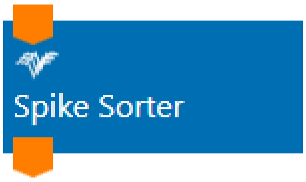
Attention: The Spike Sorter tool is currently only available on-line for the Experimenter, not for the Multi Channel Analyzer. Analysis tools can't operate on sorted units yet.

5.23.1 Description and Purpose

The Spike Sorter can analyze spike wave forms created by the Spike Detector. Spikes can be sorted in discrete units based on PCA (Principle Component Analysis) or SVD (Singular Value Decomposition) features.

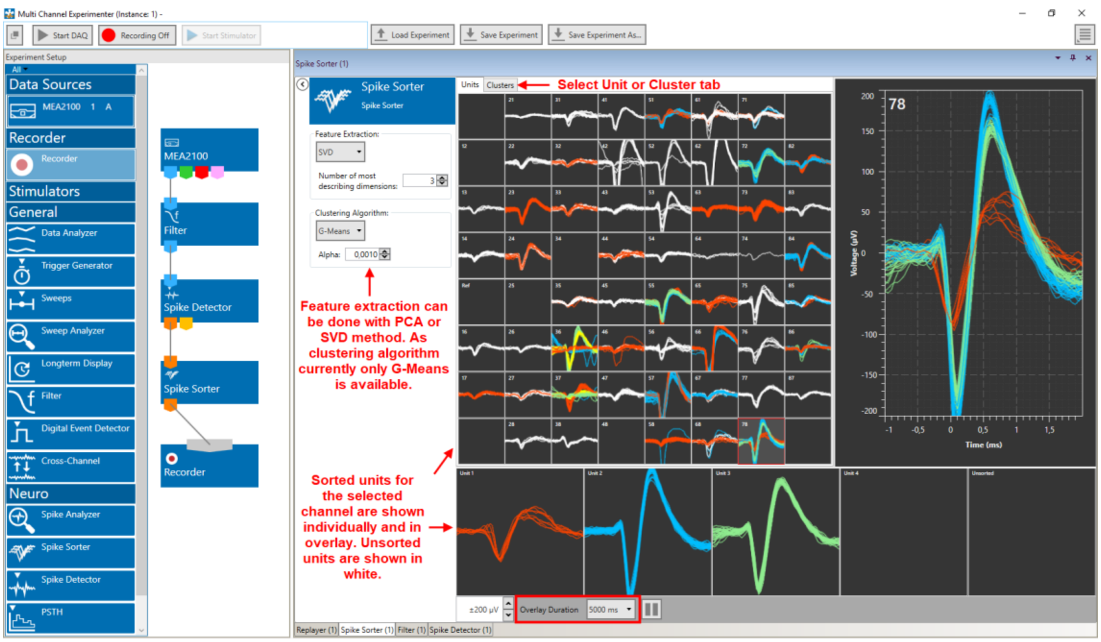
5.23.2 Data Ports and Export Options

The Spike Sorter has an orange Spike Data input and a Spike Data output. The input data must be from the Spike Detector. Sorted units can currently be connected only to the Recorder. Sorted units will be tagged accordingly, if the sorted Spike Data stream from a *.msrd file is exported with the [Multi Channel Data Manager](). There are no direct export options.

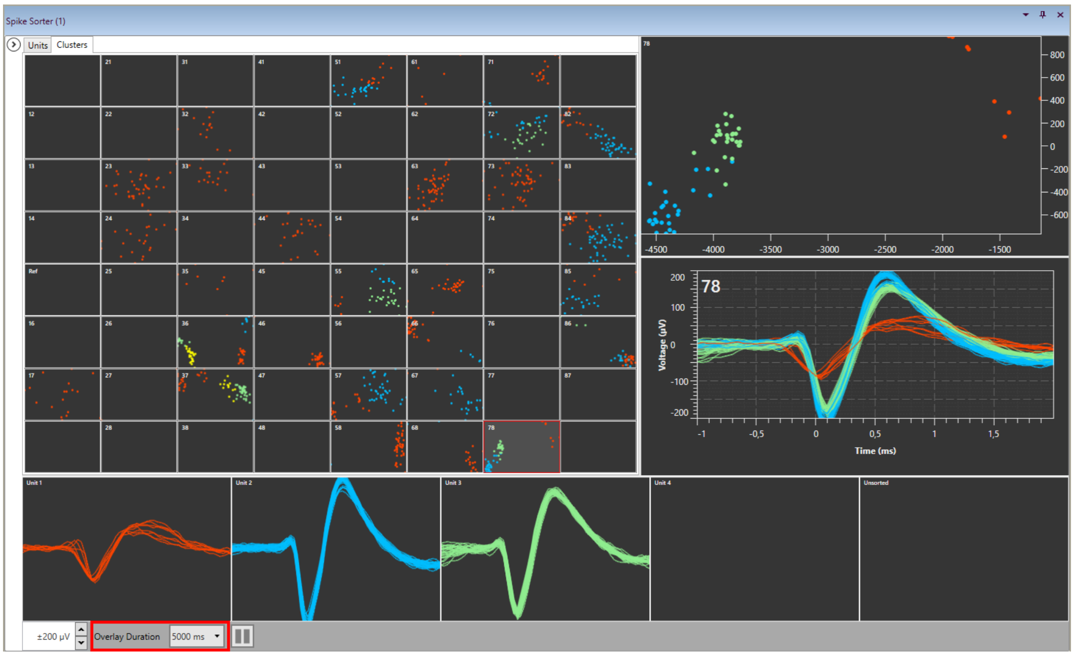


5.23.3 Operation

The Spike Sorter analyzes the waveforms of the detected spikes on all channels simultaneously, and tries to sort similar waveforms into discrete units. The sorted Units are shown in overlay in the **Units tab**, the underlaying clusters (see explanation below) can be seen in the **Clusters tab.** Each spike is shown for the time selected in the **Overlay Duration** drop down menu, and afterwards deleted from the display. This won't influence the sorting in any way. Sorted units are color coded, unsorted spikes are shown in white.



The first **50 spikes** detected after each DAQ start on each channel are used for feature extraction and clustering, they are not sorted. The **number of clusters/units per channel** is automatically determined by the G-Means algorithm, the required number of displays will be generated by the software. While the clusters are described by n dimensions, as selected in **Number of most describing dimensions** (see below) the cluster display can show only two dimensions, so the cluster separation might be better than the display view suggests.



5.23.3.1 Feature Extraction: PCA, SVD

PCA:
Principal Component Analysis (PCA) is a well-known method for **feature extraction** and dimensionality reduction. PCA computes an ordered set of orthogonal basis vectors that capture the largest variation in the data, the so-called principal components (PC). Each of the original data vectors can then be represented as a weighted sum of the PCs. Because the first few PCs will often explain most of the variance of the data, the other PCs can be discarded without losing too much information and the weighted sum of the remaining PCs should still reasonably well approximate the original data. This is called "**dimensionality reduction**". In the context of spike sorting, PCA is applied to the extracted spike waveforms. Each PC represents a common pattern in the spike waveforms. The assumption is that spikes originating from the same neuron have similar waveforms, so their weights for the different PCs will be similar. Spikes from different neurons, however, should exhibit different weight patterns. We can think of each spike as a point in n-dimensional space, where n is the number of remaining PCs. Ideally, the coordinates of spikes from the same neuron should form a **cluster** in this space, where each cluster is separated from each other cluster. These clusters are displayed in the **Cluster tab**. In the example shown above, the cluster of the red unit is well separated from the others, while green and blue show some overlap.

References: Lewicki, 1998 and Gibson, 2012.

SVD:
Singular Value Decomposition (SVD) serves a similar purpose as PCA and is closely related to it. It will also factorize a data set into a set of vectors: the left- and right-singular vectors, which represent patterns in the spike waveforms, and a set of weights, the singular values which give information about the importance of each pattern. Thus, dimensionality reduction is achieved by representing each spike by its weights for the singular vectors with the largest singular values. Similar as for PCA, the resulting clusters are shown in the Clusters tab.

Reference: Pachitariu, 2016

Number of Dimensions:
In both extraction methods, the parameter **"Number of most describing dimensions"** defines how many dimensions (PCs or singular vectors) remain after dimensionality reduction. If this is set to three, for example, only the first three dimensions explaining the most variability in the data are used. Thus, every spike is described as a 3-dimensional data point whose coordinates are the weights of its waveform for the first three PCs or singular vectors. Predicting the effects of changing the number of dimensions on the spike sorting results is not easy. Increasing the number of dimensions from three to four, for example, can be helpful if the 4th dimension contains valuable information, so that spikes originating from different neurons which show overlapping clusters in a three-dimensional space can be better discriminated. On the other hand, it is also possible that the 4th dimension doesn't really contain relevant information, and instead introduces noise and makes it harder to differentiate between clusters.

5.23.3.2 Clustering: G-Means

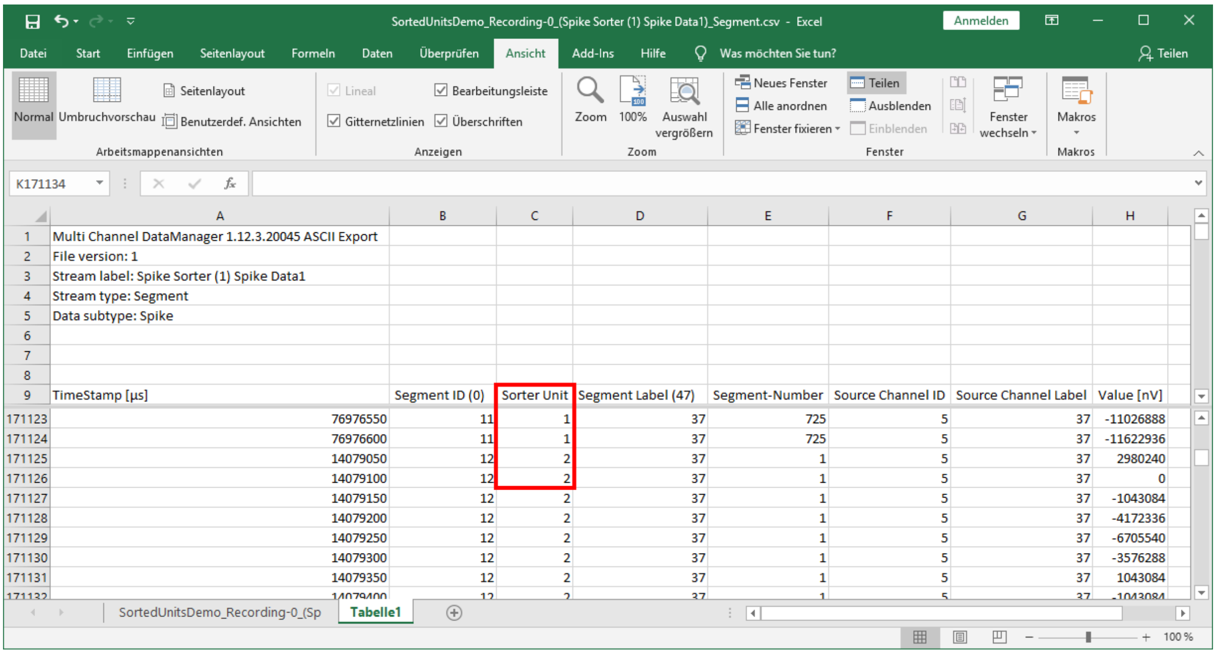
For clustering, the Spike Sorter uses the G-Means algorithm, an improved version of the k-Means algorithm. Compared to many other clustering algorithms, G-means has the advantage that it is able to estimate the number of clusters in the dataset **automatically**. This heuristic process starts by putting all data points in a single cluster and use a statistical test to determine whether the data points assigned to this cluster follow a Gaussian distribution. If the test fails, the cluster is split into two smaller clusters and the test is repeated for both new clusters. This process continues until all generated clusters pass the test and the distributions of their data points are accepted as Gaussian.

The statistical test is controlled by the **parameter Alpha**, which is the significance level for the test for Gaussianity: A smaller alpha means that the test is more restrictive and less likely to label data in a cluster as "non-Gaussian". Therefore, less splitting of clusters occurs, and fewer clusters are produced in total. A higher value for alpha has the reverse effect and will tend to increase the number of clusters. Alpha is therefore helpful to control the sensitivity of the clustering.

Reference: [Hamerly and Elkan, 2004]

5.23.3.3 Exporting Sorted Units

If a **Spike Data** stream processed by the Spike Sorter has been recorded during an experiment, this stream can be exported to other file formats with the Multi Channel Data Manager. Each detected event has the tag "Sorter Unit". Unsorted units have the tag 0, the sorted units are labelled 1 and up.



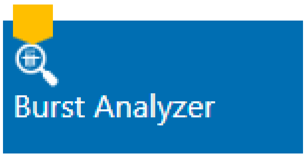
5.24 Burst Analyzer

5.24.1 Description and Purpose

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

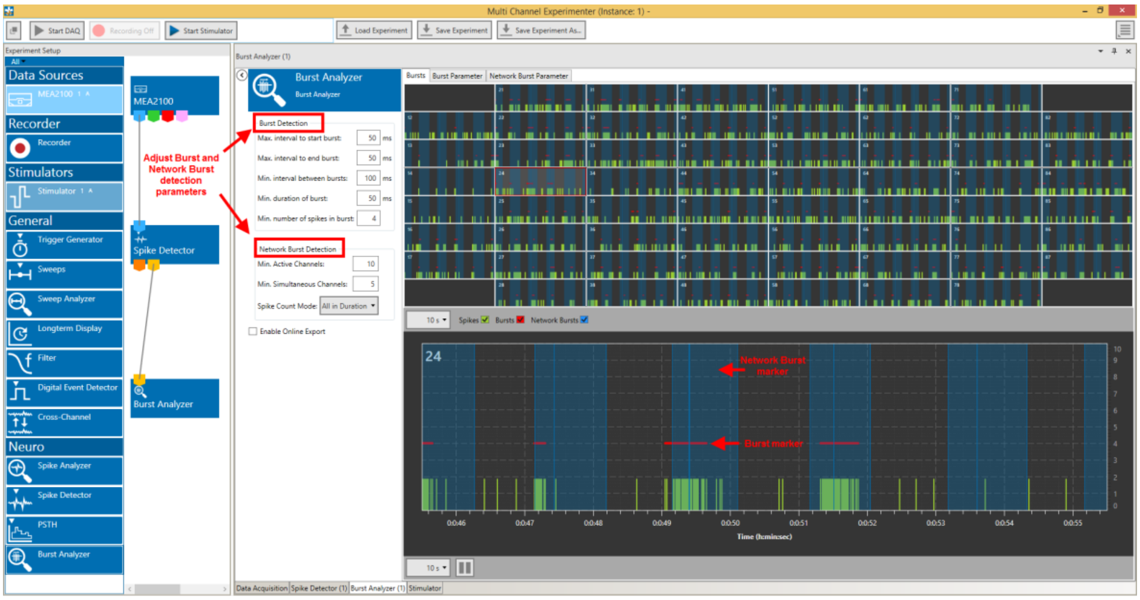
5.24.2 Data Ports and Export Options

The Burst Analyzer has a yellow Spike Time Stamps input only. The input data must be from the Spike Detector. All results can be exported online as ASCII data with the Enable Online Export function, see chapter 4.7.



5.24.3 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.



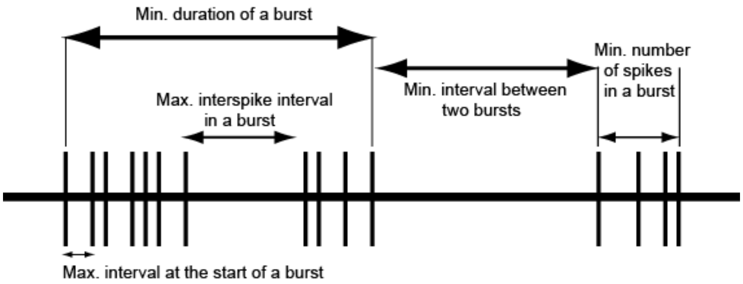
5.24.3.1 Burst Detection Parameters

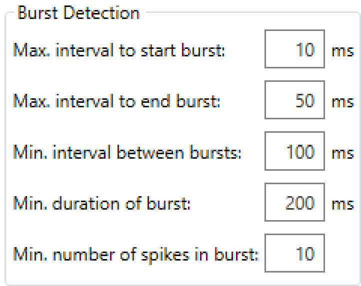
The Burst Analyzer uses the **MaxInterval Method** for detecting bursts:

- 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:





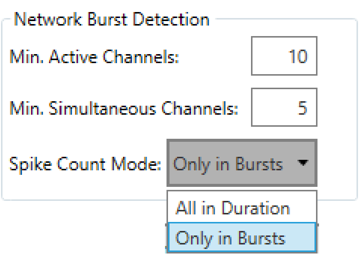
These parameters can be adjusted in the Burst Analyzer control window. Bursts are labeled in the Bursts tab with **red lines**.

5.24.3.2 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,

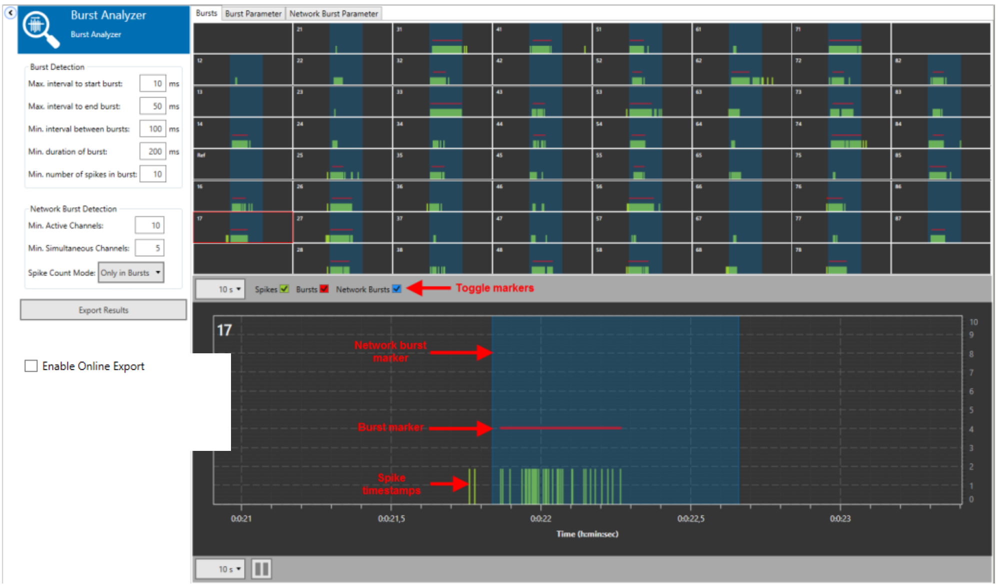
- the number of distinct bursting channels in the sequence needs to be at least the number defined for **Min. Channels Participating** and
- at some point during the sequence, there need to be at least the **Min. Simultaneous Channels** bursting at the same time.

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**.



5.24.3.3 Bursts Tab

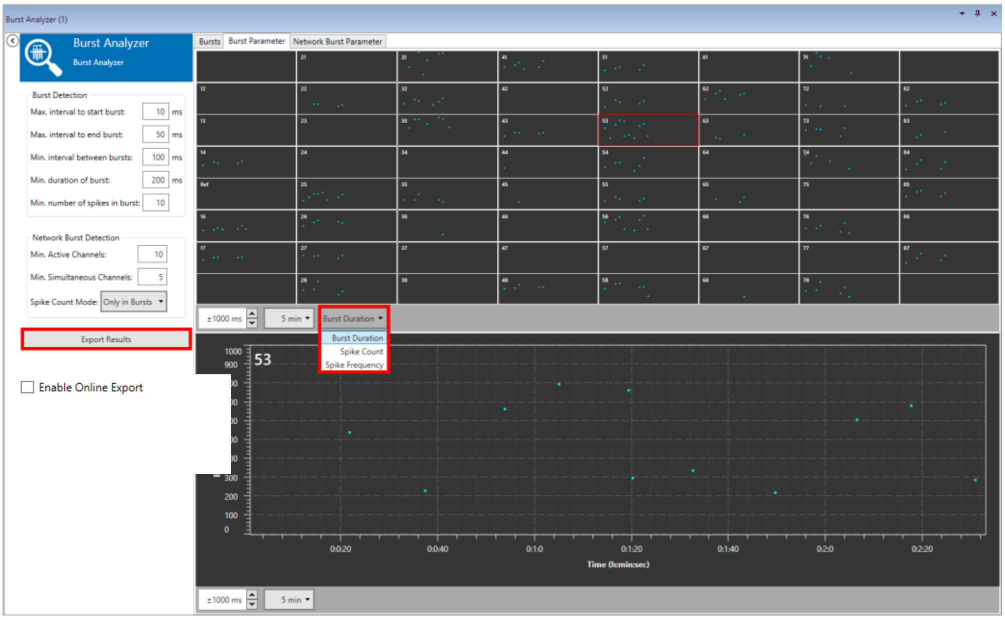
The Bursts tab shows the spike time stamps and the detected bursts as a red line on top of the events. If a network burst is detected, it's shown on all channels as a blue background.



5.24.3.4 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

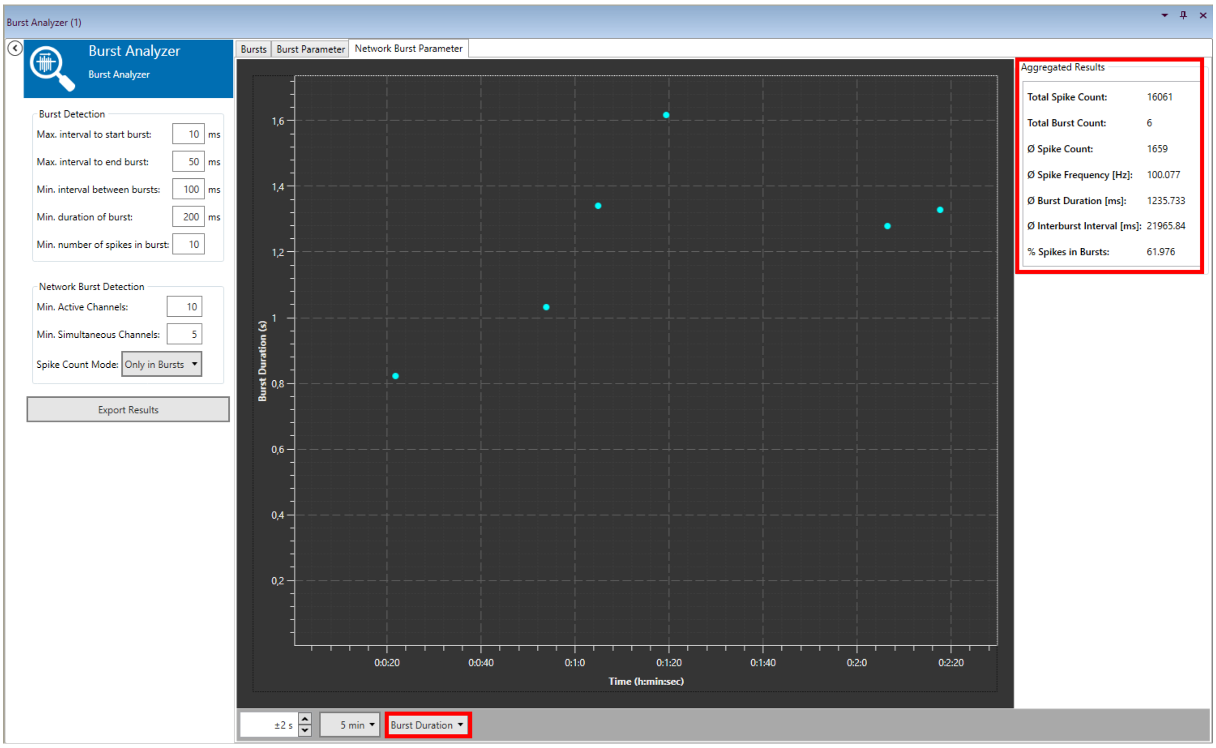


5.24.3.5 Network Burst Parameter Tab

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 influences these results.



The **Aggregated Results** for all network bursts detected in the analyzed file (or file segment) are shown as numerical results after the data acquisition is stopped. 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.

6 Index of Application Examples

Below is a list of all application examples mentioned in this manual. Many examples shown, especially in relation to specific data sources, will work just as well for other data sources.

7 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 website. 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 (*.mse) 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** which has been used, and possibly a **data file** to the support e-mail address. An upload option for the files will be provided if need be.

8 Contact Information

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

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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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