

PATCHMASTER NEXT

USER MANUAL

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1 Introduction

1.1 Disclaimer

This *Product* relies on the tools of Microsoft Windows 10 (64-bit) or MacOS 10.14 (or more recent). HEKA is not responsible for:

1. the contents of these third party products,
2. any links contained in these third party products,
3. changes or upgrades to these third party products and
4. for any consequential damages resulting from the use of these products.

This *Product* may not be reverse engineered, decompiled or disassembled without the express written consent of HEKA.

In no event shall HEKA be responsible for any incidental, punitive, indirect, or consequential damages whatsoever, (including but not limited to loss of data, privacy of data or other pecuniary loss), arising from or relating to the use, or the inability to use, this *Product* or the provision, or lack of provision, of support services.

In all cases where HEKA is liable, the extend of HEKA's liability shall be limited to the actual cost of the *Product* or to the provision of a replacement version of the *Product*.

1.2 Scope of the Program

PATCHMASTER NEXT is a versatile toolkit for electrophysiological experiments. It provides multi-channel stimulation and data acquisition with integrated online and offline analysis features.

PATCHMASTER NEXT is a driving program for amplifiers, especially with controlling features for EPC 10 USB (Single, Double, Triple and Quadro) amplifiers. Further, surrounding components like temperature controllers, perfusion systems or other third-party devices can be managed by PATCHMASTER NEXT.

PATCHMASTER NEXT originates from the PATCHMASTER software and represents the next level of our famous patch clamp software. Initially, we aim to cover the full functionality of its precursor PATCHMASTER. Once, this goal is reached, we will include more new features and alleviations to make PATCHMASTER NEXT even more powerful.

Due to the fact that PATCHMASTER NEXT is transformed to a new object oriented application framework one of the biggest changes you might immediately realize when starting PATCHMASTER NEXT the first time is difference in the graphical user interface (GUI). The new GUI will help beginners and advanced learners to accelerate the learning curve of operating the software but will also still be recognizable enough to make a smooth transition of experienced PATCHMASTER users.

1.3 Supported Hardware

PATCHMASTER NEXT does currently support the following hardware:

- EPC 10 USB Single / Double / Triple / Quadro
- LIH 8+8
- EPC 800

Note: In future there will be more devices supported by PATCHMASTER NEXT.

1.4 Supported System Software



PATCHMASTER NEXT is supported on MS Windows 10 (64-bit).



PATCHMASTER NEXT is supported on MacOS X version 10.14 (or more recent). Older MacOS X versions and MacOS 9 are not supported. Please check our website for support of the latest MacOS.

1.5 Naming Conventions

1.5.1 EPC Type Amplifiers

Throughout this manual we will address the EPC 10 USB patch clamp amplifier. We will explicitly mention other patch clamp amplifiers when required.

1.5.2 Windows Versions

Throughout this manual we will address all the supported Microsoft Windows versions as "MS Windows". We will explicitly mention the particular MS Windows versions if required.

1.5.3 Macintosh Versions

Throughout this manual we will address all the supported Macintosh versions as "MacOS". We will explicitly mention the particular Macintosh versions if required.

1.5.4 Syntax

- PATCHMASTER NEXT: Small Capitals denote a HEKA program / hardware
- `Replay`: Typewriter font denotes menu entries and chapters or filenames
- `Protocol Editor`: Sans serif font denotes window names or dialogs
- *Sine Wave*: Roman font denotes options or buttons
- [F12]: Indicates an assigned key in the software
- *Italic*: Denotes general emphasis

1.6 Installing PATCHMASTER NEXT

Please install the hardware and the software according to the “Installation Guide” which is supplied with your order. It can also be obtained from the HEKA homepage ([Installation Guide](#)).

Note: PATCHMASTER NEXT is installed in `C:/Programs/HEKA` in its own folder. The installation process does not interfere with any existing PATCHMASTER NEXT installation. However, if you have already installed an older version of PATCHMASTER NEXT you are strongly encouraged to make a backup of your user files in `Documents/HEKA` before deleting the old PATCHMASTER NEXT version on your PC.

1.7 Starting PATCHMASTER NEXT

Upon clicking on the PATCHMASTER NEXT icon the software starts. At first the settings file dialog appears:

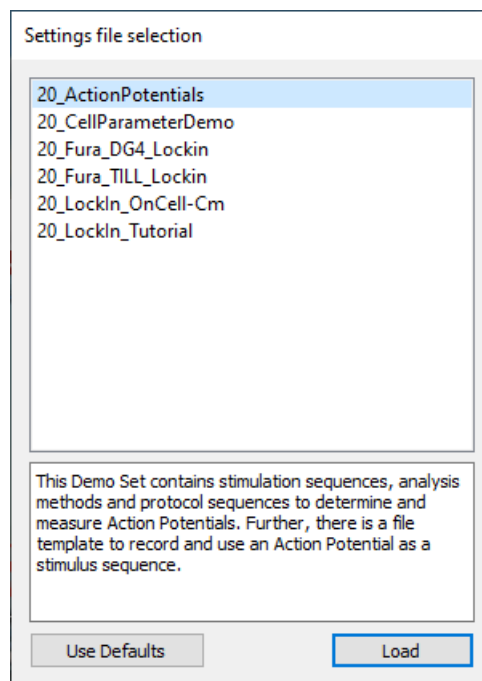


Figure 1.1: After starting Patchmaster Next the settings file dialog comes up offering starting configurations for applications.

PATCHMASTER NEXT provides several starting configurations for special applications. The user is free to select one of them to load the corresponding files or use the *Use Defaults* option to start from scratch.

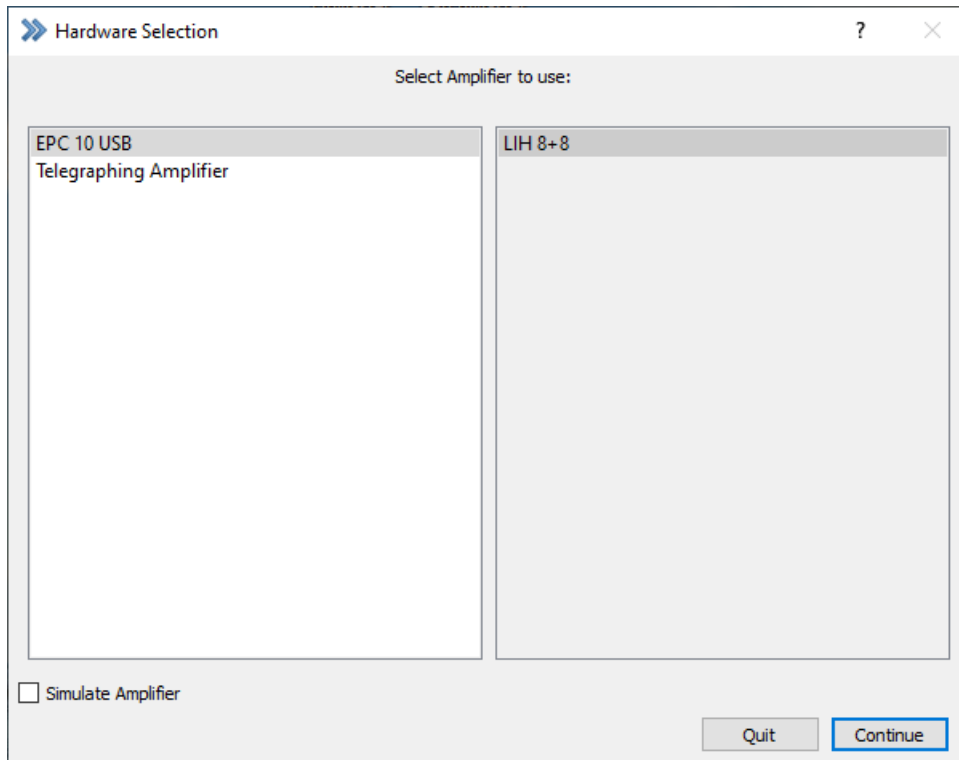


Figure 1.2: Hardware Selection dialog of PATCHMASTER NEXT. This dialog comes up if no configuration file is existing.

Select the appropriate hardware to continue the initialization process.

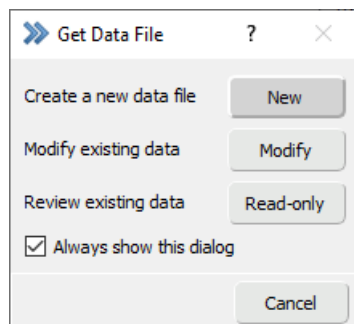


Figure 1.3: Get Data File dialog of PATCHMASTER NEXT.

Once the software is ready the Get Data File dialog pops up providing the following options:

- New: Create a new data file
- Modify: Opens an existing file in modify mode
- Read-only: Opens an existing file in write-protect mode
- Always show this dialog: Brings up this dialog every time you start PATCHMASTER NEXT

After the user made a decision in the Get File dialog, the following windows open up:

- Amplifier
- Control
- Test Pulse
- Oscilloscope
- Notebook
- Data Tree

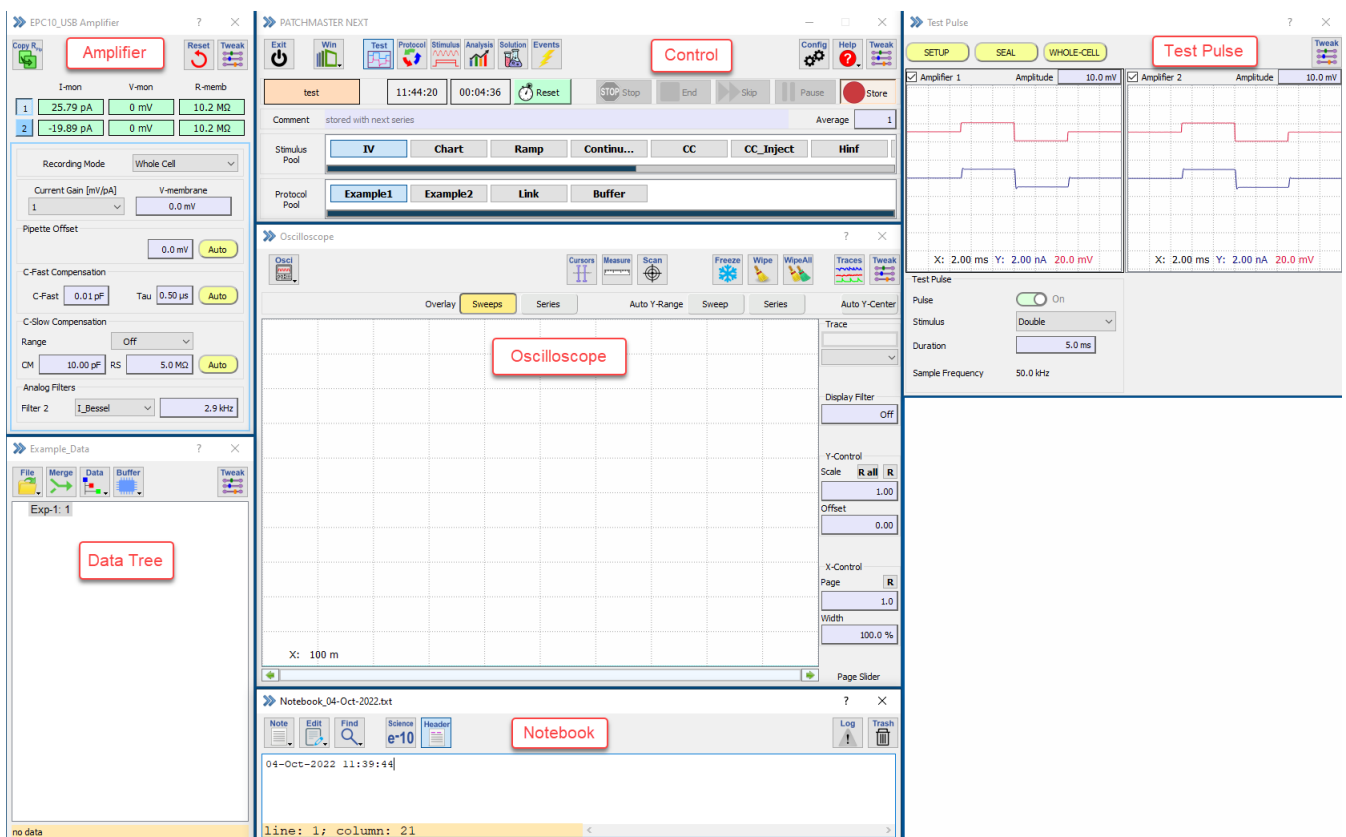


Figure 1.4: Multi window capability of PATCHMASTER NEXT.

There is no main window any more which incorporates all the windows but each of the windows are independent of each other in PATCHMASTER NEXT. One can easily drag them to other screens (multi window capability).

Note: If the window position or the window size is changed you need to save the configuration to save the changes for the next launch of PATCHMASTER NEXT.

1.8 Hierarchy Levels of PATCHMASTER NEXT

The following diagram presents a rather simplified view on the overall structure of the PATCHMASTER NEXT program. It provides a rough schematic overview about the main modules and their hierarchy ranking (Levels) illustrating the versatility of the software.

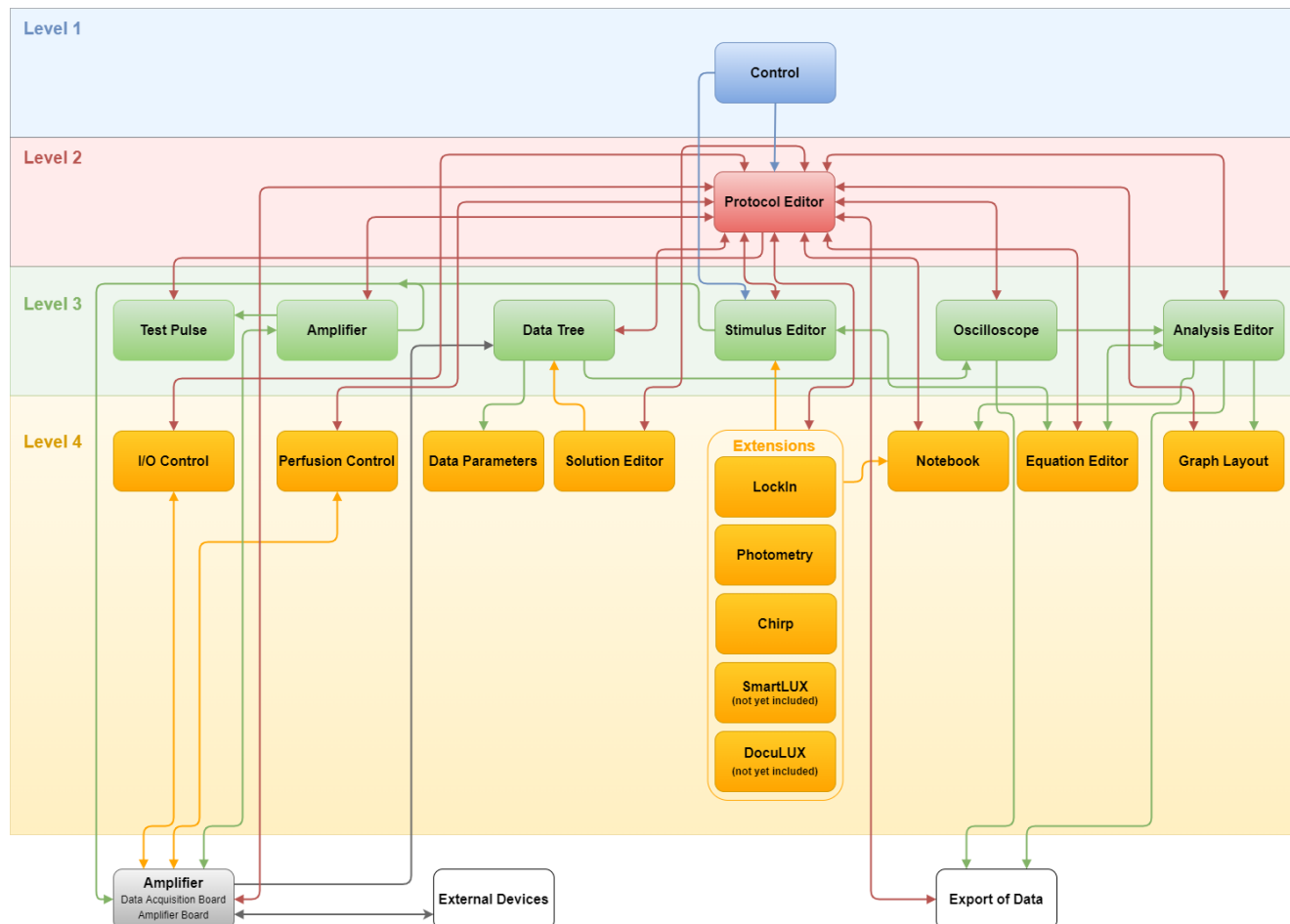


Figure 1.5: Hierarchy Diagram of PATCHMASTER NEXT. The several main windows or modules can be categorized into different hierarchy levels (color scheme). Further, their interaction with each other is shown by the connection arrows.

Level 1 (blue)

The Control window is the main window of the software. From there, you can directly execute and control all protocols and stimulation sequences of the Protocol Editor and the Stimulus Editor, respectively. Further, you get access to the Configuration (not depicted in the figure) and all other windows of PATCHMASTER NEXT.

Level 2 (red)

The Protocol Editor with its protocols is a key player of PATCHMASTER NEXT. These protocols are called by the Control window and they help to automate and standardize your experimental procedures and analysis processes. PATCHMASTER NEXT offers an extensive list of *Events* to readout, check and modify values, hardware parameters or analysis results of other modules and is capable to define conditionally actions in response.

Level 3 (green)

Most of the windows or modules are located in the third hierarchy level. This means they are important

for the general workflow of PATCHMASTER NEXT but they are under control of the Control window or the Protocol Editor.

Level 4 (orange)

These modules are the lowest level modules. They are not needed to stimulate, record, display or analyze data but they contribute to the enormous flexibility and versatility of PATCHMASTER NEXT.

The Amplifier itself is included in the diagram as well but due to the fact that it is not a software dialog it cannot be directly covered by the hierarchy levels. Also, external instances like “External Devices” and “Export of Data” are located outside the hierarchy level organization.

1.9 The PATCHMASTER NEXT Workflow

The hierarchy diagram illustrates the full complexity of PATCHMASTER NEXT. However, working with all modules might not occur on a daily basis. For simplification and to get beginners familiar with the software we start with an overview about the main objects of PATCHMASTER NEXT:

- Amplifier window for hardware control
- Test Pulse window for patch procedure
- Stimulus Editor for stimulation and data acquisition
- Data Tree for data storage
- Oscilloscope window for data display
- Analysis Editor to perform data analysis
- Protocol Editor for standardization and automation of complete experiments
- Application assistance (Extensions) such as:
 - Software LockIn
 - Photometry
 - Chirp
 - SmartLUX
 - DocuLUX

To get familiar with PATCHMASTER NEXT we will introduce several complexity levels starting with the least complex one. The intention is to simplify the workflow and interactions of the software depicted in figure 1.5 on the facing page, thereby focusing on the purpose of the complexity levels.

Note: The usage of the offered modules is not restricted to the complexity levels. The user is free to use any number of modules and in any meaningful combination or interaction. The complexity level organization is more or less arbitrary and should provide orientation when using PATCHMASTER NEXT.

1.9.1 Complexity Level: Patch

The most simplified complexity level is called “Patch”. It shows the used modules necessary to perform the most basic process of patching a cell.

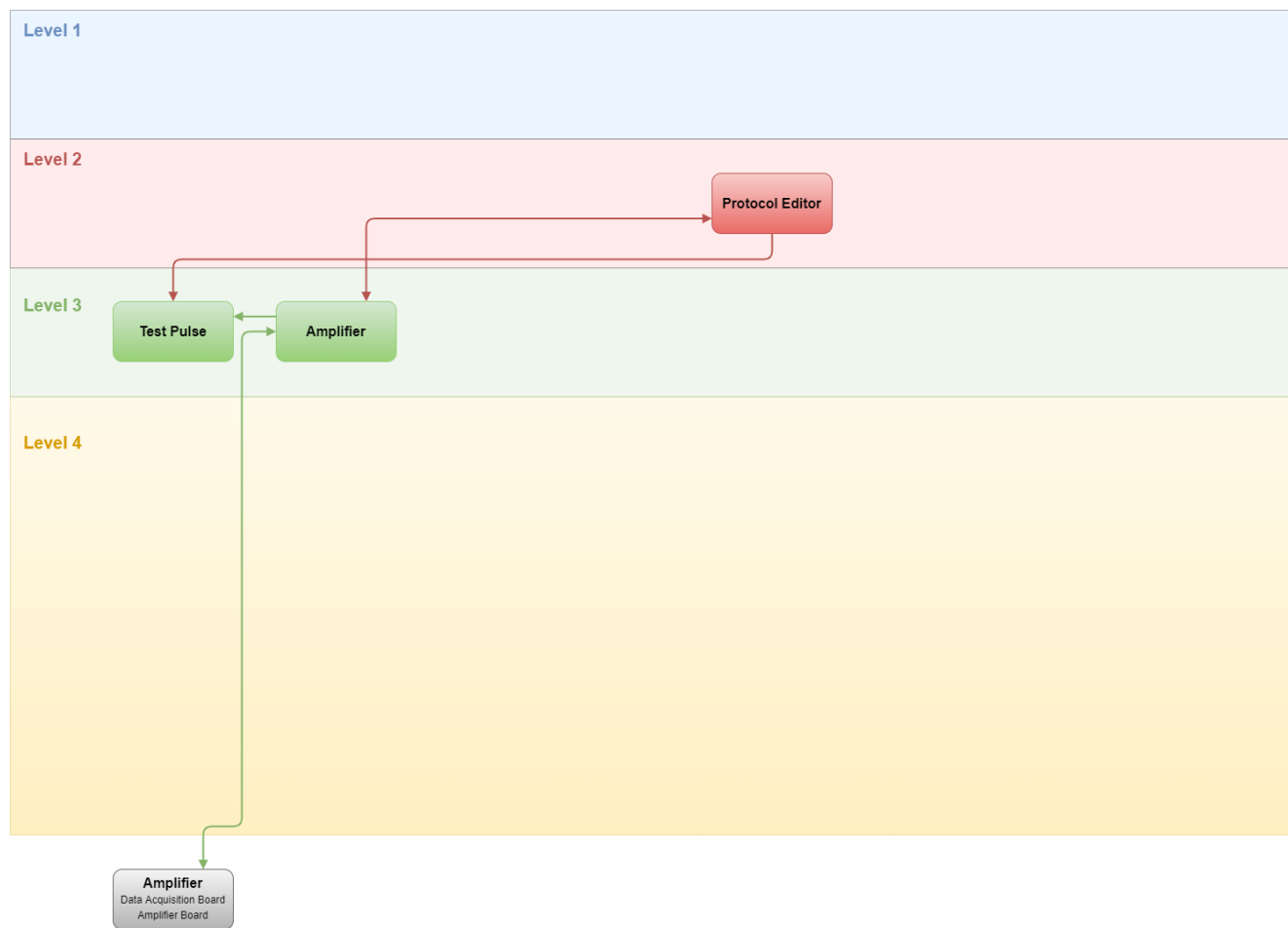


Figure 1.6: Complexity level “Patch” of PATCHMASTER NEXT. This diagram shows the modules necessary for patching a cell.

While patching a cell the main modules would be the **Test Pulse** window and the **Amplifier** window. Here, you make all the settings (e.g. test pulse, compensations, holding potential) for the patch process. These settings are directly sent to the hardware and the corresponding feedback is displayed in the **Test Pulse** window or **Amplifier** window again. The other depicted module is the **Protocol Editor**. This is necessary since during the patch process the amplifier protocols (“SETUP”, “SEAL”, “WHOLE-CELL”) are used in the **Test Pulse** window. These protocols are defined in the **Protocol Editor** and can also be edited there according to the customer wishes.

1.9.2 Complexity Level: Basic

The complexity level “Basic” shows the main modules involved when you simply want to stimulate your sample and record the data while showing them in the Oscilloscope window. This is the most straightforward way to operate Patchmaster Next.

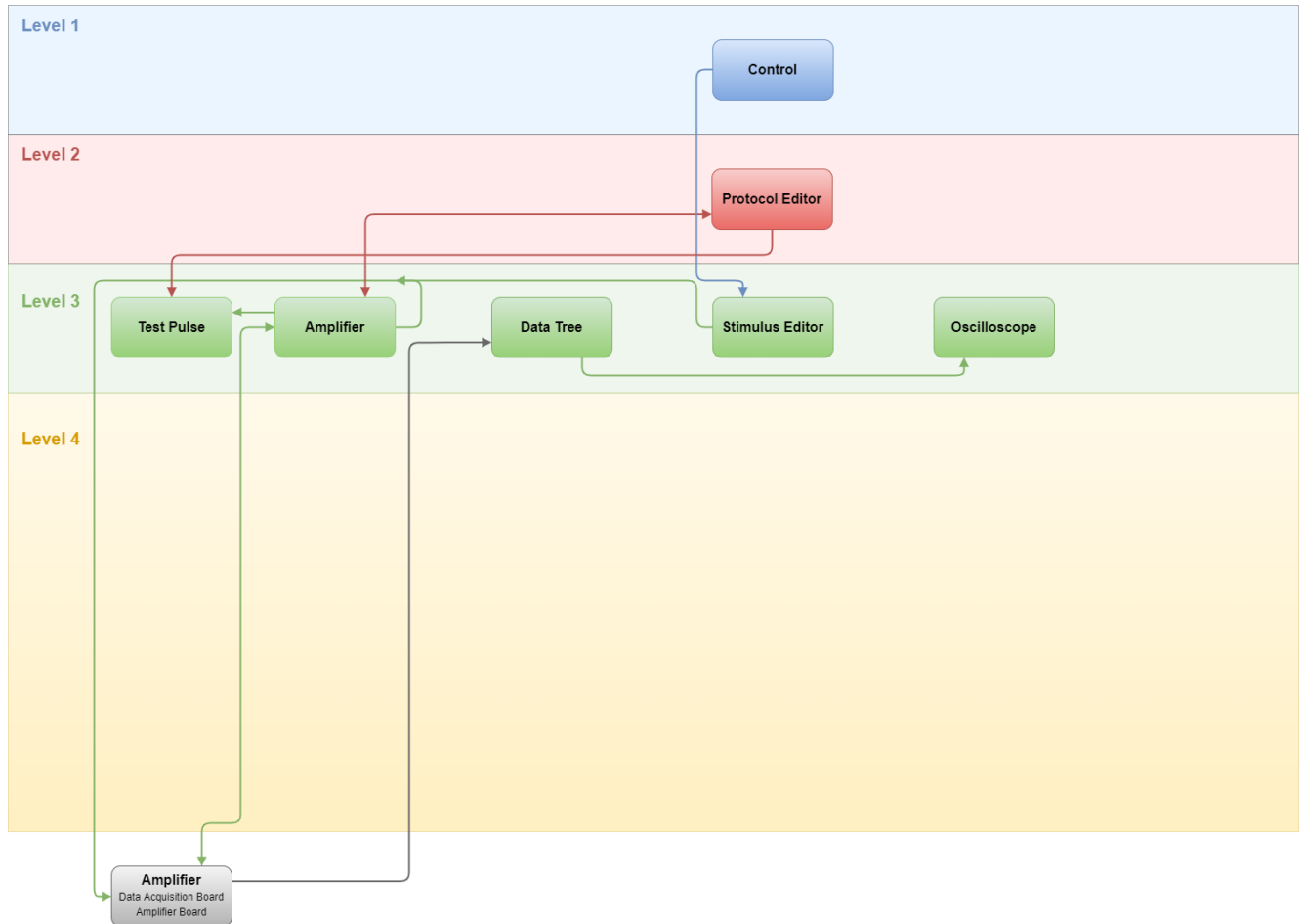


Figure 1.7: Complexity level “Basic” of PATCHMASTER NEXT. This diagram shows the modules necessary for most patch clamp experiments including the patch clamp procedure itself.

We assume that the patch process itself is accomplished. Via the Control window a predefined stimulation sequence is executed out of the Stimulus Editor. The incoming data is displayed in the Oscilloscope window and stored in the data file, hence, listed in the Data Tree window. During this process several hardware parameters might be changed which is depicted in the Amplifier window.

1.9.3 Complexity Level: Novice

The complexity level “Novice” is identical to the “Basic” level but adds three more modules into the experimental process - Analysis Editor, Graph Layout and Notebook.

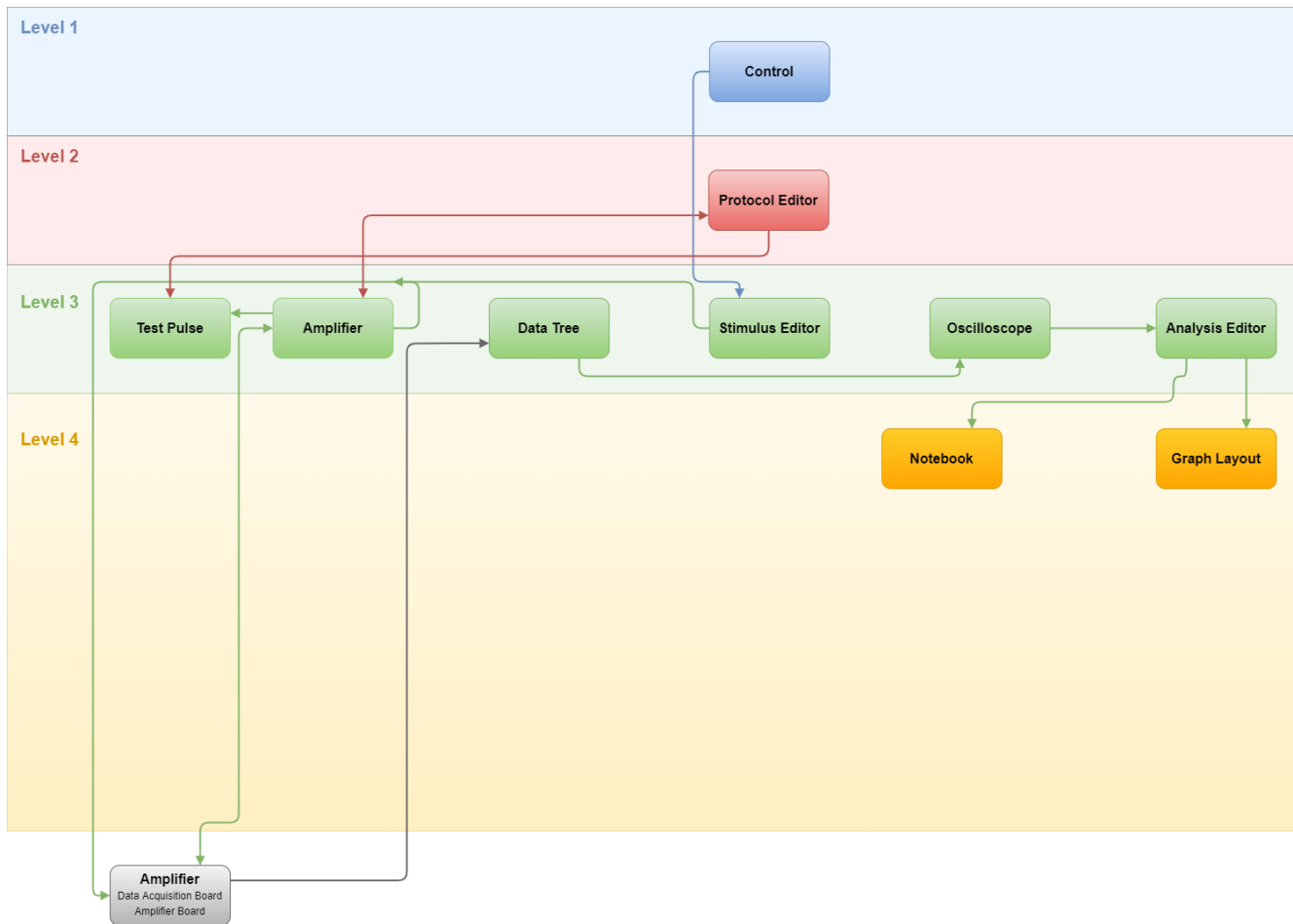


Figure 1.8: Complexity level “Novice” of PATCHMASTER NEXT. In addition to the “Basic” patch clamp complexity level the modules for data analysis are included.

While acquiring data it is possible to analyze the data online via the new modules. In the Analysis Editor one would setup the analysis functions in front of the recording. The analysis results can be displayed in analysis graphs of the Graph Layout window and/or listed in the Notebook window. Of course the analysis or re-analysis of the data can also be performed offline.

1.9.4 Complexity Level: Advanced

The complexity level “Advanced” now further includes the control of external devices, Perfusion Control and an organizing tool for your solutions - the Solution Editor.

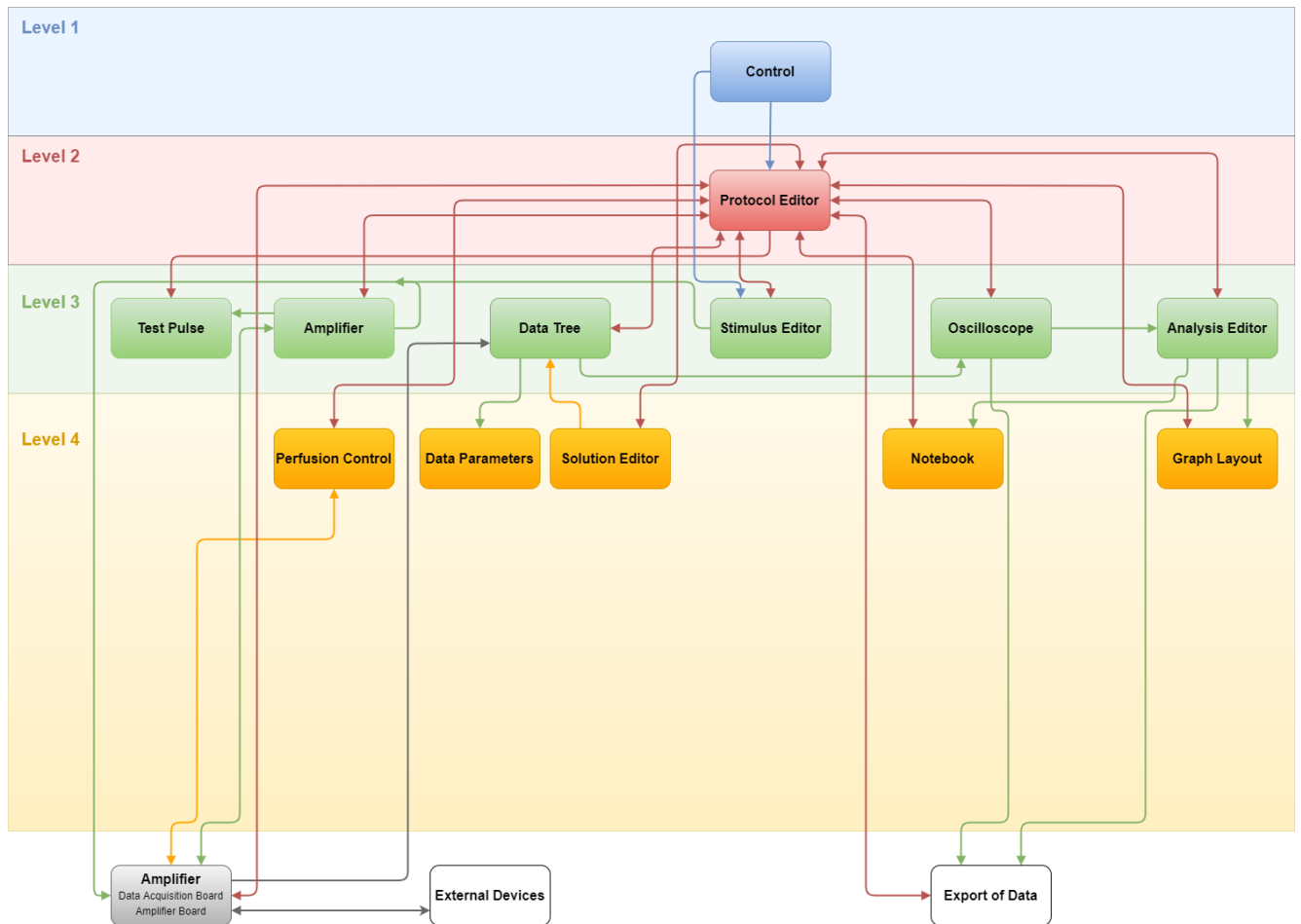


Figure 1.9: Complexity level “Advanced” of PATCHMASTER NEXT. In the “Advanced” complexity level one adds modules to organize solutions and to control external perfusion systems.

The Perfusion Control window defines e.g. the settings for your external perfusion system. In combination with the Solution Editor one can easily setup and track the solutions used during the experimental course. All the data parameters including the used solutions for each experiment is safely stored in the data file and can be checked every time in the Data Parameters window. It is not necessary any more to note all hardware settings or solution changes. If necessary the acquired data and the analysis results can be exported to other formats for further usage elsewhere.

1.9.5 Complexity Level: Expert

The complexity level “Expert” is the final complexity level PATCHMASTER NEXT can offer. Here, every module is shown and might be used by the user. The last inclusions are: Equation Editor, I/O Control window and the all extensions.

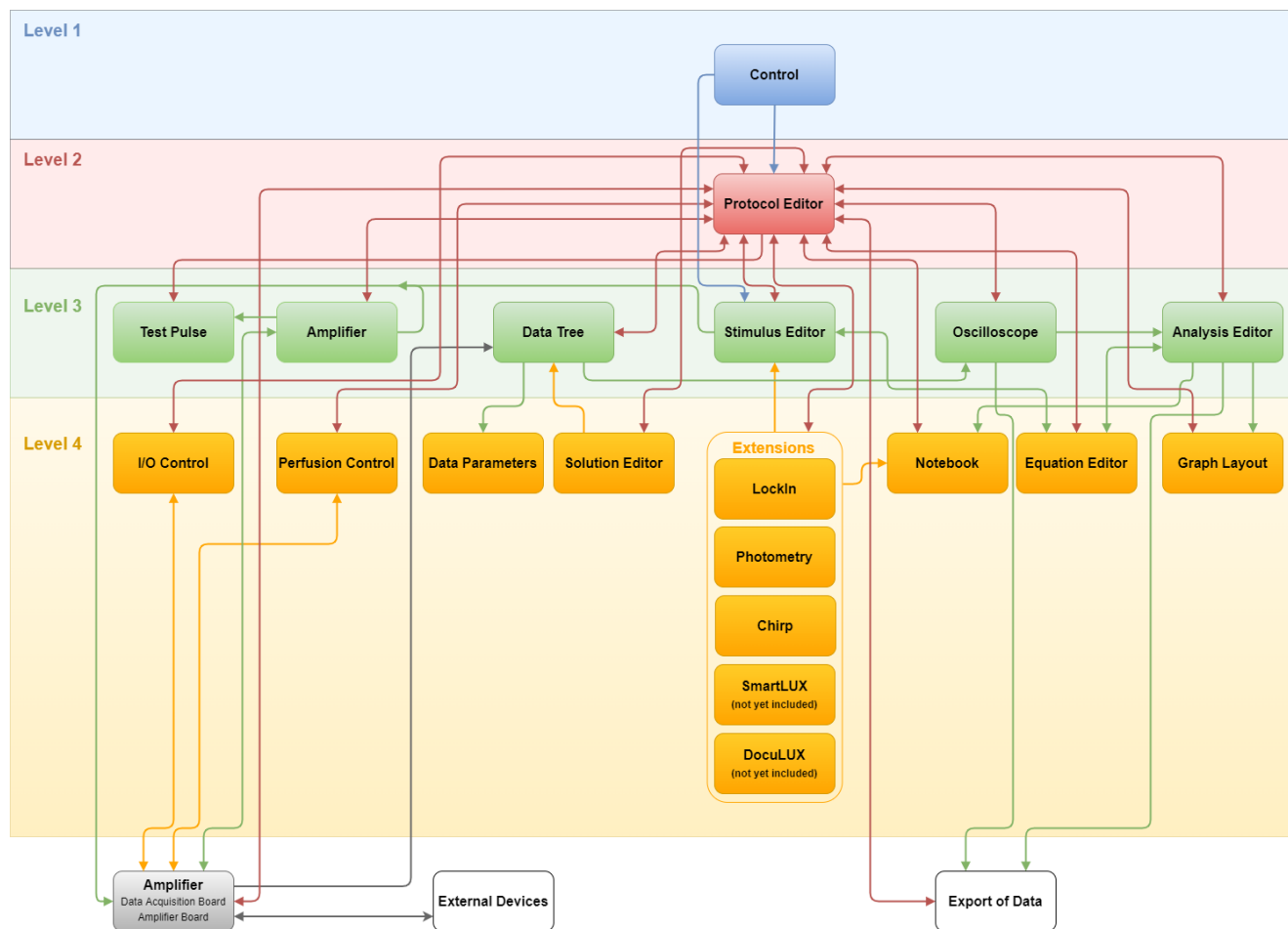


Figure 1.10: Complexity level “Expert” of PATCHMASTER NEXT. The “Expert” complexity level incorporates all modules and extensions of the PATCHMASTER NEXT software. Here, one has the full versatility of the software available.

The Equation Editor is a module which allows to compose formulas which can be used in other modules. The I/O Control window is mainly a tool to check parameters or the status of the analog or digital channels of the amplifier. The versatile software extensions like LockIn, Photometry, Chirp, SmartLUX and DocuLUX acknowledge the power level of PATCHMASTER NEXT.

1.10 Support Hotline

If you have any questions, suggestions, or improvements, please contact HEKA's support team. The best way is to send an e-mail to support@heka.com specifying as much information as possible:

- Your contact information
- The program name: e.g. PATCHMASTER NEXT
- The program version number: e.g. v1.4
- Your operating system and its version: e.g. Mac OS 10.14, Windows 10 64-bit
- Your type of computer: e.g. Intel Core i7 4 GHz with 8 GB RAM
- Your acquisition hardware, if applicable: e.g. EPC 10 USB
- The serial number and version of your amplifier, if applicable: e.g. EPC 10 USB Single, Serial Number "520000", Revision "T"
- The questions, problems, or suggestions you have
- Under which conditions and how often the problem occurs

Note: Please provide as much and as detailed informations as possible to speed up the support process. If available, please include images and data files in your e-mail.

We will address the problem as soon as possible.

Contact Address:

Multi Channel Systems MCS GmbH

Aspenhastrasse 21

D-72770 Reutlingen

Germany

Phone: +49 7121 90925-26 or +1 800 272 2775

E-mail: support@heka.com

Website: www.heka.com

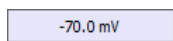
2 User Interface

The following chapter describes the user interface of PATCHMASTER NEXT. There are several dialog control types, Copy & Paste options and Right-click context menu options available.

2.1 Dialog Controls

Controls are defined as parts of the dialog that allow user input (data, options or execution of actions).

The general rules for dialog controls in this program are:



: Box item with a drop shadow for entering values.



: Box item with a drop shadow. You either enter a value directly or you use the up/down arrows to set the value.



: Box with small arrow indicating an available drop-down menu.



: Inactive tool button



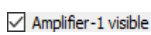
: Active tool button indicating an active dialog.



: Inactive slide button indicating a not selected parameter or option (especially in *Tweak* menus).



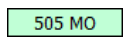
: Active slide button indicating a selected parameter or option (especially in *Tweak* menus).



: Selected or deselected option.



: Rounded rectangles are items that perform some action.



: Simple rectangles display a measured value.

Recording Mode : Plain text is for titles only.



: Plain text field to enter a text string.

2.1.1 Numerical Input

The numerical values can be entered in scientific notation (e.g., "2.3e-3", "2.3E-3") or in engineering format (e.g., "2.3m"). Numbers outside the range for engineering numbers are always displayed in scientific notation. The old value is erased as soon as the user starts to type. The table below provides an overview about engineering and scientific notation nomenclature.

Table 2.1: Engineering & Scientific Input Nomenclature

Name	Engineering	Scientific
Tera	T	E12
Giga	G	E9
Mega	M	E6
kilo	k	E3
milli	m	E-3
micro	μ / u	E-6
nano	n	E-9
pico	p	E-12
femto	f	E-15

SI Units: PATCHMASTER NEXT expects most units to be SI units, i.e., meters, seconds, amperes, or derived units like Hertz, etc. However, for convenience there are exceptions to that rule. In such cases the item title contains an identifier for what unit is to be used, e.g., "mV" if a voltage is to be entered in millivolts rather than in volts.

3 Control

The Control window is the main window of the PATCHMASTER NEXT software. It is the starting point to execute and control protocols or stimulation sequences. In addition, status information like state of the experiment, time settings or entering comments are provided.

Furthermore, the Control window gives you access to all windows of the software or other auxiliary menus.

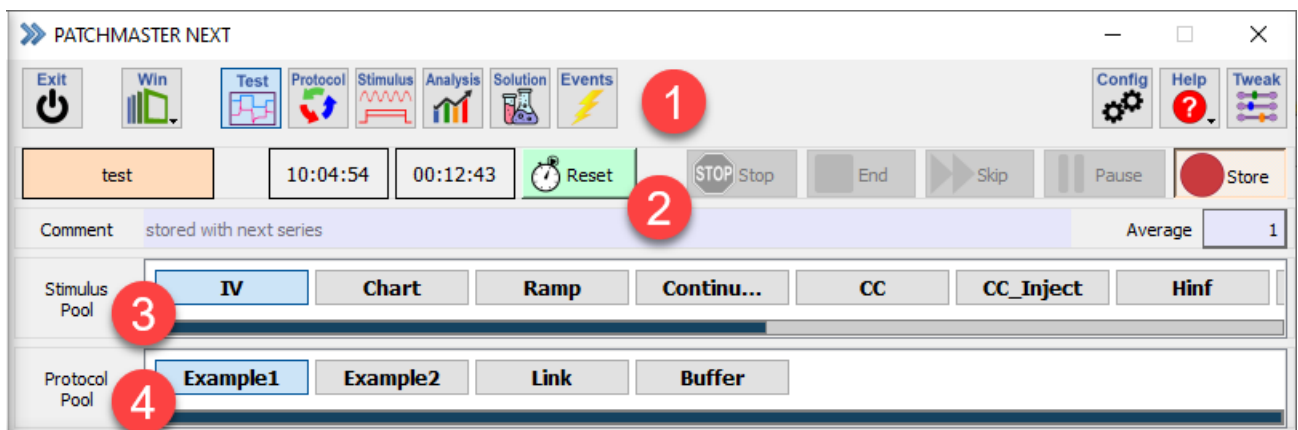


Figure 3.1: Control window of PATCHMASTER NEXT.

The Control window can be divided into four major sections:

1. Global
2. Data Acquisition Control
3. Stimulus Pool
4. Protocol Pool

All sections are described in detail below.

3.1 Global

On top of the Control window several buttons are shown which provide access to the main functions of the PATCHMASTER NEXT software.

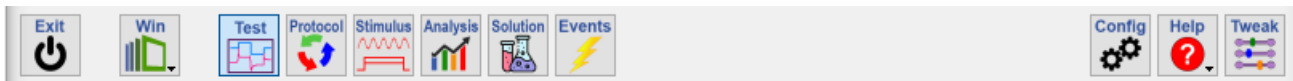
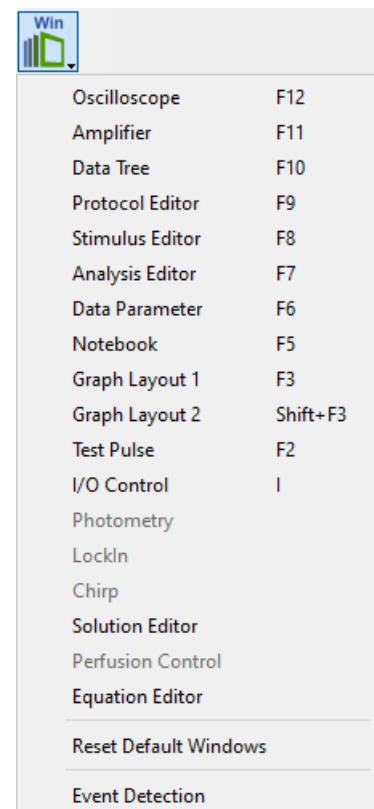


Figure 3.2: Global section of the Control window.

Exit: The *Exit* tool button closes PATCHMASTER NEXT.

Win: The *Win* (Windows) menu provides access to all main windows and dialogs of PATCHMASTER NEXT:

- Oscilloscope [F12]
- Amplifier [F11]
- Data Tree [F10]
- Protocol Editor [F9]
- Stimulus Editor [F8]
- Analysis Editor [F7]
- Data Parameter [F6]
- Notebook [F5]
- Graph Layout 1 [F3]
- Graph Layout 2 [SHIFT+F3]
- Test Pulse [F2]
- I/O Control
- Photometry
- LockIn
- Chirp
- Solution Editor
- Perfusion Control
- Equation Editor
- Reset Default Windows: Resets the default window positions of PATCHMASTER NEXT.
- Event Detection



Selecting one entry from the drop-down menu opens it (except *Reset Default Windows*). Further, the shortcut key is indicated in brackets after the window name.

Test: Opens the Test Pulse window (see chapter 5 on page 59).

Protocol: Opens the Protocol Editor window (see chapter 8 on page 133).

Stimulus: Opens the Stimulus Editor window (see chapter 7 on page 83).

Analysis: Opens the Analysis Editor window (see chapter 9 on page 163).

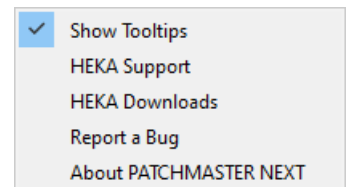
Solution: Opens the Solution Editor window (see chapter 14 on page 225).

Events: Opens the Event Detection dialog (see chapter 18 on page 239).

Config: Opens the Configuration of PATCHMASTER NEXT (see chapter 3.5 on page 22).

Help: The Help menu offers several support options for troubleshooting problems:

- Show Tooltips: Whenever the cursor of the mouse is hovering for several seconds above an entry field or button a help text is displayed.
- HEKA Support: Direct link to the support section of the HEKA website.
- HEKA Downloads: Direct link to the download section of the HEKA website.
- Report a Bug: Opens your e-mail program to report your software problem directly to our support center.
- About PATCHMASTER NEXT: Shows the splash screen of the software.



Tweak: When activating the Tweak button the Tweak section gets visible.



Figure 3.3: View options of the Tweak section.

There you have the following View options:

- Comment: The *Comment* option is either displayed or hidden in the Control window.
- Stimulation Pool: The *Stimulation Pool* is either displayed or hidden in the Control window.
- Protocol Pool: The *Protocol Pool* is either displayed or hidden in the Control window.

3.2 Data Acquisition Control

Below the *Global* section with all the buttons to get access to important PATCHMASTER NEXT windows and settings there is another area with buttons and display fields. The purpose of this section is to provide information about the data acquisition status or to control the data acquisition process.



Figure 3.4: Section for controlling the data acquisition in the Control window.

State: Current state of the experiment. All possible states are listed below:

- “acquiring”: Data acquisition is currently running
- “idle”: No task is executed by the program
- “pause”: The acquisition is paused e.g. when the *Pause* button was pressed.
- “recording”: A macro is currently recorded (*Record* function of the Protocol Editor is active).
- “Re-Initializing Amplifier: The amplifier is getting re-initialized
- “run macro”: Runs a macro command which is part of a protocol.
- “run protocol”: Runs a protocol sequence.
- “test”: Test Pulse is switched on and running.
- “wait for trigger”: The program is waiting for an external trigger via the *Trigger Input* channel.

Time: In this field the actual system time is displayed.

Timer: This item functions as a stopwatch. To reset the *Timer* time use the *Reset* button beneath. The *Timer* might be useful to keep track of the experiment (e.g., to monitor the time spent in a specific experiment mode). The *Timer* time is updated also during the Series execution. The *Timer* time value is stored at the beginning of the Sweep acquisition. The internal *Timer* tick corresponds to 1 ms.

Reset [T]: When you click *Reset*, the *Timer* time will be reset to zero and started again.

Stop [S]: *Stop* is used to stop Series acquisition or data replay. The *Stop* flag is reset, when the next target is displayed or executed. If *Stop* is pressed during acquisition of a Sweep, this particular Sweep will not be completed and the data acquired for this Sweep thus far will be discarded. All previous Sweeps of the Series will be saved; thus, there can be a Series with fewer Sweeps than specified in the Stimulus Editor. The *Stop* button can also be used while replaying data.

End [H]: This button is used similarly to *Stop*. However, during acquisition of a Sweep, this particular Sweep will be completed. The *End* button can also be used while replaying data.

Skip [K]: At the end of the actually acquired Sweep the Series is stopped and the next protocol event will be executed.

Pause/Resume [P]: This button is used to pause Series execution. The label of the button changes into “Resume” thereafter. The Series execution goes on by a click on *Resume*. *Pause* will become effective after completion of the currently acquired Sweep. The *Pause* button can also be used while replaying data; e.g., it allows inspecting a particular Sweep, when replaying a collection of Sweeps.

Store: Store is used to save the data that will be acquired from now on. In case Store is not activated but the file is opened with write permission, the last Sweep will be stored temporarily. This Sweep is marked with a "#" in its label. It will be deleted when:

- the next Sweep is acquired
- a new Group is created
- the file is closed.

Comment: Enter a Comment to the currently active Series. The Comment will be stored along the data parameters when the next Series gets acquired and the file is opened with write permission. Via the Tweak options it is possible to hide the Comment field.

Note: Be aware that the entered Comment is stored for every forthcoming Series until the entry is deleted or changed.

Average: By use of this function, a stimulation sequence can be repeated several times and only the average is stored to disk. You need to enter the number of averages acquired for one Sweep. Via the Tweak options it is possible to hide the Average field (use the option "Comment" to hide it).

Note: It is not mandatory but advised to use even numbers of averages in order to avoid possible problems with the Alternating Leak Averaging feature (see chapter 7.6.4 on page 102).

The Average function uses the Trace buffers for temporary storage. Thus, the average is limited to 4 Traces per Sweep. Also, it will overwrite whatever is in the Trace buffers at time of acquisition.

3.3 Stimulus Pool

Via the help of the Control window you can directly execute any of your stimulation sequences. The Stimulus Pool area provides an overview about all stimulation sequences available in your currently loaded stimulation sequence file (*.pgf).

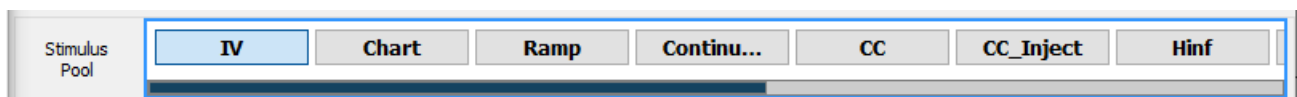


Figure 3.5: Stimulus Pool area of the Control window.

The first seven sequence names of the currently loaded stimulus sequences file are visible at a first glance. Use the scroll bar to find the stimulation sequence you want to execute. Once you select one of the stimulation sequences the Stimulus Pool gets into the focus which is indicated by a blue rectangular border around it. The execution of the stimulus sequence is started by a double-click onto the sequence box.

It is possible to hide the Stimulus Pool by the Tweak settings of the Control window.

3.4 Protocol Pool

Via the help of the Control window you can directly execute any of your protocol sequences. The Protocol Pool area provides an overview about all stimulation sequences available in your currently loaded protocol sequence file (*.pro).

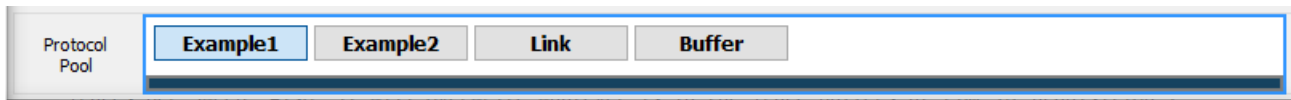


Figure 3.6: Protocol Pool area of the Control window.

The first seven sequence names of the currently loaded protocol file are visible at a first glance. Use the scroll bar to find the protocol you want to execute. Once you select one of the stimulation sequences the `Protocol Pool` gets into the focus which is indicated by a blue rectangular border around it. The execution of the protocol is started by a double-click onto the sequence box.

It is possible to hide the `Protocol Pool` by the *Tweak* settings of the Control window.

3.5 Configuration

The Configuration window is the central place to define your individual settings of PATCHMASTER NEXT, e.g. User Names, File Locations, used Hardware, Hardware settings and more.

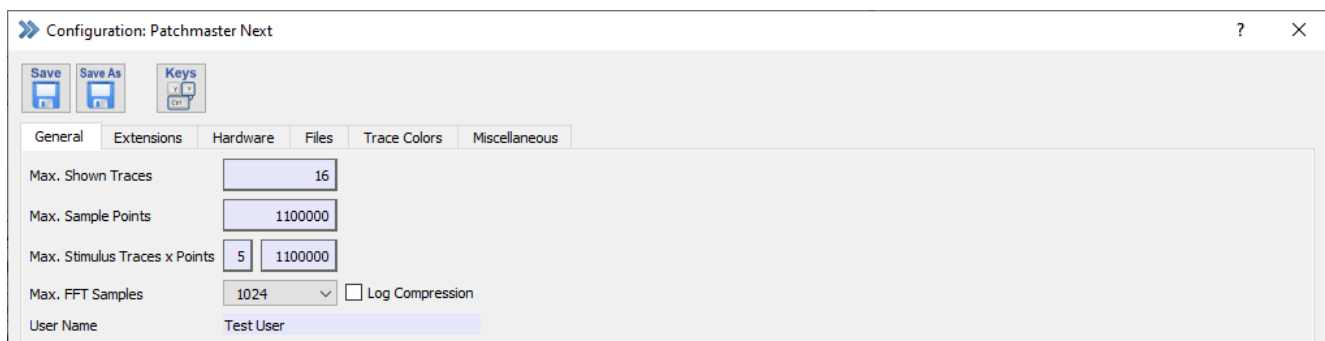


Figure 3.7: Configuration of PATCHMASTER NEXT.

Once you made any modifications you should store immediately the configuration file. This can be done in two different ways:

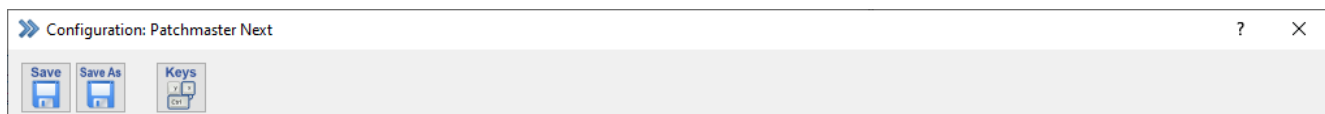


Figure 3.8: Save options of the Configuration.

Save: Saves the changes in the currently active configuration file. The name of the active configuration file is displayed at the top of the Configuration window.

Save As: The changes are saved in a new configuration file. If you decide to do so you need to enter an appropriate and not yet existing name.

If there more than one configuration file exists the Configuration Selector will open. Now, select the configuration file for saving the changes.

During your next start of PATCHMASTER NEXT you will be asked which configuration file should be loaded.

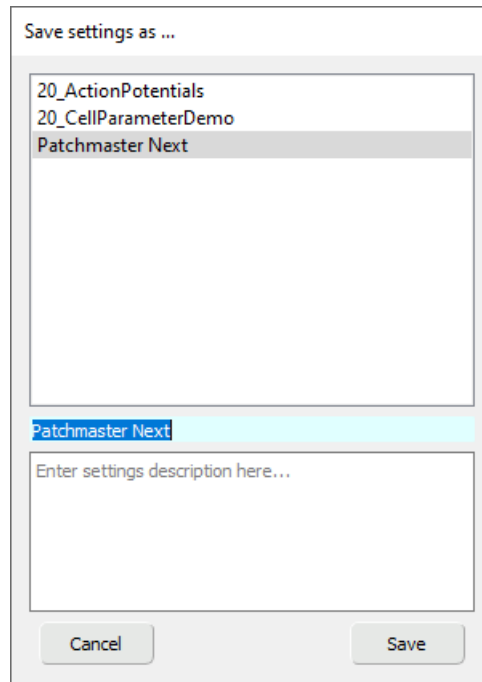


Figure 3.9: Configuration Selector of PATCHMASTER NEXT.

This process allows to store different configuration files for one setup either structured for different users or applications.

The Configuration window is separated into the following tabs:

- General
- Extensions
- Hardware
- Files
- Trace Colors
- Miscellaneous

The content in each tab is explained below.

3.5.1 General

The **General** tab of the Configuration provides the following settings:

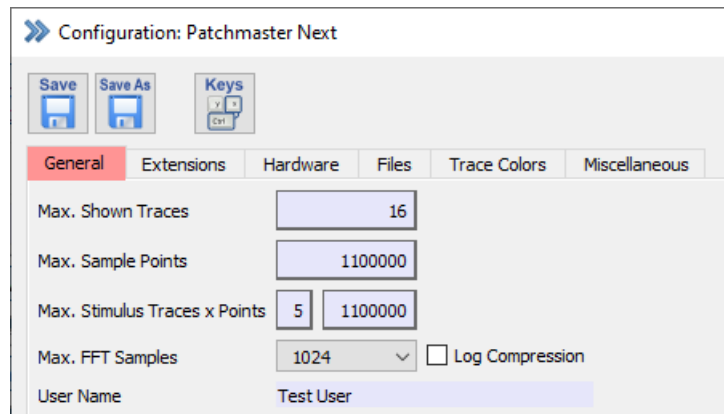


Figure 3.10: General settings of the Configuration.

Max. Shown Traces: This number defines how many Traces are used by PATCHMASTER NEXT. The user interface is adapted to show only the defined number of Traces. A small number makes the dialog and lists within PATCHMASTER NEXT shorter. The new setting only takes effect after saving the configuration file and upon restarting the program.

Max. Sample Points: Sets the maximum number of points that can be acquired in one Trace. This parameter is limited by the amount of available memory.

Max. Stimulus Traces x Points: Maximum number of stimulation Traces and their respective number of points. This parameter is limited by the amount of available memory.

Note: In case you want to drive the number of Max. Sample Points to its limit you might reduce the number of Max. Stimulus Traces to make more memory available for allocating sample points.

Max. FFT Samples: Maximum number of Fast Fourier Transformation (FFT) samples for one calculation used for a power spectrum analysis. If more data points were acquired than defined an average of the FFT calculation will be performed.

- 1024
- 4096
- 16384
- 65536
- 262144

Note: It is recommended to adapt the number of Max. FFT Samples in respect to the sampling frequency/sample interval and the frequency of interest of your power spectrum.

Log Compression: In a power spectrum analysis (FFT calculation) one gets an exponential increase of data points with increasing frequencies. To balance that unequal distribution the *Log Compression* can be used. Here, the logarithmic frequencies are divided into equal sized bins and for each bin the average value is calculated. This allows a smooth representation of the power spectrum in the analysis.

User Name: Enter a user name here. It will be used to label your data (see chapter 12.1.3 on page 211) and it will be stored together with the raw data.

3.5.2 Extensions

In the `Extensions` section of the Configuration several extensions of PATCHMASTER NEXT can be activated or deactivated.

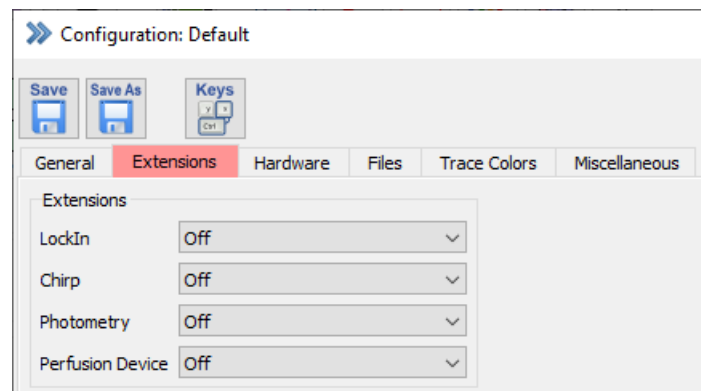


Figure 3.11: Extensions selection settings of the Configuration.

3.5.2.1 LockIn

Activate or deactivate the LockIn amplifier of PATCHMASTER NEXT. For a detailed description see chapter 19 on page 253.

3.5.2.2 Chirp

Activate or deactivate the Chirp Extension of PATCHMASTER NEXT. For a detailed description see chapter 21 on page 293 and the PATCHMASTER Tutorial "Using the Chirp Extension".

3.5.2.3 Photometry

This will activate stimulation of a monochromator and acquisition via a photo-multiplier or photo diode.

You can choose four kinds of Photometry Extensions:

- TILL / Cairn OptoScan: A "TILL Photonics" or a "Cairn OptoScan" device can be controlled via an analog output of the amplifier or any AD/DA converter supported by PATCHMASTER NEXT.
- DG4/DG5: The "DG-4/DG-5" can be controlled via the digital output of the EPC 10 USB.
- Lambda-10: The Sutter filter wheel "Lambda-10" can be controlled via the digital output of the EPC 10 USB.
- pti DeltaRAM: The High-Speed Random-Wavelength illuminator can be controlled via the digital output of the EPC 10 USB.

For details on the Photometry Extension please refer to the chapter 20 on page 279.

3.5.2.4 Perfusion Device

Select one of the perfusion devices available in the drop-down menu to control the perfusion during your experiment:

- ALA VC3-4 (Analog)
- ALA VC3-8 (Analog)
- Warner VC-8 (Analog)
- Warner VC-8 (Digital)

According to the selected device all appropriate settings are made in the PATCHMASTER NEXT software. Go to the Perfusion Control window (see chapter 15 on page 229) to set your solutions.

3.5.3 Hardware

The `Hardware` tab of the Configuration contains all settings and parameters related to the currently active hardware.

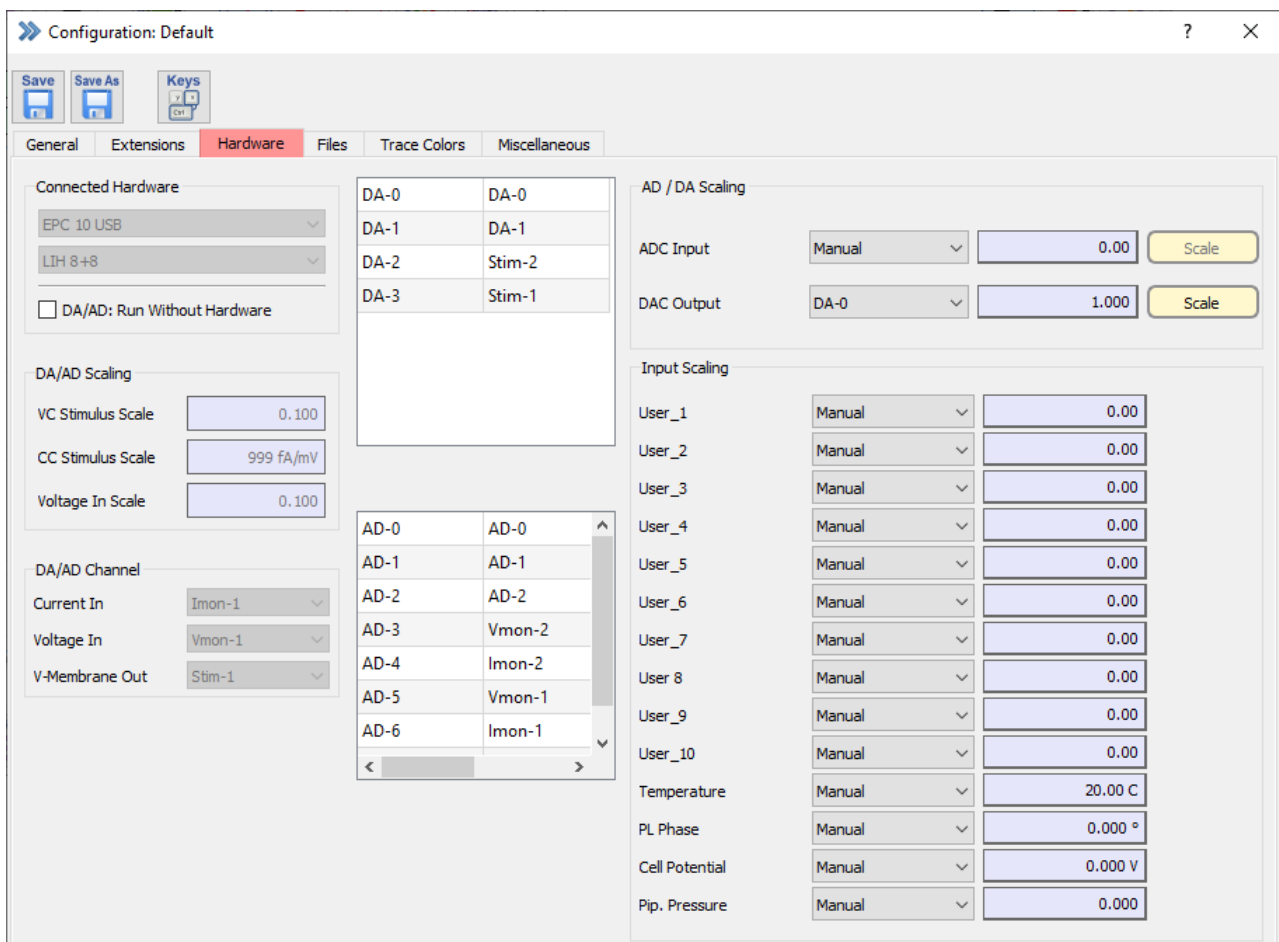


Figure 3.12: Hardware settings of the Configuration.

3.5.3.1 Connected Hardware

The currently used amplifier type and AD/DA Digitizer board are shown.

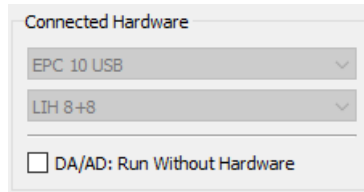


Figure 3.13: Hardware display of the Configuration.

DA/AD: Run Without Hardware: Activate the checkbox to use the program in *Demo* mode without any hardware. You can open, read and modify existing data files but you are not able to record data.

If a *Telegraphing Amplifier* is used, one needs to make several connections of AD and DA channels between the AD/DA Digitizer and the telegraphing amplifier. PATCHMASTER NEXT provides information about the necessary connections for known telegraphing amplifiers.

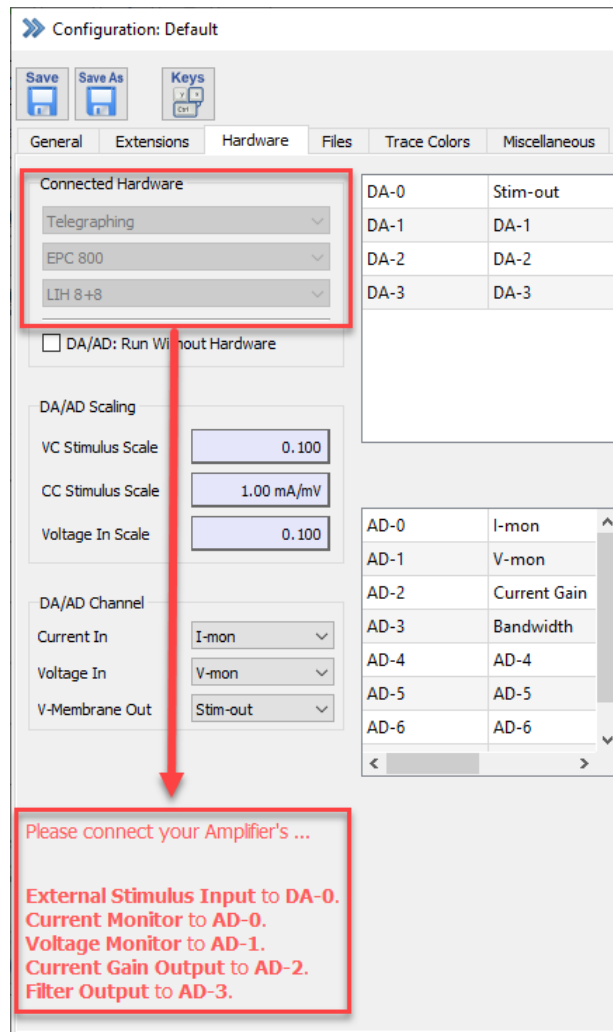


Figure 3.14: Exemplary channel connection instructions when using a Telegraphing Amplifier.

The corresponding *Gain* and *Bandwidth* tables are loaded automatically if an amplifier is selected from the list in

the start up configuration dialog.

Note: The user does only see this information if a telegraphing amplifier was selected during the start up process of PATCHMASTER NEXT.

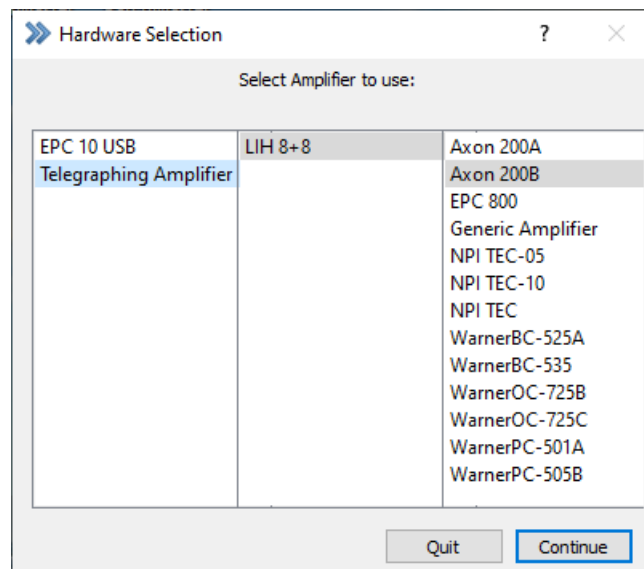


Figure 3.15: Telegraphing options in Hardware Selection dialog.

3.5.3.2 DA/AD Scaling

If an EPC 10 USB is used, *VC Stimulus Scale* and *CC Stimulus Scale* are set internally and can not be changed by the user in the Configuration.

If a telegraphing amplifier is used one can adjust the preset *VC Stimulus Scale*, *CC Stimulus Scale* or *Voltage In Scale* values to match the *External Stimulus Input Scaling* factors of the amplifier.

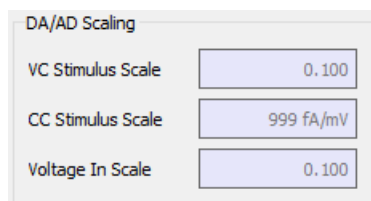


Figure 3.16: DA/AD Scaling section of the Configuration.

VC Stimulus Scale: Scaling factor of the stimulus signal in voltage clamp mode. The voltage amplitude that is set in the stimulation sequence is *divided* by this factor before output to the DA channel.

Note: The HEKA amplifiers have an internal *VC Stimulus Scale* fixed at 0.1.

CC Stimulus Scale: Scaling factor of the stimulus signal in current clamp mode. The current amplitude that is set in the PGF sequence is *divided* by this factor before to convert it to a command voltage output at the DA channel. This factor has to be adjusted with respect to the *External Stimulus Input Scaling* in current clamp mode of the controlled amplifier to guarantee that the current amplitudes specified in the PGF sequence are identically to those that the amplifier applies to the cell membrane.

Note: The EPC 800 and EPC 10 USB amplifiers feature a so-called *CC-Gain* (see chapter 4.1.1.2 on page 45) that allows current injection in different current ranges using the same command voltage range of the DA converter. The different *CC-Gain* settings set automatically the corresponding *CC Stimulus Scale* to match the conversion from mV (command voltage from DA channel) to pA (actual command current at the membrane).

Voltage In Scale: The scaling factor of the voltage input signal is fixed to 0.1 for all HEKA amplifiers. Incoming voltages have to be multiplied with this factor.

3.5.3.3 DA/AD Channel

The `DA/AD Channel` section shows the used voltage and current channel inputs (AD channels) of your hardware. The fields are read-only if an EPC 10 USB amplifier is used.

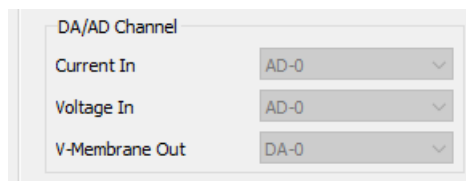


Figure 3.17: DA/AD channel section of the Configuration.

If a telegraphing amplifier is used one can modify the `Current In`, `Voltage In` or `V-Membrane Out` channels.

V-Membrane Out: Assigns the output DA channel of the stimulus.

Current In: Assigns the input AD channel for the current Trace. This input channel is scaled by the *Current Gain* (see chapter 4.1.1.2 on page 45).

Voltage In: Assigns the input AD for the voltage Trace.

3.5.3.4 Channel Assignment

The `Channel Assignment` table of the Configuration provides information for the user which DA and AD channels are already occupied by the hardware and which channels are free to use.

DA-0	Dac-0
DA-1	Dac-1
DA-2	Stim-2
DA-3	Stim-1
AD-0	AD-0
AD-1	AD-1
AD-2	AD-2
AD-3	Vmon-2
AD-4	Imon-2
AD-5	Vmon-1
AD-6	Imon-1

Figure 3.18: Example of the Channel Assignment for an EPC 10 USB DOUBLE amplifier.

In the shown example here (EPC 10 USB DOUBLE), the DA channels (Dac) "2" and "3" are used for the stimulation via the headstages. AD channels "3" and "5" are used for reading the voltage signal, AD channels "4" and "6" for reading the current signal.

3.5.3.5 AD/DA Scaling

If a free AD or DA channel is used as a signal source you can define the scaling in this section.

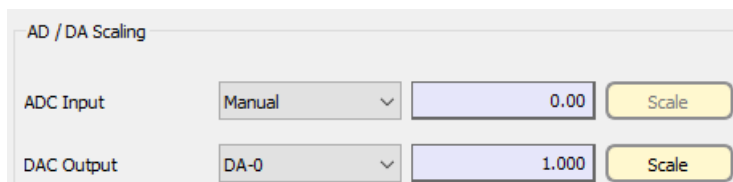


Figure 3.19: AD and DA scaling options of the Configuration.

ADC Input: If data *Traces* from AD channels are acquired in a stimulation sequence, the voltages can be scaled to convert them to appropriate units.

- Manual: The scaling factor specified in the entry field beneath is taken.
- AD Channel: When an AD channel is selected, there are several scaling options available:
 - Scaling: A scaling factor together with an offset can be specified (see example in 3.5.3.5 on page 32).

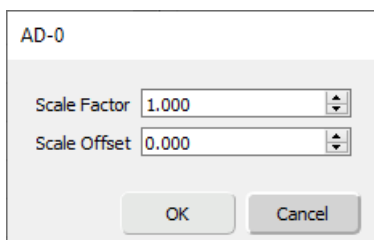


Figure 3.20: Scaling dialog of the Configuration for scaling inputs.

- Lookup Table: Use a lookup table which specifies the scaling of the incoming data. *Lookup Tables* for commonly used amplifiers are supplied.

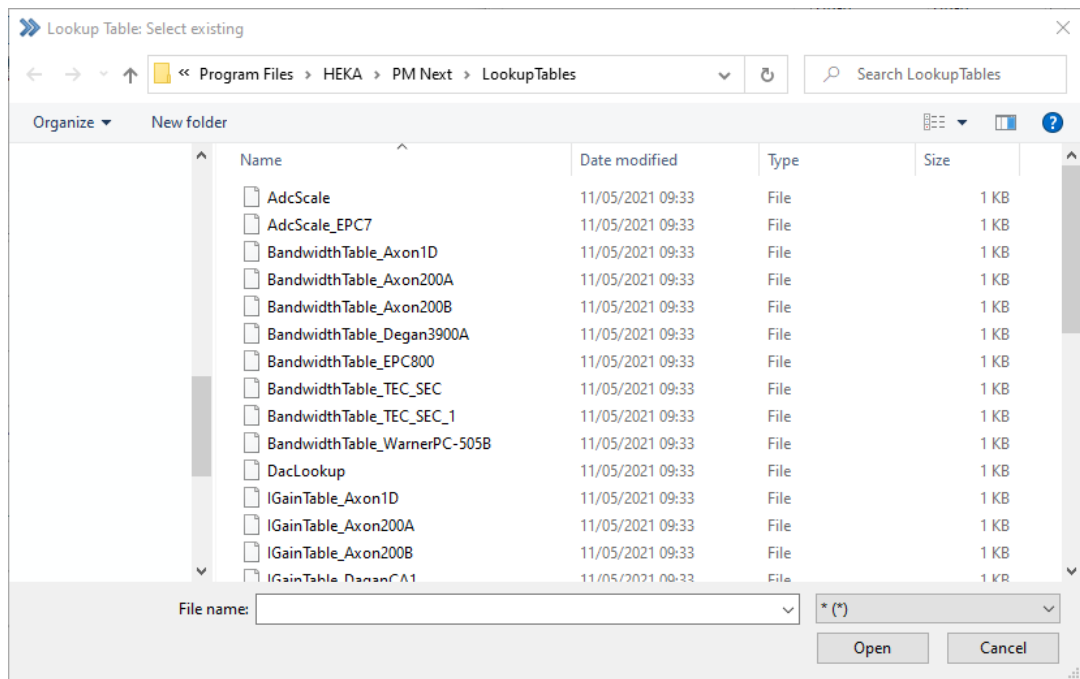


Figure 3.21: Lookup dialog of the Configuration for scaling inputs.

DAC Output: Free DA output channels can be used and scaled in a stimulation sequence.

- Equation

A detailed description how to operate the Equation Editor is provided in chapter 17 on page 235.

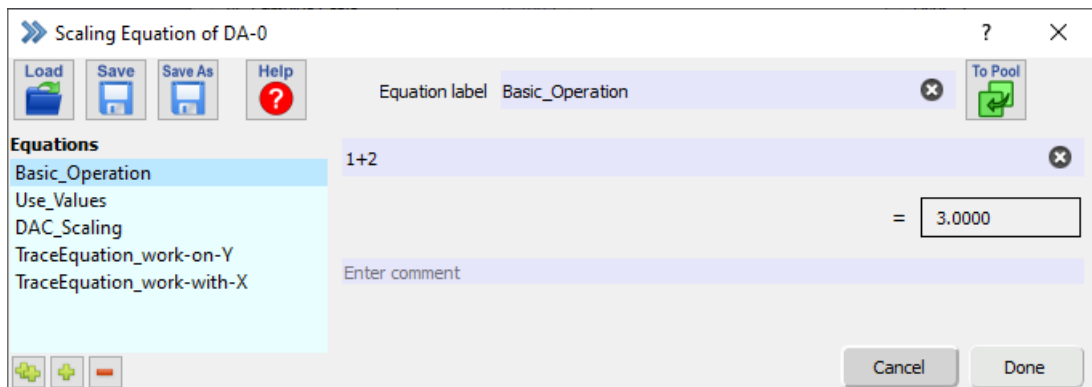


Figure 3.22: Equation Editor of the Configuration for scaling outputs.

- Lookup: Use a *Lookup Table* which specifies the scaling of the outgoing data. *Lookup Tables* for commonly used amplifiers are supplied.

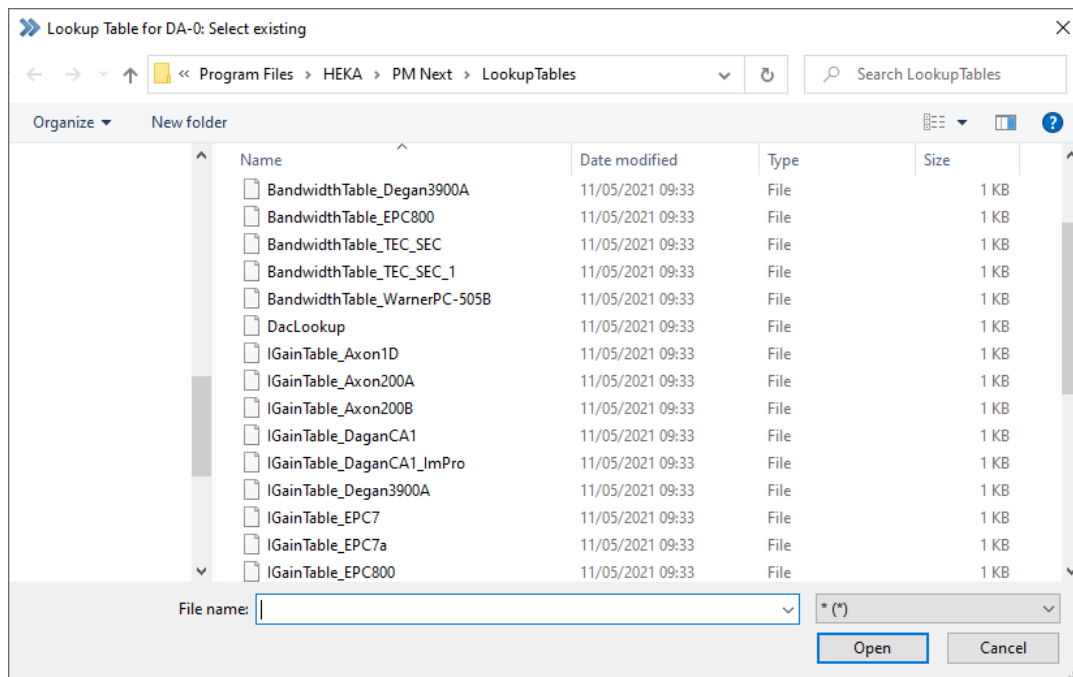


Figure 3.23: Lookup dialog of the Configuration for scaling outputs.

- Scaling

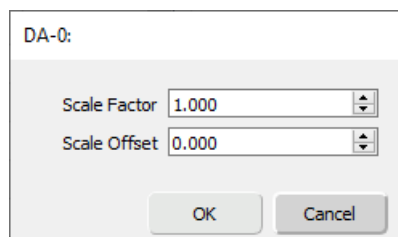


Figure 3.24: Scaling dialog of the Configuration for scaling outputs.

For direct input of scaling factors, enter a *Scale Factor* and a *Scale Offset*:

Example: A temperature controller has a measure range from 10 °C to 50 °C and a corresponding voltage output range of +/- 10 V. The correlation between measured temperature and voltage output is linear (see figure below).

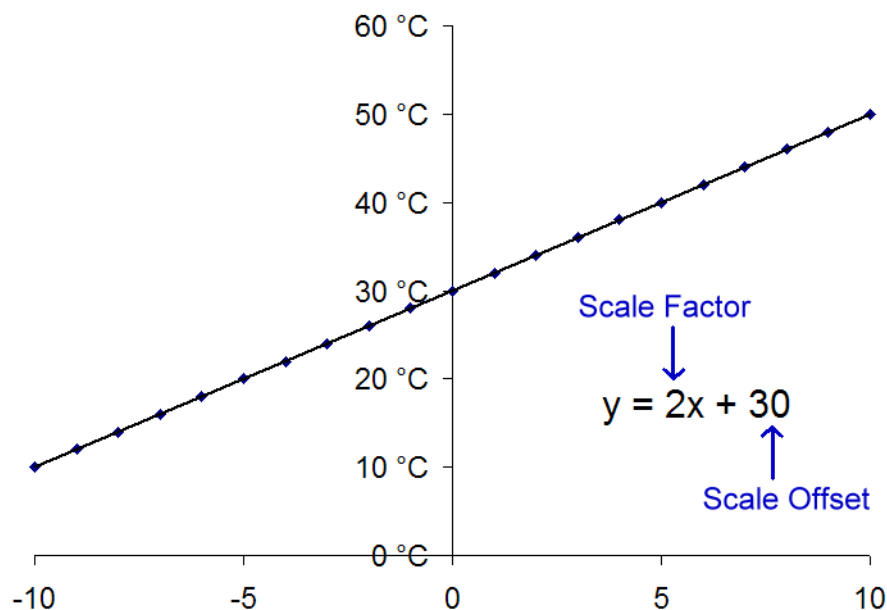


Figure 3.25: Scaling example for temperature (Scaling Factor & Scaling Offset).

In this example, *Scaling Factor* should be set to "2" and *Scale Offset* should be set to "30". If the correlation between measured signal and voltage output is not linear then a *Lookup Table* should be used. For input via a *Lookup Table*, you have to choose the file from which the program will read the data.

3.5.3.6 Input Scaling

In the *Input Scaling* section of the *Configuration* one can set up the scaling factors for different parameters such as e.g. *Temperature*, *Gain*, *R-series*.....

User 1...User 10: There are ten parameter fields reserved for user-specific assignments. These fields act like other parameter fields with the addition that parameter name and unit can be specified (e.g., "pH" and "U"). Name and unit are also stored in the output *.pul file on the level of each Sweep, so they can be changed during an experiment, if required.

Temperature: Use this parameter and specify the input channel to record the temperature from an external recording device.

PL-Phase: Via this parameter you can track the phase angle of a LockIn amplifier.

Cell Potential: Track the cell potential value e.g. from an external amplifier.

Pip. Pressure: This parameter tracks the pipette pressure value e.g. from an external device.

Parameter	Scaling	Value
User_1	Manual	0.00
User_2	Manual	0.00
User_3	Manual	0.00
User_4	Manual	0.00
User_5	Manual	0.00
User_6	Manual	0.00
User_7	Manual	0.00
User_8	Manual	0.00
User_9	Manual	0.00
User_10	Manual	0.00
Temperature	Manual	20.00 C
PL Phase	Manual	0.000 °
Cell Potential	Manual	0.000 V
Pip. Pressure	Manual	0.000

Figure 3.26: Input Scaling section of the Configuration.

For each parameter you can enter the appropriate scaling and AD channel:

- Manual: The scaling factor specified in the entry field beneath is taken.
- AD Channel: Parameter data is sampled via the specified AD channel. Also, the scaling options defined in 3.5.3.5 on page 30 are taken into consideration. The entry field is now for read-only purposes.

3.5.4 Files

In this section, the user can specify the paths for the data files and other *File-relevant* settings.

Configuration: Patchmaster Next

Save Save As Keys

General Extensions Hardware **Files** Trace Colors Miscellaneous

Data Path: C:\Users\moberhofer\Documents\HEKA\Data\

Save Settings Auto Filename [USER]_[DATE2]_[001]

Ask for Data File Startup Protocol

Save after Stop Experiment Number 1

Verify Quit Max. File Size Alert 1.00 MB

Figure 3.27: Files settings of the Configuration.

Data Path: Defines the path for storing the acquired data. Click inside to open the finder and set a new folder/path to store the data there.

Save Settings The Configuration settings will be saved automatically on exit, thus overwriting the previous settings.

Ask for Data File: When this option is enabled, PATCHMASTER NEXT will ask for a data file upon start-up.

Save after Stop: When this option is enabled, PATCHMASTER NEXT will save the data in a Sweep that have been acquired when the acquisition was terminated via the *Stop* button. With this option disabled the entire Sweep will be discarded if during its acquisition the *Stop* button was pressed.

Verify Quit: When this option is enabled, PATCHMASTER NEXT will ask to save modified setting files (*.pgf, *.pro, *.onl) when you quit the program. When it is not enabled, the Configuration settings will not be saved on quit!

Auto Filename: Allows an automatic file name generation. The file names can be generated out of a set of special components.

- [DATE=<descriptor>] = descriptor compounded by "YYYY" or "YY", "MMM" or "MM", and "DD". Characters separating the blocks are copied to the name, e.g. [DATE=DD-MMM-YYYY] = 31-Dec-2015
- [DATE] or [DATE1] = DD-MMM-YYYY (e.g. "31-DEC-2015")
- [DATE2] = YYYY-MM-DD, international custom data format, directly sorts according to dates e.g. "2015-12-31"
- [DATE3] = YYYYMMDD, analog to [DATE2], but without hyphens e.g. "20151231"
- [DATE4] = YYMMDD, e.g. "151231"
- [TIME] or [TIME1] = HH-MM-SS (e.g. "24-59-59")
- [TIME2] = HHMMSS, analog to [TIME1], but without hyphens (e.g. "245959")
- [USERxx] = username, "xx" is a number giving the maximal number of characters. No "xx" means maximal allowed characters, presently 32
- [000] = an incrementing counter, each digit being a placeholder. The given number is the starting value of the counter. The counter will increment until a unique filename is created
- [NAME] = a placeholder for the data file name

The components are composed to a file name by the insertion of an underscore "_".

A typical entry could look like: HERG_[DATE]_[001] = "HERG_31-Dec-2015_001.dat"

Do not use the following characters:

- path separators: "/", "\", ":" (unacceptable)
- extension separator: "." (unacceptable)
- blank: " " (compatibility problems, danger of oversight)

Once a data file is created the defined *Auto Filename* is displayed in the text box on the right side.

Startup Protocol: Here, you can enter the name of a protocol that is executed after the end of program initialization.

Experiment Number: This number can be used to identify experiments. It is automatically incremented when a new experiment is generated with *File* → *New Experiment*.

Max. File Size Alert: This feature will not limit the size of any file written to disk, but can be used as a reminder so that no files are created that e.g., do not fit onto a CD. A warning is written to the Notebook if the size of the raw data file exceeds this value. Entering a very large number will - in practice - suppress any warnings.



The maximum file size is limited to 2 GB. If you exceed this number during data acquisition you have to consider data loss.

3.5.5 Trace Colors

In this section you can check and modify the *Trace Color* definitions of each Trace.

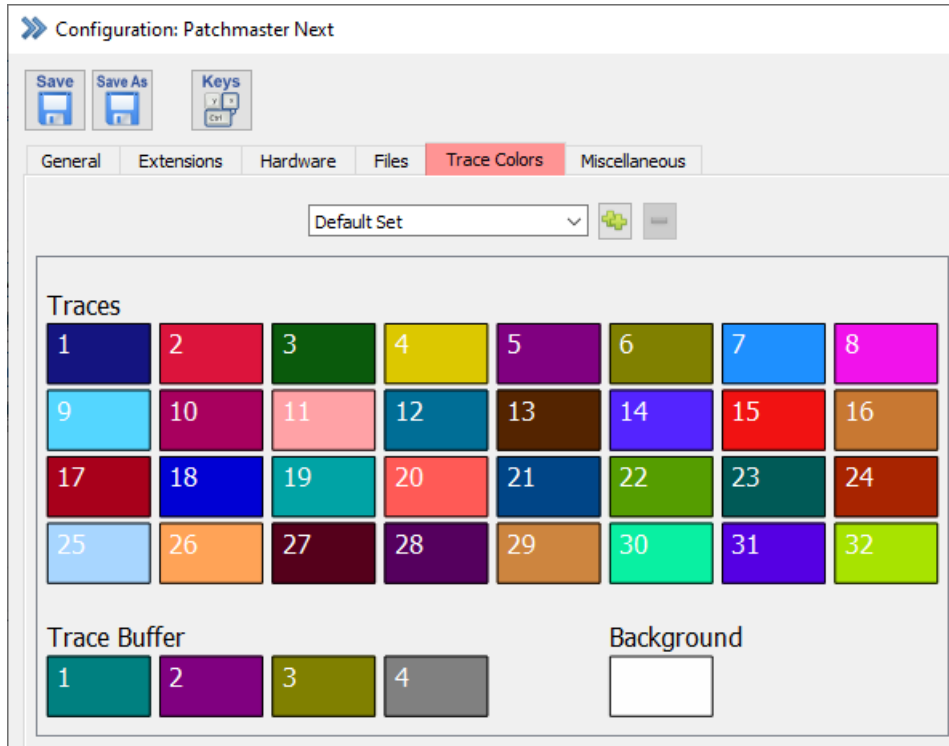


Figure 3.28: Overview of the Trace Colors Editor.

At the top of the tab there is a selection list to select the defined and stored trace color sets.

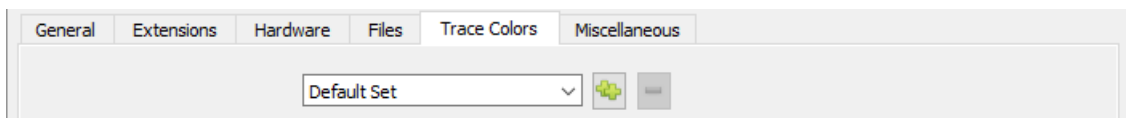


Figure 3.29: File modification options of the Trace Colors Editor.

Once you change one of the default settings a new color set will be created automatically. To change its name you need to click inside the displayed name and modify it.

Using the button with the two green plus signs duplicates the selected color setting. The red minus sign button deletes the selected Trace color setting.

Each Trace color and each Buffer Trace color is represented by a colored rectangle indicating the currently set Trace color. To change the color assignment click into one of these colored rectangles. Now, the color finder opens and one can define a new color for the selected Trace.

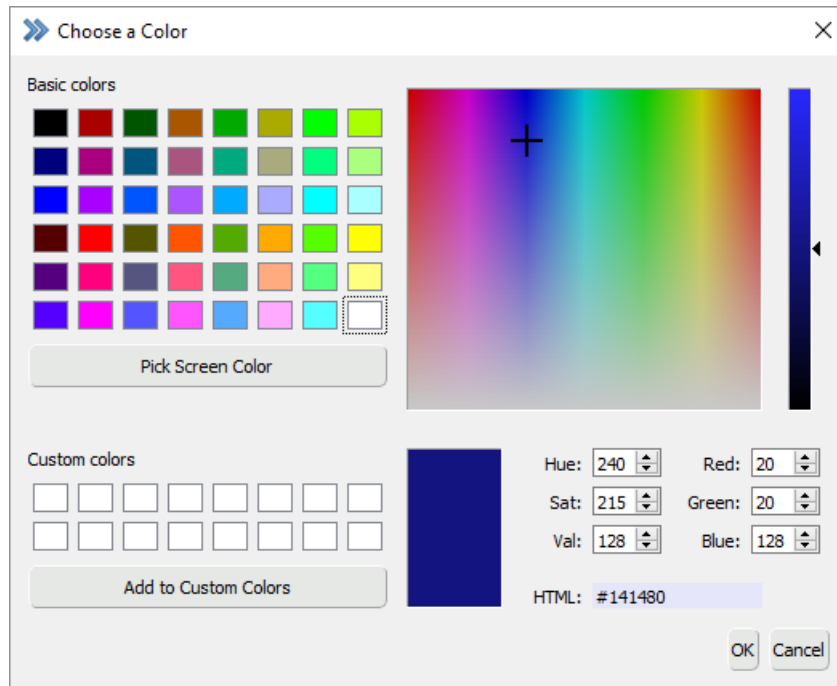


Figure 3.30: Selecting a new Trace color in the color finder.

3.5.6 Miscellaneous

Settings which do not fit into the first three tabs are collected in the `Miscellaneous` tab of the Configuration.

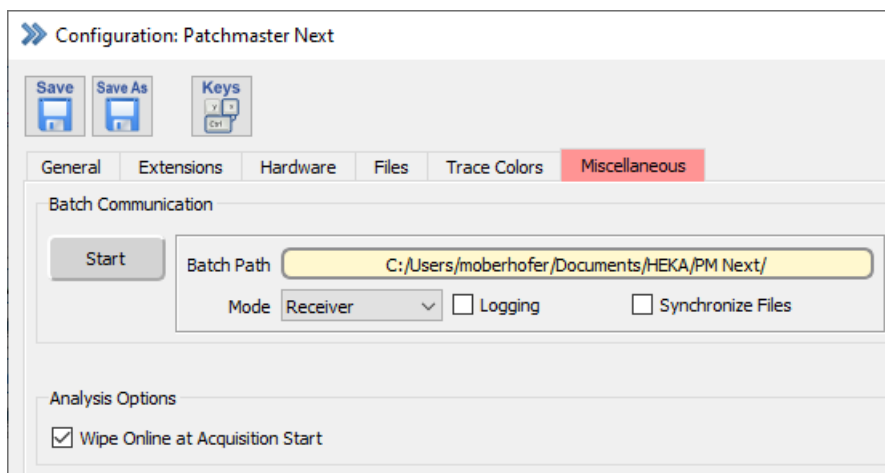


Figure 3.31: Miscellaneous settings of the Configuration.

3.5.6.1 Batch Communication

The *Batch Communication* allows PATCHMASTER NEXT to communicate with other, third-party, software programs. All necessary settings to work with this communication protocol can be found here. Further information can be found in the PATCHMASTER tutorial *Controlling PATCHMASTER*.

- **Start/Stop:** By pressing this button you either start or stop the *Batch Communication* process.
- **Batch Path:** The working path for the *Batch Communication* is defined here. By default it is set to the home path of the PATCHMASTER NEXT installation.

- Mode:
 - Sender: PATCHMASTER NEXT is configured to serve as *Sender*, thereby controlling another program.
 - Receiver: PATCHMASTER NEXT is configured to serve as *Receiver* and will wait for command inputs of other applications.
 - Polling Sender: The *Sender* is continuously checking for updates of the *Receiver*, thereby, avoiding a loss of commands in the communication.
 - Polling Receiver: The *Receiver* is continuously checking for updates of the *Sender*, thereby, avoiding a loss of commands in the communication.
- Logging: If the checkbox is activated the communication via the *Batch Communication* interface is written to the Notebook.
- Synchronize Files: With this option the *Sender* application will send the "FileOpened" message, when a *.dat file, *.pgf file, *.pro file or *.onl file is opened, and the "FileUpdated" message, when the data file is updated. The "FileClosed" message is send, when the data file is closed. The *Receiver* will then open, update, or close the same data file as the *Sender* application.

3.5.6.2 Analysis Options

Wipe Online at Acquisition Start: If this option is enabled the content of the Graph Layout windows are wiped before the data acquisition starts.

3.5.7 Keys

The Keys button opens the `Shortcut Keys Editor`. In this editor you can check for shortcut key definitions hardwired already by default and further you can edit some shortcut keys of functions or buttons in different windows of `PATCHMASTER NEXT`.

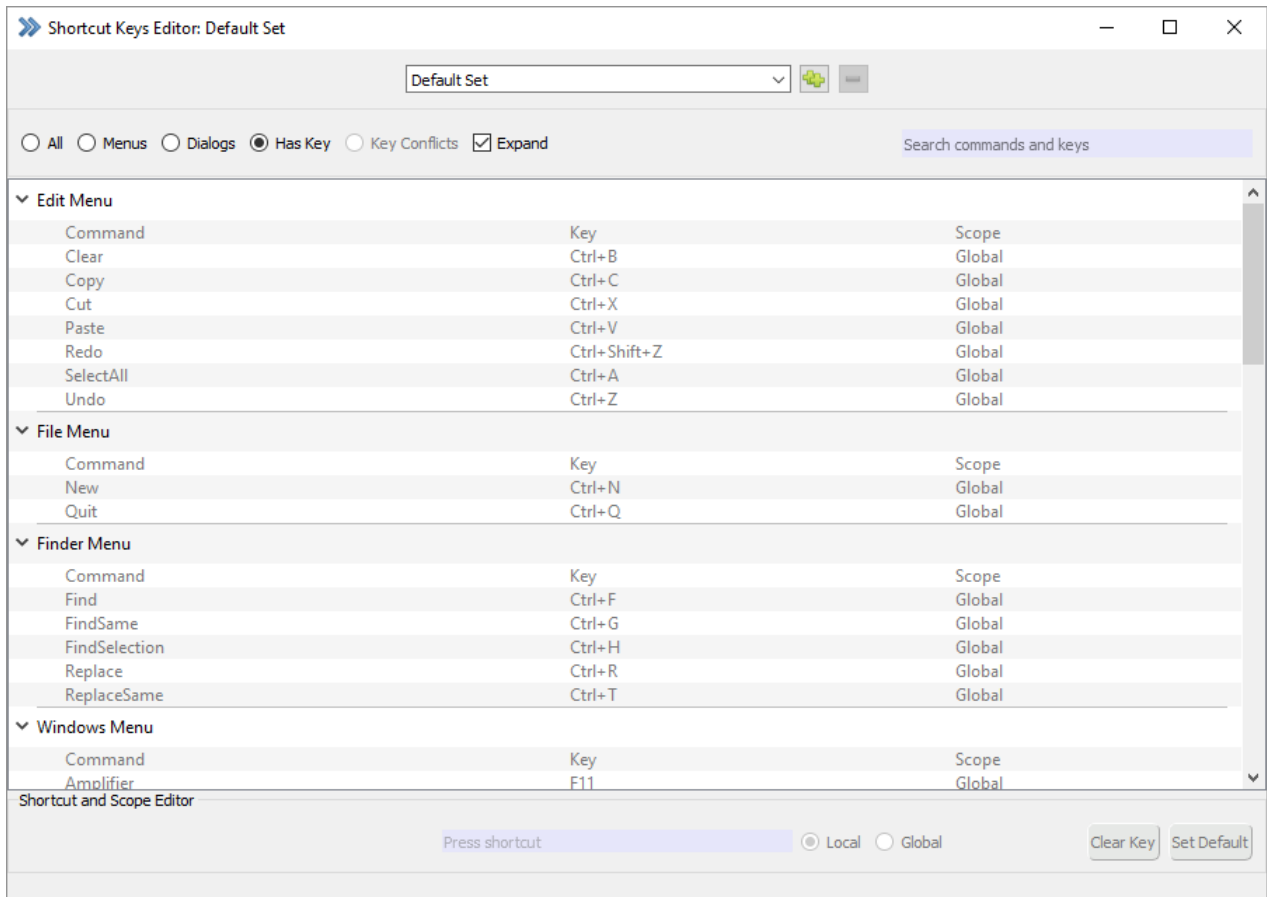


Figure 3.32: Overview of the Shortcut Keys Editor.

At the top of the tab there are some options to modify the key settings:

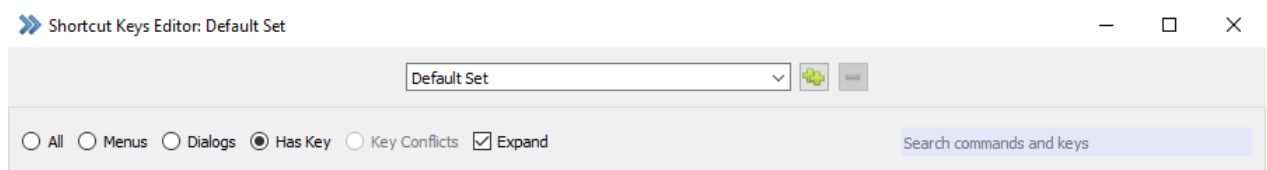


Figure 3.33: File modification options of the Shortcut Keys Editor.

Once you change one of the default settings a new key file will be created automatically. To change its name you need to click inside the displayed name and modify it.

Using the button with the two green plus signs duplicates the selected key setting. The red minus sign button deletes the selected key setting.

Below this area there are some radio buttons which allow to filter the commands listed in the Shortcut Keys Editor:

All: Lists all commands of all dialog and menus.

Menus: Lists all commands of all menus.

Dialogs: Lists all commands of all dialog.

Has Key: Lists all commands of all dialog and menus which have an assigned key (active by default).

Key Conflicts: Lists the conflicts in the key assignment if there are any of those in the key file.

Expand: If this checkbox is inactive (active by default) all the expanded sections for the dialog and menus are shrank together.

Search Field: Either enter the command name or search for a defined key in the key file.

Shortcut and Scope Editor: If one wants to edit an existing and editable key you need first to select the dialog or menu and select the command in the list:

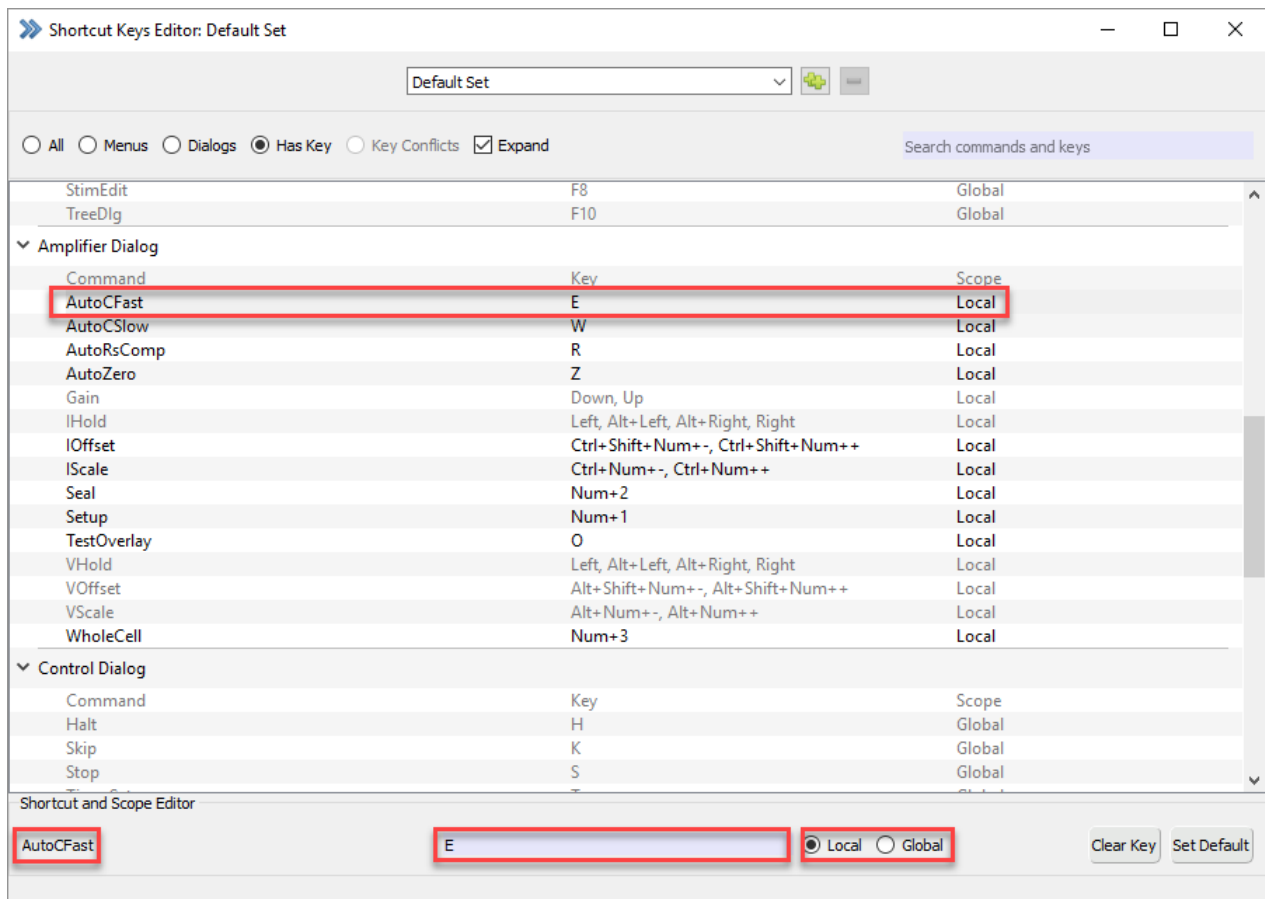


Figure 3.34: Editing shortcut keys in the Shortcut Keys Editor.

In our example we selected the *Amplifier Dialog* command *AutoCfast* which has the assigned key “E”. *Scope* means if this key does only work when the appropriate window is selected (“Local”) or if the key can work even if a different window is in focus (“Global”).

At the bottom of the *Shortcut Keys Editor* the name of the selected command is listed and its assigned key. If necessary one can now change the key entry in the entry field and change the scope of the key.

The changes are automatically saved in the active key file.

Clear Key: Deletes the key definition and the scope of the selected command.

Set Default: Resets the key definition and the scope of the selected command to the default settings.

Note: Please be aware that key settings for commands listed in grey font colors cannot be changed.

4 Amplifier

The **Amplifier** window is used for controlling, adjusting and displaying the amplifier settings and parameters. It is the key window of PATCHMASTER NEXT for the patch clamp process and monitoring your recording conditions.

The window appearance can differ depending on the type of amplifier (e.g. EPC 10 USB SINGLE, DOUBLE, QUADRO) you are controlling with PATCHMASTER NEXT.

The following screenshot provides an example how the Amplifier window will look like when you start PATCHMASTER NEXT with the default settings (using an EPC 10 USB amplifier):

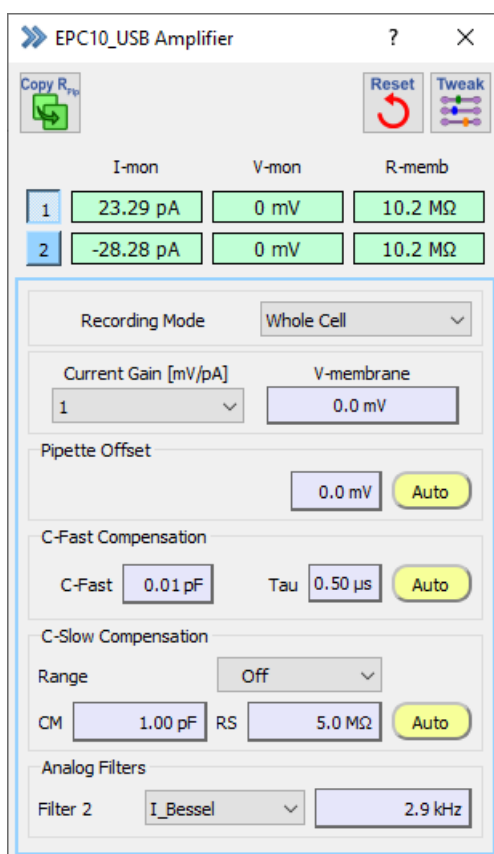


Figure 4.1: Amplifier window of PATCHMASTER NEXT.

For a better orientation we divided the Amplifier window into three sections:

1. Global Section
2. Amplifier Selection
3. Amplifier Parameters

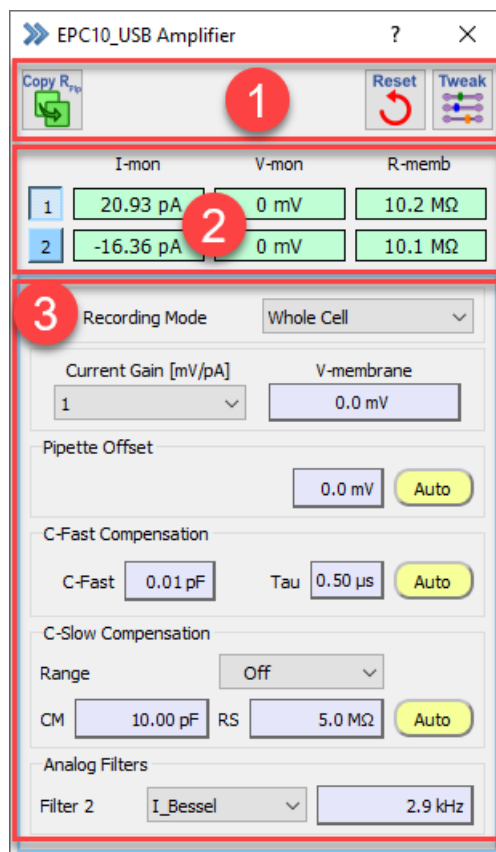


Figure 4.2: The three sections of the Amplifier window.

4.1 Global Section

There are three icon buttons in the Global Section of the Amplifier window. The *Tweak* button opens an extension in the Amplifier window, therefore, the functionality will be explained in a separate sub-chapter (see 4.1.1 on the next page).

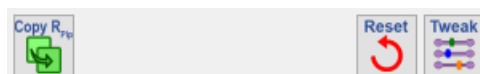


Figure 4.3: Global section of the Amplifier window.

Copy R_{pip} : Via this icon button one copies the value of the *R-memb* (membrane resistance) field of the active amplifier into the *R-pip* (pipette resistance) data parameter field. One can use this function to store the pipette resistance value in the data file before forming a seal.

Reset: This function resets most hardware settings of the EPC 10 USB (exceptions: *LJP*, *Pipette Offset*, *V-membrane*, *CM* and *RS* values.).

4.1.1 Tweak

When pressing the *Tweak* button an extension of the Amplifier window opens which provides access to further hardware settings.

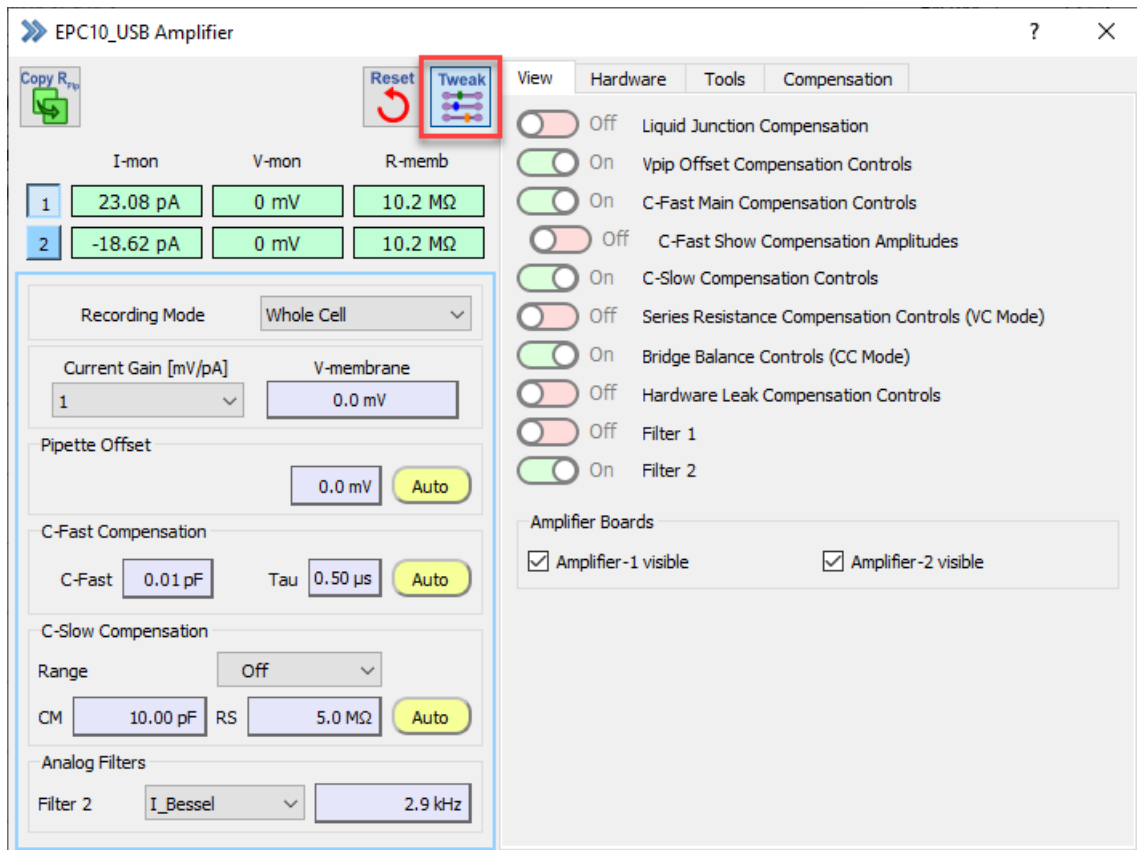


Figure 4.4: Tweak extension of the Amplifier window.

4.1.1.1 View Tab

By setting the sliders to either “On” or “Off” one can customize the parameters displayed in the Amplifier window. Select only the parameters needed for your experiment to keep the Amplifier window as short as possible. The individual parameters are explained in detail in the Amplifier Selection section (see 4.3 on page 51).

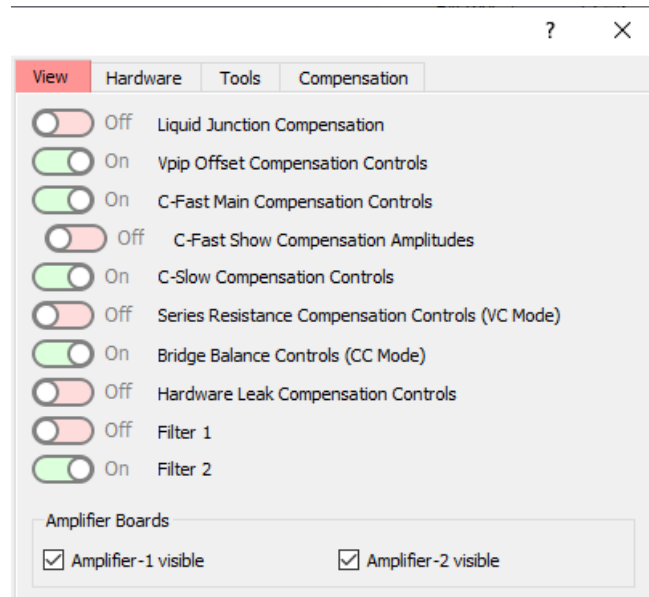


Figure 4.5: View tab of the Amplifier window.

Amplifier Boards: In the *Amplifier Boards* section one can deselect amplifier boards not needed for the experiment. E.g. if one is using an EPC 10 USB DOUBLE amplifier and only one amplifier board is needed one could deactivate the display of the Amplifier Parameters in the Amplifier window.

4.1.1.2 Hardware Tab

In the `Hardware` tab one can either enable or define specific hardware features or settings. In contrast to the `View` tab enabling features do not affect the display of amplifier parameters in the Amplifier window but extending existing hardware functions.

Note: The selections made here do only apply for the active amplifier in the Amplifier window. Hence, changes you want to make need to be repeated for each individual amplifier.

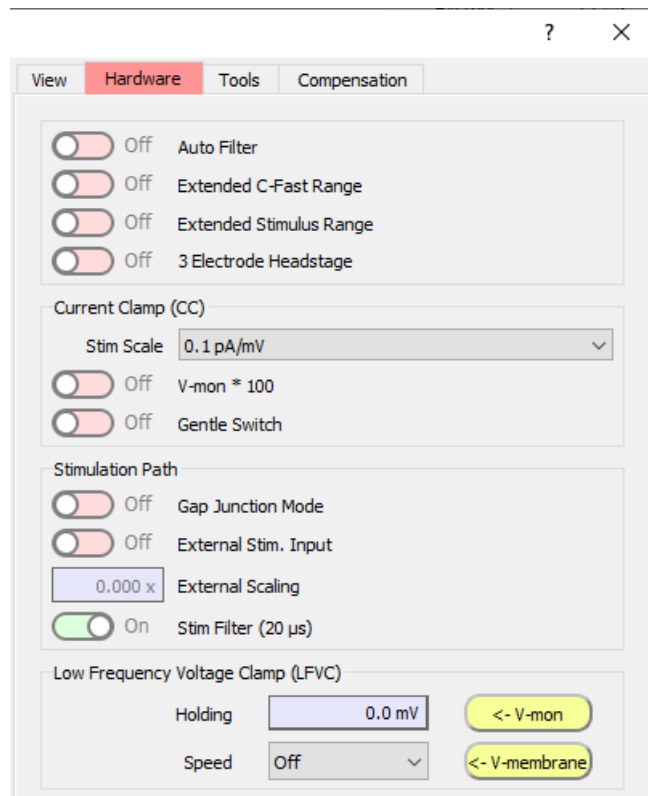


Figure 4.6: Hardware tab of the Amplifier window.

Hardware Options

Auto Filter: Enables the *Auto Filter* option. When active this automatically sets the hardware *Filter 1* and *Filter 2* according to the chosen sample interval.

Extended C-Fast Range: Activates the extended C-Fast compensation range. The C-Fast range can be extended by a factor of about 10x. With this extended compensation range, the noise in lipid bi-layer experiments can be significantly reduced, by fully compensating for the huge and fast capacitance in these experiments.

Extended Stimulus Range: Activate this option to increase the stimulus range of the amplifier in voltage clamp mode to ± 2 V and in current clamp mode to ± 5 μ A.

3 Electrode Headstage: If you want to use a 3 Electrode Headstage (S-PROBE HEADSTAGE you have to activate this option. In the 3 Electrode Mode you can measure the potential difference between a third electrode (bath sense) and the ground (ground) electrode without having a current flow through the reference electrode (bath sense). The reference electrode (bath sense) stays in equilibrium. The potential difference is added to the command potential for the working electrode.

Current Clamp (CC)

Current Clamp Stimulus Scale: For the current clamp mode you have four scaling ranges available:

- 0.1 pA/mV
- 1 pA/mV
- 10 pA/mV
- 100 pA/mV

When the Current Clamp Stimulus Scale is set to 1 pA/mV, a command pulse of 1 mV gives rise to 1 pA in current clamp mode. This relationship holds regardless of the *Current Gain* setting. The maximal output current in current clamp mode is 1 / 10 / 100 / 1000 nA, respectively (x5 in *Extended Stimulus Range*).

Note: *The scaling influences the precision of the zero point adjustment. The minimal step size ("granularity") is 0.03125 (1/32), 0.3125, 3.125, 31.25 pA (with increasing range).*

The Current Clamp Stimulus Scale range cannot be switched in current clamp mode. Please go back to voltage clamp mode and use the to switch to another range.

V-mon * 100: The measured voltage can be amplified by a factor of 10 or 100 before digitization (default: "10"). In the *V-mon * 100* setting the voltage range is ± 100 mV instead of 1 V, therefore the resolution is 10x better. This is particularly useful in field potential measurements, where small voltage deflections are to be resolved.

Note: *This feature is available only with EPC 10 revision F (or newer) amplifiers.*

Gentle Switch: This control selects between the two alternate modes of switching between voltage and current clamp and back. If this option is "Off" *I-membrane* will be set to 0 pA when switching to current clamp mode. If set to "On" *I-membrane* will be set to inject the holding current required to keep the membrane voltage constant while switching to current clamp mode. Thus, *V-mon* will be identical to the original *V-membrane* commanded under voltage clamp. In analogy, upon returning to voltage clamp, *V-membrane* will be set according to the membrane potential under current clamp and rounded to the next 2 mV.

Stimulation Path

Gap Junction Mode: This procedure allows simultaneous stimulation of multiple amplifiers on basis of a single sample of the digitizer clock. This is very important e.g. for studying Gap Junctions.

Note: *This option is only available if one uses an EPC 10 USB Double, Triple or Quadro amplifier.*

Ext. Stim. Input Select this option to switch on the external stimulus input. The external input will be added to the internal stimulus (holding potential/holding current and stimulus generated by the Stimulus Editor.)

It is strongly recommended to set External to "OFF" (i.e., equal to zero) if no external stimulator is connected to the "External Stim. Input". This will prevent pick-up of external noise.

External Scaling: It is possible to feed external stimulus signals into an EPC 10 USB patch clamp amplifier. Therefore, you need to connect the external stimulus source to either the "External Stim. Input CC" or "External Stim. Input VC" on the front panel of the EPC 10 USB amplifier. The signal is internally added to the holding current or holding potential of the EPC 10 USB. The entry field provided here, defines the scaling factor (range: $\pm 0.1 - 1x$) for the external stimulus signal.

When using an EPC 10 USB Single amplifier this option is always disposable but inactive. For users of an EPC 10 USB Double, Triple or Quadro amplifier one needs to activate "TestDA to Stim" first (see 4.1.1.2).

Note: *The internal V-membrane is not affected by changing the external scale factor. If the user sets the holding potential externally (e.g., with a stimulator or another computer), then the scaling will affect the holding potential.*

Stimulus Filter: The stimulus applied by the amplifier is filtered by a 2-pole Bessel filter to reduce the amplitude of fast capacitance transients when the speed of potential changes is not critical. Two settings are available:

- 2 μ s

- 20 μs

Usually a setting of 20 μs is sufficient, unless very fast currents such as from voltage-gated Na^+ channels are studied.

Low Frequency Voltage Clamp (LFVC)

Holding: Entry field for the desired membrane potential which should be set by the Low Frequency Voltage-Clamp mode.

V-membrane: Copies the values of the *V-membrane* parameter into the *Holding* field of LFVC.

Speed: Here you can select the speed of the feedback for LFVC. The LFVC mode is a modified current clamp mode that allows for the measurement of potential deflections, such as action potentials or synaptic potentials, while the average potential is kept constant at a value chosen by the user (*Holding*). The circuit thus works like a current clamp for fast signals and like a voltage clamp for low frequency signals. To achieve this, the measured membrane potential is low-pass filtered and compared to the *Holding* potential. Then a current is injected into the cell to keep the membrane potential at the chosen LFVC potential. Since the cell does not distinguish currents entering through the pipette from currents crossing the membrane, the LFVC circuit can be considered an additional membrane conductance. Various time constants for the LFVC can be selected (1, 3, 10, 30, 100). These time constants describe the speed of regulation.

The effective feedback speed for the five possible response settings depends on the *Current Gain* range. In the medium *Gain* range, 1-100 is approximately the time in seconds, whereas in the high *Current Gain* range it is a hundred times faster.

Note: A LFVC value of "100" means slow compensation, whereas a LFVC value of "1" means fast compensation!

For further information we refer to:

Peters F, Gennerich A, Czesnik D, Schild D (2000). Low frequency voltage clamp: recording of voltage transients at constant average command voltage. J Neurosci Meth 99, 129-135.

V-mon: Copies the value of the *V-mon* parameter into the *Holding* field of LFVC.

4.1.1.3 Tools Tab

In the **TOOLS** tab essential amplifier functions such as calibrating or testing the amplifier.

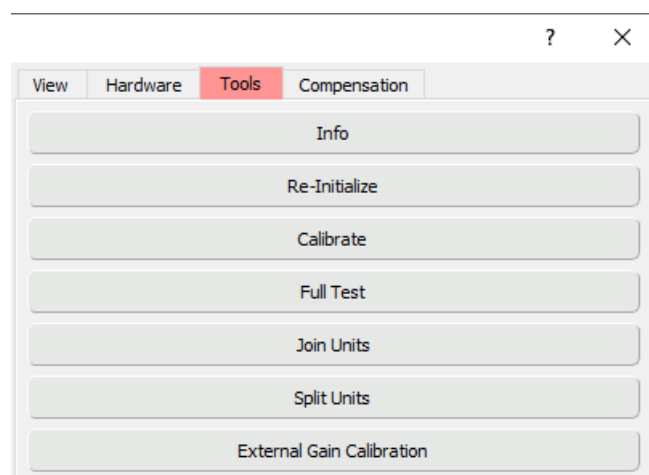


Figure 4.7: Tools tab of the Amplifier window.

Info: Displays the relevant information about the currently connected and initialized EPC 10 USB amplifier like revision letter, serial number and technical features.



Figure 4.8: Amplifier info dialog of the Tweak menu.

Re-Initialize: Via this function one re-initializes the EPC 10 USB patch clamp amplifier.

Calibrate: This function starts the calibration routine of the EPC 10 USB amplifier. Follow the instructions of PATCHMASTER NEXT. You need to shield the headstage/headstages and during the calibration process you need the model cell MC 10. For an EPC 10 USB Single amplifier the whole procedure will take around 10 minutes. When the process has finished a new scale file and a new C-fast table is written into the EEPROM of the EPC 10 USB amplifier.

***Note:** We recommend to switch on the amplifier at least on hour in front of performing the calibration process.*

Full Test: This function starts the testing procedure of the EPC 10 USB amplifier. Follow the instructions of PATCHMASTER NEXT. You need to shield the headstage/headstages at the beginning of the testing procedure. During the testing procedure you will also need up to five BNC cables and the model cell MC 10.

***Note:** We recommend to switch on the amplifier at least on hour in front of performing the testing procedure.*

Join Units: Via this function you can join two hardware units together. E.g. you can join any two EPC 10 USB amplifiers (Single, Double, Triple, Quadro) or one EPC 10 USB amplifier (Single, Double, Triple, Quadro) and one stand-alone LIH 8+8 or two LIH 8+8 units. All combinations can be handled with one PATCHMASTER NEXT instance. Follow the instructions of PATCHMASTER NEXT to join two hardware units.

Split Units: Use this function to split two previously joined hardware units (EPC 10 USB/LIH 8+8.)

***Note:** Whenever two units are joined together this “mode” is written into the hardware itself. If one wants to use these devices in any other configuration or as a single unit again it is essential to execute the Split operation in advance.*

External Gain Calibration: Enter here gain correction factors for the low (top), medium (middle) and high (bottom) Gain range.

Please be aware that any changes here will modify the scale files of your amplifier and therefore may lead to erroneous results if misused.

4.1.1.4 Compensation Tab

In the `Compensation` tab detailed settings for the compensation functions are listed.

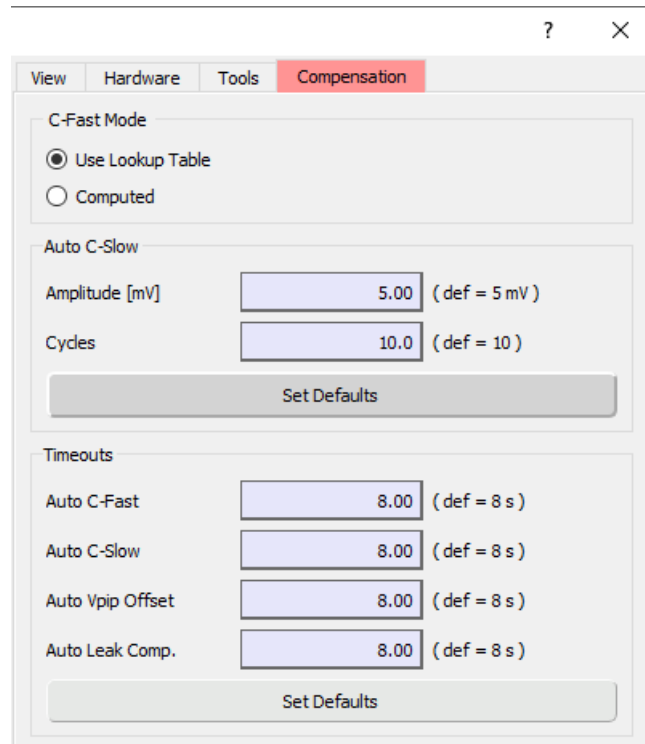


Figure 4.9: Compensation tab of the Amplifier window.

C-Fast Mode

Two options are available:

- Use Lookup Table: Results in faster compensation procedure than the “Computed” mode.
- Computed: Preferable when compensating large capacitance.

Auto C-Slow

Two parameters of the Auto C-Slow setting can be modified:

Amplitude [mV]: Defines the amplitude of the voltage pulses used to compute C-Slow and G-Series. The minimum and maximum numbers are “1” and “20”, respectively. The default value is “5” mV.

Cycles: Defines the number of voltage pulses to used to compute C-Slow and G-Series. The minimum and maximum numbers are “4” and “40”, respectively. The default entry is “10”.

Timeouts

Four “Timeout” parameters can be edited:

Auto C-Fast: Time after an Auto C-Fast compensation will be stopped. The default value is “8 s”, the minimum value is “1 s”.

Auto C-Slow: Time after an Auto C-Slow compensation will be stopped. The default value is “8 s”, the minimum value is “1 s”.

Auto Vpip Offset: Time after an Auto Vpip Offset compensation will be stopped. The default value is “8 s”, the minimum value is “1 s”.

Auto Leak Comp.: Time after Auto Leak compensation will be stopped. The default value is “8 s”, the minimum value is “1 s”.

Note: If the compensation routine does not find appropriate parameters within the timeout period, the settings in the amplifier dialog will remain unchanged.

4.2 Amplifier Selection

Depending on the type of EPC 10 USB amplifier, you will either get one, two, three or four amplifier selection rows. If one has joined two EPC 10 USB amplifiers, the number can increase up to eight amplifier selections.

	I-mon	V-mon	R-memb
1	254 fA	0 mV	507 MO
2	-1.43 pA	0 mV	---

Figure 4.10: Amplifier Selection of an EPC 10 USB Double amplifier.

The amplifier marked in red is the active amplifier (indicated by the active numerical button on the left). When switching to another amplifier, the `Amplifier` window updates all `Amplifier Parameters` (see 4.3 on the facing page) to show the state of the selected amplifier. The state of each amplifier is independent of the settings of the other amplifiers. This allows e.g., to keep the first amplifier in cell-attached mode with a high *Current Gain*, while reading at the same time from the second amplifier in the whole-cell mode with a medium *Current Gain* setting.

On the right to the amplifier button there are display fields showing three main amplifier parameters.

I-mon: Displays the DC current monitor signal.

V-mon: Displays the actually measured pipette voltage after correcting for liquid junction potentials and offsets (provided the zero current potential has been set correctly). This may differ (temporarily) from the holding voltage (e.g., during long stimulation pulses) as it indicates the average sum of *V-membrane* and the scaled stimulus voltage.

R-memb: The seal resistance *R-memb* is determined from the current sampled during the baseline and the second half of the *Test Pulse*. *R-memb* can be encoded into a tone using the *Sound* (see 5.3.5 on page 67).

Computing *R-memb* when a *Test Pulse* is applied:

$$R_{memb} = \frac{V_{test}}{I_{test} - I_{leak}} \quad (4.1)$$

Membrane resistance is computed using the difference between the pipette current before and during the first *Test Pulse* amplitude. This algorithm is quite insensitive to problems such as pipette offsets, reversal potential, and liquid junction potential.

I-mon, *V-mon* and *R-memb* are continuously updated, even when the `Amplifier` window is not in front (*Test Pulse* needs to be switched on, see 5.3.1 on page 65), during the wait period between the acquisition of `Sweeps` and `Series`, and in current clamp mode. *I-mon* and *R-membrane* are also computed and updated after every acquired `Sweep`.

Clipping: The *I-mon* field gets a red background when there is saturation of the amplifier in the current monitor circuitry. Like the *Clipping* LED on the EPC 10 USB main unit, this is a warning that excess artifacts or noise may occur due to the saturation of the amplifier.

Note: This indicator may appear to be more sensitive than the LED on the EPC 10 USB. It is not; it just latches the clipping status longer than the LED light.

Note: In the case of an EPC 10 USB Double, Triple or Quadro amplifier, the clipping state of non-active amplifiers is also indicated; it is not just limited solely to the active amplifier.

4.3 Amplifier Parameters

To see all *Amplifier Parameters* one needs to activate all of them in the View tab of the Tweak menu (see 4.1.1.1 on page 43). Each amplifier board has its own set of *Amplifier Parameters* which are set and stored independent of each other.

Figure 4.11: Amplifier Parameters of an EPC 10 USB amplifier.

Recording Mode

PATCHMASTER NEXT tries to use the physiological convention whenever stimulating or acquiring data: therefore, a downward current always reflects a cationic influx into the cell. However, for this to work properly you always have to instruct the program about the actual recording configuration.

- In Out: Inside-Out configuration (voltage clamp), the stimulus and the acquired data are automatically inverted.
- On Cell: Cell-Attached configuration (voltage clamp), the stimulus and the acquired data are automatically inverted.

Note: The automatic inversion applies to all voltages (including *V*-membrane and the stimuli from the *Stimulus Editor*). As for the liquid junction potential, the polarity is always set correctly, regardless of the recording mode.

- Out Out: Outside-Out configuration (voltage clamp).
- Whole Cell: Whole-Cell configuration (voltage clamp).
- C-Clamp: Current Clamp mode.

Current Gain

Sets the scaling of the current monitor output. For EPC 10 USB amplifiers, the range is 0.005 to 2000 mV/pA and can be set by pressing the UP and DOWN arrow keys or by selecting it directly in the drop-down menu with the mouse. The *Current Gain* setting automatically selects one of the three available current measuring feedback resistors in the probe (5 M Ω , 500 M Ω , and 5 G Ω), corresponding to low, medium and high *Current Gain* ranges. The table below summarizes the main features and limitations of the *Current Gain* ranges:

Table 4.1: Current Gain ranges of the EPC 10 USB amplifier.

	Low	Medium	High
Feedback Resistor	5 M Ω	500 M Ω	50 G Ω
Current Gain Range	0.005-0.2 mV/pA	0.5-20 mV/pA	50-2000 mV/pA
I_{max}	$\pm 2 \mu\text{A}$	$\pm 20 \text{ nA}$	$\pm 200 \text{ pA}$
C-Slow Ranges	30, 100, 1000 pF	30, 100, 1000 pF	30, 100 pF
Current Clamp	yes	yes	no
R_s Compensation	yes	yes	yes

The lowest range may be used for experiments (e.g., bi-layers, loose-patch, or large cells) in which large currents need to be delivered (up to about 2 μA). Capacitance compensation of up to 1 nF is available and R_s compensation can be used for R_s values down to 10 Ω in this range.

In the medium *Current Gain* range, the background noise is larger than in the high *Current Gain*, but up to about 20 nA can be recorded. This range is used mainly for whole-cell recordings.

The high *Current Gain* range is intended for single channel recording or low noise whole-cell recordings. It has a very low noise level, but this is obtained at the expense of a maximum current limit of about 200 pA. The current clamp mode can not be used in the high *Current Gain* range and the 1000 pF range for *C-Slow* compensation is not available.

Note: When switching from voltage clamp to current clamp in high *Current Gain*, then the *Current Gain* is set to the next highest valid *Gain* before changing to current clamp.

V-membrane / I-membrane

Sets the desired holding voltage in voltage clamp mode and the holding current in current clamp mode. The maximum holding potential and current range is $\pm 1000/2000 \text{ mV}$ and $\pm 1/5 \mu\text{A}$, respectively (depending on the revision letter of your amplifier). The value can be set by dragging the mouse or by typing. The *V-membrane* is corrected for liquid junction potentials and voltage offsets. *V-membrane* is converted to *I-hold* in current clamp mode.

Liquid Junction Potential

Liquid Junction (LJ) is a variable, to be set by the user, which allows to correct for *Liquid Junction* potentials and other offsets. It works in conjunction with the *Pipette Offset* (see chapter 4.3) operation. An online correction requires an *Pipette Offset* operation to be performed before seal formation and LJ to be set to an appropriate value. No correction is performed if $LJ = 0$.

LJ can be adjusted by dragging the mouse or typing after a double-click.

LJ should be 0 mV when using identical pipette and bath solutions. It may be changed to any desired value within ± 200 mV in case asymmetrical solutions are used. For the standard *Liquid Junction* potential correction, the polarity of the entered value should be such that it represents the potential of the bath with respect to the pipette solution. For example, if the pipette solution contains glutamate or aspartate (with chloride in the bath), then the polarity of LJ should be positive (+10 mV). After an *Auto V_{pip} Offset* operation, *V_{membrane}* will be changed to -10 mV (in *Whole Cell* and *Out Out* recording modes) or +10 mV (for *On Cell* and *In Out* recording modes), which corresponds to the true zero current potential.

Pipette Offset

Pipette Offset: Displays the offset voltage (a voltage that is added to *V_{membrane}* to obtain the pipette command voltage). It can be set either by the *Auto Pipette Offset* operation or by manually dragging the mouse after clicking into the item. It is not recommended to change *Pipette Offset* manually, because this interferes with the software features for automatic offset correction.

Auto Pipette Offset: The *Auto Pipette Offset* button calls a procedure for automatic zeroing of the pipette current. Thereby, an offset voltage to the pipette potential is systematically varied until pipette current is zero. The range of *Pipette Offset* is 400 mV. *Auto Pipette Offset* is typically performed before seal formation. It only works when a pipette is inserted into the bath.

The *Auto Pipette Offset* procedure interacts with the variable LJ to provide for online correction of *Liquid Junction* potentials and other offsets. This requires that *V_{membrane}* is set to the value of LJ (for *On Cell* and *In Out* recording modes) or to the opposite polarity of LJ (for *Whole Cell* and *Out Out* recording modes), before the actual zeroing operation is performed. *Auto Pipette Offset* does this automatically and leaves *V_{membrane}* at that value.

Note: Pipette Offset is not changed by the Reset function.

C-Fast Compensation

C-Fast Amplitudes: The *C-Fast compensation* circuit is used to cancel fast capacitive currents that charge the pipette and other stray capacitance (range: 0-15 pF). With nothing connected to the probe input, cancellation is typically obtained at a setting of 1-1.5 pF due to the residual input capacitance of the current measuring amplifier. The compensation can be performed manually by dragging the mouse or typing.

Tau determines the time constant of *C-Fast* (up to 8 μ s). The value of *Tau* may be adjusted by dragging the mouse, or typing, or automatically by selecting the *Auto* function.

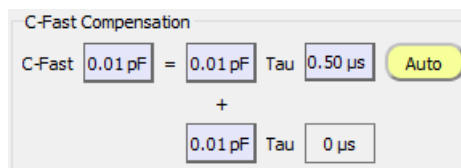


Figure 4.12: C-Fast Compensation options.

In PATCHMASTER NEXT we display all internal *C-Fast Compensation* parameters:

- The total C-Fast amplitude (C-Fast Total)
- The filtered C-Fast amplitude (C-Fast Amplitude 2)
- The unfiltered C-Fast amplitude (C-Fast Amplitude 1)
- The Tau value of the filtered C-Fast amplitude (Tau 2)
- The Tau value of the unfiltered C-Fast amplitude (Tau 1)

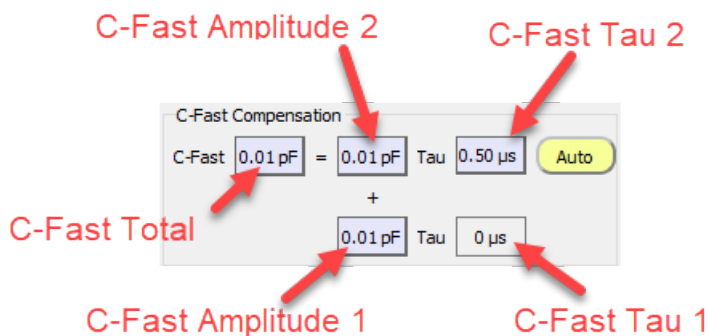


Figure 4.13: C-Fast Compensation parameters.

If *C-Fast Total* is changed, then *C-Fast Amplitude 1* and *C-Fast Amplitude 2* are changed by the same ratio.

If *C-Fast Amplitude 1* and *C-Fast Amplitude 2* are changed individually, *C-Fast Total* is changed as a sum of *C-Fast Amplitude 1* and *C-Fast Amplitude 2*.

By default, *C-Fast Tau 1* is set to "0".

In case *C-Fast Show Compensation Amplitudes* is switched off in the *View* tab of the *Amplifier* window (see chapter 4.1.1.1 on page 43), only the *C-Fast Total* amplitude and the real *Tau* value are shown in the *C-Fast Compensation* section of the *Amplifier* window.

Note: The range of the fast capacitance can be extended to 0-80 pF if an EPC 10 USB amplifier with revision letter 'N' is used.

Auto C-Fast: The *Auto* function performs an automatic compensation of *C-Fast* and *Tau*. The procedure uses a routine that applies a number of small pulses (5 mV), averages the resulting currents and fits an exponential to deduce the capacitance compensation values required to cancel the current. During this procedure, some parameters are changed temporarily: *Series Resistance Compensation* is turned off, the stimulation is interrupted and the *Current Gain* is internally set to an appropriate value (0.2, 2 or 50 mV/pA, depending on the effective *Current Gain* range). The values are updated in the respective displays. If the automatic compensation routine fails to converge to ideal adjustment, the background of the *Auto* button will become red to indicate the error. *Auto* compensation works best with coated pipettes in the cell-attached configuration with *C-Slow* turned off.

C-Slow Compensation

Range: Selects the range for slow capacitance compensation:

- Off: Turns cancellation off.
- 30 pF: Small cells.
- 100 pF: Small and medium-sized cells.

- 1000 pF: Large cells (low and intermediate *Current Gain* range only).

The program will prevent oscillations by limiting compensation according to the selected range and the effective capacitance values ($R\text{-Series} * C\text{-Slow} > 5 \mu\text{s}$). Selecting the 1000 pF range will limit the *Current Gain* adjustment to a maximum of 20 mV/pA.

CM: This is used to cancel slow capacitive currents that charge the cell membrane in the whole-cell configuration. The 30, 100 and 1000 pF ranges actually allow capacitance values to be compensated in the ranges of 0.12-30 pF, 0.4-100 pF and 4-1000 pF, respectively. The adjustment range is also limited by the program in order to make the time constant greater than 5 μs to prevent oscillations. The *C-Slow* value may be adjusted manually by dragging the mouse, or typing, or automatically by selecting the *Auto* function. Auto-compensation will adjust *C-Slow* as well as *R-Series*.

RS: Adjusts the resistance in series with the slow capacitance (range: 0.1 M Ω - 10 G Ω) to determine the time constant of the *C-Slow* transient and for *R_S Compensation*. Adjustment is limited by the capacitance values and the range as described above. The value can be changed manually by dragging the mouse, or typing, or automatically by clicking the *Auto* button.

Note: The value of R-Series is used by the R_S Comp circuitry as the measure of series resistance.

Further information can be found in: Sigworth FJ, Affolter H, Neher E (1995). *Design of the EPC-9, a computer-controlled patch-clamp amplifier. 2. Software. J Neurosci Meth* 56, 203-215.

Auto C-Slow: Selecting this function performs an automatic compensation of *C-Slow* and *R-Series*. The procedure uses a routine that applies short trains of square-wave pulses (number and amplitude of these pulses are specified in the Compensation tab of the Amplifier window (see chapter 4.1.1.4 on page 49) by *Cycles* and *Amplitude*), averages the resulting currents, and fits an exponential to deduce the compensation values required to cancel the current. During this procedure, some parameters are changed temporarily: *R_S Comp* is turned off, the stimulation is interrupted and the *Current Gain* is internally set to an appropriate value (0.2, 2 or 50 mV/pA, depending on the effective *Current Gain* range). If the *C-Slow Range* is set to 'Off', it will automatically be set to the highest possible range, i.e., the 1000 pF range in the low and medium *Current Gain* ranges, and the 100 pF range in the high *Current Gain* range.

Auto-compensation works best when *C-Fast* is canceled beforehand in the cell-attached configuration. The compensation may be improved by alternating cycles of *Auto C-Fast* and *Auto C-Slow*. A failed compensation is indicated by a red background of the *Auto* button.

Note: It is necessary that reasonable estimates for C-Slow and R-Series are supplied before the Auto C-Slow is started. Otherwise, the Auto-compensation may fail.

Series Resistance Compensation

Off: Sets the amount of compensation as well as the *Prediction* value to "Off".

Series Resistance Compensation Value: The series resistance compensation corrects for membrane voltage errors under conditions of high access resistance between pipette and cell interior. The amount of compensation can be changed by dragging the mouse or typing (range: 0 – 95%). One can either set manually a value for the series resistance compensation or a value is set here e.g. by the *Auto* series resistance compensation function.

Auto Series Resistance Compensation: A click on the *Auto* button automatically adjust the series resistance compensation setting to optimize the speed of a voltage jump at the cell membrane. The procedure tries to keep overshooting response to a minimum, since such overshoot leads to increased noise and introduces higher frequency components into the current signal. The two parameters for the automatic compensation routine can be set in the Compensation tab of the Amplifier window (see chapter 4.1.1.4 on page 49).

Series Resistance Compensation Mode: The compensation is based on the value of *Series Resistance Compensation Value* and will be effective only when series resistance compensation is not off, i.e., set to a speed value. The following settings determine the speed of feedback compensation:

- Off: Turns compensation off
- 100 μ s: Slow compensation
- 10 μ s: Medium speed compensation
- 5 μ s: Fast compensation
- 2 μ s: Very fast compensation

The choice of speed depends on the recording time constant and the degree of compensation desired. Fast series resistance compensation requires more critical adjustment of the controls but provides the maximum voltage clamp speed. In fast modes, Filter 1 will be automatically switched to its HQ 30 kHz setting.

Prediction: Switches "On" or "Off" the series resistance compensation *Prediction* by selecting/deselecting the checkbox. The *Prediction* accelerates the charging of the membrane capacitance by imposing large, transient voltages on the pipette when step changes are commanded. The compensation of the *Prediction* is set independent of the series resistance compensation value. The value for the *Prediction* can be set in the entry field to the right of the checkbox. Further information about this option can be found in the in the EPC 10 USB manual.

Bridge Compensation: In current clamp mode, series resistance compensation will act as a bridge compensation. Optimal bridge compensation will be achieved when using *Auto C-Slow* in whole-cell mode (to update the R_S value), then switching to current clamp mode and set R_S Comp to 100%.



Figure 4.14: Bridge Compensation Options in Current Clamp Mode.

Hardware Leak Compensation

Leak Conductance: This controls a hardware *Leak Compensation* which uses the inverted and scaled stimulus signal and adds it to the current monitor outputs. Leak can be adjusted manually by dragging the mouse, or typing, or automatically by clicking on the *Auto* button. The maximal values attainable depend on the *Current Gain* range. They are 2 nS, 200 nS and 20 μ S for high, medium, and low ranges, respectively. The injection time constant is 100 μ s.

Note: Data acquisition in PATCHMASTER NEXT does not consider a possible hardware "Leak" correction of the EPC 10 USB. The "Leak" value is stored and displayed together with the series parameters if Leak was activated in the stimulation sequence (i.e., Leak \geq 0.1 pS). Be aware of the consequences using Leak Comp, i.e., of the fact that the current measurements are not absolute values anymore. Because of this and because capacitive artifacts cannot be subtracted by this type of leak subtraction, it is advised to use the P/n leak subtraction instead whenever possible.

Auto Leak Compensation: Automatically determines and corrects the leak conductance.

Analog Filters

Filter 1: Controls an analog 6-pole low-pass filter in the current monitor pathway. The pop-up menu provides for the following settings:

- Bessel 100 kHz
- Bessel 30 kHz
- Bessel 10 kHz

- HQ 30 kHz

Under most conditions a 10 kHz bandwidth is more than ample, and the filtering reduces the high-frequency noise substantially.

When series resistance compensation is active PATCHMASTER NEXT sets an appropriate *Filter 1* according to the speed value of the series resistance compensation.

Table 4.2: Filter 1 setting according to the defined Series Resistance Compensation speed

R_s Comp	Filter 1
2 μ s	HQ 30 kHz
5 μ s	Bessel 30 kHz
10 μ s	Bessel 10 kHz
100 μ s	Bessel 10 kHz

Filter 2: Controls an analog 4-pole low-pass filter for current monitor 2 (*Imon2*) and the voltage monitor (*Vmon*). *Filter 2* is in series with *Filter 1*. Dragging the mouse or typing allows fine adjustment from 0.1 – 16 kHz in 0.1 kHz steps (guaranteed accuracy 0.5 – 15 kHz). The following filter characteristics can be selected from the pop-up menu:

- I_Bessel
- I_Butterworth
- Bypass (with amplifier of Revision 'N' and later)
- V_Bessel
- V_Butterworth

The -3 dB point is given in both cases. *Bessel* is the best characteristic for general use; the *Butterworth* response rolls off more rapidly with frequency and is mainly useful for power spectral analysis. The setting of the *Filter 2* control is the actual effective bandwidth of the series combination of *Filter 1* and *Filter 2*.

If you want to use an external filter rather than *Filter 2*, you can proceed as follows (example given for an EPC 10 USB Single amplifier): Connect current monitor 1 (*Imon1*) to the external filter input, feed the filter output back into one of the AD channels, and select this channel as input channel.

Note: Filter 2 is disabled when Imon1 is selected. This is to prevent the user to mistake the Filter 2 bandwidth with the active filter bandwidth used for the sampled data, i.e., Filter 1 bandwidth. To change Filter 2 in this case, select Imon2 first. If you want to use Filter 1 only, then you can choose Bypass.

5 Test Pulse

By default the Test Pulse window is displayed when starting PATCHMASTER NEXT with the default settings. If the window is not shown you can open it from the Windows menu (see 3.1 on page 18) or the short-cut icon (see 3.1 on page 19) in the Control window.

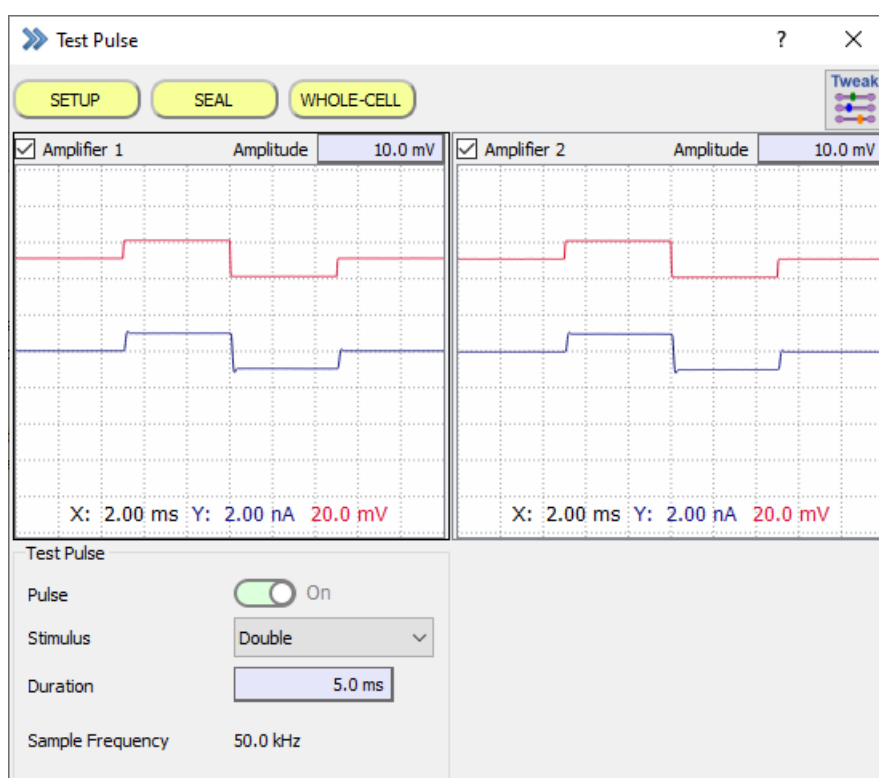


Figure 5.1: Test Pulse window of PATCHMASTER NEXT.

For a better orientation we divided the Test Pulse window into three sections:

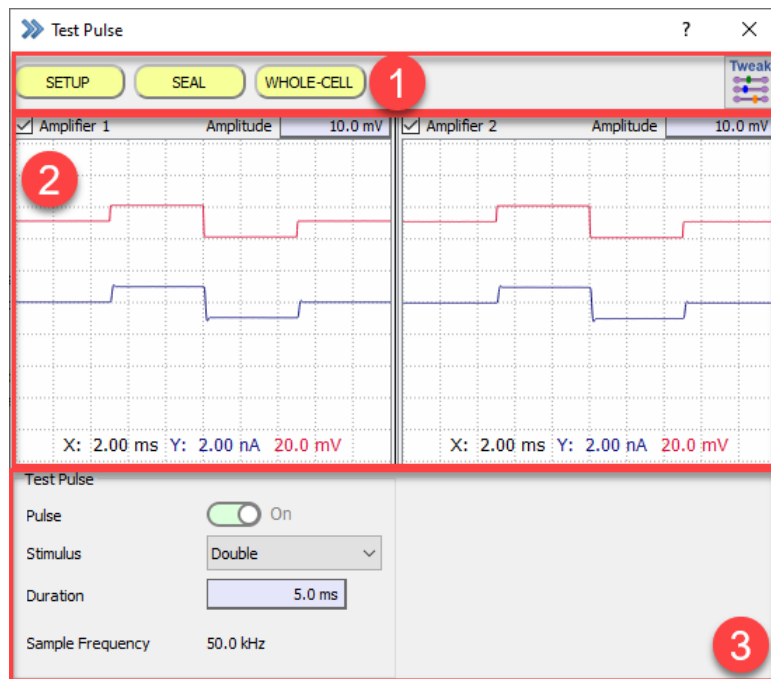


Figure 5.2: The three sections of the Test Pulse window.

1. Global Section
2. Test Pulse Viewports
3. Test Pulse Settings

5.1 Global Section

There are several icon buttons in the Global Section of the Test Pulse window. The functionality of the `Test Pulse Protocols` and the `Tweak` button will be explained in the chapters below.



Figure 5.3: Test Pulse Protocols of the Test Pulse window.

5.1.1 Test Pulse Protocols

Three predefined protocols are displayed as yellow buttons. These protocols can be used to easily adjust the amplifier for different recording conditions.

SETUP: Resets all parameters (with the exception of Liquid Junction and Vpip Offset), sets the *Recording Mode*, the *Current Gain*, the initial *C-Slow* and *R-Series* estimates and defines *Test Pulse* parameters. This protocol should be applied after the pipette has entered the bath.

SEAL: Sets the *Recording Mode*, the *Current Gain* and invokes an *Auto C-Fast* compensation. The protocol should be applied sometime during the sealing process.

WHOLE-CELL: Sets the *Recording Mode*, the *Current Gain*, the initial *C-Slow* and *R-Series* estimates and invokes an *Auto C-Slow* compensation. The protocol should be applied after opening of the cell.

Note: All three protocols are default suggestions and can potentially be modified by the user.

5.1.2 Tweak

When pressing the *Tweak* button an extension of the *Test Pulse* window opens which provides access to further settings.

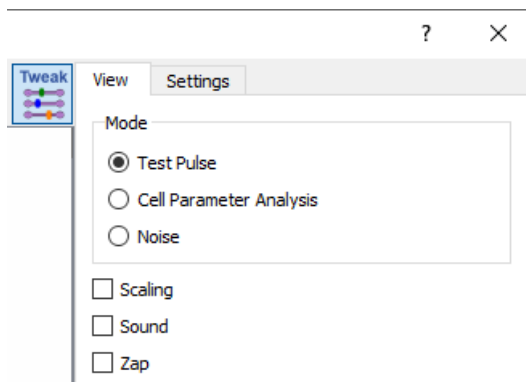


Figure 5.4: Tweak options of the Test Pulse window.

5.1.2.1 View Tab

Depending on the selection made in the *View* Tab a set of specific parameters are displayed in the *Test Pulse* Setting area of the *Test Pulse* window.

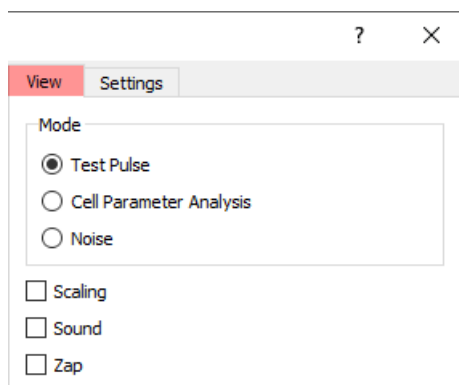


Figure 5.5: View Tab of the Test Pulse window.

Mode: By default the *Mode* is set to *Test Pulse*. This means a defined *Test Pulse* is added to the holding potential and applied to the pipette. The current response is sampled and displayed together with the applied voltage signal in a separate display which is called *Test Pulse Viewport*.

- **Test Pulse:** Runs the conventional *Test Pulse* mode where a voltage pulse is applied and the current response is measured.
- **Cell Parameter Analysis:** Runs either a ramp-shaped voltage pulse for e.g. Bilayer measurements, or a single unidirectional square-shaped voltage pulse for Whole Cell measurements.
- **Noise:** The RMS noise value is calculated.

When selecting the Cell Parameter Analysis one needs to take care of correct software and hardware settings. Therefore, we recommend to start PATCHMASTER NEXT with the Cell Parameter demo set.

Scaling: Shows the *Scaling* parameters for the *Test Pulse* mode in the *Test Pulse Settings* area.

Sound: Shows the *Sound* parameters for the *Test Pulse* mode in the *Test Pulse Settings* area.

Zap: Shows the *Zap* parameters for the *Test Pulse* mode in the *Test Pulse Settings* area.

5.1.2.2 Settings Tab

Here, test pulse parameters are available for some *Test Pulse* window features which are sufficient to adjust once and therefore do not get displayed in the *Test Pulse Setting* area.

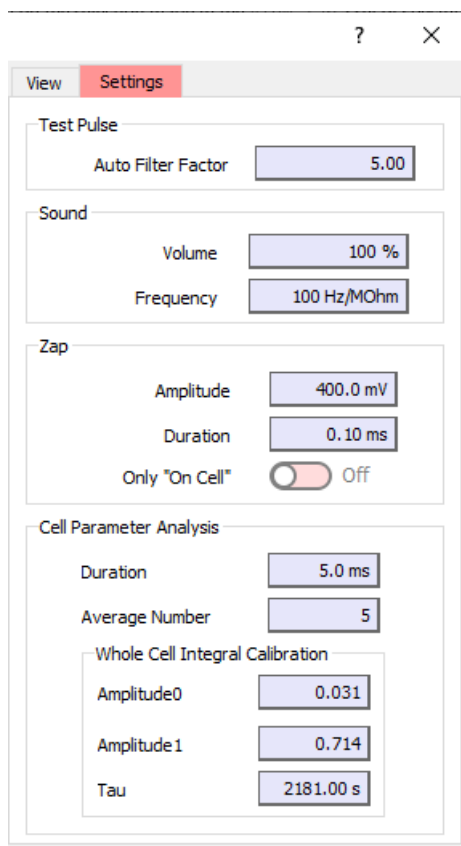


Figure 5.6: Settings of the Test Pulse window.

Test Pulse

Auto Filter Factor: The EPC 10 USB hardware filter is set according to the sample interval of the built-in *Test Pulse* using this *Auto Filter Factor*. Of course, this only is active when *Auto Filter* is activated in the *Amplifier* window (see chapter 4.1.1.2 on page 45). The *Auto Filter Factor* can be turned off by setting the value to zero.

Sound

Volume: Enter the sound volume here (in %).

Frequency: Specify the frequency in relation to the *R-membrane* value (Hz/M Ω).

Zap

Amplitude: Define the amplitude of the *Zap* pulse. The range of the amplitude is ± 1000 mV.

Duration: Define the duration of the *Zap Pulse*. The range of the duration is 0.01 to 1000 ms.

Only "On Cell": If this option is active it restricts the "Zap Pulse" application to the "On Cell" recording mode only.

Cell Parameter Analysis

Duration: Define the length of the applied test pulse of the *Parameter Fit* in the range of 0.1 - 1000 ms.

Average Number: Enter the number of averaged test pulses for the calculation of the cell parameter.

Amplitude0: Hardware parameter for *Amplitude0* value of the integral calculation. This is a fixed value which can be fine-tuned if necessary.

Amplitude1: Hardware parameter for *Amplitude1* value of the integral calculation. This is a fixed value which can be fine-tuned if necessary.

Tau: Hardware parameter for *Tau* value of the integral calculation. This is a fixed value which can be fine-tuned if necessary.

5.2 Test Pulse Viewport

The *Test Pulse Viewports* displays only the *Test Pulse*, it does not replay any recorded data. Further, there is a right-click context menu offering the following options:

- Copy: Copies the displayed data into the clipboard.
- Print: Enables to print the displayed data on your local printer.
- Grid:
 - None: No grid is displayed.
 - Grid Only: Only the grid is displayed.
 - Bars Only: Only scale bars are displayed.
 - Grid + Labels: Shows the grid and labels of the Y- and X-axis.
 - Grid + Values: Shows the grid and the values of the Y- and X-axis.
 - Show Zero Line: Shows the Y-axis zero line of each Trace.

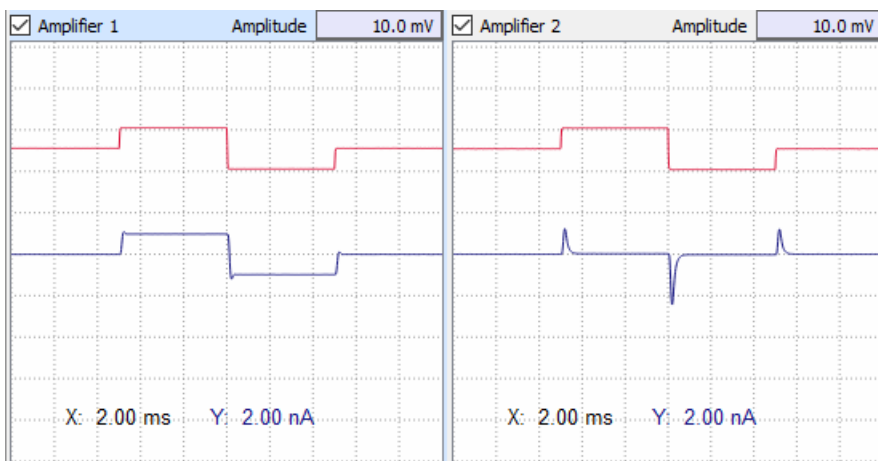


Figure 5.7: Test Pulse Viewports of the Test Pulse window.

Amplifier Selection: By left-clicking onto the viewport of each amplifier the corresponding amplifier is selected. This selection is synchronized with the amplifier selection in the Amplifier window (see chapter 4.2 on page 50) and vice versa.

Amplitude: Enter a value for the Test Pulse amplitude in the range of ± 100 mV.

Note: Short Test Pulses will require a high sampling rate, i.e., a high filter bandwidth. If the Auto Filter Factor option is activated (4.1.1.2 on page 45), PATCHMASTER NEXT automatically selects the appropriate current input channel after Filter 1 or Filter 2 and sets the filter bandwidth (= Sampling Rate / Auto Filter Factor). For bandwidths greater than approximately 9 kHz, PATCHMASTER NEXT automatically selects the Filter 1 signal as input, otherwise PATCHMASTER NEXT selects the Filter 2 signal. Therefore, it can happen that the channel selection automatically toggles between the Filter 1 signal and the Filter 2 signal if Test Pulses are followed by a sequence from the Stimulus Editor or vice versa.

5.3 Test Pulse Settings

The Test Pulse Settings is an area for parameters depending on the selections made in the View tab.

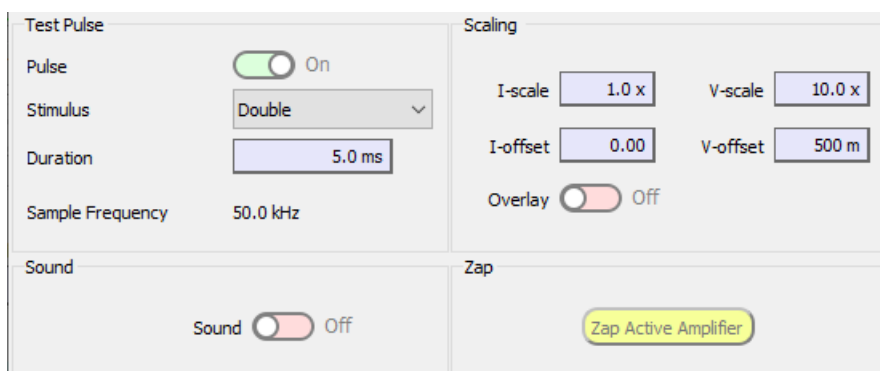


Figure 5.8: Parameter settings of the Test Pulse window.

5.3.1 Test Pulse Parameter

The parameters defined in this area are applied to all amplifiers.

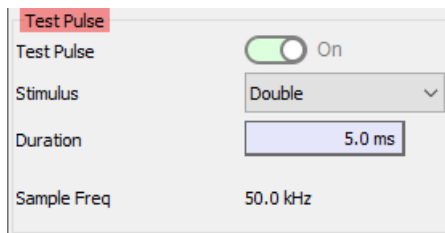


Figure 5.9: Test Pulse parameter of the Test Pulse window.

Test Pulse: Turn on or off the *Test Pulse*.

Stimulus: There are four options available:

- No Stimulus: No test pulse stimulus is applied but the current response is still measured.
- Single: A mono pulse pattern is generated and the current signal is sampled and displayed.
- Double: A bipolar pulse pattern is generated and the current signal is sampled and displayed.
- Ramp: A triangle-shaped ramp pulse pattern is applied.

Duration: Enter a value for the Test Pulse Duration in the range of 0.1 - 1000 ms.

Sample Frequency: Display field of the current *Sample Frequency*. The *Sample Frequency* is adjusted in dependence of the set *Duration* to sample at least 1000 data points.

5.3.2 Cell Parameter Analysis

The parameters defined in this area are applied to all amplifiers.

When selecting the Cell Parameter Analysis one needs to take care of correct software and hardware settings. Therefore, we recommend to start PATCHMASTER NEXT with the Cell Parameter demo set.

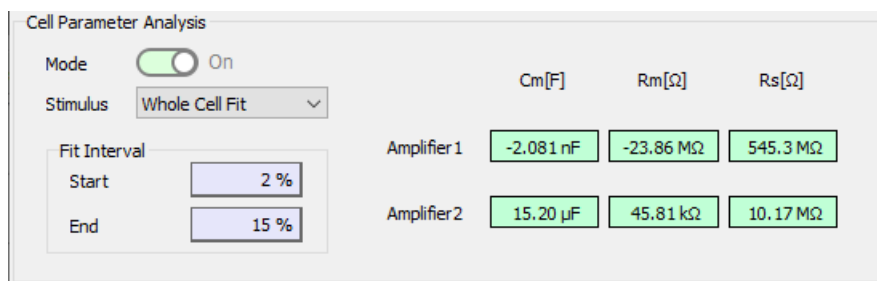


Figure 5.10: Cell Parameter Analysis settings of the Test Pulse window.

Mode: Turn on or off the *Cell Parameter Analysis*.

Stimulus: There are three options available:

- **Bilayer/On Cell:** A triangular-shaped ramp stimulus pattern is applied. This option can be used when either experimenting with bilayers or in the on cell recording mode.
- **Whole Cell Fit:** A singular test pulse is applied. This option can be used when experimenting in whole cell mode.
- **Whole Cell Integral:** A singular test pulse is applied. This mode calculates the integral from the capacitive artifact while the DC component is subtracted. The parameters are predefined and measured in respect to the model cell. One can further self-tune these values with the model cell to get more accurate values (see chapter 5.1.2.2 on page 63).

Fit Interval

Start: Defines the start point for the fitting routine.

End: Defines the end point for the fitting routine.

Note: Fit Interval option are not available in Whole Cell Integral mode.

5.3.3 Noise Mode

The parameters defined in this area are applied to all amplifiers.



Figure 5.11: Noise Settings of the Test Pulse window.

Noise: If selected, the RMS noise is continuously measured and updated in the field usually labeled R-memb. For the determination of the noise level, no pulses are output and current is sampled via the active AD channel using the current filter settings (usually the current monitor 2 output, so that the bandwidth is determined by Filter 2).

Note: Make sure to turn off the Auto Filter option (see chapter 5.4.10 on page 55) when you plan to measure noise in the Amplifier window. When noise of the amplifier is to be determined, usually a fixed bandwidth of 3 kHz (Filter 2) is selected.

Duration: Enter a value in the range of 0.1 - 1000 ms for the noise calculation.

Sample Frequency: Display field of the current *Sample Frequency*. The *Sample Frequency* is adjusted in dependence of the set *Duration* to sample at least 1000 data points.

5.3.4 Scaling

Using *Test Pulses* are thought to be used for amplifier tuning, i.e., to adjust *Gain* and capacitance cancellation to achieve a maximal dynamic range of the recorded signal. Thus, without display gain, one can easily see when the input signal saturates the AD converter. If, however, amplification is needed one can use the scaling options provided.

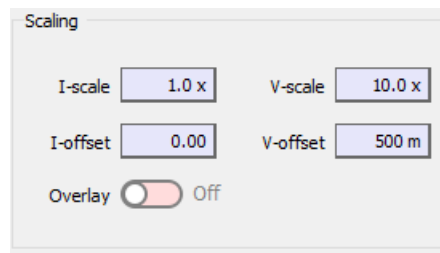


Figure 5.12: Scaling settings of the Test Pulse window.

The parameters defined in this area are applied to all amplifiers.

I-scale: Set the scaling of the input current of the *Test Pulse*.

V-scale: Set the scaling of the input voltage of the *Test Pulse*.

I-offset: Adjust the offset of the input current signal of the *Test Pulse*.

V-offset: Adjust the offset of the input voltage signal of the *Test Pulse*.

Overlay: When active all applied *Test Pulses* are overlaid in the Test Pulse Viewport.

5.3.5 Sound

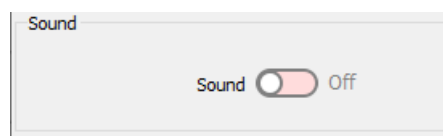


Figure 5.13: Sound settings of the Test Pulse window.

Sound: Activates the sound generator of the data acquisition interface board which reflects changes in the membrane resistance (R_{membrane}) by changing sound frequencies. To use this function one needs to plug in loud-speaker at the rear panel of the amplifier or the stand-alone data acquisition interface.

5.3.6 Zap

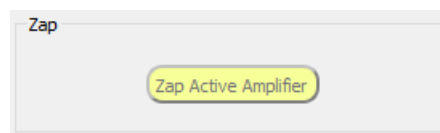


Figure 5.14: Zap settings of the Test Pulse window.

Zap Active Amplifier: Press this button to apply a specified “Zap” pulse to the active headstage of the patch clamp amplifier.

6 Oscilloscope

The purpose of the Oscilloscope window is either to monitor the incoming data during a running acquisition process or to review acquired data. The data Traces of a Sweep are displayed versus the time of the recording. To review data one needs to open the according data file and select the data in the Data Tree (see chapter 11 on page 195). Further, the Oscilloscope window provides tools to select, scale and measure the depicted data.

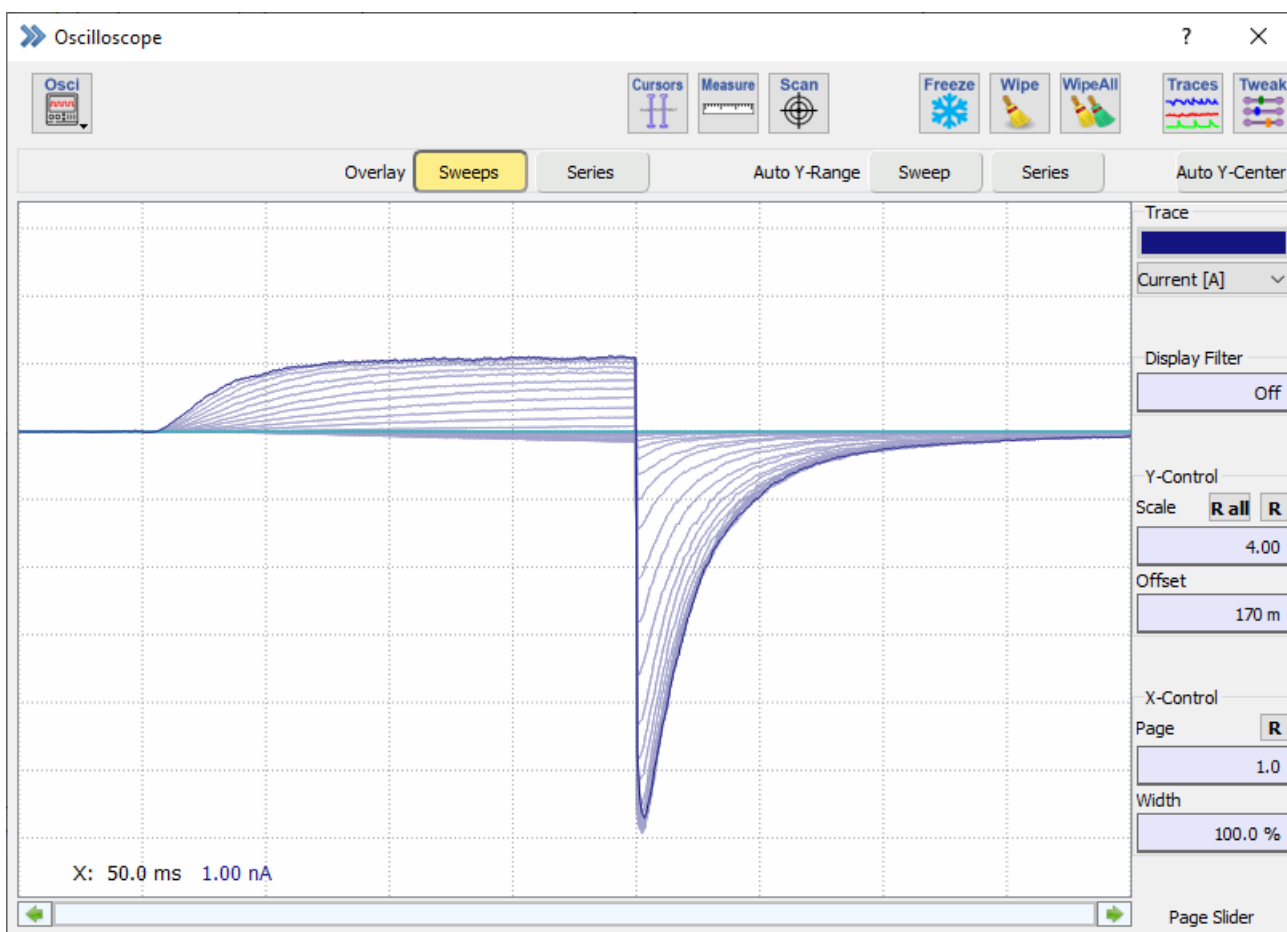


Figure 6.1: The Oscilloscope window of PATCHMASTER NEXT.

One can divide the Oscilloscope window into three main sections:

- Global Section
- Data Display
- Oscilloscope Controls

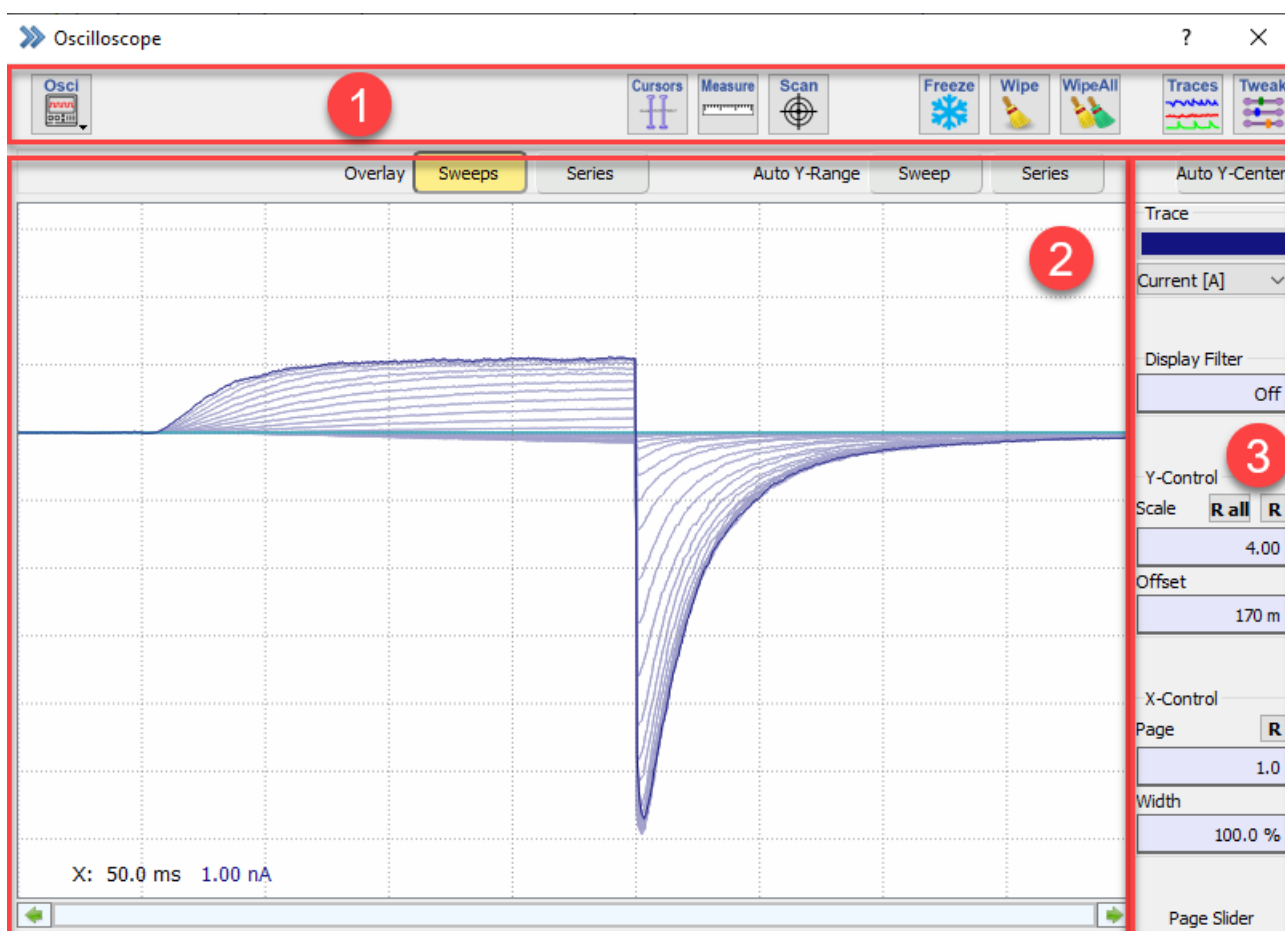


Figure 6.2: The three sections of the Oscilloscope window.

Each of these sections will be explained in the following chapters.

6.1 Global Section

There are several icon buttons in the Global Section of the Oscilloscope window. The functionality of these icons will be explained in the following sections below.



Figure 6.3: The Global Section of the Oscilloscope window.

Oscilloscope Menu: The `Oscilloscope` menu contains the display options which are relevant for the data display. The options are explained in detail in chapter 6.1.1 on page 74.

Cursors: When activating this button the cursor position of the currently active analysis function is shown. Either you change the cursor position in the Oscilloscope window directly or you open the Analysis Editor (see chapter 9.3.1.1 on page 169) to change the cursor position. The changes are taken over in both windows, independent where you made them.

Note: Cursors cannot be moved if the Analysis Function in the Analysis Editor does not provide cursor settings.

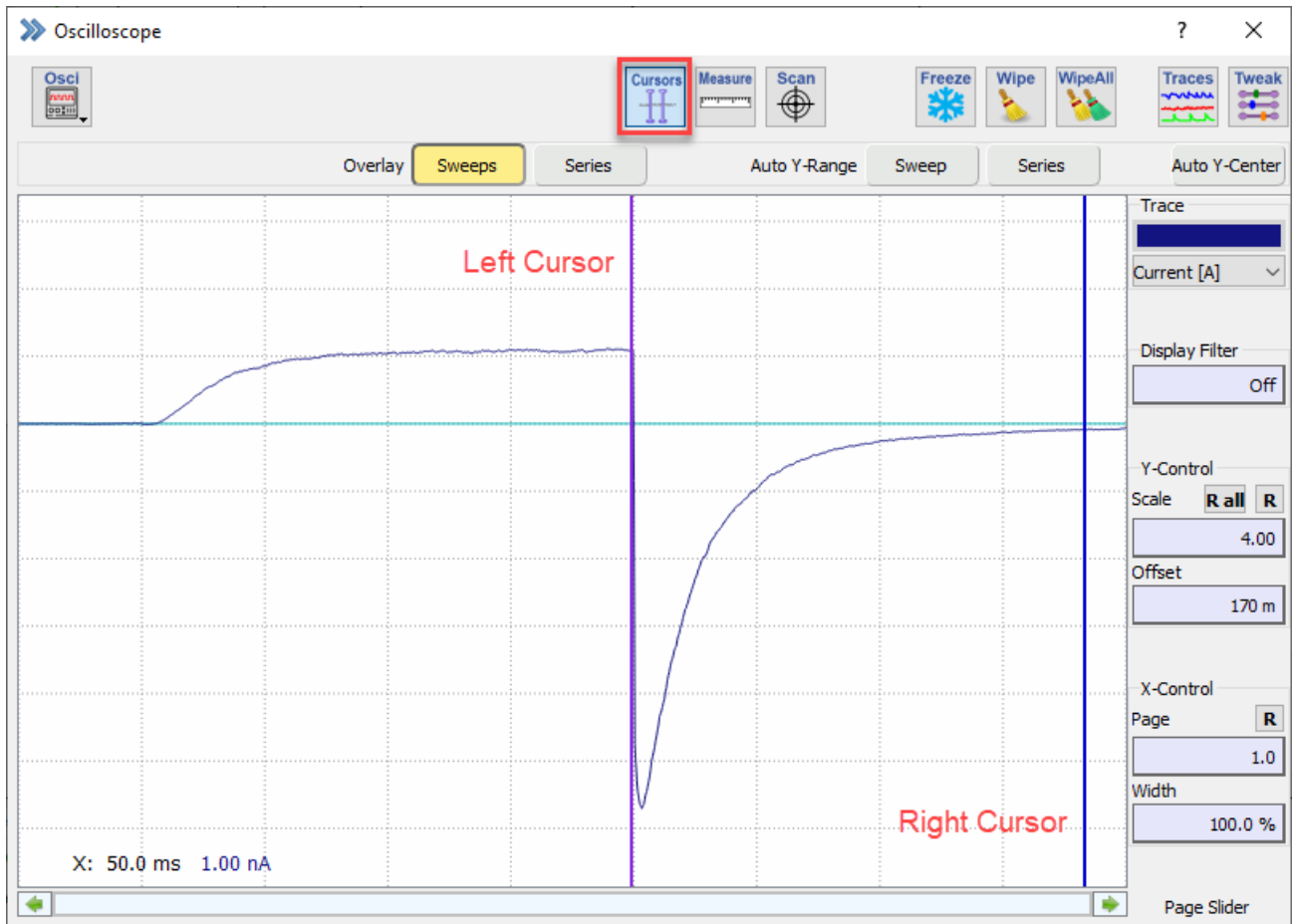


Figure 6.4: The Cursors option of the Oscilloscope window.

Measure: This function allows to measure amplitudes and duration of the displayed data [NUM+ 8]. If the function is enabled an extension bay of the Oscilloscope window gets visible at the bottom:

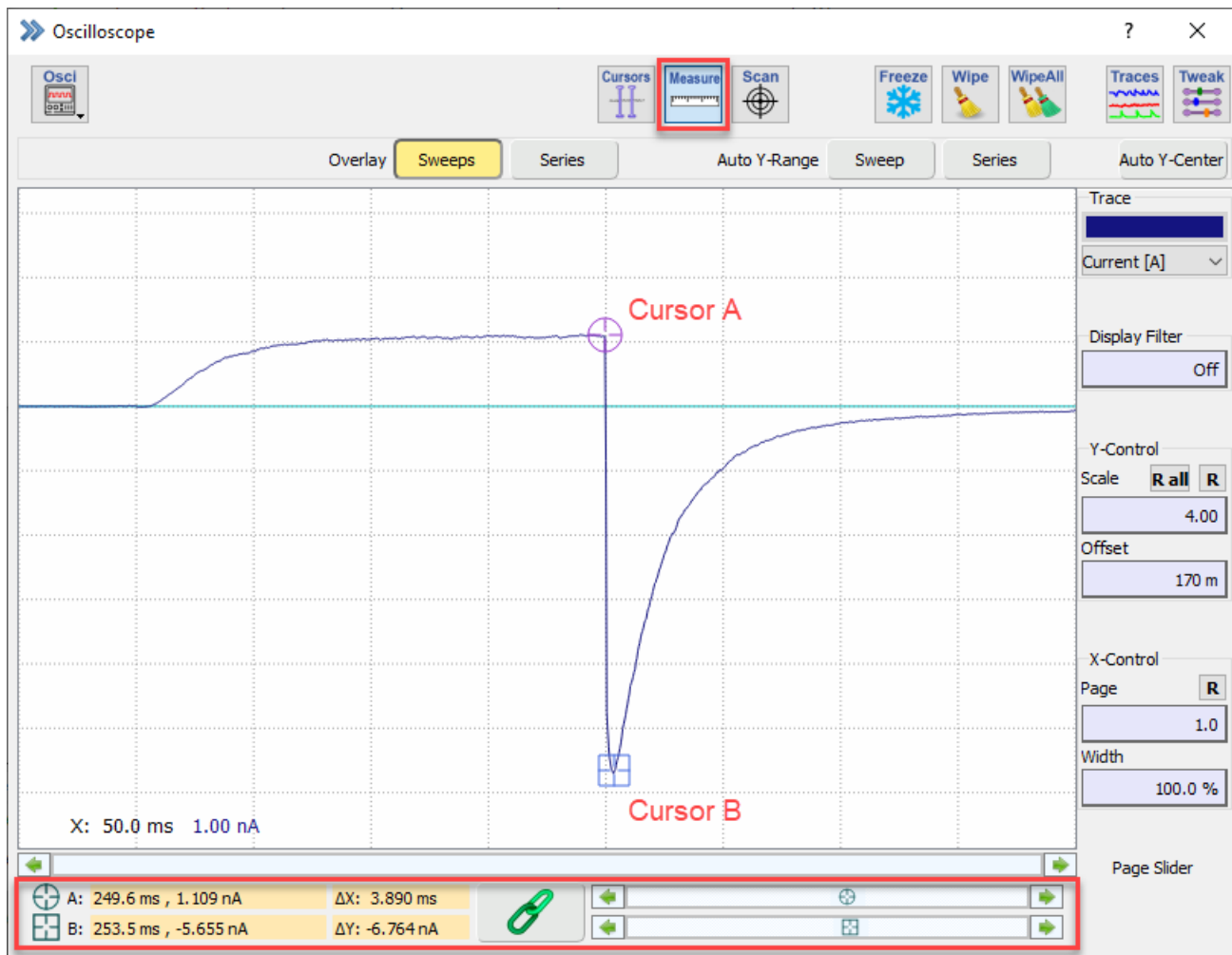


Figure 6.5: The Measure functionality of the Oscilloscope window.

Two cursors (Cursor A & Cursor B) can be used to manually measure relative or absolute amplitudes. Relative changes indicated by ΔX or ΔY are calculated between both cursor positions. The *Chain Link* button allows to fix the distance of both cursors to each other. The corresponding values of the cursor positions are displayed in the text fields with the orange background. Once you right-click on it a context menu opens offering two options to extract the values:

- Copy to Clipboard
- Write to Notebook

Note: Please be aware that each of these orange text fields offers this functionality to extract the values.

Instead of moving the cursors with the mouse you can also use the corresponding sliders beside the *Chain Link* button.

Scan: The *Scan* function does also provide two cursors (Cursor A & B) but in opposition to the *Measure* function the cursors are sticking to the selected Traces and cannot freely moved inside the *Data Display* section. This means the cursors can be moved along the selected Traces point by point [NUM+ 7].

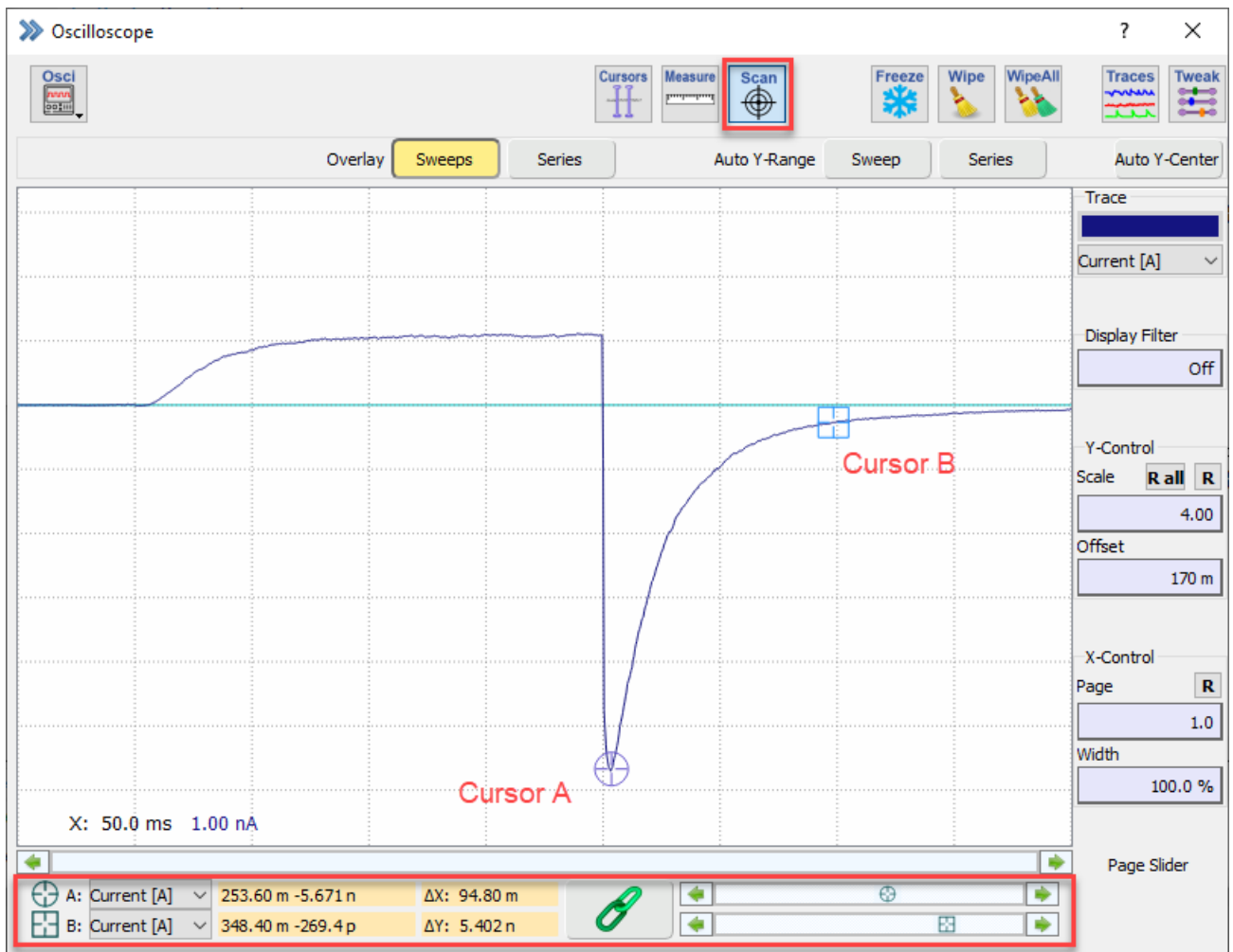


Figure 6.6: The Scan functionality of the Oscilloscope window.

The Trace selection option for each cursor is indicated in the lower left corner of the extension bay. All other functionality of the *Scan* option is identical to the *Measure* option (see above).

Freeze: This button freezes the present content of the data displayed in the Oscilloscope window. Either press the *Freeze* button again to unfreeze the display or acquire new data.

Wipe: Clears the *Data Display* section of the Oscilloscope window [D].

WipeAll: Clears the *Data Display* section of the Oscilloscope window and the *Graph Layout* [BACKSPACE].

Traces: This button opens the *Trace Properties* dialog (see chapter 6.1.2 on page 75).

Tweak: The *Tweak* button uncovers some more scaling or display options which are explained in chapter 6.1.3 on page 77.

6.1.1 Oscilloscope Menu

This menu contains all display options which are relevant for the data display.

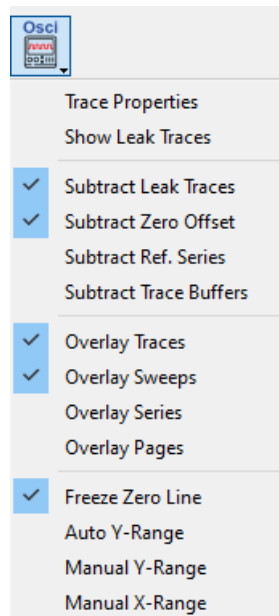


Figure 6.7: The Display options of the Oscilloscope window are concentrated in one menu.

Trace Properties: Via this selection the `Trace Properties` dialog opens which allows to define individual display settings of Traces and Buffers (see chapter 6.1.2 on the next page).

Show Leak Traces: Shows the P/n leak pulse signals in the Oscilloscope window like a Trace. As a prerequisite you have to activate the `Leak Subtraction` in the Stimulus Editor (see chapter 7.6.1 on page 99).

Subtract Leak Traces: Subtracts the P/n leak responses from the measurement signals.

Subtract Zero Offset: Subtracts the zero offset from the measured signals. The zero offset is the mean value, calculated from the `Zero` segment. The `Zero` segment is defined in the Stimulus Editor (see chapter 7.6.1 on page 98). Usually, the zero offset value is determined online, but it can also be computed offline by use of the `Recompute Zero Offset` function from the `Data` menu (see chapter 11.1.2 on page 197).

Subtract Zero in the Display menu is kept "On" even if the conditions for Zero Subtraction are not met. If one wants to see the subtracted Trace offline, one has to switch to another Trace in the Data Tree and then back to the just acquired Trace. A simple "Wipe" or "Redraw" of the displayed data is not sufficient.

Subtract Ref. Series: If this option is checked, then the `Reference Series` is subtracted from all replayed data (see chapter 6.1.2 on the next page).

Subtract Trace Buffers: If this item is checked, then the Trace Buffer is subtracted from all replayed Traces (see chapter 6.1.2 on the facing page).

Overlay Traces: Overlay of all Traces of a selected Sweep.

Overlay Sweeps: Overlay of all Sweeps of a Series.

Overlay Series: Overlay of all Series, i.e. overlay of all.

Overlay Pages: During continuous acquisition data is displayed in page with overlay.

Freeze Zero Line: This checkbox determines the behavior of the display scaling in the Oscilloscope window. When `Freeze Zero Line` is selected, the Oscilloscope window behaves like an oscilloscope, i.e. when the display

gain is changed the zero line stays at its position. When deselected, display scaling is performed with reference to the center of the display. I.e., when *Y-offs* is not zero, the zero line may alter its position on the screen.

Auto Y-scale: The Y-scaling is automatically adapted to the replayed data to fit minimal and maximal data of a Series onto the screen. The range of the Y-scaling depends on the *Gain* settings.

Manual Y-Range: The Y-scale is fixed to the manually defined range. In the Oscilloscope window the controls *Y-scale* and *Y-offs*. turn into *Y-max* and *Y-min*.

Note: Auto Y-scale has priority over Manual Y-scale.

Manual X-Range: The X-scale is fixed to the manually defined range. In the Oscilloscope window the controls *Start Time* and *End* turn into *X-min* and *X-max*.

6.1.2 Trace Properties

The Trace Properties dialog provides access to certain display settings of individual Traces and Buffers. The dialog is divided into two parts:

- Trace Settings
- Buffer Settings

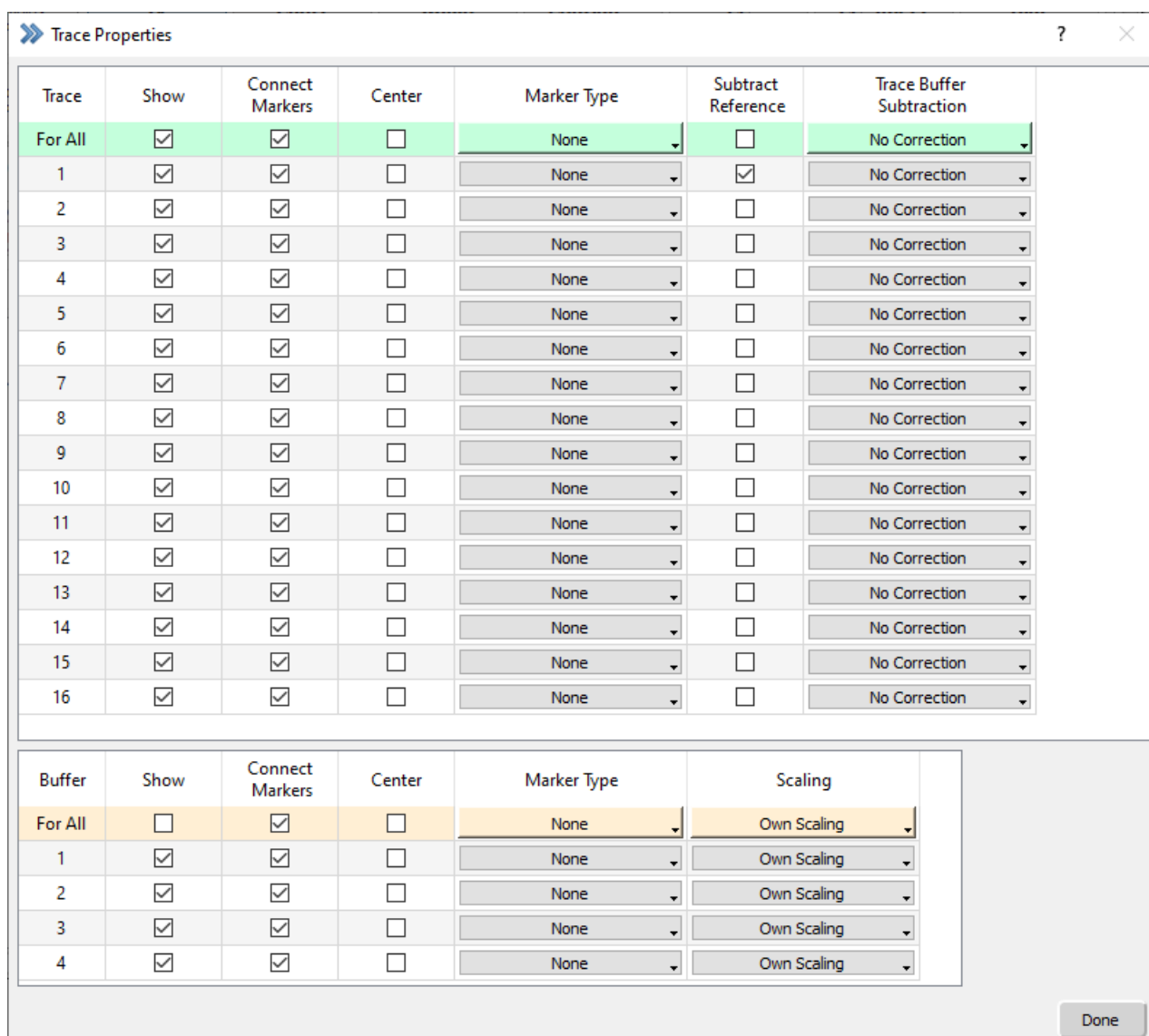


Figure 6.8: The Trace Properties dialog allows to edit the display settings of Traces.

The settings and functions for both parts are almost identical except of the scaling options provided for the Buffer Traces.

The first row, marked by green background color (orange for Buffer Traces), enables to extend the settings to all other Traces listed.

Trace: Up to 16 Traces and 4 Buffers can be individually modified.

Note: The number of Traces is defined in the Configuration (see chapter 3.5.1 on page 24).

Show: Here, one can decide if a Trace shall be displayed (Show) in the Oscilloscope window or not.

Connect Markers: When option *Connect Markers* is checked then the markers are connected by lines.

Center: If this option is set the Traces get centered during acquisition. Therefore the left 10% of the first page are brought to the center of the display.

Marker Type: Markers can be used for plotting data points e.g. if LockIn or FURA data are acquired. The following *Marker Types* are available:

- Dot

- Plus
- Star
- Diamond
- Cross
- Square
- Circle
- Triangle Up
- Triangle Down

Subtract Reference: If *Subtract Reference* is checked for a Trace, then the *Reference Series* is subtracted from that Trace. A *Reference Series* can be defined by use of the *Reference Series - Set* option in the *Data Tree* (see chapter 11.1.2 on page 197). The application of the *Subtract Reference* option can be controlled globally via the *Subtract Ref. Series* option in the *Display Options* (see chapter 6.1.1 on page 74).

Trace Buffer Subtraction: If *Trace Buffer Subtraction* is set to one of the four available Trace Buffers then the contents of the chosen Buffer are subtracted from that Trace. This can be used for subtracting a control Trace from other Traces. The application of the *Trace Buffer Subtraction* option can be controlled globally via the *Subtract Trace Buffers* option in the *Display Options* (see chapter 6.1.1 on page 74).

Scaling: The section for the trace properties of the Buffers do not contain any reference correction settings. Instead one can define the scaling behavior of the Buffer content. With this option, the display scaling of a Trace Buffer can be coupled to the display scaling of a certain Trace.

Note: The Trace Properties settings are stored in the PATCHMASTER NEXT configuration file.

Note: The color of Traces can be set in the Configuration dialog (see chapter 3.5.5 on page 36).

6.1.3 Tweak

The options provided by the *Tweak* button visualizes some additional display and scaling functions of the Oscilloscope window:

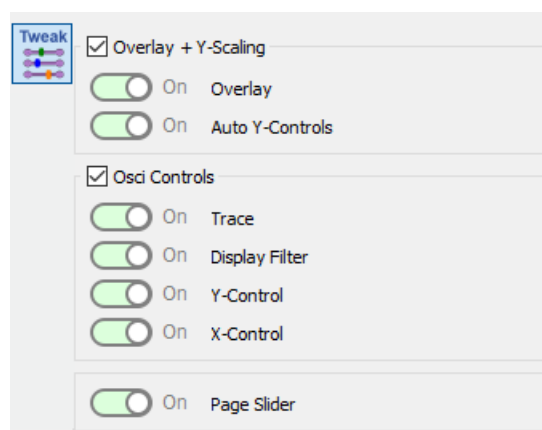


Figure 6.9: Tweak options of the Oscilloscope window.

6.1.3.1 Overlay + Y-Scaling

The checkbox needs to be active to get the parameters displayed in the Oscilloscope window.

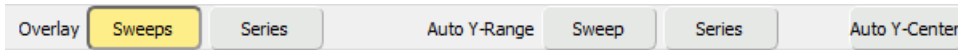


Figure 6.10: Overlay + Y-Scaling options of the Oscilloscope window.

Overlay: The *Overlay Sweeps* displays all Sweeps of a Series without erasing the screen in between Sweeps. The next Series, however, will erase the screen.

The *Overlay Series* displays all incoming Sweeps without erasing the screen. This allows Sweeps of different Series to be computed. The screen can be wiped by pressing *Wipe* (see chapter 6.1 on page 73).

Auto Y-Controls: Pressing the *Auto Y-Range Sweep* button calculates the maximum Y-scaling factors to display the selected Trace of a Sweep as large as possible in the Oscilloscope window. The basis for the calculation are the *Y-min* and *Y-max* values (see chapter 12.1.5 on page 213) of the selected Trace of the Sweep.

Pressing the *Auto Y-Scale Series* button calculates the maximum Y-scaling factors to display the selected Traces of a Series as large as possible in the Oscilloscope window. The basis for the calculation are the *Y-min* and *Y-max* values (see chapter 12.1.5 on page 213) of the Traces of the Series.

The *Auto Y-Center* button automatically centers the active Trace on the screen.

6.1.3.2 Osci Controls

The checkbox for *Osci Controls* need to be active in the *Tweak* menu to get the activated parameters displayed in the Oscilloscope window. The Oscilloscope controls help to properly scale and display the data in the *Data Display* section.

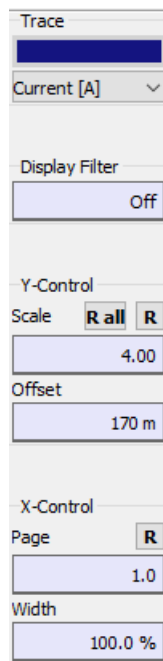


Figure 6.11: Oscilloscope Control section of the Oscilloscope window.

Trace: Selects the Trace to which the scaling setting applies. The color of the selected Trace is displayed in the *Trace Color* field.

Note: The names of the Traces are taken over from the Stimulus Editor.

Display Filter: Shows the currently selected bandwidth of a digital non-lagging Gaussian low-pass filter (i.e. software filter). The -3dB cutoff frequency is specified in Hertz. This allows to reduce the original bandwidth by the factor 0.0025 (1/400). It is for display purposes only; no changes to the data are performed.

Y-Control: For each Trace you can control the display scaling. The value of 1 corresponds to full scale of ± 10.24 V. Pressing NUM+ from the numeric keypad (take care not to have NUMLOCK activated!) increases the display scaling by a factor of 2 and NUM- decreases it by a factor of 2. It is also possible to auto-adjust (*Auto Y-scale*) or fix the Y scaling (*Manual Y-scale*) of the Oscilloscope window (see chapter 6.1.1 on page 74).

For each Trace you can control the offset of the zero line. Offsets of Traces may be between -1 and 1 relative to full scale of display (default = 0). SHIFT + NUM+ and SHIFT + NUM- (from the numeric keypad) increase and decrease the display offset by "0.2".

Via the *Reset* options you can either reset the *Scale* of the active Trace (*R*) or of all Traces (*R all*) to the default values.

X-Control The *Page* shows the display page number during replay of continuous data Sweeps or when time axis (*Width*) is chosen to be less than 100%. By using the *Page Slider* in the *Data Display* one can scroll horizontally through the current Sweep. Dragging the page number scrolls the data forward or backward; entering a page number will display that particular page.

The *R* button resets the *Page* and the *Width* values.

Note: The length of one Page is defined by the total length of all segments which are set in front of the Continuous segment in the stimulation sequence.

Note: The full time scale provided for Sweep display is based on the first Trace within a Series. Alternatively, one can use the Manual X-Range option (see chapter 6.1.1 on page 74).

Note: If one sets the Width to e.g. 20 % when a Continuous segment is used (see chapter 7.8 on page 117) the Page size will be decreased to 20 %.

6.2 Data Display

While acquiring or replaying data the data itself is shown in the Data Display section of the Oscilloscope window.

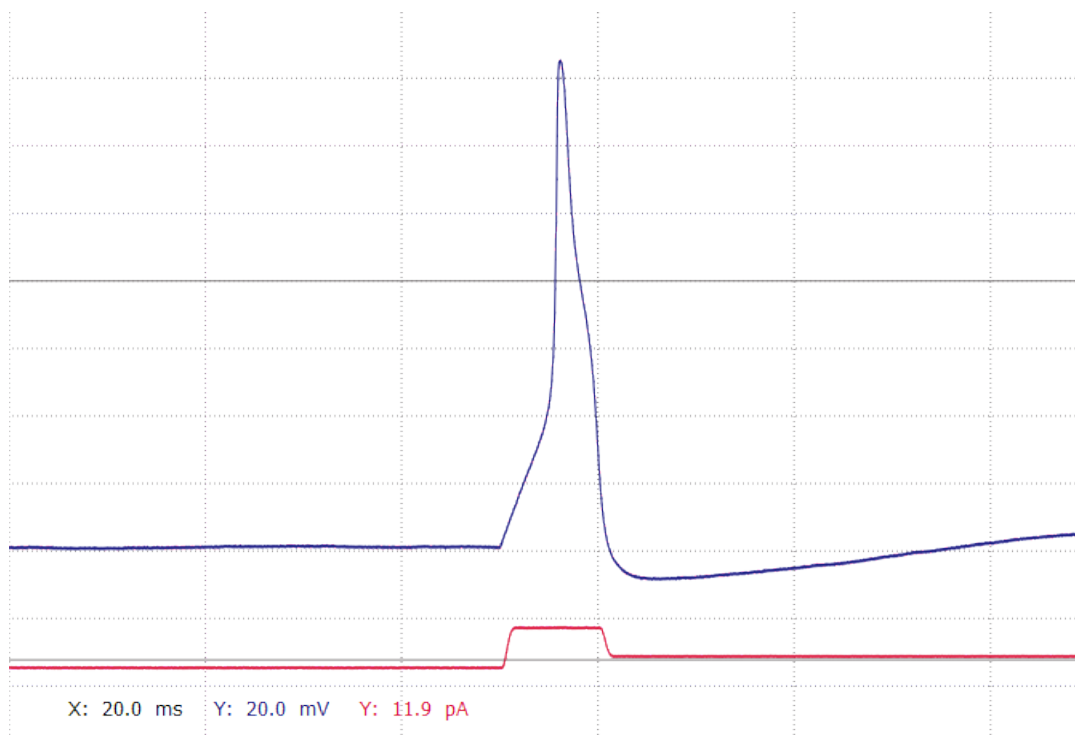


Figure 6.12: Data Display section of the Oscilloscope window.

The Trace colors match with the color of the units at the bottom. There will be one label for the X-axis unit and one label for each acquired Trace (Y-axis unit).

Scaling and display options are either available in the Global Section (see chapter 6.1 on page 70) or the Oscilloscope Controls (see chapter 6.1.3.2 on page 78).

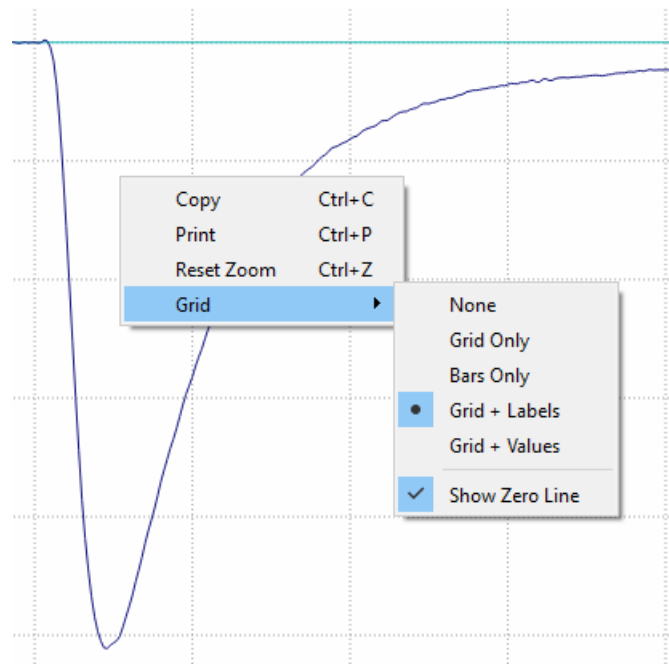


Figure 6.13: Right-click context menu of the Data Display.

By a right-click on the `Data Display` section a context menu gets visible offering the following options:

- **Copy:** Copies the displayed data into the clipboard.
- **Print:** Enables to print the displayed data on your local printer.
- **Reset Zoom:** Resets the Y- and X-settings of the `Oscilloscope Controls`. This option does only get active if any zooming of the data was done before.
- **Grid:**
 - **None:** No grid is displayed.
 - **Grid Only:** Only the grid is displayed.
 - **Bars Only:** Only scale bars are displayed.
 - **Grid + Labels:** Shows the grid and labels of the Y- and X-axis.
 - **Grid + Values:** Shows the grid and the values of the Y- and X-axis.
 - **Show Zero Line:** Shows the Y-axis zero line of each Trace.

Zooming: It is possible to set the display scaling in the `Oscilloscope` window by zooming into a screen region with the mouse while pressing the left mouse button. When you release the mouse button, the marked area will be set to fill the `Oscilloscope` window screen. The scaling has to be done for each Trace separately.

If you want to display data that is outside the active screen area, you can either enter some values into *Scale* or *Width* in the `Oscilloscope Controls`.

6.3 Using the Numeric Keypad to control the Oscilloscope

For a fast and intuitive operation of the Oscilloscope window settings and parameters one can use the predefined keys for the numeric keypad. These key assignment can be modified and stored in the Keys Editor (see chapter 3.5.7 on page 39).

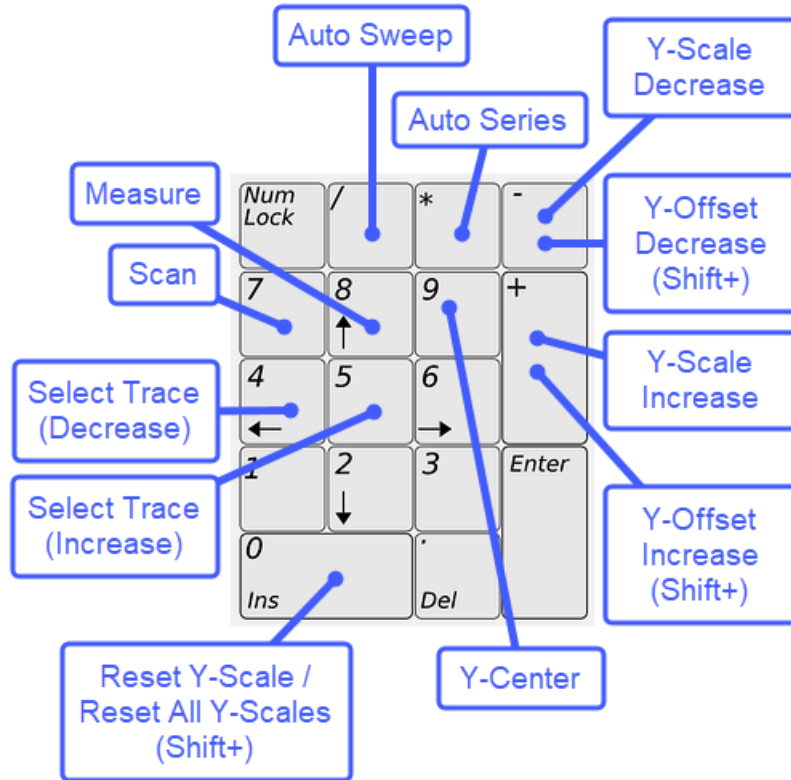


Figure 6.14: Assigned Keys for controlling the Oscilloscope window.

Note: Please check if the numeric keys feature of your keyboard is active.

7 Stimulus Editor

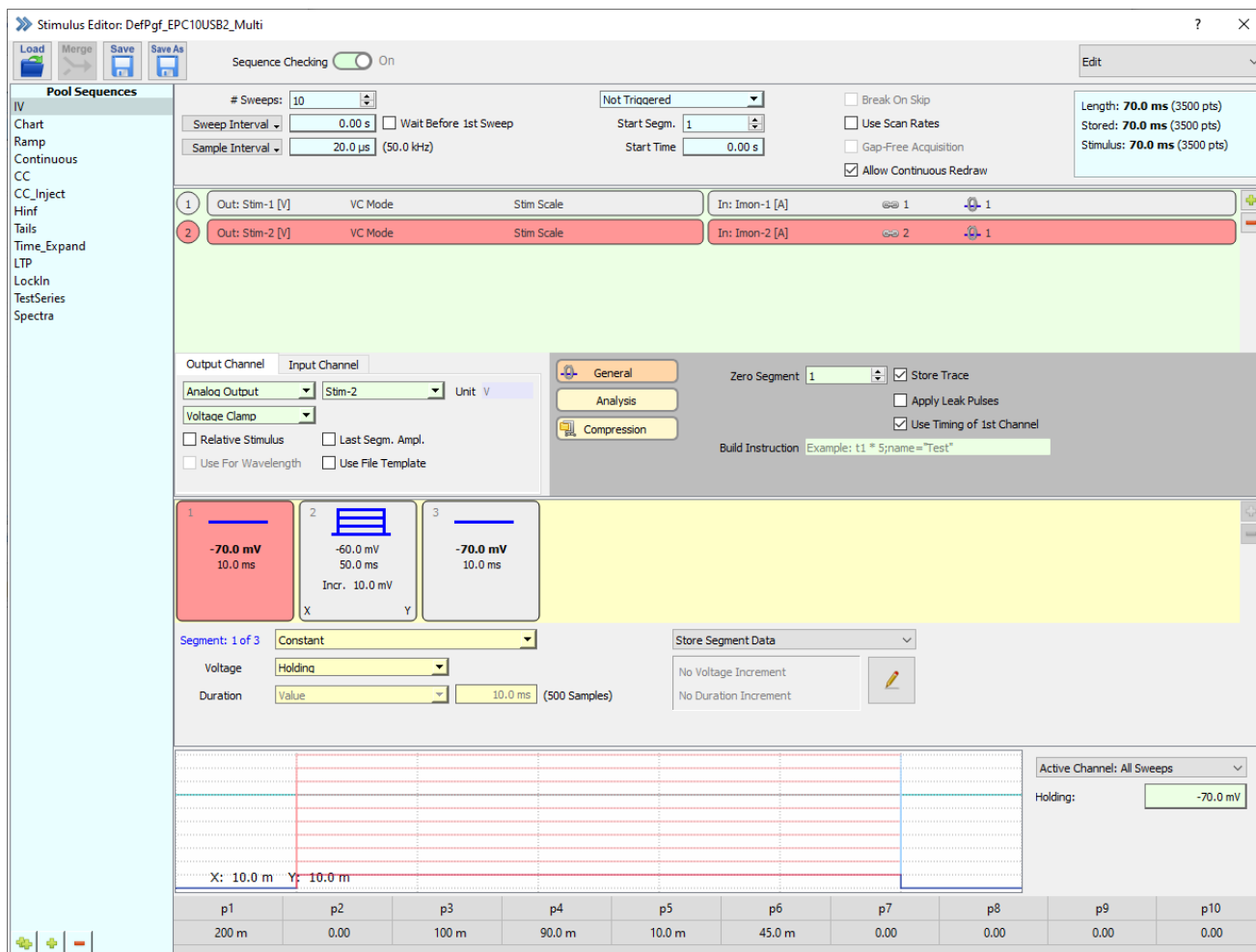


Figure 7.1: Stimulus Editor window of PATCHMASTER NEXT.

The Stimulus Editor window defines the layout of stimulation pulse patterns. On the level of stimulation, they are called "sequence" throughout this manual. The result of a sequence stimulation, i.e. the families of sampled data *Traces*, are called a *Series*. Entries that are not default (e.g. adjustable parameters) are highlighted in bright background colors (*active* parameters), the rest is shown in dim background colors (*inactive* parameters). Because of this automatic feature, the color of individual editable controls cannot be changed in this dialog. All other controls can be modified.

For a better orientation we divided the Stimulus Editor window into ten sections:

1. Global Section

2. Stimulus Pool
3. General Stimulus Settings
4. Channel View
5. Channel Settings
6. Parameter Section
7. Segment View
8. Segment Settings
9. Cartoon Section
10. PGF Parameter Section

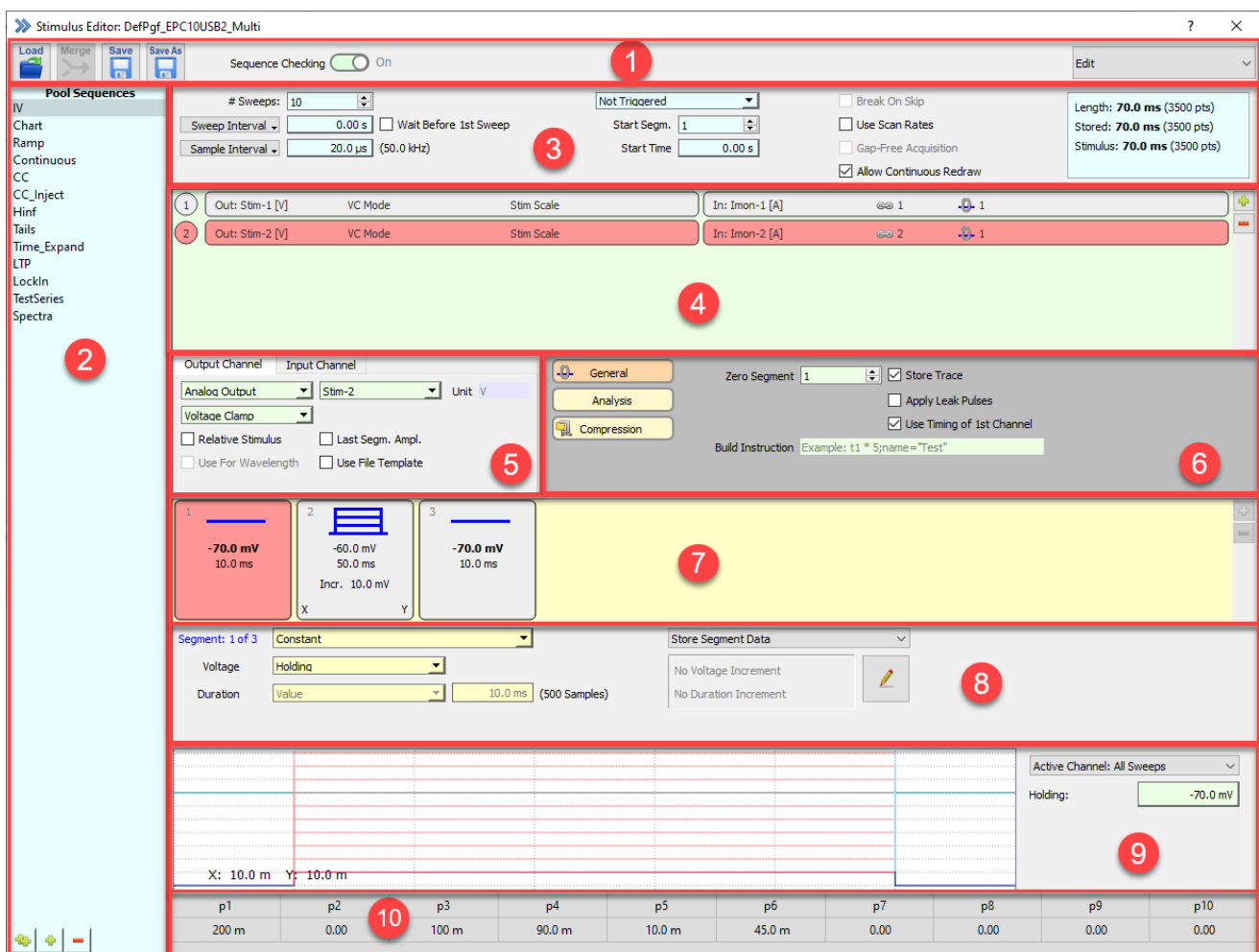


Figure 7.2: The ten sections of the Stimulus Editor window.

In addition, the three hierarchy levels of the Stimulus Editor provide some orientation:

1. Stimulus Level: Indicated by blue background colors
2. Channel Level: Indicated by green background colors
3. Segment Level: Indicated by yellow background colors

7.1 Global Section

The upper area of the Stimulus Editor window which we call here `Global Section` provides several options to organize the Stimulus Editor.

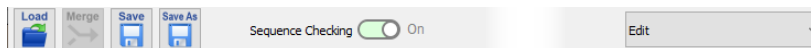


Figure 7.3: Global Section of the Stimulus Editor.

Load: Loads a Stimulus Editor file (*.pgf) with its stimulation sequences.

Merge: Loads another Stimulus Editor file (.pgf) into the currently opened file. In case a stimulation sequence name is not unique, you are prompted to either skip this stimulation sequence, mark it invalid, or give it a new name.

Save: Saves the stimulation sequences of the Stimulus Pool in the currently opened Stimulus Editor file.

Save As: Saves the stimulation sequences of the Stimulus Pool in a new Stimulus Editor file. The File Finder will open and you need to select a new destination to save the file and enter as well a name for the new Stimulus Editor file.

Sequence Checking: This option determines whether a check is performed for any inconsistencies that might occur when entering values. When this option is disabled, the validity checking of the sequence editing is suspended. This is convenient when one wants to perform multiple changes, especially when some intermediate steps would result in a (temporarily) faulty sequence. When this option is enabled, the active sequence is checked after any modification of segments and when storing, switching, or leaving the Stimulus Editor. If the input is faulty, the user is notified and the last operation is canceled, until the sequence is valid. The checking should be done at least at the end of the sequence input, before executing or storing a sequence.

Mode: The Stimulus Editor can be used in two different modes:

1. **Edit:** This is the default operating mode of the Stimulus Editor. It allows you to edit your stimulation sequences.
2. **Review:** This mode is for review purposes only and allows to check the stimulation sequences which was used for the selected Series of the Data Tree. It can only be enabled if there is a data file opened in the Data Tree.

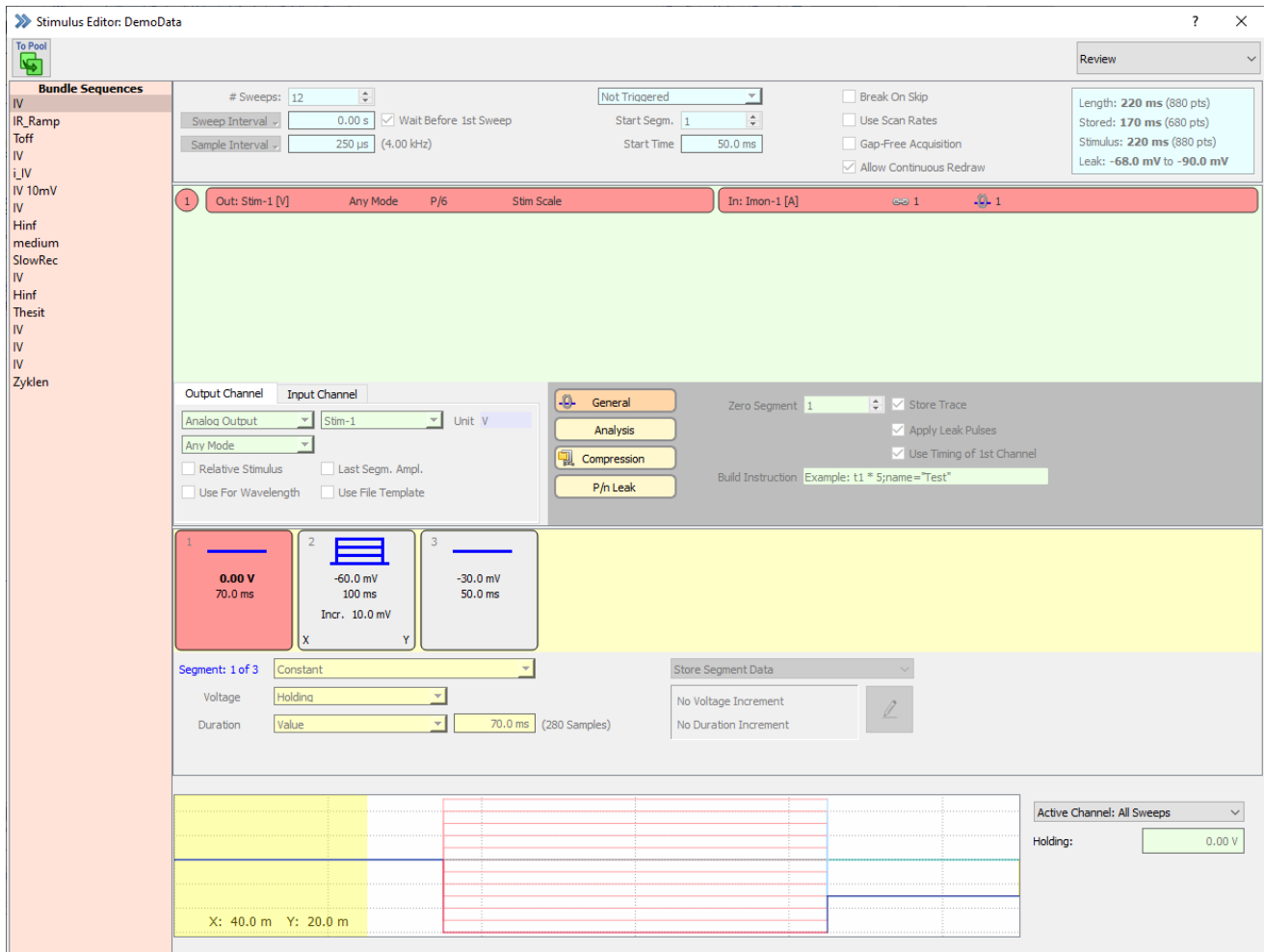


Figure 7.4: *Review Mode* of the Stimulus Editor. All settings are inactive and the Stimulus Pool indicates the currently selected stimulation sequence of the loaded data file.

To Pool: In the *Review Mode* all parameters and settings of the selected stimulation sequence are in read-only mode. One exception is the *To Pool* button on the upper left corner of the *Global Section* of the Stimulus Editor. It can be used if you want to copy the currently selected stimulation sequence to your loaded *.pgf file.

7.2 Stimulus Pool

On the left side of the Stimulus Editor the Stimulus Pool is shown. It is a list of stimulation sequences stored in the currently loaded Stimulus Editor file. A stimulation sequence is selected by clicking on it.

There are four options available to modify the Stimulus Pool content:

- **Edit Name:** Double-click inside the stimulation sequence name to change its name.
- **Duplicate Sequence:** Use the small button at the bottom with the two green plus signs to copy the selected stimulation sequence. The name is taken over but an index number is added.
- **Add new Sequence:** Use the small button at the bottom with one green plus sign to create a new and empty stimulation sequence. The new stimulation sequence will be label “New Sequence”.
- **Delete Sequence:** Use the small button at the bottom with the red minus sign to delete the selected stimulation sequence from the Stimulus Pool.

Note: All these options are also available via a right-click context menu.

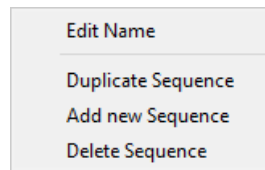


Figure 7.5: Right-click context menu of the Stimulus Pool.

7.3 General Stimulus Settings

The `General Stimulus Settings` section contains all settings which targets the whole stimulation sequence.

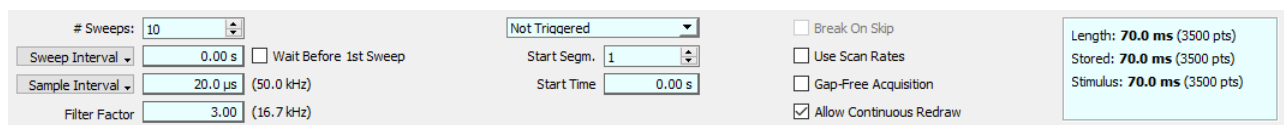


Figure 7.6: General Stimulus Settings of the Stimulus Editor.

Sweeps: Determines the number of Sweeps within one stimulation sequence, e.g., how many times the specified pulse pattern is run.

Sweep Interval: The first Sweep of a sequence has a waiting period during which the stimulation template is computed and loaded to the AD/DA interface. Then the first Sweep is acquired. The next Sweep will start a defined *Sweep Interval* duration after the beginning of the first Sweep. Defining a *Sweep Interval* is useful if you are using constant length stimulation pulses.

Sweep Delay: When you define stimulation pulses with changing length you should use the *Sweep Delay*. Here, the time between the end of a stimulation pulse and the beginning is kept constant, independent of the length of the stimulation pulse.

Wait before 1st Sweep: Leave this checkbox inactive if you want to start with your stimulation sequence without a delay. If activated PATCHMASTER NEXT will wait the defined *Sweep Interval* time before executing the stimulation sequence.

Sample Interval: The timing of data acquisition can be set either as the *Sample Interval* (in seconds) or the *Sample Rate* (in Hz). Use the drop-down menu to select your preferred setting. Whatever you prefer, the value for the second option is always displayed beneath the entry field for your convenience.

Sampling Rate: The timing of data acquisition can be set either as the *Sample Interval* (in seconds) or the *Sample Rate* (in Hz). Use the drop-down menu to select your preferred setting. Whatever you prefer, the value for the second option is always displayed beneath the entry field for your convenience.

Note: If the Sample Interval is so long as to yield only one point per segment, PATCHMASTER NEXT asks whether the segment duration should be fixed, e.g., set to a higher value.

Filter Factor: The *Filter Factor* is used to define the automatic filter setting relative to the sample rate. This option gets active and visible in the Stimulus Editor when the *Auto Filter* option in the Amplifier window is activated (see chapter 4.1.1.2 on page 45).

Example: for *Sample Interval* = 250 μ s (4 kHz) and *Filter Factor* = 4, a filter cutoff frequency closest to 1 kHz (= 4 kHz / 4) will be selected.

Trigger Mode: Determines if and how the data acquisition is triggered by an external TTL pulse. The default is *Not Triggered*, which means that stimulation is immediately elicited by the user within PATCHMASTER NEXT. Otherwise you have to activate the stimulation sequence execution by one or more external triggers.

The following options are available in the selection list:

- Not Triggered: PATCHMASTER NEXT does not wait for any external triggers but start to execute the stimulation sequence immediately.
- Trigger Series: One trigger is needed to execute the full stimulation sequence.
- Trigger Sweeps (Main & Leaks): A trigger is required for the main pulse and for each applied single pulse.
- Trigger Sweep (Main Only): One trigger is required to start the complete Sweep, main as well as leak pulses.

Note: If one wants to use the Trigger Mode together an EPC 10 USB patch clamp amplifier you need to connect your external trigger device to the Trigger Input connector at the front panel of the EPC 10 USB.

Start Segment: Defines the segment for the start of data acquisition.

Start Time: Defines the time in the set *Start Segment* for data acquisition.

Note: Start Seg and Start Time are referring to the start of the data acquisition (storing of data). The full stimulation template is always output.

Break On Skip: Terminates the acquisition of the Sweep if the user presses the *Skip* button in the Control window (see chapter 3.2 on page 20). The acquisition then continues with the next Sweep.

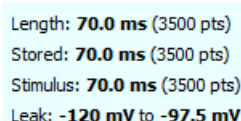
Note: The delay between the break condition and leaving the acquisition routine can be up to 50 ms. Further the total Trace length needs to be >1 s.

Use Scan Rates: Ramp segments can be specified either by their duration or by their *Scan Rate* (V/s). If the *Scan Rate* is given by the user, the duration will be calculated (see also chapter 7.8 on page 118).

Gap Free Mode: This option allows a timely precise acquisition of Sweeps without any gap in between. If you do not want to modify the recording settings during acquisition we highly recommend to select this option.

Allow Continuous Redraw: Check this option to allow continuous display of the data in the Oscilloscope during acquisition. Minimum required Sweep length is 1 second to activate this function. This option is active by default.

Sweep Information: The *Sweep Information* area on the right side of this section is a read-only field showing the length of the executed Sweep and stored data as determined by the timing, compression and segments settings.



Length: **70.0 ms** (3500 pts)
 Stored: **70.0 ms** (3500 pts)
 Stimulus: **70.0 ms** (3500 pts)
 Leak: **-120 mV to -97.5 mV**

Figure 7.7: Sweep Information of the General Stimulus settings.

- Length: Denotes the total duration of the longest Trace of the Sweep in “ms” and “points” (stored or not stored).

Note: If the total Sweep length exceeds Max. Sample Points (see chapter 3.5.1 on page 24) a warning appears. If you would like to acquire longer Sweeps or a Sweep at higher time resolution, you might have to increase the Max. Sample Points parameter in the Configuration.

- **Stored:** Denotes the total duration stored of one Sweep of the given sequence in “ms” and “points”.

Note: All Traces contribute to the Sweep size but not to the Sweep Length. Length and Stored duration may be different when a Start Segm. and Start Time are set.

- **Stimulus:** Denotes the duration of the selected stimulus signal in ‘ms’ and ‘points’. This can be shorter than the Sweep length, e.g., a short trigger impulse.
- **Leak:** The minimum and maximum voltage which will be applied during the leak pulses is shown here. The calculation of the minimum and maximum values of the leak pulses is:

$$L(i) = (V(i) - V_h) * size + L_h \quad (7.1)$$

i=pulse number, V(i)=voltage of segment i, L(i)=voltage of leak segment, V_h=holding potential, L_h=leak holding, size=leak size;

Example: i=2, V(2)=-50 mV, L(2)=voltage of leak segment, V_h=-70 mV, L_h=-120 mV, size=0.25;

$$L(2) = (-50mV + 70mV) * 0.25 - 120mV = \underline{-115mV} \quad (7.2)$$

7.4 Channel View

In the Channel View section of the Stimulus Editor the defined output and input channels of the selected stimulation sequence are listed.

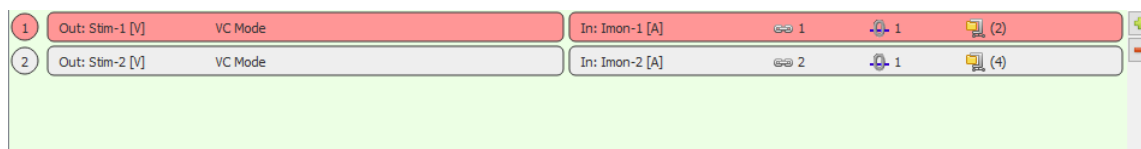


Figure 7.8: Channel View section of the Stimulus Editor.

The number in front of each line defines the channel number. The output channels are listed on the left side, the input channels on the right side of the section.

To add or remove a channel row one can use the small plus button (green) or minus button (red) on the right side. If one adds e.g. another channel via the green plus button the following dialog appears:

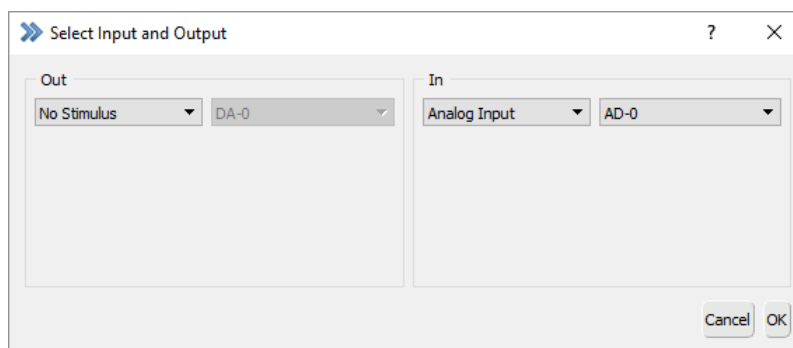


Figure 7.9: Adding a new channel to the stimulation sequence.

The settings for this dialog are identical to the ones you can set via the two Output Channel and Input Channel tabs which are explained in the chapter Channel Settings (see 7.5 on the next page).

The currently selected channel is highlighted with a red background color:



Figure 7.10: A selected channel is highlighted with a red background color.

A channel which is not stored with the data file is highlighted with dotted border lines around the boxes:

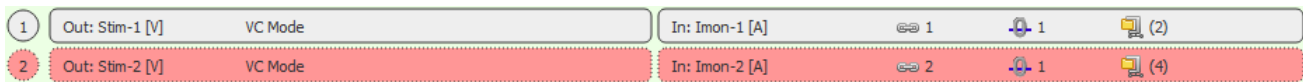


Figure 7.11: Non-stored channels are highlighted with dotted box borders.

A right-click on one of the output channels opens a context menu providing the following options:

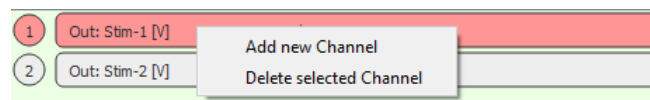


Figure 7.12: Right-Click context menu of output channels.

- Add new Channel: Opens the *Select Input and Output* dialog (see above).
- Delete selected Channel: Deletes the selected Channel.

A right-click on one of the input channels opens a context menu providing the following options:

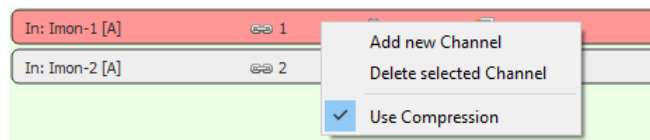


Figure 7.13: Right-Click context menu of input channels.

- Add new Channel: Opens the *Select Input and Output* dialog (see above).
- Delete selected Channel: Deletes the selected Channel.
- Use Compression: Activates the *Compression* settings for the selected input channel. The *Compression* parameters are explained below in chapter 7.6.3 on page 101.

7.5 Channel Settings

In the *Channel Settings* section of the *Stimulus Editor* one defines the output and input channels of the stimulation sequence.

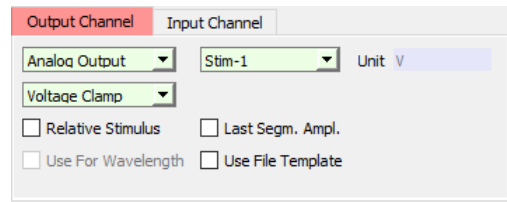


Figure 7.14: Channel settings section of the Stimulus Editor.

7.5.1 Output Channels

All settings listed in the `Output Channels` tab are related with the selected output channel.

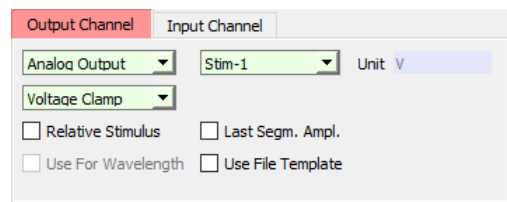


Figure 7.15: Output Channel options of the Stimulus Editor.

Output:

- No Stimulus: No output is defined for this channel.
- Analog Output: An analog output channel can be defined from the selection list beneath:
 - DA-0 / Stim-4: Digital/Analog channel "0" or Stimulation channel "4".
 - DA-1 / Stim-3: Digital/Analog channel "1" or Stimulation channel "3".
 - DA-2 / Stim-2: Digital/Analog channel "2" or Stimulation channel "2".
 - DA-3 / Stim-1: Digital/Analog channel "3" or Stimulation channel "1".

Depending on the used hardware the list entry is either label "DA-X" or "Stim-X". "DA-X" means that this is a freely available digital analog output channel which can be accessed on the front panel of the EPC 10 USB. Channels labeled "Stim-X" address the headstage of the individual amplifier board.

- Digital Word: A decimal value is converted into a binary value. This setting is especially useful when you want to trigger different or multiple digital output channels in a stimulation sequence.
- Digital Output: Sets one of the 16 digital output channels which can be either activated or inactivated in your stimulation sequence.
 - DigOut-0...DigOut-15

Digital outputs are mainly used for controlling (triggering) other devices, such as perfusion systems, filter wheels, flash lamps, or others. The EPC 10 USB amplifier (and the LIH 8+8) are equipped with 16 digital output lines. These outputs can be accessed from the rear panel of the amplifier. The first three of the 16 available digital outputs can be accessed also from the front panel of the amplifier (interface). The BNC outputs ("Trigger-Outputs") "Out-0", "Out-1", "Out-2" correspond with the out channels "DigOut-0", "DigOut-1", and "DigOut-2" in the Pulse Generator dialog of PATCHMASTER NEXT.

There are two ways to control the digital lines:

1. If only one digital line is intended to be used, then one of the "Digital Output" lines ("DigOut-0" to "DigOut-15") can be selected as output channel. In the `Segments` section of the Stimulus Editor the state of the digital line can be set either to "1" (active = TTL level), "-1" (inactive = 0 V), or to "0" (don't change, use the state that is set in the I/O control dialog).
2. The *Digital Word* output allows to switch several digital lines simultaneously. What channels are switched is defined in the `Segments` section by the corresponding bit value. If *Voltage* in the `Segments` section is set to "7", then the first three digital lines are turned on: $1 \times 10^2 + 1 \times 10^1 + 1 \times 10^0 = 4 + 2 + 1 = 7$.

Note: For such trigger channels it is suggested to switch from *Common Timing* to *Separate Timing* (see chapter 7.6.1 on page 99). With this setting, the duration and number of segments is completely independent from the first (main) channel.

Unit: Sets the unit of the output channel. The default settings are either "V" for voltage or "A" for current. Some channels settings allow to edit the unit entry.

Recording Mode: Stimulation sequences can be restricted to *Voltage Clamp* or *Current Clamp* modes. The following options are available:

- Voltage Clamp: Restricts the output channel to voltage clamp mode.
- Current Clamp: Restricts the output channel to current clamp mode.
- Any Mode: Allows the stimulus to be executed in voltage clamp or current clamp mode. This is a useful setting when using the photometry extension.

Relative Stimulus: The voltage or current applied to the output channel is relative to the set *V-membrane* or *I-membrane* value in the Amplifier window.

Last Segm. Ampl.: At the end of the Sweep, *V-membrane*/*I-membrane* is set to the amplitude of the last segment of the stimulation sequence, e.g., -90 mV, if this was the potential of the last segment. Normally, the potential is reset to the holding potential/current.

Use For Wavelength: This checkbox gets available once the `Photometry` extension is activated in the Configuration (see chapter 3.5.2.3 on page 25). Amplitudes of segments can then be entered in "nm".

Use File Template: When activating this checkbox the `File Template Editor` is opened. The functions and parameters of this dialog are explained in the section below (see chapter 7.5.1.1). Once the option is activated another button called "Edit Template" will appear.

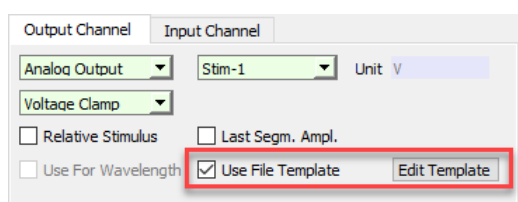


Figure 7.16: Once a file template is defined for the stimulus output in the Stimulus Editor the button "Edit Template" appears which allows to re-open the File Template Editor to modify or change the file template.

7.5.1.1 File Template Editor

The `File Template Editor` is a very useful tool if you want to use e.g. already recorded data as a stimulus template for your next recordings. Further, one can use modified traces which are stored in the buffers or import other data in the ASCII or Igor format.

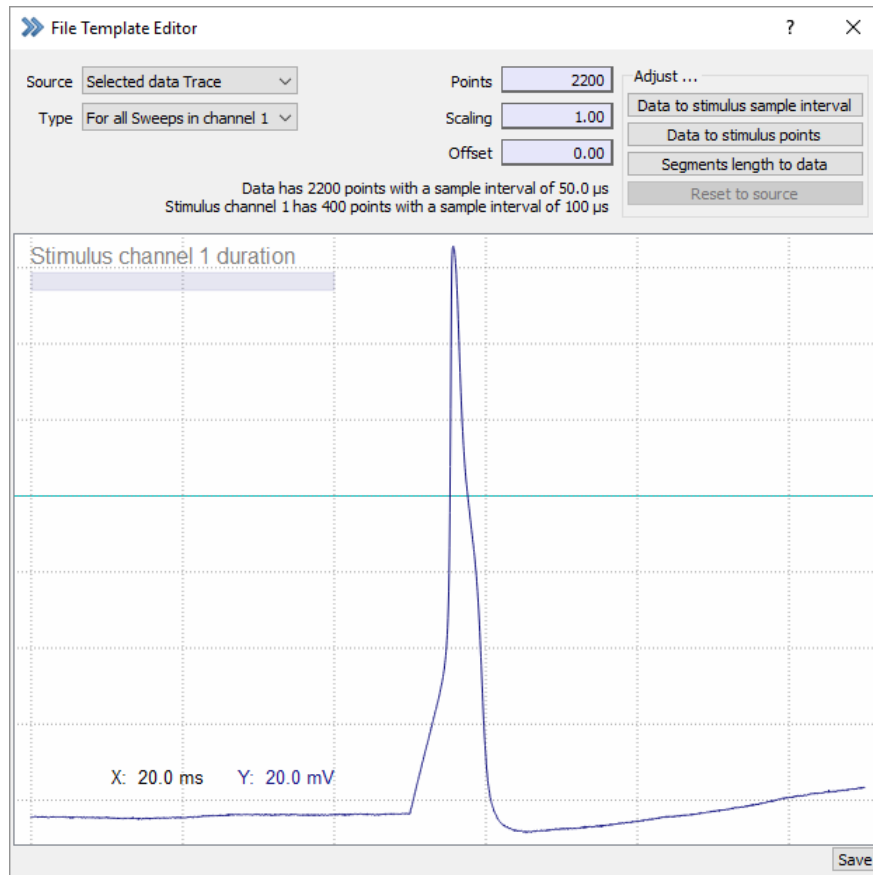


Figure 7.17: File Template Editor of the Stimulus Editor.

For a better orientation we divided the File Template Editor into four sections. They are:

1. Source Selection
2. Data Adjustment
3. Stimulus Indicator
4. Stimulus Template Preview

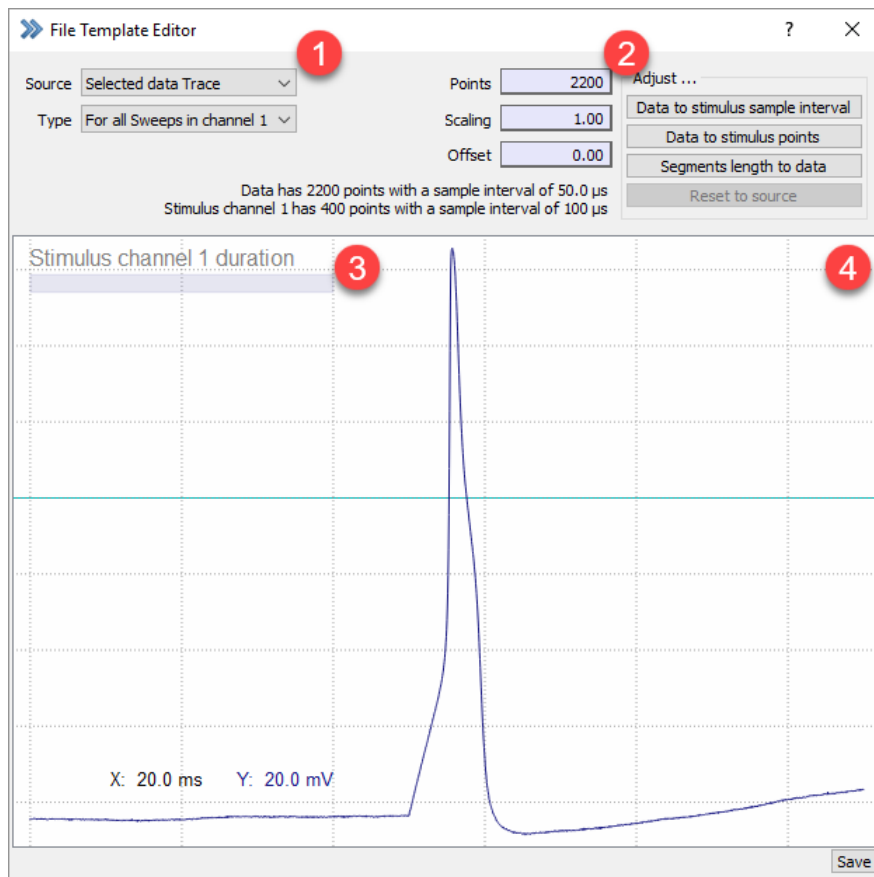


Figure 7.18: Sections of the File Template Editor.

Source Selection

Source: Select the source for the file template. The following options are available:

- No Data: This option is selected automatically if no data is displayed in the Oscilloscope window which could be used as a file template. However, starting from here one can load ASCII or Igor files.
- Existing Template File: Once a selection for a file template was made and stored via the “Save” button this option will be selected.
- Buffer 1...4: Uses the buffer content as a file template. The Buffers can be used to store data from other data files.
- Selected Data Trace: Uses the currently selected and displayed data trace as a file template.
- Import ASCII: Select this option to load an ASCII file which should be used as a file template.
- Import Igor: Select this option to load an Igor file which should be used as a file template.

Type: Specify if the file template should be used for one Sweep only or for all Sweeps of the active DA output channel. Two options are available:

1. For all Sweeps in channel X: Applies the file template for all Sweeps of the selected DA channel output.
2. For One Sweep: The file template is only output for one Sweep. Once you select this option another entry field below is created where one can specify the Sweep index number of the corresponding file template.

Data Adjustment

Points: Display and entry field for the number of data points of the currently selected file template.

Scaling: Display and entry field for scaling the currently selected file template.

Offset: Display and entry field for applying an offset to the selected file template.

Note: While applying changes either to the file template or the stimulus sequence the information provided below the Offset field provides useful information about the current status. Further, the Stimulus Indicator described below provides useful graphic information about the duration of file template and stimulus template.

Data to stimulus sample interval: Adjusts the number of data Points available to the selected Sample Interval of the stimulation sequence. However, even if the Sample Interval is now equal there can still be a mismatch of data points between file template and stimulus sequence pattern. A second click on this option resets the previous adjustment.

Data to stimulus points: Adjusts the number of data points of the file template to the number of stimulus points provided by the stimulation sequence. This could also mean that the number of Points of the file template is changed e.g. by cutting off data points at the end of the file template. Use *Reset to source* to undo the adjustment.

Note: This option does not adjust any difference in the Sample Interval of file template and stimulus sequence.

Segments length to data: Adjusts the stimulus sequence duration to match the number of Points of the file template.

Note: This option does not adjust any difference in the Sample Interval of file template and stimulus sequence.

Reset to source: Resets all changes made to the file template.

Stimulus Indicator

The *Stimulus Indicator* shows the defined stimulus template length of your selected PGF sequence (total duration time of all segments) in relation to the selected stimulus file template. In best case the *Stimulus Indicator* does match the length of the stimulus file template.

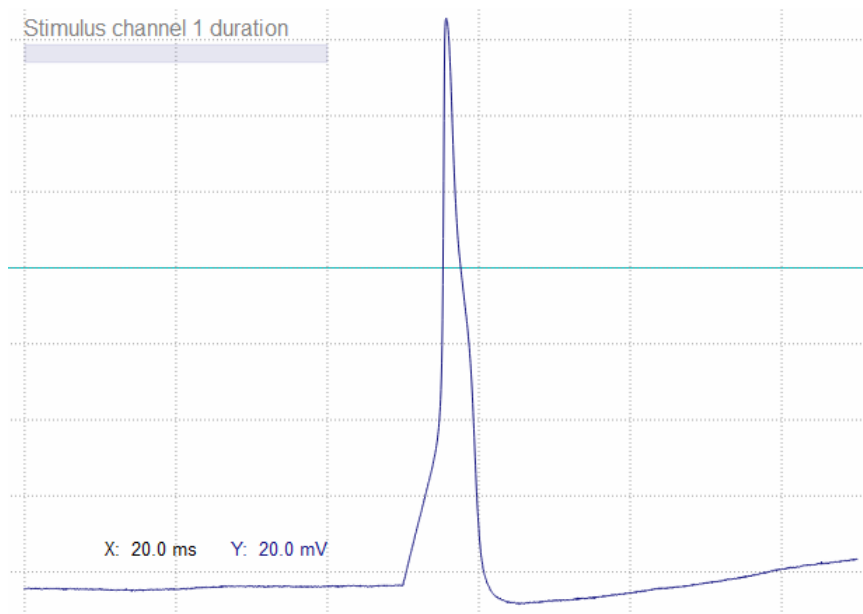


Figure 7.19: The Stimulus Indicator of the File Template Editor shows the relation between the duration of the selected stimulation sequence and the file template duration.

Stimulus Template Preview

In the *Stimulus Template Preview* the loaded stimulus template is depicted. Further, one can see the relation of the stimulus and the stimulus channel (*Stimulus Indicator*).

Save:

Use the *Save* option to save the template for the stimulation sequence as a *.tpl in your "PM Next" folder (C:\Users\Username\Documents\HEKA\PM Next).

7.5.2 Input Channel

The *Input Channel* section allows you to set the properties of the AD input, e.g., the acquired data. DA and AD settings are basically independent from each other. Their reference is only given by the variable *Linked Channel* in the AD settings (see chapter 7.6.2 on page 101). This allows you to associate several AD inputs to the same DA stimulation.

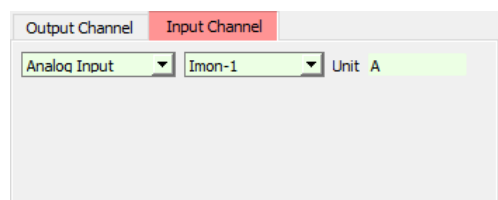


Figure 7.20: Input Channel options of the Stimulus Editor.

Input:

- Off: No input is defined for this channel.
- Analog Input: An analog input channel can be defined from the selection list beneath:
 - AD-0 / Vmon-4: Analog/Digital channel "0" or Voltage Monitor "4".

- AD-1 / Vmon-3: Analog/Digital channel "1" or Voltage Monitor "3".
- AD-2 / Imon-3: Analog/Digital channel "2" or Current Monitor "3".
- AD-3 / Vmon-2: Analog/Digital channel "3" or Voltage Monitor "2".
- AD-4 / Imon-2: Analog/Digital channel "4" or Current Monitor "2".
- AD-5 / Vmon-1: Analog/Digital channel "5" or Voltage Monitor "1".
- AD-6 / Imon-1: Analog/Digital channel "6" or Current Monitor "1".
- AD-7 / Imon-4: Analog/Digital channel "7" or Current Monitor "4".

Depending on the used hardware the list entry is either label "AD-X" or "Vmon-X"/"Imon-X". "AD-X" means that this is a freely available analog input output channel which can be accessed on the front panel of the EPC 10 USB. Channels labeled "Vmon-X" or "Imon-X" address either the current or voltage monitor of of the individual amplifier boards.

- Digital Word: Records the sum of activated digital input channels in one data trace.
- Digital Input: Defines one of the 16 digital inputs as an input channel.
 - DigIn-0...DigIn-15

The EPC 10 USB amplifier (and the LIH 8+8) are equipped with 16 digital input lines. These inputs can be accessed from the rear panel of the amplifier.

- Virtual Trace: A virtual Trace is created to store data coming e.g. from a source different from an ADC, e.g., external data loaded via the *Import Trace* option, or data which generate virtual Traces. The following virtual Traces are available:
 - LockIn_CM
 - LockIn_GM
 - LockIn_GS
 - LockIn_Real (Y)
 - LockIn_Imag (Y)
 - LockIn_DC
 - LockIn_Average
 - LockIn_CV
 - LockIn_GP
 - LockIn_Admit (Y)
 - LockIn_Phase
 - LockIn_Real (Z)
 - LockIn_Imag (Z)
 - LockIn_Impedance (Z)
 - Chirp_Average
 - Chirp_Phase
 - Chirp_Admit (Y)
 - Chirp_Real (Y)
 - Chirp_Imag (Y)
 - Chirp_Real (Z)
 - Chirp_Imag (Z)
 - Chirp_Impedance (Z)

- Photo_W1
- Photo_W2
- Photo_W3
- Photo_Ratio
- Photo_Ca

Note: Please note that usability of these traces depends on the activation of these extensions in the *Configuration*. A description of their purpose is given in the individual chapters dealing with these extensions.

Unit: Sets the unit of the input channel. The default settings are either “V” for voltage or “A” for current. Some channels settings allow to edit the unit entry.

7.6 Parameter Section

On the right side beneath the output and input channel definitions there is another section which we call “Parameter Section” offering e.g. “General” channel settings or “Analysis” settings.

7.6.1 General

The “General” settings contains options which do not fit into any other category.

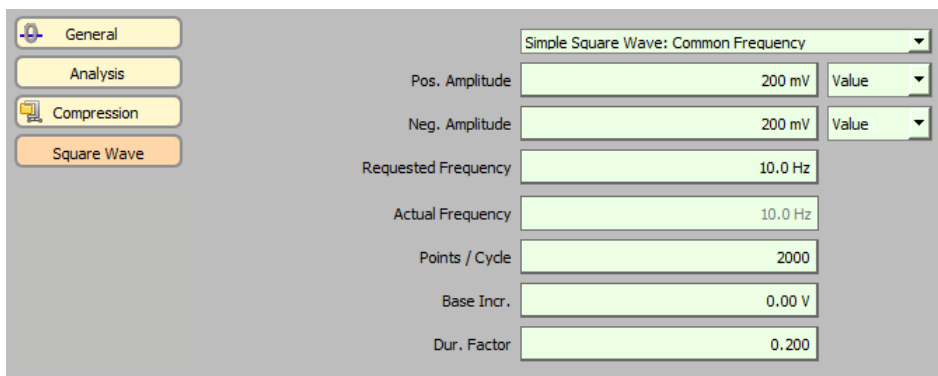


Figure 7.21: General settings of the Stimulus Editor.

Zero Segment: Defines the number of the segment on which the base of the *Zero Offset* will be calculated. Usually the *Zero Offset* is calculated from the second half of that segment to avoid contamination by tails of capacitive transients. In order to specify a custom range for the *Zero Offset* calculation please specify a *Start Segment* and a *Start Time* >0. Then the *Zero Offset* will be calculated between *Start Time* and end of the segment.

Note: When the segment number is set to “OFF”, no *Zero Offset* subtraction will be calculated.

Store Trace: If enabled, the data acquired for this channel will be stored.

Note: In case *Store* is not activated but the file is opened with write permission, the last *Sweep* will be stored temporarily. This *Sweep* is marked with a “#” in its label. It will be deleted when the next *Sweep* is acquired or a new *Group* is created or the file is closed.

Apply Leak Pulses: If selected, leak templates are output (see chapter 7.6.4 on page 102). By activating this option the Leak Traces storing option is set by default to "Store Average".

Note: The P/n Leak section is hidden in the Stimulus Editor as long as this flag is not activated.

Use Timing of 1st Channel: This option is only available for channel number "2" or higher. If activated it allows to define separate channel timing for each channel. If this option is not selected all channel will have the timing defined in channel number "1".

Build Instructions: *Build Instructions* allow to compute a virtual Trace from other Traces and inputs. Therefore, you can use e.g. mathematical operations in a kind of formula to perform the calculations. A very simple example would be to create a virtual trace plotting the resistance change during the recording. For that operation one would use the voltage trace divided by the current trace.

The *Build Instruction* string starts with the mathematical operation. The formula is terminated by a semicolon (;). Then a list with additional instructions all separated by a comma (,) can follow.

Mathematical Operations

- An: Anodic integral of Trace with index n; Example: 'A1'
- Bn: Trace buffer with index n; Example: 'B1'
- Cn: Cathodic integral of Trace with index n; Example: 'C1'
- Dn: Differential of the Trace with index n; Example: 'D1'
- In: Integral of the Trace with index n; Example: 'I1'
- M: Mean of a Trace. It must be followed by a bracket enclosing 3 numbers, separated by comma:
 1. The Trace count of the Trace from which to compute the mean
 2. The time offset (in [s]) from the beginning of the stored Trace data.
 3. The time span (in [s]) over which to compute the mean.
 Example: 'M(2,0.0,0.005)' (Mean of Trace 2 with no offset and a calculated mean along 5 ms)
- Tn: Trace with index n; Example: 'T1'
- Vn: Value with index n; Example: 'V1'
- Zn: Zero data of Trace with index n; Example: 'Z1'
- Operators: +, -, *, /
- Brackets: ()

Other Assignments

- Name="String" (separated from the math operation by a semicolon ';').
Example: 'T1; Name="Current"' (Trace 1 is labeled "Current" in the Data Tree)
- No LockIn: With this build instruction one can suppress the the LockIn computation of the trace.
Example: ';No LockIn'
- S: Stands for "Shift". It must be followed by a bracket enclosing 2 numbers, separated by comma:
 1. The source Trace index

2. The number of samples by which to shift the source Trace

Example: 'S(2,50)' = Trace with index "2" shifted by 50 samples to the beginning of the Trace. Negative numbers shift the data towards the end of the trace. The undefined data will be set to invalid (NaN). Invalid data points are not displayed.

- Slave: Copying the Trace data from the "slave" PATCHMASTER NEXT instance via the batch communication protocol. The equal sign must immediately follow "slave". The given number is the Trace count of the Trace to get.

Example: ';Slave=2' (The source Trace with Trace count = "2" is read).

- StimSwp: Only Sweeps defined in the list will output the respective segment amplitudes. The other Sweeps will use the amplitude of segment '1' for all segments. Values are to be separated by commas, and Sweep ranges can be defined by a hyphen.

Example: ';StimSwp(1,3-5,7-9)' (Sweeps 1,3,4,5,7,8 and 9 will use the specified segment amplitudes).

- Tcount: Trace count with a value between 1 and 16.

Example: ';TCount=5' (Virtual Trace gets Trace index "5" in the Data Tree)

- VC-Switch/CC-Switch: Use this build instruction for rapid switching of Recording Modes with a Sweep. The number defines the segment for the switch. **Example:** ';VC-Switch=2' or ';CC-Switch=3' (Switches the Recording Mode to Voltage Clamp in segment "2" or to Current Clamp in segment "3").

- Yrange=real: The "Yrange" sets the scaling of the Oscilloscope display. The value of "Yrange" corresponds to the total length of the Y-axis. 1/10 of "Yrange" corresponds to the scale bar displayed in the Oscilloscope window. **Example:** ';Yrange=1E-6' (Defines the Y-axis range to 1E-6).

- Yrange([N],[factor]). Sets the "Y-range" of a Trace proportional to the value of another Trace. "N" is the Trace index of the source Trace and "factor" is the factor to multiply.

Example: ';Yrange(1,0.5)' (Targets Trace 1 and the Y-range is scaled by the factor "0.5").

Note: The total length of the Build Instruction is limited to 128 characters.

Note: No other operator priority is considered besides the brackets.

Further examples:

Example 1: V1*T1;Name="m1", Yrange=1E-9

Trace 1 is multiplied with Value 1. The name of the new virtual Trace is "m1" and the total range of the Y-axis is 1e-9.

Example 2: V1*T1+(T6-T7);Name="Baseline",Yrange=1E-6

Trace 1 is multiplied with Value 1 and the difference between Trace 6 and Trace 7 is added. The name of the new virtual Trace is "Baseline" and the total range of the Y-axis is 1e-6.

Example 3: I1; Name="Charge", TCount=5, Yrange=1E-5

Trace 1 is integrated. If Trace 1 is a current Trace we get the charge. The new virtual Trace gets the name "Charge", the total range of the Y-axis is 1e-5 (i.e. 10 μC). The Trace # is set to "5".

The *Build Instruction* can also be used to give a particular Trace a name (label). This is convenient for having the name of the Trace displayed in the Data Tree.

It is also possible to specify an equation as a build instruction similar to the equation handling in the Analysis Editor (see chapter 9.4.1.8 on page 186), the Buffer menu (see chapter 11.1.3 on page 200) or the Equation Editor dialog (see chapter 17 on page 235). It is important to put the equation in double-quotes and start at the first character.

Example 4: "t[1]-t[2]" or "sin(y)".

7.6.2 Analysis

The “Analysis” channel setting section contains options related to the analysis of the data.

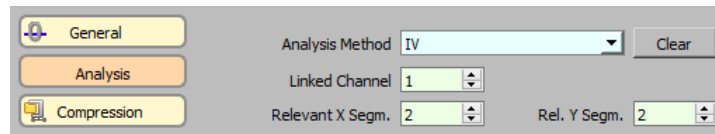


Figure 7.22: Analysis settings of the Stimulus Editor.

Analysis Method: Select an *Analysis Method* from the selection list which should be executed automatically during the data acquisition. Note that *Automatic Stimulus Control* has to be activated in the Analysis Editor to activate the link.

Clear: Via this button one does reset the *Analysis Method* selection. In that case no *Analysis Method* is selected.

Linked Channel: Number of the stimulus channel that is associated with this input. This setting is important for later analysis of the Trace. It links the stimulus segments to the recorded Trace therefore providing information about the underlying stimulus pattern of this input channel.

Relevant X Segm.: The *Rel. X Segm.* (relevant X segment) specifies a segment of interest that is mainly used as X-axis reference for later analysis.

Rel. Y Segm.: The *Rel. Y Segm.* (relevant Y segment) specifies the segment where the analysis is performed (e.g., determination of peak current).

For the measurement of an h-infinity curve, for example, the relevant X segment would be the conditioning segment of variable voltage, while the relevant Y segment would be the test segment, where the peak current is determined.

7.6.3 Compression

The compression factor for an input channel should be chosen such that the compression interval (or range) covers the complete period of interest. The number of data points divided by the number of compressed data points yields the compression factor.

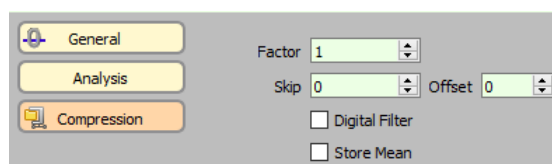


Figure 7.23: Compression parameters of the Stimulus Editor.

Factor: Enter the *Compression Factor* for the data compression.

Skip: With the *Skip* option, a specified number of points of the compression interval can be skipped before the compression starts. In a compression interval, you can only skip data points at the beginning of the interval.

Offset: With this option one can influence the start of the compression interval. A negative offset moves the start of the compression interval to the left and a positive offset to the right. A negative offset essentially adds dummy data points on the negative time axis. Since compression starts at the first recorded point, the compression range is moved to the left (towards smaller times) with respect to the data points.

Digital Filter: If selected, the raw data are filtered digitally before compression. The filter factor is given by the compression factor. Example: If a Trace is acquired with at sampling rate of 5 kHz and the compression factor is

"10", then the digital filter is set to 500 Hz. With a compression factor of "5" the filter bandwidth is increased to 1 kHz.

Store Mean: If this checkbox is activated all data points of the compression interval are averaged. If this checkbox is left unchecked one point per compression interval is taken. All data is stored as 4-byte real.

Note: For an example describing the use of *Skip* and *Offset* compression functions please refer to the chapter *High Speed Fluorescence Measurements in the PATCHMASTER Tutorial*.

7.6.4 P/n Leak

The "P/n Leak" channel setting section contains all options for the leak subtraction (P/n subtraction) of incoming data.

Note: To get this section displayed one needs to activate the *Apply Leak Pulses* option in the *General settings* (see chapter 7.6.1 on page 99).

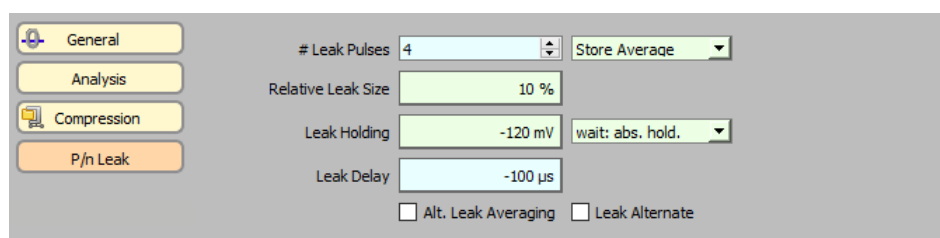


Figure 7.24: P/n Leak channel settings of the Stimulus Editor.

Leak Pulses: Define the number of leak pulses which should be applied before or after the Sweeps. An arbitrary number of leak pulses are supported.

Leak Storage: Via this selection list one defines if and how the leak pulses should be stored with the data file. The following options are available:

- No Leak: No leak correction is applied and no leak traces are stored.
- Store Average: Executes all leak pulses and stores the average leak response.
- Store Each: Executes all leak pulses and stores every leak response separately.
- Store None: Executes the leak pulses but the leak responses are not stored.

Note: The main data are always stored with leak correction performed. I.e., to replay non-corrected data, the leak responses have to be stored.

Note: The leak traces storing method is set by default to "Store Average".

Relative Leak Size: Determines the amplitude of the leak pulses relative to the main pulse in [%]. The negative sign determines if the relative amplitude is negative or positive before applied to the *Leak Holding*.

Leak Holding: Specifies the potential from which leak pulses are generated.

Note: It is important to set the *Leak Holding* to a potential which does not cause e.g. any channel activity and which does allow leak pulses not activating any channels, too.

Wait: The *Wait* option defines what potential should be applied during waiting periods of the leak pulses. The following options are available:

- wait = abs. hold: Keeps the cell potential at the chosen absolute holding potential during wait periods.

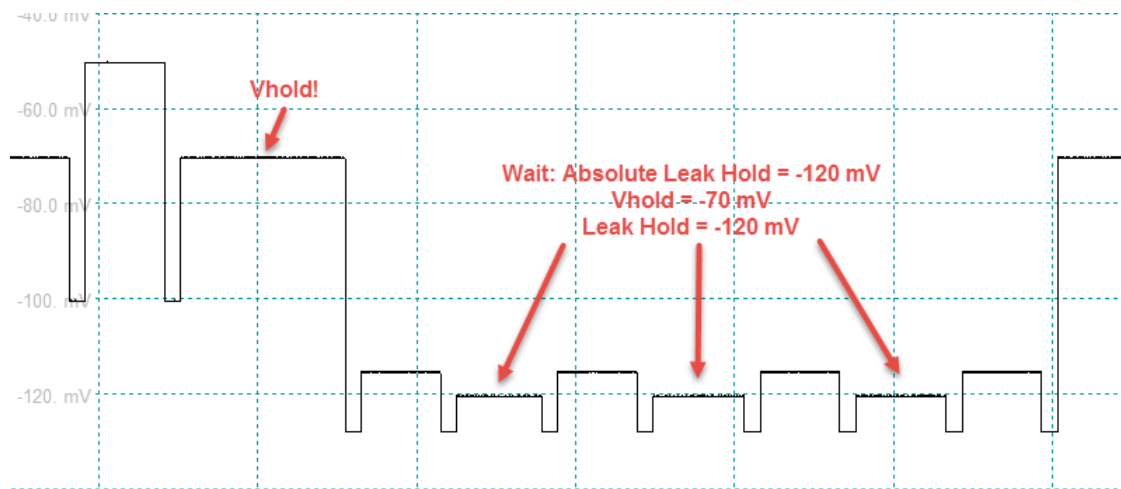


Figure 7.25: P/n Leak Pulses (4) with "wait = abs. hold" enabled.

- wait = rel. hold: Keeps the cell potential at the chosen relative holding potential during wait periods.

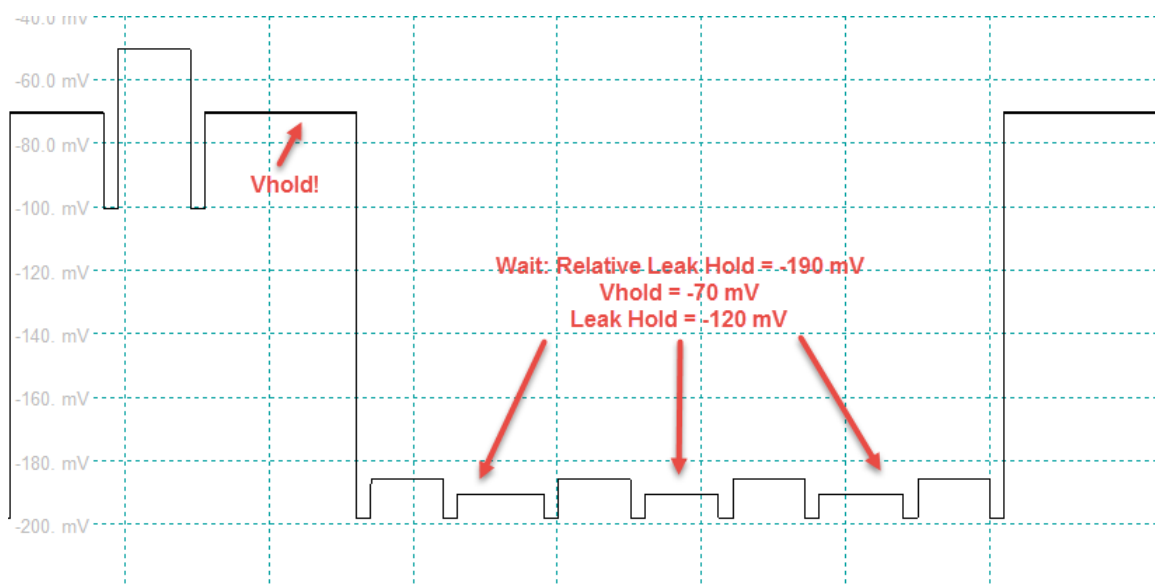


Figure 7.26: P/n Leak Pulses (4) with "wait = rel. hold" enabled.

- wait = abs. leak hold: Keeps the cell potential at the chosen absolute leak holding potential during wait periods.

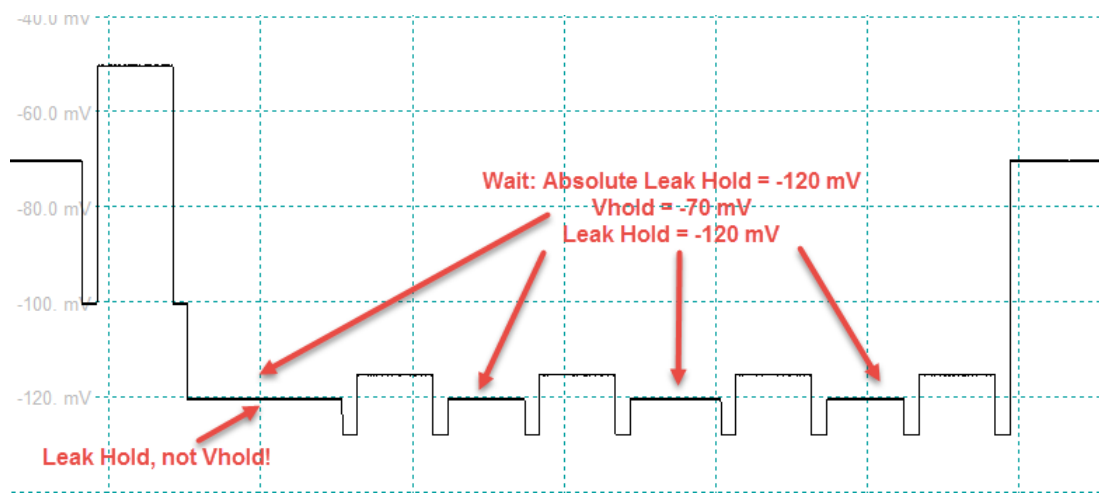


Figure 7.27: P/n Leak Pulses (4) with "wait = abs. leak hold" enabled.

Leak Delay: *Leak Delay* defines a waiting time between the leak pulses and the main pulse. In between leak pulses no extra waiting time is supported. P/n pulses precede the test pulse if a negative *Leak Delay* is supplied or they follow it if *Leak Delay* ≥ 0 .

Alt. Leak Averaging: This procedure was intended to be used with the average function provided in the Control window (see chapter 3.2 on page 20). The *Average* function acquires a given number of averages acquired for one Sweep. Only the average is stored to disk. *Alt. Leak Average* provides the option to generate leak pulses of alternating polarity while averaging. That means, if the number of averages in the Protocol Editor is set to "4", then the polarity of the leak pulses for the 2nd and 4th average is inverted. Averaging of the leak pulses thereby eliminates slow capacitive currents arising from the jump from holding to *Leak Hold*.

Note: An even number of Sweeps to average is required to give correct results. The entire leak template of every other Sweep is then inverted.

Leak Alternate: If *Leak Alternate* is on, leak pulses of alternating polarity are generated.

The following figure illustrates a pulse template.

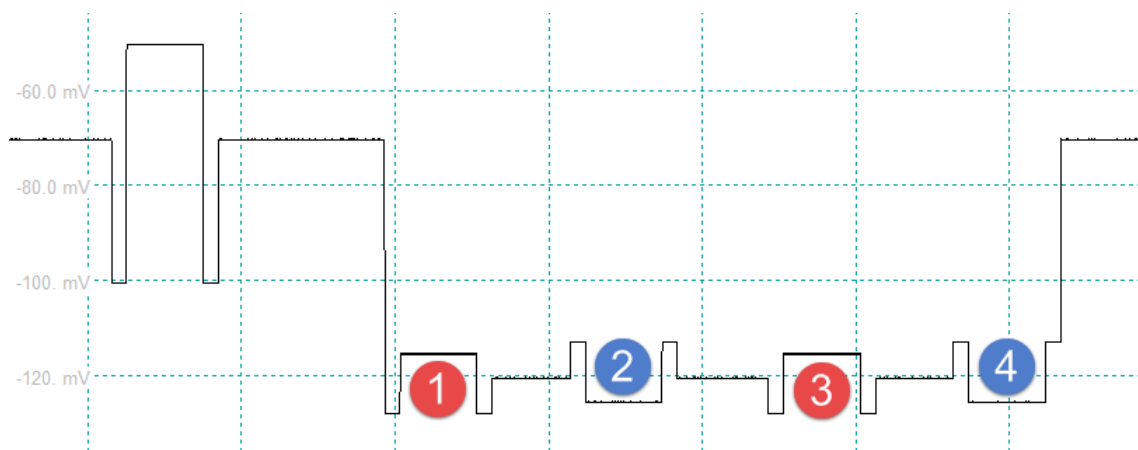


Figure 7.28: P/n Leak Pulses (4) with "Leak Alternate" enabled.

Leak Example:

If executing a stimulation sequence with such *P/n Leak Correction* settings the leak pulses will look like this:

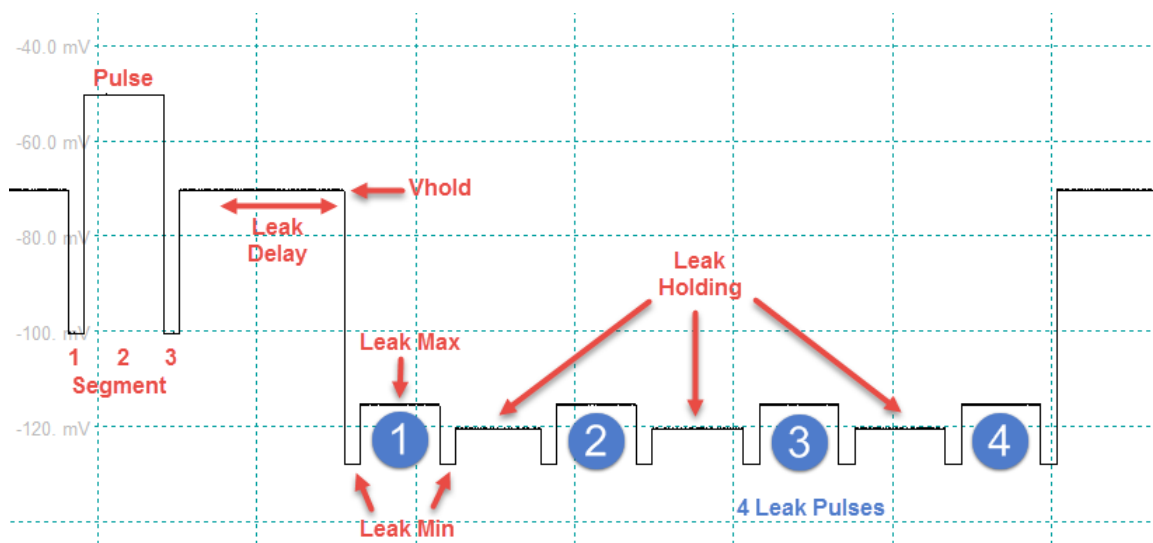


Figure 7.29: Classical P/4 protocol with 4 leak pulses scaled down to a fourth of the original pulse amplitude. In the classical P/n protocol the *Leak Size* is always equal to 1 divided by the *No of Leaks*. This is not necessary with the PATCHMASTER NEXT software: the leak pulses are summed and scaled with a factor of $1/(No\ of\ Leaks * Leak\ Size)$.

Note: You can improve the signal-to-noise ratio if you increase *Leak Size* and *No of Leaks*, but be sure that the leak pulses do not reach the activation level of the channels you want to study.

Note: The scaling of leak Traces depends on the scaling of the parent Trace (Trace to which the leak Trace belong). Therefore you have to select the respective parent Trace in the *Oscilloscope* window when you want to scale the leak Traces.

7.6.5 Sine Wave

The “Sine Wave” parameter section contains all sine wave parameters. These parameters get visible if one segment class is set to “Sine Wave” (see chapter 7.8 on page 117).

There are three sine wave modes available which are explained in the next chapters:

- Simple Sine Wave: Common Frequency
- Simple Sine Wave: Separate Frequency
- LockIn Sine Wave

The detailed settings for the *Chirp* mode itself are described in chapter 19 on page 253.

7.6.5.1 Simple Sine Wave: Common Frequency

Use this setting if all sine wave segments within a Sweep should have the same frequency.

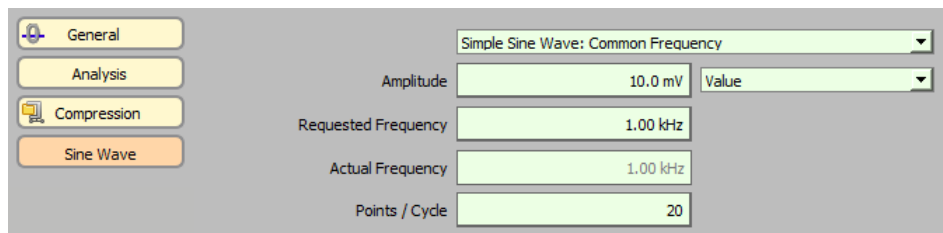


Figure 7.30: Simple Sine Wave: Common Frequency parameters of the Stimulus Editor.

Amplitude: One can enter the size of the sine wave amplitude in the entry field when the selection box beneath is set to *Value*. The defined *Amplitude* is half of the peak-to-peak amplitude.

Value or PGF-Parameter: If *Value* is selected the amplitude of the sine wave can be entered in the field *Amplitude*. Alternatively, the *Amplitude* can be determined by a *PGF-Parameter*. This allows to automatically change the *Amplitude* of the sine wave between different series acquisitions.

Requested Frequency: Define the desired frequency of the sine wave in Hertz (Hz).

Actual Frequency: Only certain frequencies are possible because they are generated by dividing a fundamental clock frequency by an integer. Nevertheless, the *Actual Frequency* is usually within a couple of percent of the *Requested Frequency*.

Points / Cycle: Number of sample points per full wave length. This number is calculated and can not be edited. It is a display value only and gives the number of points per sinus wave which is the cycle length divided by the sample interval.

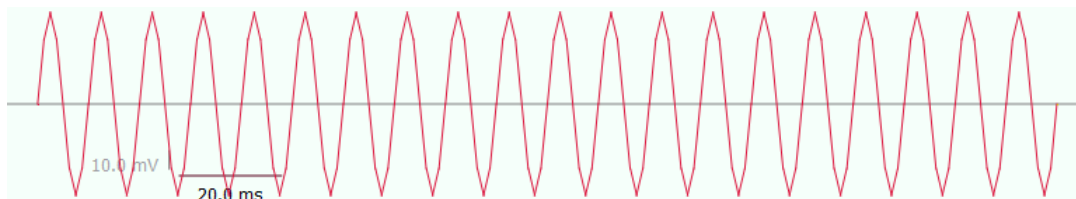


Figure 7.31: Example of a Simple Sine Wave with Common Frequency (10 mV Amplitude, 100 Hz Actual Frequency).

7.6.5.2 Simple Sine Wave: Separate Frequency

Use this setting if the frequency of the sine wave is determined by the segment duration. Each segment will contain one wave cycle only. This option allows to create a Sweep containing wave cycles of different frequencies.

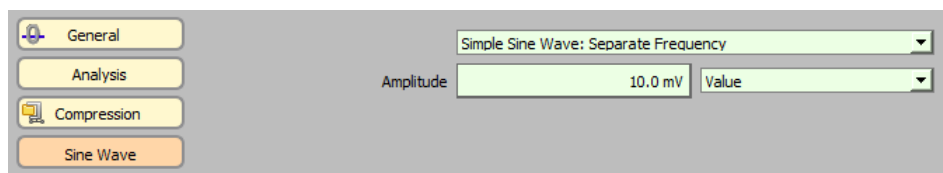


Figure 7.32: Simple Sine Wave: Separate Frequency parameters of the Stimulus Editor.

Amplitude: One can enter the size of the sine wave amplitude in the entry field when the selection box beneath is set to *Value*. The defined *Amplitude* is half of the peak-to-peak amplitude.

Value or PGF-Parameter: If *Value* is selected the amplitude of the sine wave can be entered in the field *Amplitude*. Alternatively, the *Amplitude* can be determined by a *PGF-Parameter*. This allows to automatically change the *Amplitude* of the sine wave between different series acquisitions.



Figure 7.33: Example of a simple sine wave with separate frequency (2 Segments with 10 and 50 ms Duration, 10 mV Amplitude).

7.6.5.3 LockIn Sine Wave

Use this setting if the sine wave should be analyzed with the software LockIn Extension.

Figure 7.34: LockIn Sine Wave Parameters of the Stimulus Editor.

Note: For the LockIn Sine Wave to be an active option, the LockIn Extension must first be turned on in the Configuration (see chapter 3.5.2.1 on page 25).

Amplitude: One can enter the size of the sine wave amplitude in the entry field when the selection box beneath is set to *Value*. The defined *Amplitude* is half of the peak-to-peak amplitude.

Value or PGF-Parameter: If *Value* is selected the amplitude of the sine wave can be entered in the field *Amplitude*. Alternatively, the *Amplitude* can be determined by a *PGF-Parameter*. This allows to automatically change the *Amplitude* of the sine wave between different series acquisitions.

Requested Frequency: Define the desired frequency of the sine wave in Hertz (Hz).

Actual Frequency: Only certain frequencies are possible because they are generated by dividing a fundamental clock frequency by an integer. Nevertheless, the *Actual Frequency* is usually within a couple of percent of the *Requested Frequency*.

Points / Cycle: Number of sample points per full wave length. This number is calculated and can not be edited. It is a display value only and gives the number of points per sinus wave which is the cycle length divided by the sample interval.

Skip Cycles: When a sine wave segment begins, there is a "capacitive" transient current response just as there is a transient response when a voltage step is given. In order to prevent this from causing an artifact in the C_m Trace, sine wave cycles are skipped at the beginning of each sine wave segment. If the frequency of the sinusoidal wave is chosen appropriately, then the transient should decay within a single cycle.

Note: A value of "1" Skip Cycle is recommended.

Average Cycles: If a value larger than '1' is entered, the specified number of cycles will be averaged. This reduces the noise and the time resolution (number of data points) in the results.

Total Cycles: Indicates the total number of all cycles.

Reversal: Applicable in the *Sine + DC* mode. Since the DC current is used in processing estimates of the equivalent circuit parameters, the reversal potential must be known (see Gillis, 1995). For our purposes, *Reversal* is the zero current potential extrapolated from the slope conductance about *V-membrane*, which is not necessarily the same as the actual zero current potential. If you expect a membrane conductance to be activated during the course of the experiment, set *Reversal* to the zero current potential of the activated conductance. This setting is actually not very critical if G_m is low.

Note: A value of zero is often used in the common situation where G_m is low and the actual reversal potential is unknown.

The detailed settings for the *LockIn* mode itself are described in chapter 19 on page 253.

7.6.6 Square Wave Parameters

The "Square Wave" parameter section contains all square wave parameters. These parameters get visible if one segment class is set to "Square Wave" (see chapter 7.8 on page 117).

There are three square wave modes available which are explained in the next chapters:

- Simple Square Wave: Common Frequency
- Simple Square Wave: Separate Frequency
- LockIn Square Wave

7.6.6.1 Simple Square Wave: Common Frequency

Use this setting if all square wave segments within a Sweep should have the same frequency.

Parameter	Value	Unit
Pos. Amplitude	10.0	mV
Neg. Amplitude	0.00	V
Requested Frequency	10.0	Hz
Actual Frequency	10.0	Hz
Points / Cycle	2000	
Base Incr.	0.00	V
Dur. Factor	0.200	

Figure 7.35: Simple Square Wave: Common Frequency parameters of the Stimulus Editor.

Pos. Amplitude: One can enter the size of the square wave amplitude in the entry field when the selection box beneath is set to *Value*. The defined *Amplitude* is half of the peak-to-peak amplitude.

Neg. Amplitude: If a *Dur. Factor* unequal zero is used, the negative amplitude of the square wave can be entered.

Note: This entry field will not appear if Dur. Factor is set to "0".

Value or PGF-Parameter: If *Value* is selected the amplitude of the square wave can be entered in the field *Amplitude*. Alternatively, the *Amplitude* can be determined by a *PGF-Parameter*. This allows to automatically change the *Amplitude* of the square wave between different Series acquisitions.

Requested Frequency: Define the desired frequency of the square wave in Hertz (Hz).

Actual Frequency: Only certain frequencies are possible because they are generated by dividing a fundamental clock frequency by an integer. Nevertheless, the *Actual Frequency* is usually within a couple of percent of the *Requested Frequency*.

Points / Cycle: Number of sample points per full wave length. This number is calculated and can not be edited. It is a display value only and gives the number of points per square wave which is the cycle length divided by the sample interval.

In contrast to the sine wave the square wave allows to use different positive and negative amplitudes as well as different half cycle times. By the use of the following two parameters, e.g. stimulation trains can be easily parameterized.

Base Incr.: Defines an increment amplitude which causes a baseline shift of the square waves ("Staircase" effect).

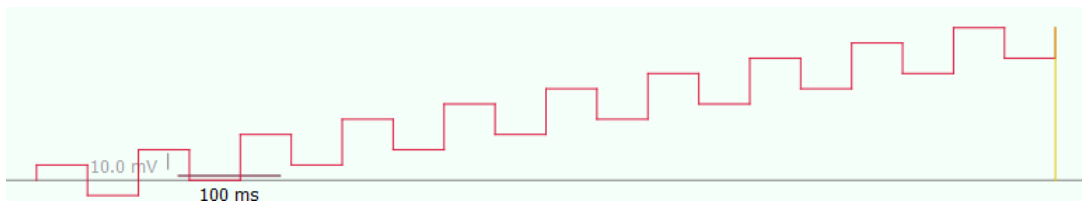


Figure 7.36: Example of a square wave with common frequency and "staircase" effect (Pos. Amplitude of 10 mV, Actual Frequency of 10 Hz and a Base Incr. of 10 mV).

Dur. Factor: Factors between 0 and 1 are allowed. A factor of '0.5' means that both half cycles have the same duration. A factor smaller than '0.5' shortens the positive half cycle of the square wave.

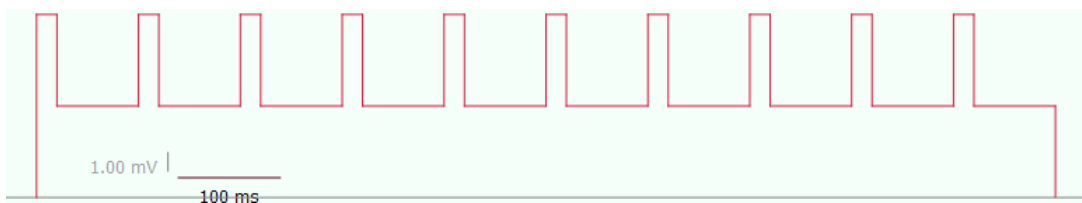


Figure 7.37: Example of a square wave with common frequency and usage of Dur. Factor (Pos. Amplitude of 10 mV, Neg. Amplitude of 5 mV, Actual Frequency of 10 Hz and a Dur. Factor of "0.2").

7.6.6.2 Simple Square Wave: Separate Frequency

Use this setting if the frequency of the wave is determined by the segment duration. Each segment will contain one wave cycle only. This option allows to create a *Sweep* containing wave cycles of different frequencies.

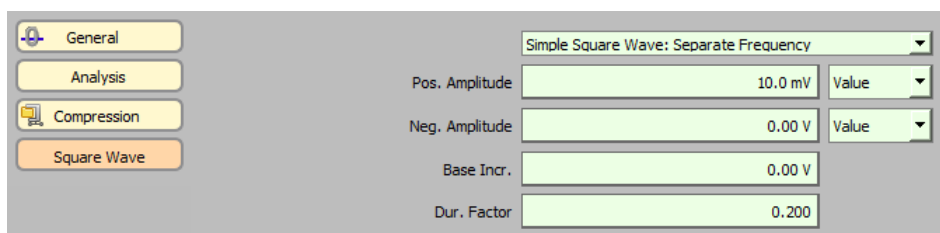


Figure 7.38: Simple Square Wave: Separate Frequency parameters of the Stimulus Editor.

Pos. Amplitude: One can enter the size of the square wave amplitude in the entry field when the selection box beneath is set to *Value*. The defined *Amplitude* is half of the peak-to-peak amplitude.

Neg. Amplitude: If a *Dur. Factor* unequal zero is used, the negative amplitude of the square wave can be entered.

Note: This entry field will not appear if *Dur. Factor* is set to "0".

Base Incr.: Defines an increment amplitude which causes a baseline shift of the square waves ("staircase" effect).

Dur. Factor: Factors between 0 and 1 are allowed. A factor of '0.5' means that both half cycles have the same duration. A factor smaller than '0.5' shortens the positive half cycle of the square wave.

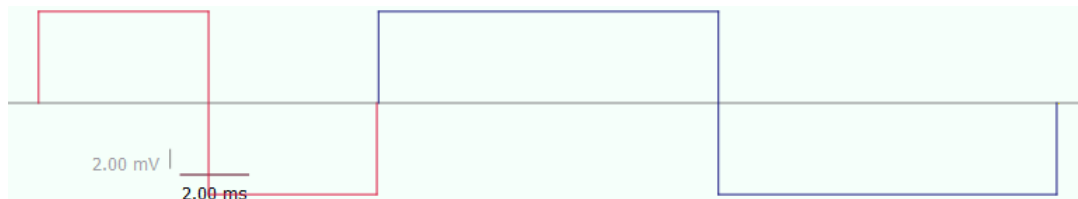


Figure 7.39: Example of a simple square wave with separate frequency (2 Segments with 10 and 20 ms Duration, 10 mV Pos. Amplitude).

7.6.6.3 LockIn Square Wave

Use this setting if the square wave should be analyzed with the *LockIn Extension*.

Figure 7.40: LockIn Square Wave parameters of the Stimulus Editor.

Note: For the *LockIn Square Wave* to be an active option, the *LockIn Extension* must first be turned on in the *Configuration* (see chapter 3.5.2.1 on page 25).

Pos. Amplitude: One can enter the size of the square wave amplitude in the entry field when the selection box beneath is set to *Value*. The defined *Pos. Amplitude* is half of the peak-to-peak amplitude.

Value or PGF-Parameter: If *Value* is selected the amplitude of the square wave can be entered in the field *Pos. Amplitude*. Alternatively, the *Pos. Amplitude* can be determined by a *PGF-Parameter*. This allows to automatically change the *Pos. Amplitude* of the square wave between different Series acquisitions.

Requested Frequency: Define the desired frequency of the square wave in Hertz (Hz).

Actual Frequency: Only certain frequencies are possible because they are generated by dividing a fundamental clock frequency by an integer. Nevertheless, the *Actual Frequency* is usually within a couple of percent of the *Requested Frequency*.

Points / Cycle: Number of sample points per full wave length. This number is calculated and can not be edited. It is a display value only and gives the number of points per square wave which is the cycle length divided by the sample interval.

Skip Cycles: When a square wave segment begins, there is a "capacitive" transient current response just as there is a transient response when a voltage step is given. In order to prevent this from causing an artifact in the C_m Trace, square wave cycles are skipped at the beginning of each square wave segment. If the frequency of the square wave is chosen appropriately, then the transient should decay within a single cycle.

Note: A value of "1" Skip Cycle is recommended.

Average Cycles: If a value larger than '1' is entered, the specified number of cycles will be averaged. This reduces the noise and the time resolution (number of data points) in the results.

Total Cycles: Indicates the total number of all cycles.

Reversal: Applicable in the *Sine + DC* mode. Since the DC current is used in processing estimates of the equivalent circuit parameters, the reversal potential must be known (see Gillis, 1995). For our purposes, *Reversal* is the zero current potential extrapolated from the slope conductance about *V-membrane*, which is not necessarily the same as the actual zero current potential. If you expect a membrane conductance to be activated during the course of the experiment, set *Reversal* to the zero current potential of the activated conductance. This setting is actually not very critical if G_m is low.

Note: A value of zero is often used in the common situation where G_m is low and the actual reversal potential is unknown.

The detailed settings for the *LockIn* mode itself are described in chapter 19 on page 253.

7.6.7 Chirp Wave Parameters

The "Chirp Wave" parameter section contains all chirp wave parameters. These parameters get visible if one segment class is set to "Chirp Wave" (see chapter 7.8 on page 117).

There are three sine wave modes available which are explained in the next chapters:

- Linear Chirp
- Exponential Chirp
- Spectroscopy Chirp

The *Chirp* wave's characteristic is either an increasing or decreasing frequency over time. This frequency modulation can be in a linear, exponential or spectroscopic manner. An example for a *Linear Chirp* and an *Exponential Chirp* is given below:

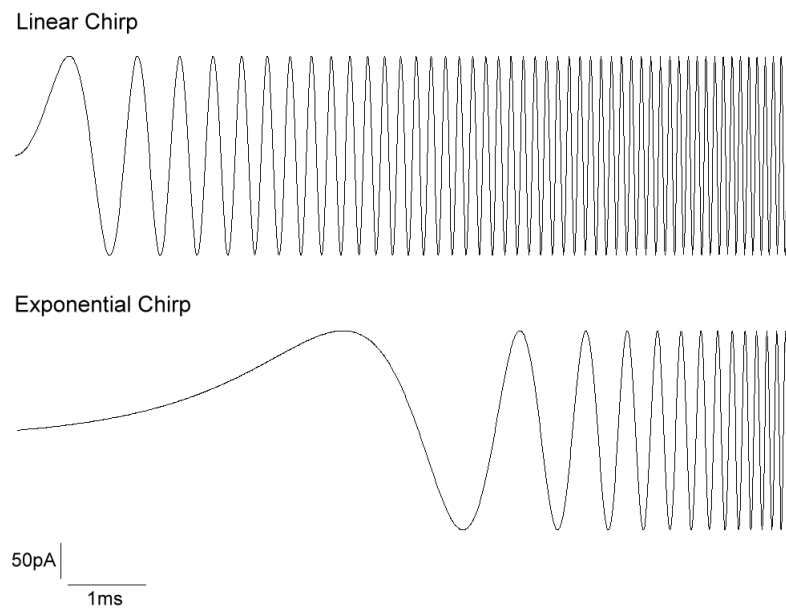


Figure 7.41: Example of Linear Chirp and Exponential Chirp waves.

The detailed settings for the *Chirp* mode itself are described in chapter 21 on page 293.

7.6.7.1 Linear Chirp

Use this setting if the chirp frequency should change in a linear manner.

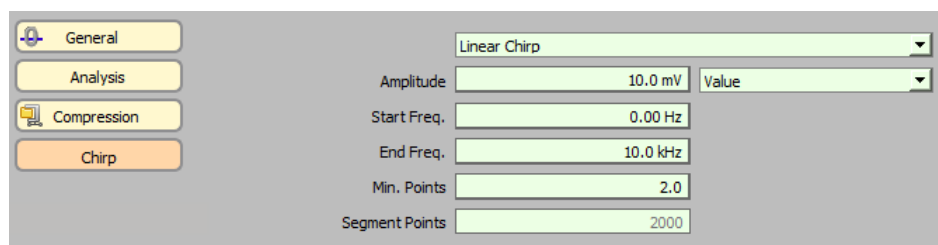


Figure 7.42: Linear Chirp parameters of the Stimulus Editor.

Amplitude: One can enter the size of the chirp wave amplitude in the entry field when the selection box beneath is set to *Value*. The defined *Amplitude* is half of the peak-to-peak amplitude.

Value or PGF-Parameter: If *Value* is selected the amplitude of the chirp wave can be entered in the field *Amplitude*. Alternatively, the *Amplitude* can be determined by a *PGF-Parameter*. This allows to automatically change the *Amplitude* of the chirp wave between different Series acquisitions.

Start Frequency: Defines the frequency where the chirp wave starts.

End Frequency: Defines the frequency where the chirp wave ends.

Min. Points / Cycle: Indicates the minimum number of sample points per full wave length for the *End Frequency*.

Segment Points: Indicates the total amount of points for the *Chirp* segment.

7.6.7.2 Exponential Chirp

Use this setting if the chirp frequency should change in an exponential manner.

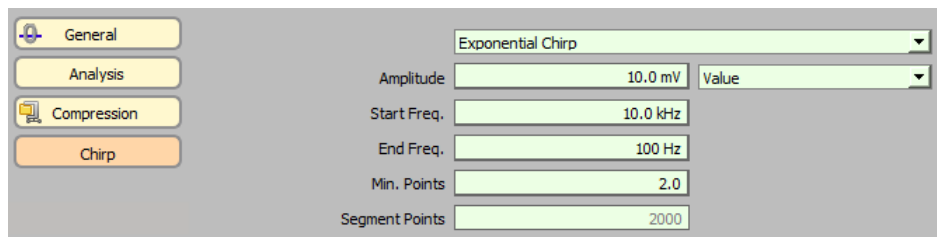


Figure 7.43: Exponential Chirp parameters of the Stimulus Editor.

Amplitude: One can enter the size of the chirp wave amplitude in the entry field when the selection box beneath is set to *Value*. The defined *Amplitude* is half of the peak-to-peak amplitude.

Value or PGF-Parameter: If *Value* is selected the amplitude of the chirp wave can be entered in the field *Amplitude*. Alternatively, the *Amplitude* can be determined by a *PGF-Parameter*. This allows to automatically change the *Amplitude* of the chirp wave between different Series acquisitions.

Start Frequency: Defines the frequency where the chirp wave starts.

End Frequency: Defines the frequency where the chirp wave ends.

Min. Points / Cycle: Indicates the minimum number of sample points per full wave length for the *End Frequency*.

Segment Points: Indicates the total amount of points for the *Chirp* segment.

7.6.7.3 Spectroscopy Chirp

Use this setting if the *Chirp* wave should be analyzed with the *Chirp Extension*.

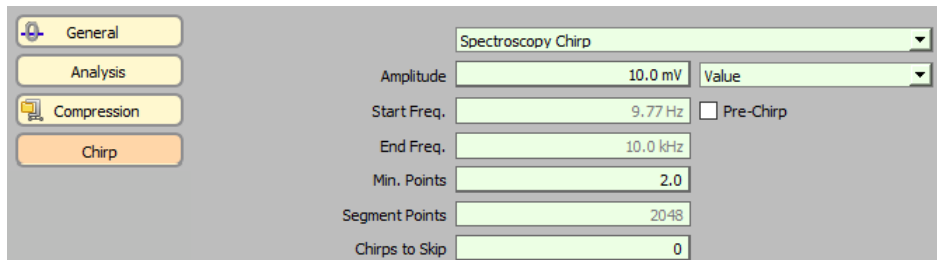


Figure 7.44: Spectroscopy Chirp parameters of the Stimulus Editor.

Note: For the *Spectroscopy Chirp* to be an active option, the *Chirp Extension* must first be turned on in the *Configuration* (see chapter 3.5.2.2 on page 25).

Amplitude: One can enter the size of the chirp wave amplitude in the entry field when the selection box beneath is set to *Value*. The defined *Amplitude* is half of the peak-to-peak amplitude.

Value or PGF-Parameter: If *Value* is selected the amplitude of the chirp wave can be entered in the field *Amplitude*. Alternatively, the *Amplitude* can be determined by a *PGF-Parameter*. This allows to automatically change the *Amplitude* of the chirp wave between different Series acquisitions.

Start Frequency: Defines the frequency where the chirp wave starts.

Pre-Chirp: When activation this option, the segment before the *Chirp* segment is filled with appropriate *Chirp* stimulus data for an optimized swing in effect. The effect of the use of a pre-chirp is comparable to record two chirp segments and skip the first chirp. However, the pre-chirp segment can be of much shorter time and therefore facilitates the measurement especially if spectra with very low frequencies are recorded. The pre-chirp segment has to be skipped in order to provide an accurate chirp analysis.

End Frequency: Defines the frequency where the chirp wave ends.

*Note: End Freq. = Min. Points / Cycle * Sampling Freq.*

Min. Points / Cycle: Indicates the minimum number of sample points per full wave length for the *End Frequency*.

Segment Points: Indicates the total amount of points for the *Chirp* segment.

Chirps to Skip: Defines the number of skipped chirp segments.

7.6.8 Photometry Wave Parameters

If the option *Use For Wavelength* is active for a DA output channel, then the *Wavelength* button will appear in the `Parameter Section`.

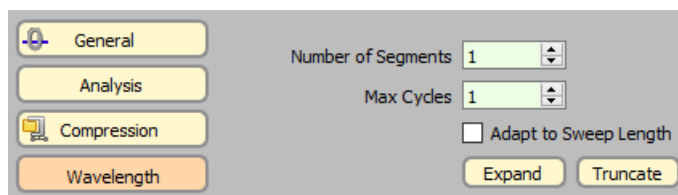


Figure 7.45: Photometry parameters of the Stimulus Editor.

Number of Segments: The number of creating a single cycle for a measurement. Typically there are three segments used for a ratio-metric measurement. Segment '1' and '2' for the wavelength setting and a third segment for a waiting time.

Max Cycles: As input determines how many times the first *Number of Segments* are duplicated with the *Expand* command. In case *Adapt to Maximal Sweep Length* is checked, then it shows how many cycles have been fit into the stimulus.

Adapt to Sweep Length: If checked, then the number of cycles is calculated by the software in respect to the duration of the longest output channel.

Expand: Creates the complete photometry stimulus. The first *Number of Segments* are duplicated until the *Maximal Sweep Length* or the *Number of Cycles* is reached.

Truncate: Removes all segment except the first *Number of Segments*. This option should be used to clean the stimulus before changing any parameters in the photometry stimulus. After parameter adjustment an *Expand* creates the new stimulus.

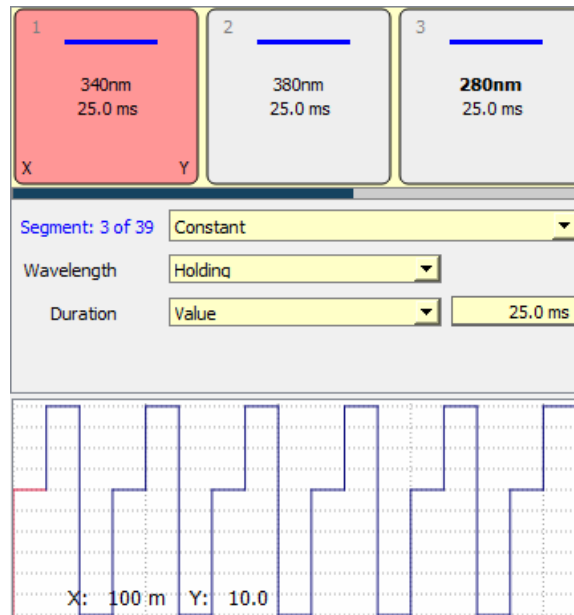


Figure 7.46: Example of Photometry stimulation sequence.

In this example a single cycle consists of 3 segments. You have just to edit the first three segments. Set the *Number of Segments* to "3". A click on *Expand* adds 36 additional segments to the PGF sequences. The basis for the calculation is the length of the first Trace (here: 1s). The resulting PGF sequence then consists of $13 * 3 = 39$ segments which results in a total length of 975 ms. *Truncate* removes all segments except the initial 3 segments.

7.7 Segment View

In the *Segment View* section of the *Stimulus Editor* the defined segments of the stimulation sequence of the the selected channel are displayed. A stimulation sequence consists of an arbitrary number of segments.

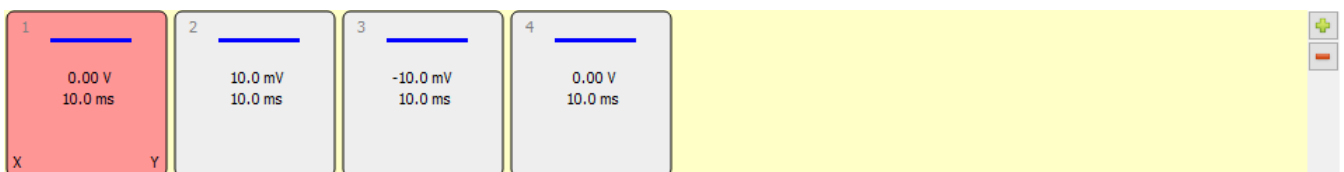


Figure 7.47: Segment View of the Stimulus Editor.

The position of the segments can be easily changed by the drag-and-drop functionality. Selected segments are displayed with a red background.

Via the two button on the right side of this section (green plus sign & red minus sign) it is easily possible to add or remove individual segments.

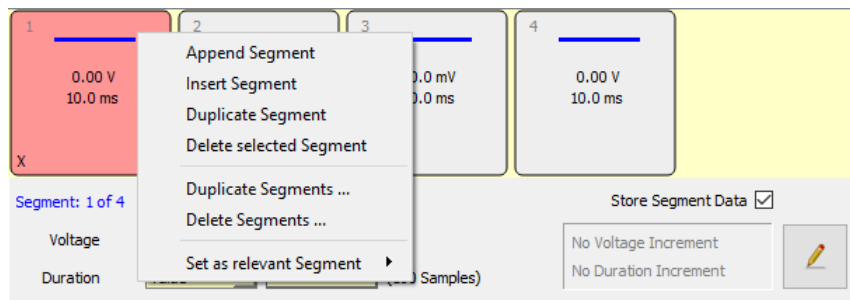


Figure 7.48: Context menu option of the Segment View.

The right-click context menu provides the following options:

- **Append Segment:** Add another segment to the end of the segment list (equals the green plus button functionality on the right side).
- **Insert Segment:** Inserts another segment after the currently selected segment.
- **Duplicate Segment:** Creates a copy of the selected segment and inserts it after the selected segment.
- **Delete selected Segment:** Deletes the selected segment (equals the red minus button functionality on the right side).
- **Duplicate Segments...:** Creates multiple copies of a number of segments. First, you have to enter how many segments you want to copy. The counting starts with the selected segment going up. Second, you have to specify how often you want to copy these segments. They will be inserted after the last segment which was defined in the first step.
- **Delete Segments...:** Deletes a specified number of segments. The counting starts with the selected segment going up.
- **Set as relevant Segment**
 - X: Sets the relevant X-Segment to this segment
 - Y: Sets the relevant Y-Segment to this segment
 - X and Y: Sets the relevant X- and Y-Segment to this segment

The segment boxes offer information about the *Segment Number*, *Segment Class*, *Potential/Current Amplitude*, *Duration*, *Increment* and *Relevant X/Y-Segments*.

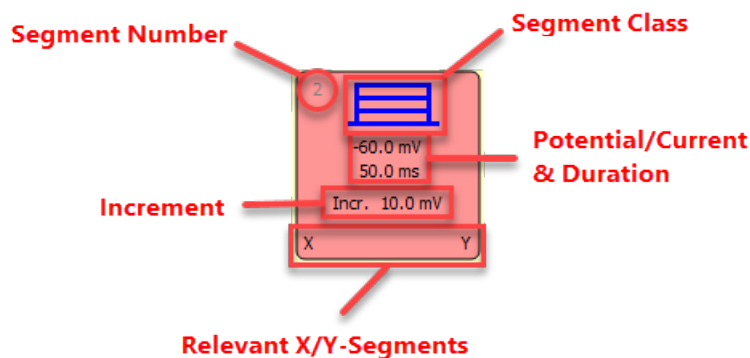


Figure 7.49: Each segment has its own Segment Box displaying the selected segment options.

The potential or current amplitude value is displayed in bold letters if this value represents the holding value from the Amplifier window.

If a segment is set to “non-stored” the border is drawn with a dotted border line instead of a solid one.

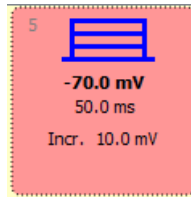


Figure 7.50: Segments which are not stored show a dotted border frame.

7.8 Segment Settings

In the Segment Settings section of the Stimulus Editor the main settings for the segment definitions are provided.

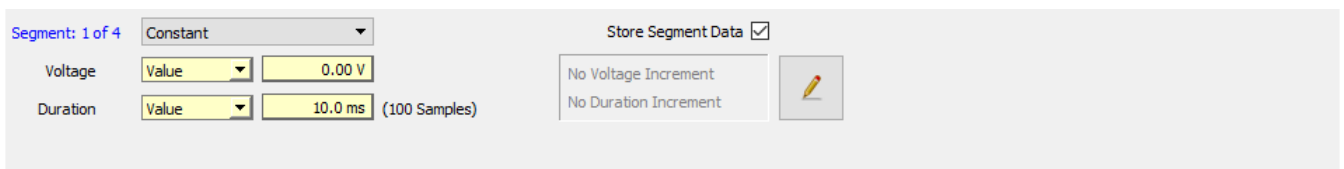


Figure 7.51: Segment settings of the Stimulus Editor.

Segment Number: The number of the currently selected segment is given in blue letters in relation to the total numbers of set segments.

Segment Class:

The following *Segment Classes* are available:

- **Constant:** In this segment a constant amplitude will be applied.
- **Ramp:** A *Ramp* segment starts with the amplitude of the previous segment and ramps to the amplitude of the *Ramp* segment.
- **Continuous:** This segment allows long-term recordings. The amplitude is held at a constant level.

Note: Please be aware that this Segment Class either needs to be the last segment in your stimulation sequence or the only one.

- **Sine Wave:** Defines a segment with sine wave characteristics. Amplitude and cycle duration are defined in *Sine Wave* parameters (see chapter 7.6.5 on page 105).
- **Square Wave:** Defines a segment segment with rectangular square wave characteristics. Amplitude and cycle duration are defined in *Square Wave* parameters (see chapter 7.6.6 on page 108).
- **Chirp:** Defines a segment with a chirp wave frequency. Chirp wave characteristics are defined in *Chirp Wave* parameters (see chapter 7.6.7 on page 111).

Voltage: The *Voltage* of a segment is either a numeric value in [V] (*Value*), a holding value at time of sequence execution (*Holding*), or a value from the *PGF parameters* list (*p1-p10*). The *Voltage* field may turn into "Current" in current clamp mode or into "Wavelength" when the segments are used for photometry stimulation is used.

The valid amplitude range is a function of the given DA output voltage range of the interface and the appropriate stimulus scaling factors (e.g. *VC: Stim Scale* in *Voltage Clamp* mode, *CC:Stim Scale* (or *CC-Gain*) in current clamp or the wavelength to voltage conversion formalism of the `Photometry Extension`.)

Duration: Duration of a segment, entered in [s]. The value may be adjusted by dragging the mouse or typing the number. For segment duration, *PGF parameters* can also be used (p1-p10). Make sure that the duration of segments are even multiples of the *Sample Interval*.

On the right side of the entry field the number of samples for the selected segment is indicated.

Scan Rate: The *Scan Rate* option is only available if *Use Scan Rates* is activated (see chapter 7.3 on page 88). This option is especially useful when using *Ramp* segments.

Figure 7.52: Scan Rate option of the Segment settings.

The user can enter a defined value into the field *Scan Rates* whereby the duration of the segment will be calculated or vice versa. The *Scan Rate* is calculated as [V/s].

Store Segment Data: Defines if the data of a segment is stored or not. There are several options available:

- Don't Store Segment Data: The data of the segment is not stored.
- Store Segment Data: The data of the segment is stored.
- Don't Store, but use in first Sweep template: The data of the segment is not stored but the stimulus is output for the first Sweep.
- Don't Store, but use in last Sweep template: The data of the segment is not stored but the stimulus is output for the last Sweep.

Non-stored segments are displayed with a dotted border line. The data for the segment are sampled but removed before the Trace gets stored to the Data Tree.

Note: A non-stored segment must not be a *StartSegment* or a *Relevant Y-segment*.

Note: There are some important consequences when using *P/n leak correction* (see chapter *P/n Leak*, 7.6.4 on page 102). Non-stored segments are not used for the *P/n* template. Thus, for proper leak correction it is recommended to insert a constant segment of the same value as the non-stored segment before and after that segment. The duration of these inserted segments should be sufficient to settle capacitive transients. Since *P/n* signals are generated for these inserted constant segments, *P/n leak correction* will work.

7.8.1 Increments

The display field indicates whether an amplitude increment (current or voltage) or a time increment for the selected segment is set.

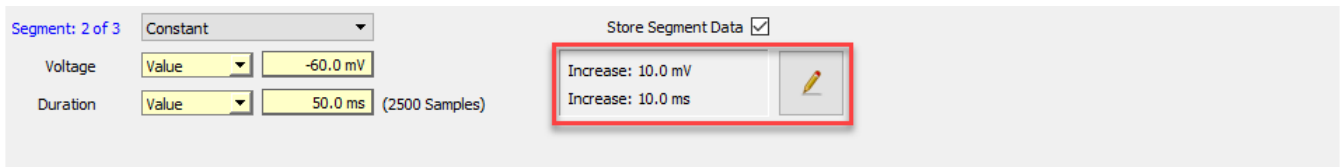


Figure 7.53: Increment area of the Segment Settings. Use the button to enter the Segment Editor.

To add or edit the increment settings please press the button with the pen to enter the Segment Editor.

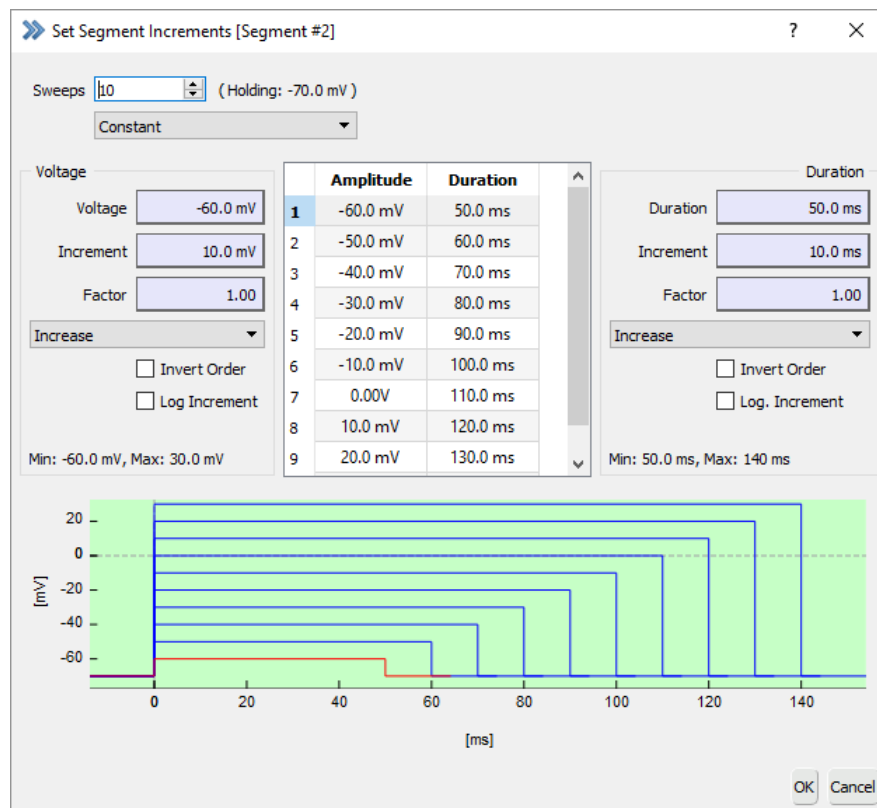


Figure 7.54: Segment Editor of the Segment Settings section.

Sweeps: This entry field is identical to the number of Sweeps entry field of the General Stimulus Settings section (see chapter 7.3 on page 87) which means in both fields one can change the number of Sweeps of the stimulation sequence. It is especially useful having the option to change the number of Sweeps in the Segment Editor because it helps to define and visualize the increment pattern.

On the right side of the entry field the currently set holding potential (or holding current) is displayed (see chapter 4.3 on page 52).

Note: We recommend to have at least two Sweeps for using the increment mode.

Segment Class: One can change the segment class of the segment from the drop-down menu. See chapter 7.8 on page 117 for a detailed description of the Segment Classes.

Increment Table: In the middle of the Segment Editor there is a table showing the order of increments. One can select individual rows of the table which highlights the Sweep in the Increment Cartoon (see 7.8.1 on the next page.)

	Amplitude	Duration
1	-60.0 mV	50.0 ms
2	-50.0 mV	50.0 ms
3	-40.0 mV	50.0 ms
4	-30.0 mV	50.0 ms
5	-20.0 mV	50.0 ms
6	-10.0 mV	50.0 ms
7	0.00V	50.0 ms
8	10.0 mV	50.0 ms
9	20.0 mV	50.0 ms
10	30.0 mV	50.0 ms

Figure 7.55: Increment Table of the Segment Editor.

Increment Cartoon: The *Increment Cartoon* provides a preview about the increment definition. The active Sweep index (see 7.8.1 on the preceding page) is highlighted in red colors.)

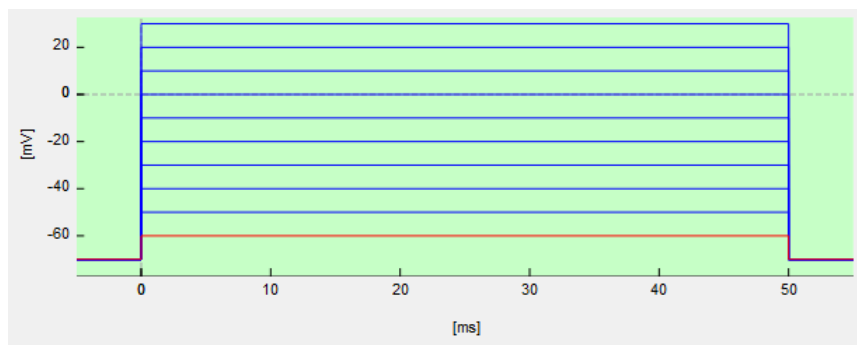


Figure 7.56: Increment Cartoon of the Segment Editor.

With a right-click on the *Increment Cartoon* a context menu opens providing the following options:

- Overlay: All increment steps are drawn overlaid.

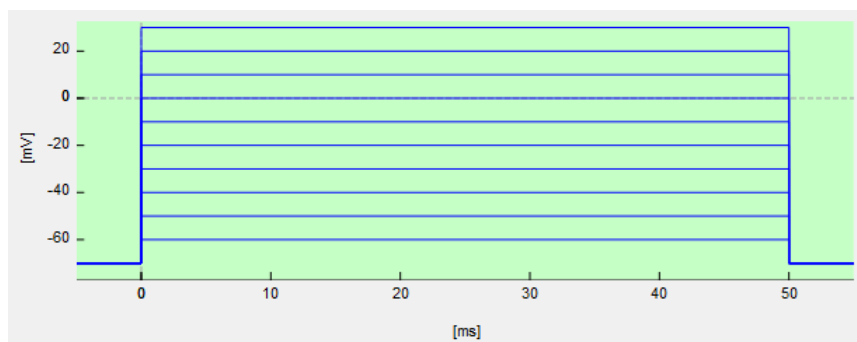


Figure 7.57: Overlay Mode of the Segment Editor cartoon area.

- Concatenated: All increments steps are drawn concatenated.

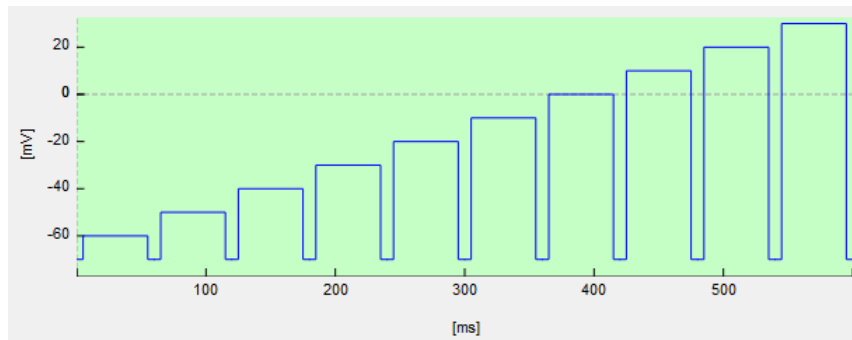


Figure 7.58: Concatenated Mode of the Segment Editor cartoon area.

7.8.1.1 Voltage/Current Increment

In this section one can set the voltage/current increments of this segment. Depending if the recording mode was set to current clamp or voltage clamp mode the section title is labeled “Current” or “Voltage”.

Figure 7.59: Voltage/Current Increment section of the Increment Editor.

- Voltage/Current: Enter the voltage or current where the increment starts.
- Increment: Enter the voltage or current step (increment) which should be applied for the next Sweeps.
- Factor: Depending on the *Increment* mode the *Factor* is computed either with *Increment* or *Voltage*.

Increment Modes:

The *Increment Modes* define the increment calculation and they can influence the order of the applied Sweeps. The selection list provides the following *Increment Modes*:

- Increase: The *Increment* value is added to the *Voltage/Current* value of the segment. The Sweep order is linear: 1-2-3-4-5-6-7-8-9-10.

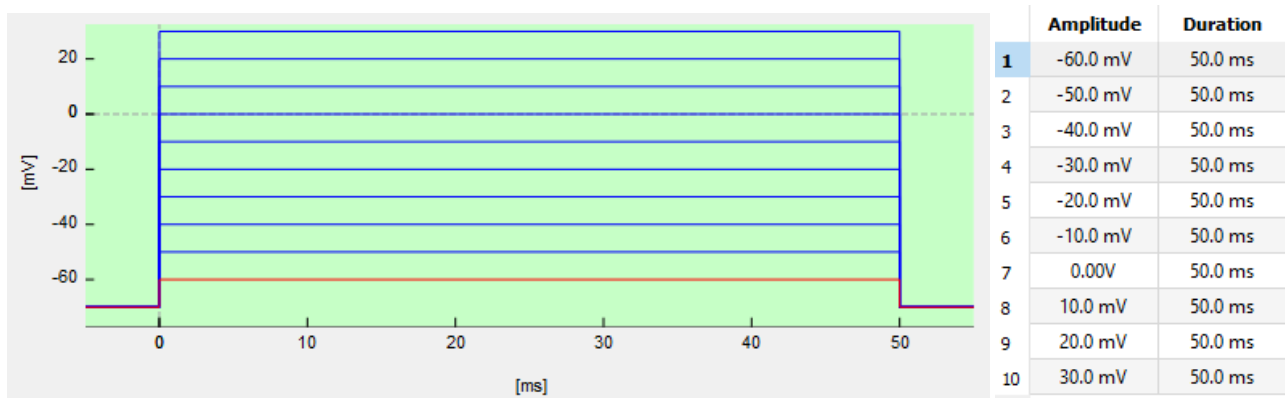


Figure 7.60: Example for Increase mode of Voltage/Current Increment section. Starting potential of the first Sweep is -60 mV ending at +30 mV after ten iterations (10 Sweeps, Voltage = -60 mV, Increment = 10 mV, Factor = 1).

- Interleave: The *Increment* value is added to the *Voltage/Current* value of the segment. The Sweep order is interleaved: 1-3-2-5-4-7-6-9-8-10.

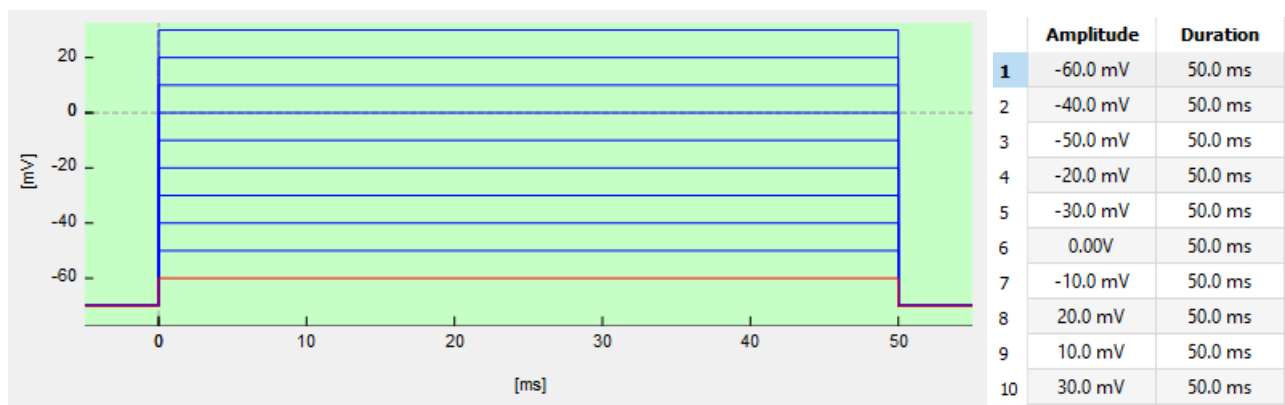


Figure 7.61: Example for Interleave mode of Voltage/Current Increment section. Starting potential of the first Sweep is -60 mV ending at +30 mV after ten iterations (10 Sweeps, Voltage = -60 mV, Increment = 10 mV, Factor = 1).

- Alternate: The *Increment* value is added to the *Voltage/Current* value of the segment. The Sweep order is alternated: 1-10-2-8-3-7-4-5-6.

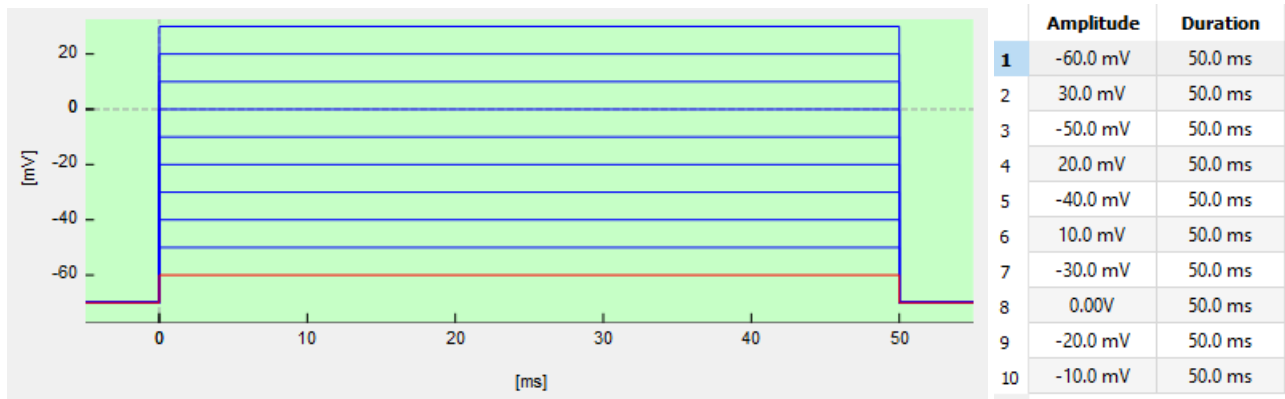


Figure 7.62: Example for Alternate mode of Voltage/Current Increment section. Starting potential of the first Sweep is -60 mV ending at -10 mV after ten iterations (10 Sweeps, Voltage = -60 mV, Increment = 10 mV, Factor = 1).

- Toggle Increment: The *Increment* value is added and subtracted in an alternate way thereby increasing the increment in positive and negative directions. In our example below the voltage values for each Sweep are: 1:(-60 mV), 2:(-50 mV), 3:(-70 mV), 4:(-40 mV), 5:(-80 mV), 6:(-30 mV), 7:(-90 mV), 8:(-20 mV), 9:(-100 mV), 10:(-10 mV)

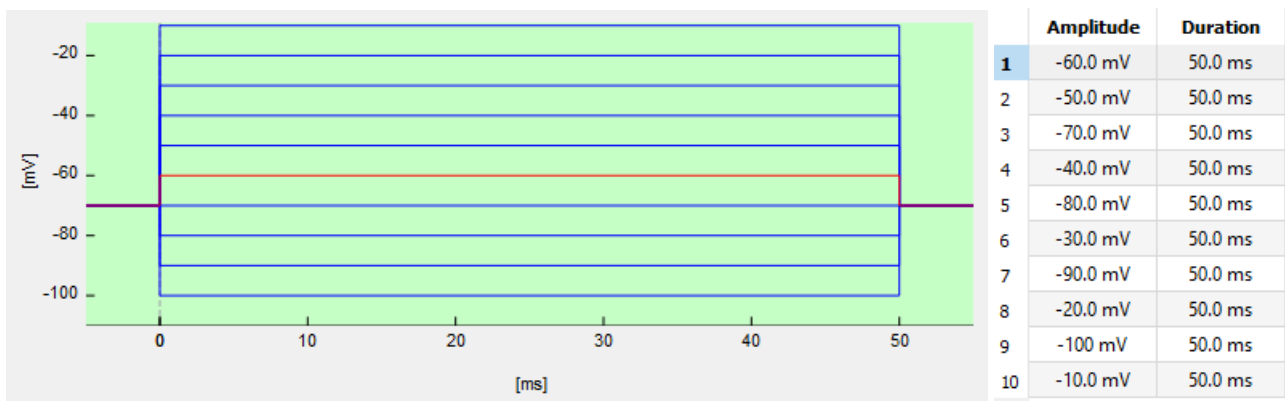


Figure 7.63: Example for Toggle Increment mode of Voltage/Current Increment section. Starting potential of the first Sweep is -60 mV ending at -10 mV after ten iterations (10 Sweeps, Voltage = -60 mV, Increment = 10 mV, Factor = 1).

- Toggle Potential: The *Increment* toggles around the starting *Voltage/Current* value. Only two voltage/current values are applied: The *Voltage/Current* value and the *Voltage/Current* value processed with the *Increment* value. There is no increase or decrease in the increment itself. In our example below the voltage values for each Sweep are: 1:(-60 mV), 2:(-70 mV), 3:(-60 mV), 4:(-70 mV), 5:(-60 mV), 6:(-70 mV), 7:(-60 mV), 8:(-70 mV), 9:(-60 mV), 10:(-70 mV)

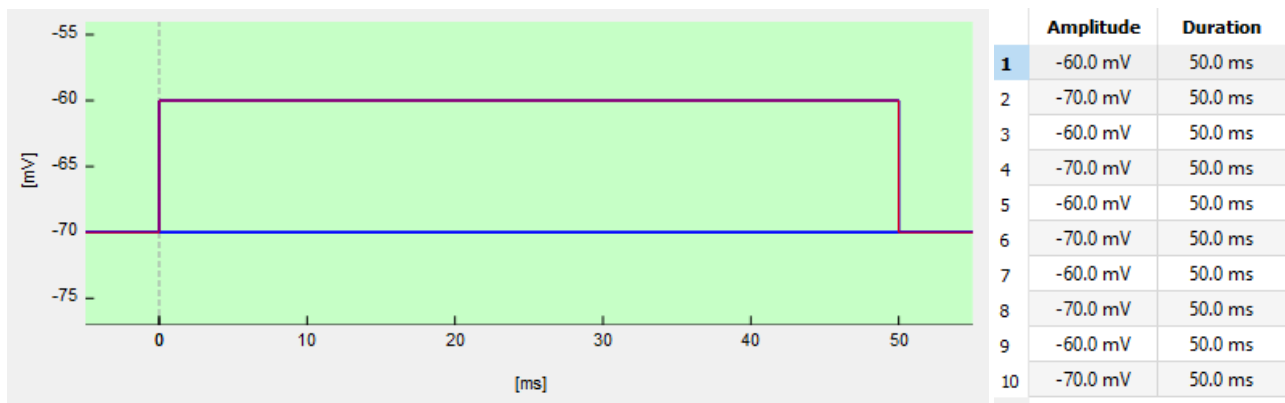


Figure 7.64: Example for Toggle Potential mode of Voltage/Current Increment section. Starting potential of the first Sweep is -60 mV ending at -70 mV after ten iterations (10 Sweeps, Voltage = -60 mV, Increment = 10 mV, Factor = 1).

Special Cases

Besides the usage of the basic *Increment* modes there are several special cases of increment creation.

Factor: When using a *Factor* the voltage step calculation is as follows (i =Sweep Index):

$$V_i = Voltage * Factor^{(i-1)} + (i - 1) * Increment \quad (7.3)$$

Note: If the Voltage is set to "0" the Factor is not applied but only the increment will determine the voltage step calculation.

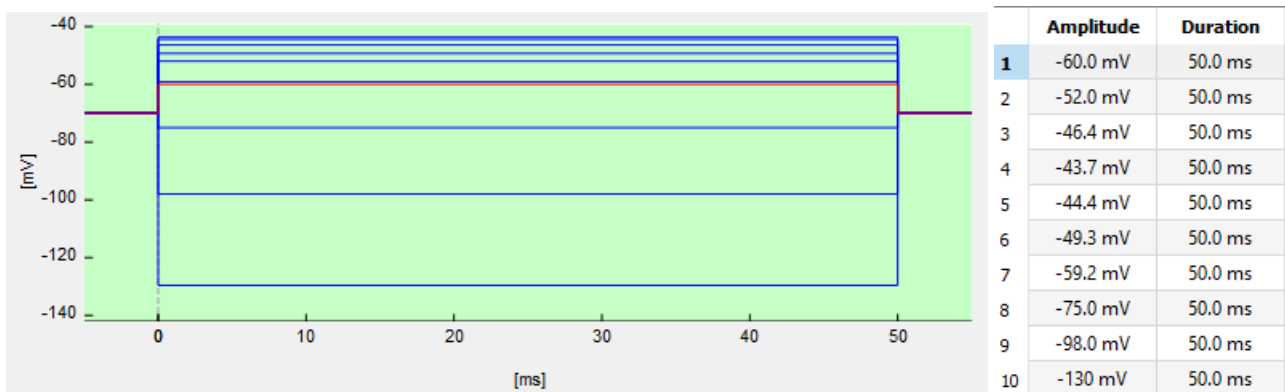


Figure 7.65: Example for voltage step calculation by using the Factor. Starting potential of the first Sweep is -60 mV ending at -130 mV after ten iterations (10 Sweeps, Voltage = -60 mV, Increment = 20 mV, Factor = 1.2, Increment Mode = Increase).

Note: The entered value for the Factor can be between "0.01" and "10".

Invert Order: With this option one can invert the order of the applied Sweeps. For the *Increase* mode the order would be now: 10-9-8-7-6-5-4-3-2-1.

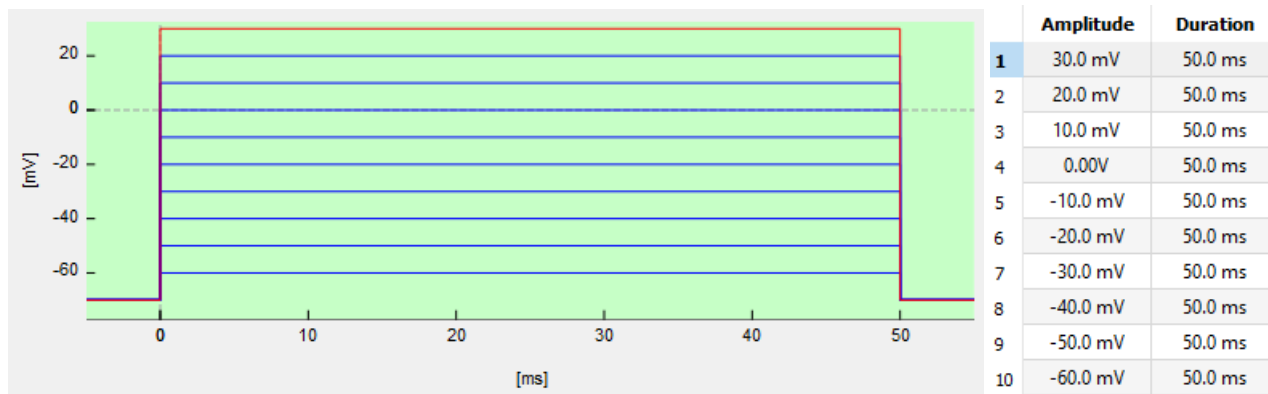


Figure 7.66: Example for Invert option of Voltage/Current Increment section. Starting potential of the first Sweep is +30 mV ending at -60 mV after ten iterations (10 Sweeps, Voltage = -60 mV, Increment = 10 mV, Factor = 1).

Log Increment: When using *Log Increment* the voltage step calculation is as follows (*i*=Sweep Index):

Factor = 1

$$V_i = Voltage + (i - 1) * Increment \tag{7.4}$$

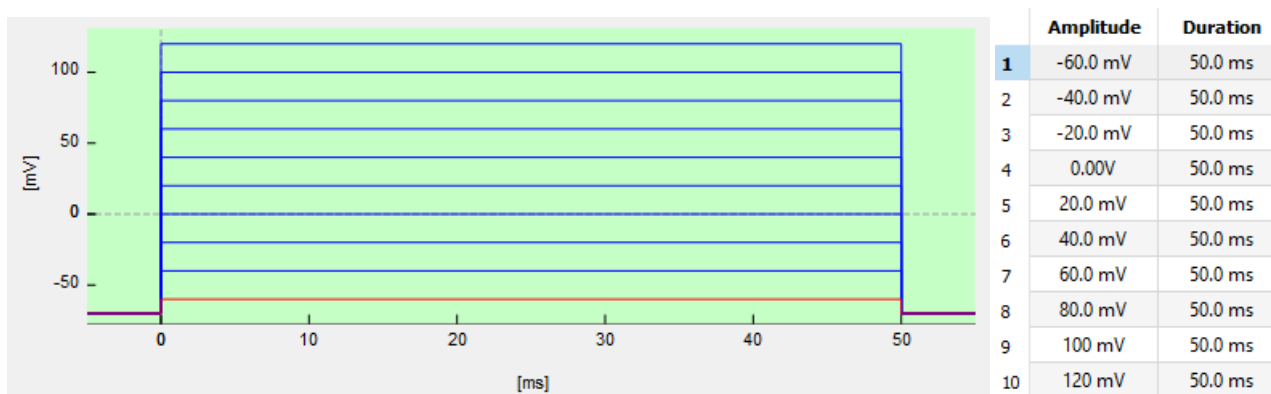


Figure 7.67: Example for voltage step calculation by using the Log Increment option and Factor set to “1”. Starting potential of the first Sweep is -60 mV ending at +120 mV after ten iterations (10 Sweeps, Voltage = -60 mV, Increment = 20 mV, Increment Mode = Increase).

Factor $\neq 1$

$$V_i = \text{Voltage} + \text{Increment} * \text{Factor}^{i-2} \quad (7.5)$$

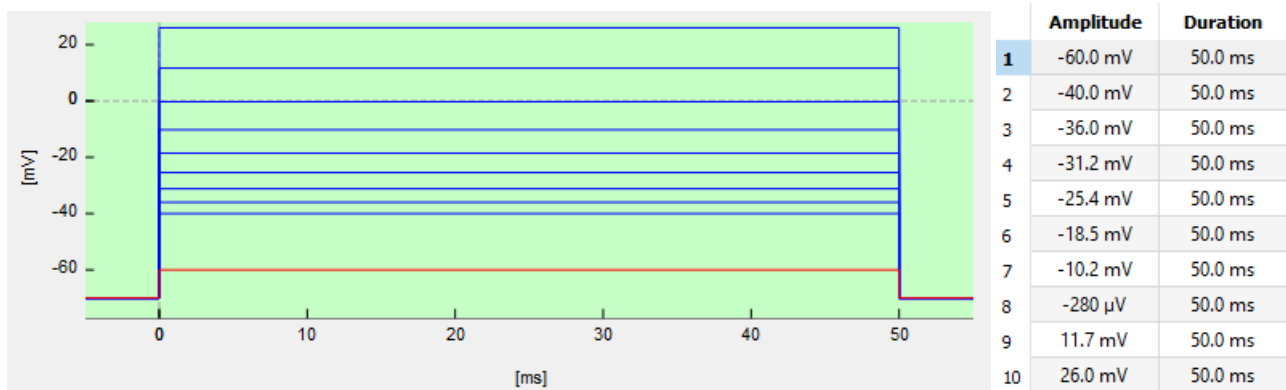


Figure 7.68: Example for voltage step calculation by using the Log Increment option and Factor set to “1.2”. Starting potential of the first Sweep is -60 mV ending at +26 mV after ten iterations (10 Sweeps, Voltage = -60 mV, Increment = 20 mV, Increment Mode = Increase).

Note: The Log Increment is based on the linear increment step. Therefore, the Increment cannot be “0”.

Increment Range: In this display field one gets a kind of summary about the minimum and maximum voltage/current amplitude applied in this segment, thereby defining the range of the incrementing steps.

7.8.1.2 Duration Increment

In this section one can set the duration increments of a segment.

Figure 7.69: Duration Increment section of the Increment Editor.

- Duration: Enter the duration where the increment starts.
- Increment: Enter the duration step (increment) which should be applied for the next Sweeps.
- Factor: Depending on the *Increment* mode the *Factor* is computed either with *Increment* or *Duration*.

Increment Modes:

The *Increment Modes* define the increment calculation and they can influence the order of the applied Sweeps. The selection list provides the following *Increment Modes*:

- Increase: The *Increment* value is added to the *Duration* value of the segment. The Sweep order is linear: 1-2-3-4-5-6-7-8-9-10.

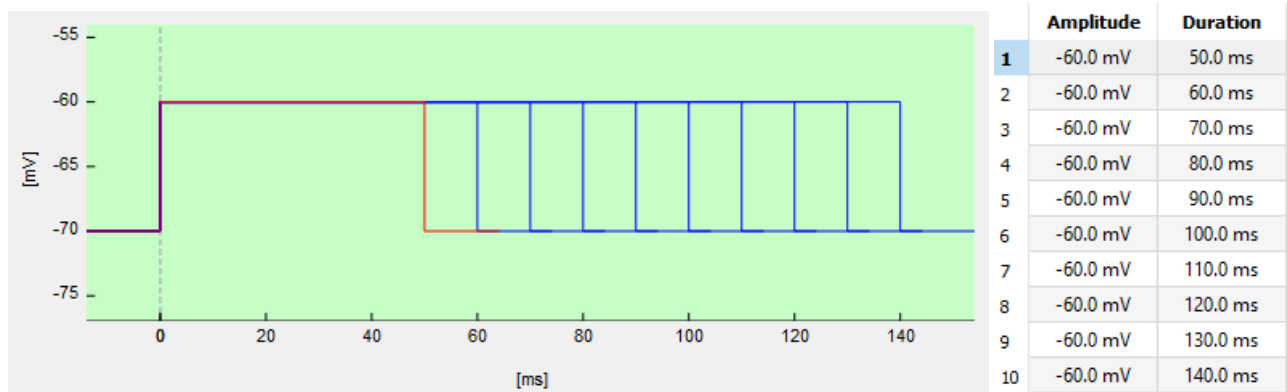


Figure 7.70: Example for Increase mode of Duration Increment section. Starting duration of the first Sweep is 50 ms ending at 140 ms after ten iterations (10 Sweeps, Duration = 50 ms, Increment = 10 ms, Factor = 1).

- Interleave: The *Increment* value is added to the *Duration* value of the segment. The Sweep order is interleaved: 1-3-2-5-4-7-6-9-8-10.

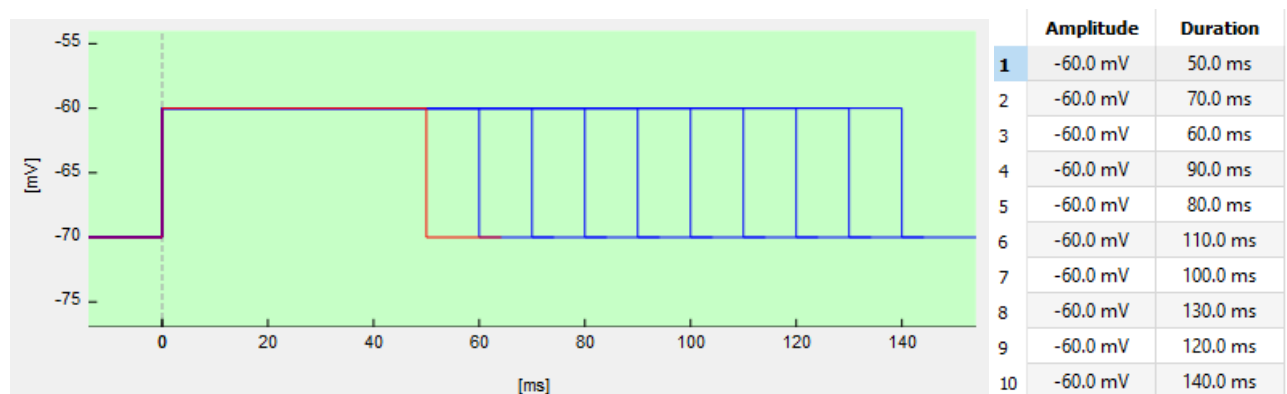


Figure 7.71: Example for Interleave mode of Duration Increment section. Starting duration of the first Sweep is 50 ms ending at 140 ms after ten iterations (10 Sweeps, Duration = 50 ms, Increment = 10 ms, Factor = 1).

- Alternate: The *Increment* value is added to the *Duration* value of the segment. The Sweep order is alternated: 1-10-2-8-3-7-4-5-6.

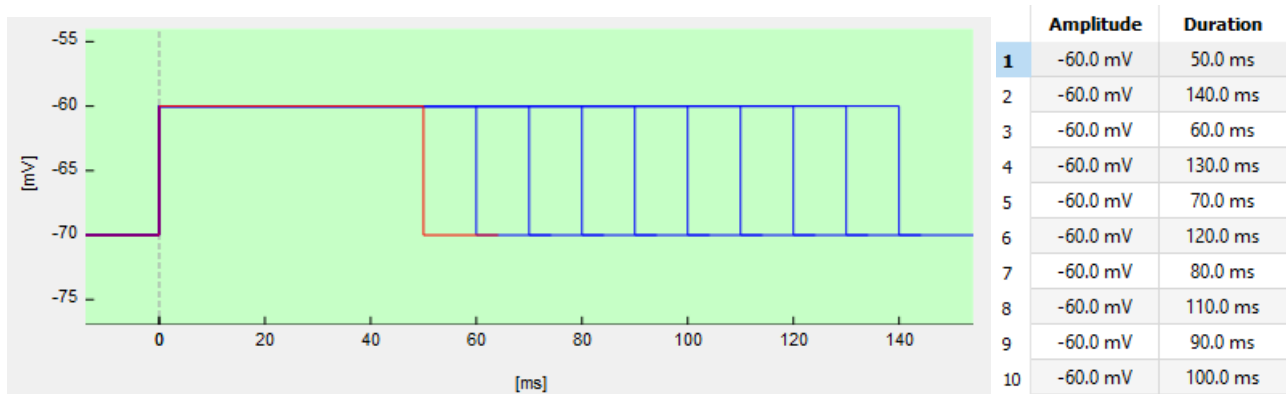


Figure 7.72: Example for Alternate mode of Duration Increment section. Starting potential of the first Sweep is 50 ms ending at 140 ms after ten iterations (10 Sweeps, Duration = 50 ms, Increment = 10 ms, Factor = 1).

Special Cases

Besides the usage of the basic *Increment* modes there are several special cases of increment creation.

Factor: When using a *Factor* the voltage step calculation is as follows (i=Sweep Index):

$$T_i = Duration * Factor^{(i-1)} + (i - 1) * Increment \tag{7.6}$$

Note: If the *Duration* is set to “0” the *Factor* is not applied but only the *increment* will determine the duration step calculation.

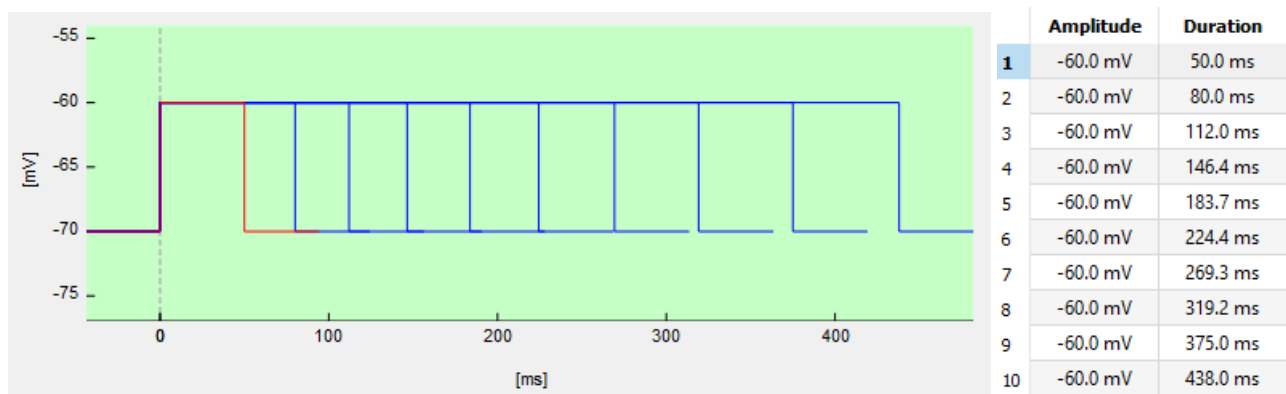


Figure 7.73: Example for duration step calculation by using the *Factor*. Starting duration of the first Sweep is 50 ms ending at 438 ms after ten iterations (10 Sweeps, Duration = 50 ms, Increment = 20 ms, Factor = 1.2, Increment Mode = Increase).

Note: The entered value for the *Factor* can be between “0.01” and “10”.

Invert Order: With this option one can invert the order of the applied Sweeps. For the *Increase* mode the order would be now: 10-9-8-7-6-5-4-3-2-1.

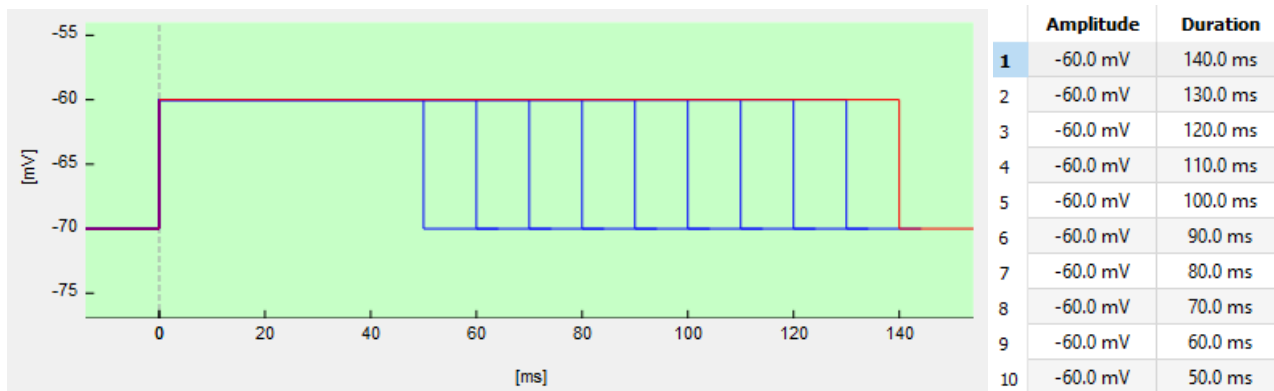


Figure 7.74: Example for Invert option of Duration Increment section. Starting duration of the first Sweep is 140 ms ending at 50 ms after ten iterations (10 Sweeps, Duration = 50 ms, Increment = 10 ms, Factor = 1).

Log Increment: When using *Log Increment* the voltage step calculation is as follows (i =Sweep Index):

Factor = 1

$$V_i = Voltage + (i - 1) * Increment \tag{7.7}$$

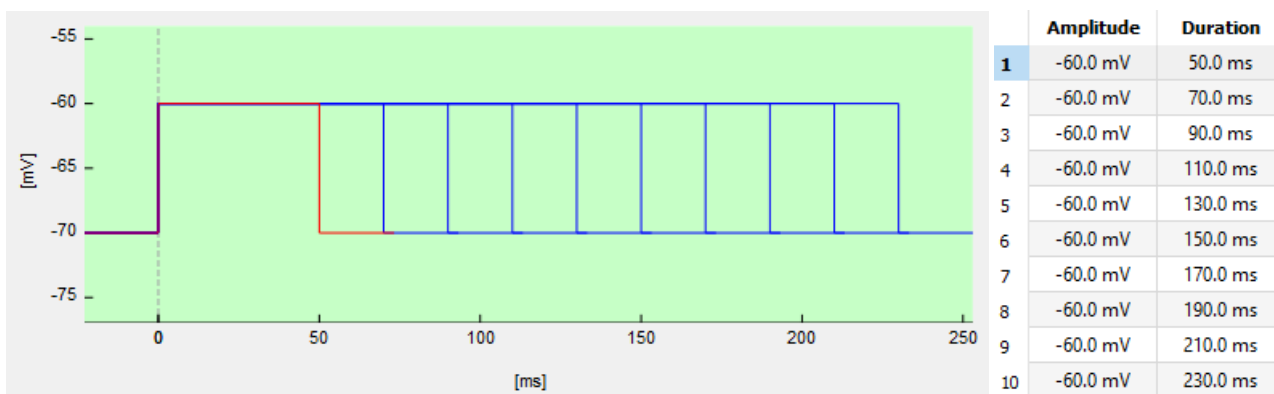


Figure 7.75: Example for duration step calculation by using the Log Increment option and Factor set to "1". Starting duration of the first Sweep is 50 ms ending at 230 ms after ten iterations (10 Sweeps, Duration = 50 ms, Increment = 20 ms, Increment Mode = Increase).

Factor $\neq 1$

$$V_i = \text{Voltage} + \text{Increment} * \text{Factor}^{i-2} \quad (7.8)$$

Note: The Log Increment is based on the linear increment step. Therefore, the Increment cannot be "0".

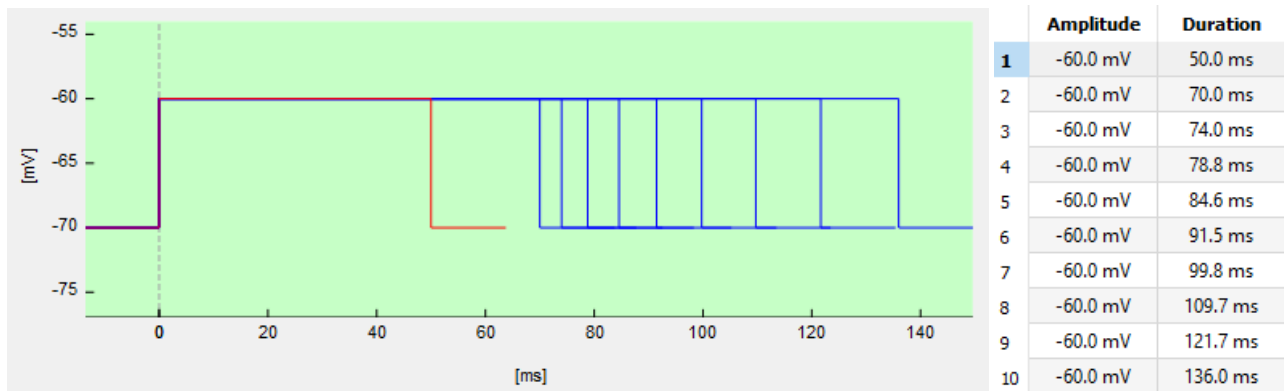


Figure 7.76: Example for duration step calculation by using the Log Increment option and Factor set to "1.2". Starting duration of the first Sweep is 50 ms ending at 136 ms after ten iterations (10 Sweeps, Duration = -50 ms, Increment = 20 ms, Increment Mode = Increase).

Increment Range: In this display field one gets a kind of summary about the minimum and maximum duration applied in this segment, thereby defining the range of the incrementing time steps.

7.9 Cartoon Section

In the Cartoon Section of the Stimulus Editor one gets an graphical overview about the stimulation sequence.

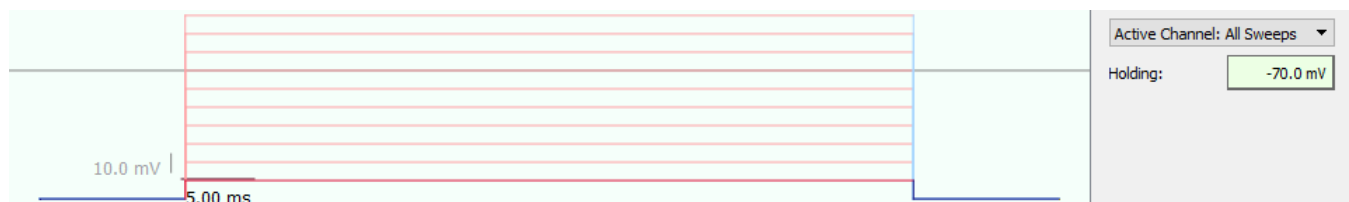


Figure 7.77: Cartoon Section of the Increment Editor.

After each editing operation, the stimulus template is refreshed to reflect the changes made. Sweeps other than the first one are shown as dashed lines.

If parts of the pulse pattern exceeds the DA limits, the corresponding warning message, e.g. "Segment: Voltage too large, maximum is: + 1.023 V" appears in the Notebook/Log window. Segments drawn in red color refer to the relevant Y-segment. The zero line of the Y-axis (current or voltage) is drawn in gray color.

Further, there is a right-click context menu available:

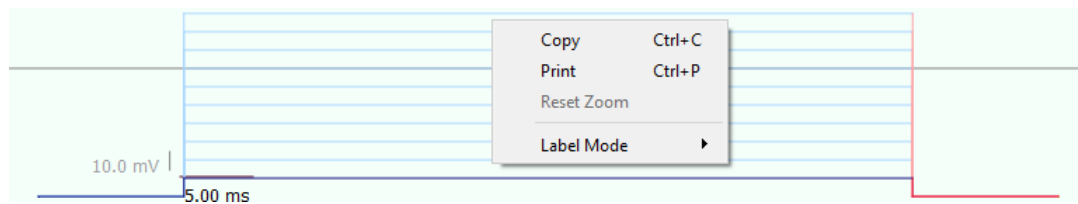


Figure 7.78: Context menu of the Cartoon Section.

- Copy: Creates a copy of the *Cartoon* in the clipboard [CTRL+C]
- Print: Prints the *Cartoon* with your local printer [CTRL+P]
- Label Mode: Defines the type of graph scaling.
 - Labels Only: Only two calibration bars are displayed.
 - Grid + Labels: A grid and the units/divisions are drawn.
 - Grid + Values: Draws a labeled grid.
 - No Labels: Neither labels nor a grid is shown.

Preview Mode: Via the selection list on the right side one can select what shall be displayed in the *Cartoon Section*:

- Active Channel: All Sweeps: Displays the settings of the active channel for all Sweeps. Recommended if you use increment modes in your settings.
- Active Channel: First Sweep: Displays the settings of the active channel for the first Sweep.
- Active Channel: Last Sweep: Displays the settings of the active channel for the last Sweep.
- All Channels: All Sweeps: Displays the settings of all channels for all Sweeps. Recommended if you use *Increment* modes in your settings.
- All Channels: First Sweep: Displays the settings of all channels for the first Sweep.
- All Channels: Last Sweep: Displays the settings of all channels for the last Sweep.

Holding: This control displays the presently selected membrane potential/membrane current. It is **only** used for the sequence cartoon as reference and can be changed without actually affecting the membrane potential/current in the Amplifier window.

7.10 PGF-Parameters

At the bottom of the Stimulus Editor the *PGF-Parameters* section is located. These global variables can be used for stimulus sequence editing. They are also accessible from other parts of the software like the Protocol Editor.

p1	p2	p3	p4	p5	p6	p7	p8	p9	p10
0.1	0	0.1	0.09	0.01	0.045	0	0	0	0

Figure 7.79: PGF-Parameters section of the Stimulus Editor.

Use the *PGF-Parameters* as variables in the segment settings for *Voltage/Current* or *Duration*. This allows you to change multiple settings in one or more sequences with changing only one parameter. PATCHMASTER NEXT

provides 10 *PGF-Parameters* which will be stored with the Stimulus Editor file (*.pgf). Instead of *p1* to *p10* you can assign use specific names to make the parameter input more intuitive.

Example: Rename the PGF-Parameters “p1” and “p2” to “Voltage” and “Duration”, respectively. Now, you enter the “Voltage” value in [V] and the “Duration” in [s]. In the select-list for Voltage and Duration you can now select the PGF-Parameter names “Voltage” and “Duration” for the active segment. It is possible to do this for further segments or define one or several segments in this way and creating copies of them afterwards.

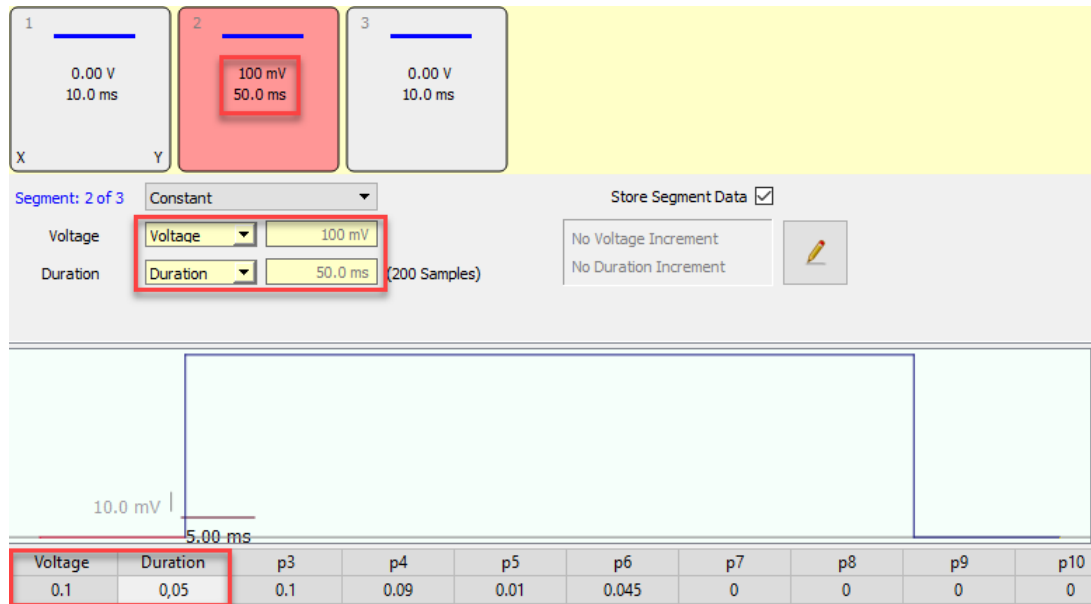


Figure 7.80: Example for PGF-Parameter settings in a stimulation sequence.

Note: The stimulus amplitude is given relative to 1 V in voltage clamp mode or relative to 1 nA in current clamp mode. Thus, to obtain a stimulus amplitude of 100 pA in current clamp mode you have to enter '100m' (= 0.1 * 1 nA).

8 Protocol Editor

The overall aim in good laboratory practices is to work under reproducible experimental conditions. Data collected from different personnel and at different setups should have the same conditions that they can be used for data pooling. User errors should be prevented as much as possible.

In order to accomplish the above mentioned points you can create an experimental protocol script in the Protocol Editor. By the use of such a protocol script the experimental approach is very much standardized. The Protocol Editor gives you access to all parts of the PATCHMASTER NEXT program. You can get input from these parts, make decisions, and control other parts of the program.

The Protocol Editor is the central part of PATCHMASTER NEXT, keeping the experimental cycle running. E.g. the Protocol Editor starts a data acquisition. Data are recorded, stored and analyzed by the Analysis Editor. Results from the Analysis Editor can be feed back into the Protocol Editor, e.g. making a decision in a conditional "If"-statement. Furthermore, the Protocol Editor can react on input from external devices or the user.

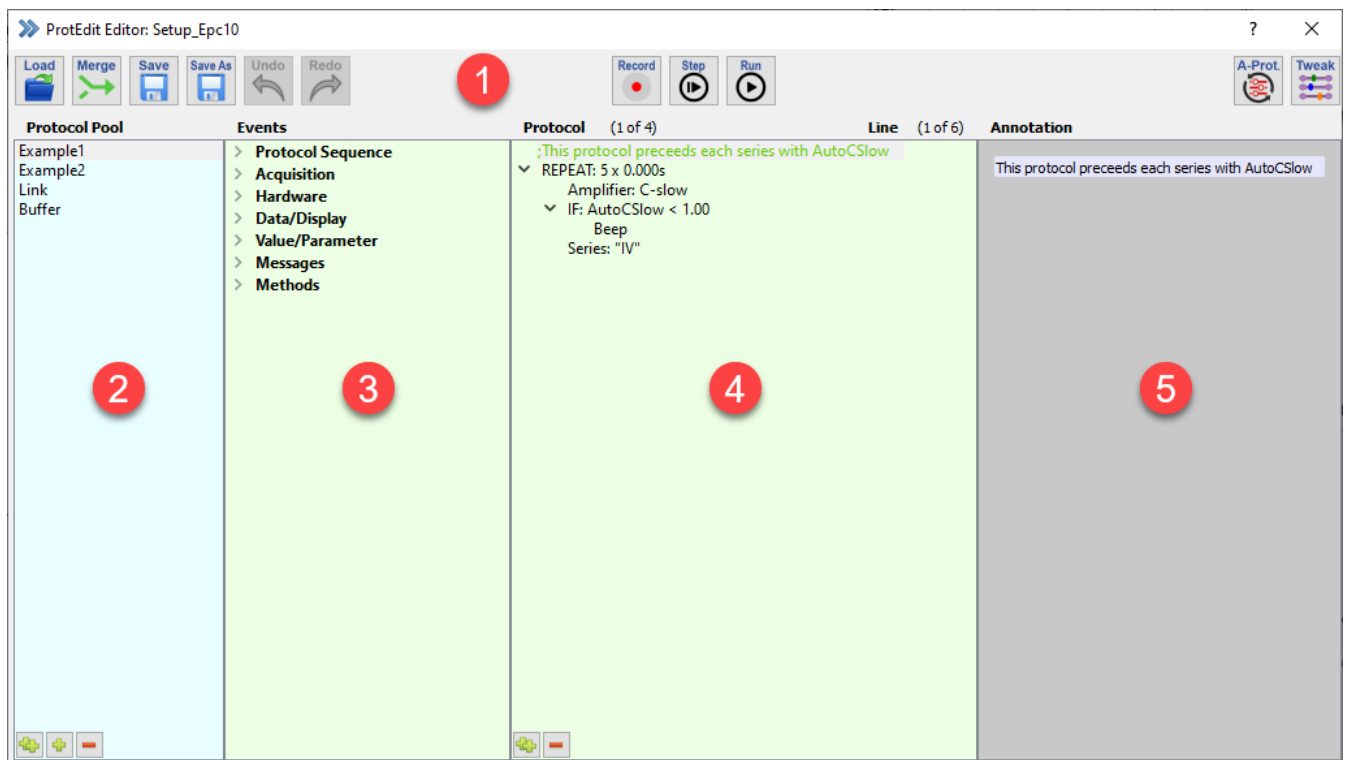


Figure 8.1: Protocol Editor window of PATCHMASTER NEXT.

With protocols, complex experimental procedures can be set up. During protocol execution no opening/closing of windows is necessary, thus the execution is pretty fast. Furthermore, in protocols it is possible to use conditional events, depending on e.g., analog or digital input, analysis results or key commands.

The Protocol Editor window consists of five sections:

1. Global Section
2. Protocol Pool
3. Event Pool
4. Protocol Sequence Section
5. Event Parameter Section

The Protocol Editor does also have two hierarchy levels:

1. Protocol Level: Indicated by blue background colors
2. Event Level: Indicated by green background colors

The hierarchy level colors provides orientation when defining parameters in a protocol sequence.

8.1 Global Section

The upper area of the Protocol Editor window which we call here “Global Section” provides several buttons to organize the Protocol Editor.



Figure 8.2: Global section of the Protocol Editor.

Load: Loads a Protocol Editor file (*.pro) with its protocols.

Merge: Loads another Protocol Editor file (.pro) into the currently opened file. In case a protocol name is not unique, you are prompted to either skip this protocol, mark it invalid, or give it a new name.

Save: Saves the protocols of the Protocol Pool in the currently opened Protocol Editor file.

Save As: Saves the protocols of the Protocol Pool in a new Protocol Editor file. The File Finder will open and you need to select a new destination to save the file and enter as well a name for the new Protocol Editor file.

Undo: Undo the last change made in the Protocol Editor file since it was last loaded.

Redo: If one used the *Undo* function before one can redo the latest modification made in the protocol sequence.

Record: This button activates the macro recording function. In the following the command lines for all activated events are recorded and inserted into the active protocol. Press the *Record* button again to finish the macro recording.

Step: Steps to the next event line in the events list and executes the event.

Run: Runs the protocol script through to its end.

A-Prot.: In the Test Pulse Window there are the three test pulse protocols named *SETUP*, *SEAL* and *WHOLE-CELL* which are essential for the patch clamp procedure (see chapter 5.1.1 on page 60). To have a better orientation in the Protocol Editor we introduced a new display mode like we did it in the Stimulus Editor. Now, by default, the custom-made protocols are shown for the user. By clicking onto the A-Prot. icon one can switch the display mode to show the amplifier protocols.

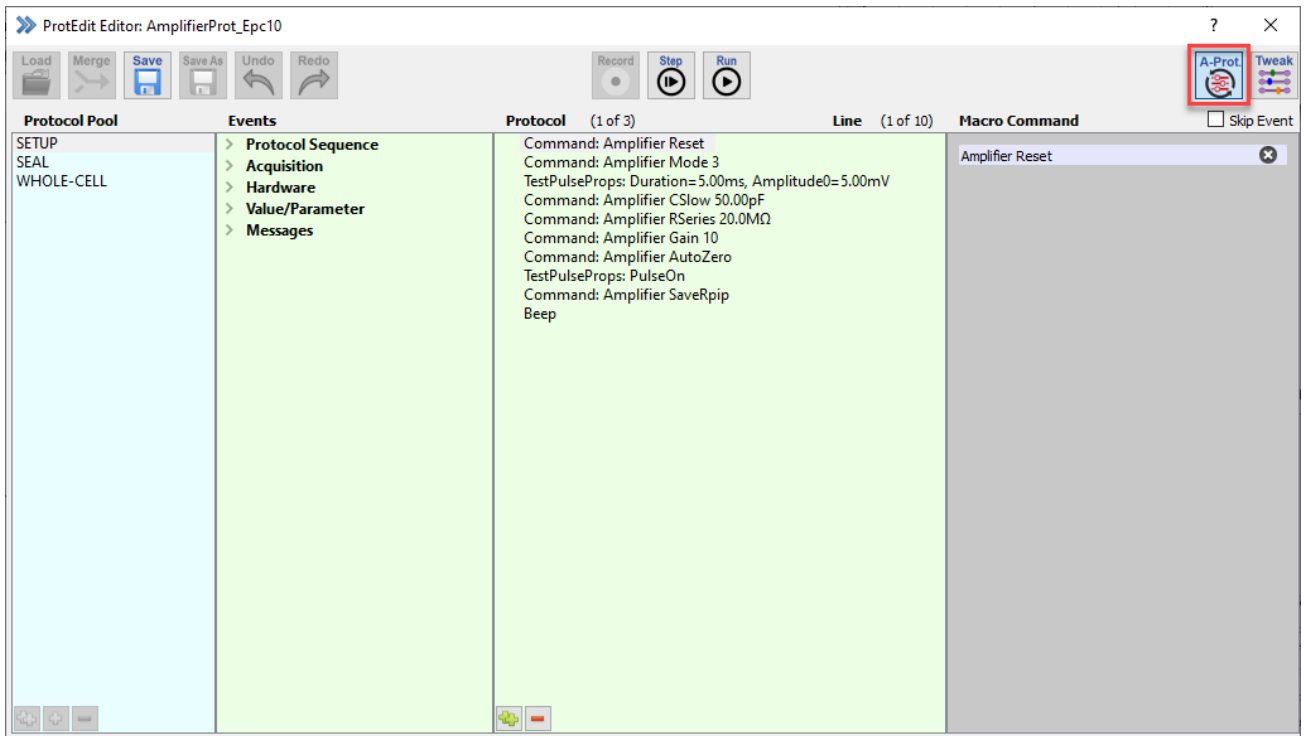


Figure 8.3: Test Pulse Protocol display mode of the Protocol Editor.

Tweak: By clicking on the *Tweak* button another extension bay of the Protocol Editor opens which shows the stack of operations performed in the loaded Protocol Editor file. This stack is the basis for the function of the *Undo* and *Redo* button.

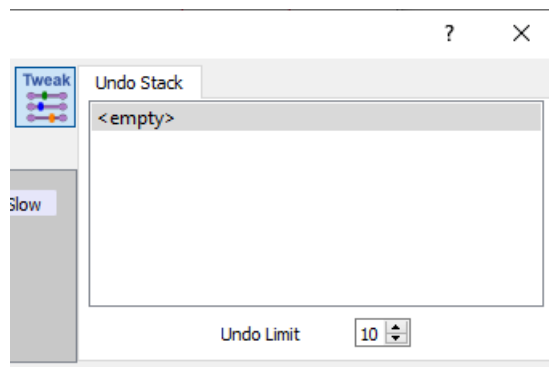


Figure 8.4: Tweak section of the Protocol Editor.

8.2 Protocol Pool

On the left side of the Protocol Editor the Protocol Pool is shown. It is a list of protocols stored in the currently loaded Protocol Editor file. A protocol is selected by clicking on it.

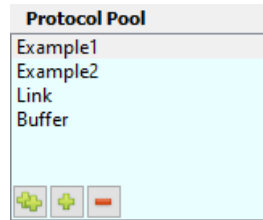


Figure 8.5: Protocol Pool of the Protocol Editor.

There are four options available to modify the Protocol Pool content:

- Edit Name: Double-click inside the protocol sequence name to change its name.
- Duplicate Protocol: Use the small button at the bottom with the two green plus signs to copy the selected protocol sequence. The name is taken over but an index number is added.
- Add new Protocol: Use the small button at the bottom with one green plus sign to create a new and empty protocol sequence. The new protocol sequence will be label "New Protocol".
- Delete Protocol: Use the small button at the bottom with the red minus sign to delete the selected protocol sequence from the Protocol Pool.

These options are also available via a right-click context menu.

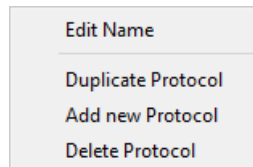


Figure 8.6: Right-click context menu of the Protocol Pool.

Moving Protocols: Via drag-and-drop it is easily possible to change the position of the selected protocol in the Protocol Pool.

8.3 Event Pool and Event Parameters

The `Event Pool` contains all pre-defined events which can be used in a protocol sequence. You can either double-click on a event to insert it into a protocol sequence or you can drag and drop the event to move it to a certain position of your protocol sequence.

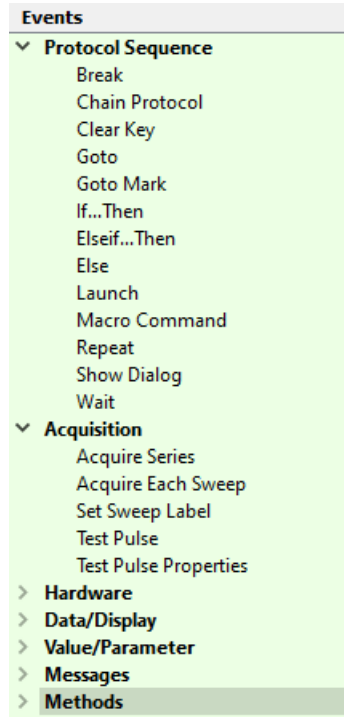


Figure 8.7: Event Pool of the Protocol Editor.

The Event Parameters get visible on the right side of the Protocol Editor when the event is inserted into a protocol sequence and if it is selected:

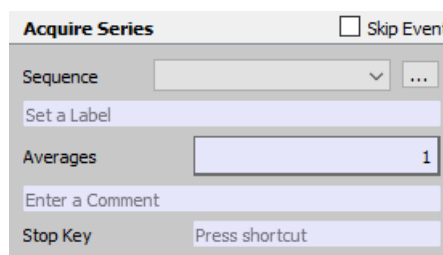


Figure 8.8: Event Parameters section of the Protocol Editor.

Each of these events can be “skipped” in the protocol sequence by activating the checkbox *Skip Event* on top of the `Event Parameter` section

The available events are categorized into seven groups in the `Event Pool`.

8.3.1 Protocol Sequence Group

The events of the `Protocol Sequence` group are important for the control of the protocol script itself.

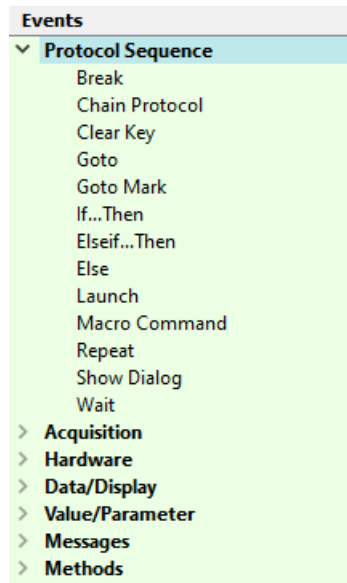


Figure 8.9: Protocol Sequence group of the Event Pool.

8.3.1.1 Break

Use to terminate a repeat loop or the protocol.

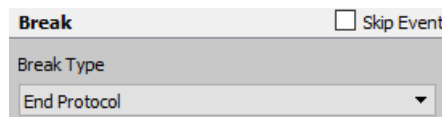


Figure 8.10: Break event of the Event Pool.

There are three *Break Types* options for the *Break* event:

- End Protocol: Stops the protocol execution completely at this point.
- End Repeat Loop: Ends the execution of a repeat loop (e.g. *Acquire Each Sweep...*) and jumps out of it to the following events. If this is used outside of a loop, the execution jumps to the end of the protocol.
- End If Loop: Ends the execution of an IF-THEN statement and jumps out of it to the following events. If this is used outside of a loop, the execution jumps to the end of the protocol.

8.3.1.2 Chain Protocol

This event is used to link one protocol sequence to another protocol sequence.

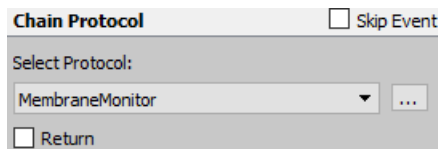


Figure 8.11: Chain Protocol event of the Event Pool.

Select Protocol: Select a protocol in the drop-down menu where you want to link to.

Edit Protocol: Jumps to the selected protocol sequence named in the field *Select Protocol*.

Return: If this option is not checked the protocol sequence will stop after executing the selected protocol. If the *Return* option is enabled the protocol sequence will come back to the main protocol and will be continued.

8.3.1.3 Clear Key

Normally a keystroke is stored during the execution of a protocol until a new key is pressed. In case the key buffer has to be cleared, the *Clear Key* event can be used. This is typically used when a protocol should react once on a single key stroke (e.g. execute an acquisition). In case the key would not be cleared the protocol would react repeatedly until another key is entered.

8.3.1.4 Goto

Use to jump to a *Goto_Mark*.

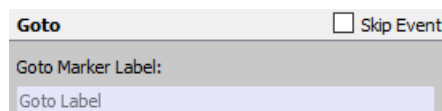


Figure 8.12: Goto event of the Event Pool.

Goto Label: Enter the name of the mark that denotes the destination of the Goto jump (see below).

8.3.1.5 Goto_Mark

Creates a *Goto_Mark*.

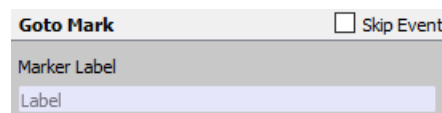


Figure 8.13: Goto Mark event of the Event Pool.

Label: Enter the name of the mark for a possible destination of a GOTO jump.

8.3.1.6 If..Then

Allows the definition of conditional events. Note that the list of available channel variables depends upon the chosen source.

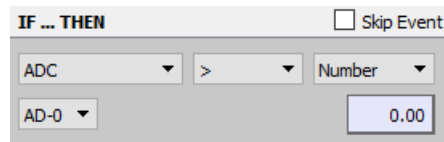


Figure 8.14: "If...Then" event of the Event Pool.

Left Source:

- ADC: AD channel to be read
 - Digital In: Digital Input
 - Parameter: Choose from a list of parameters, e.g., *Imon*, *C-Slow*...
 - Analysis: Choose from the list of available functions (1...16)
 - Value: Use one of the *Values 1...16* that can be defined in the event *Set Value*. In addition, results from the *Analysis* can be stored in these "Values" (see below). Therefore, the "Values" constitute an important interface between the Protocol Editor and the Analysis.
 - Key: Input a key command
 - Icon Value: Reads the value of an icon.
 - Repeat Count: Enter a number that will be compared with the actual *Repeat Count*. If the *Repeat Count* is... then...
 - Sweep Count: Enter a number that will be compared with the actual *Sweep Count*. If the *Sweep Count* is... then...
 - Last Sweep: If this condition is true
 - Sweep Aborted: In case a Sweep has been aborted with a *Stop* command or condition in the stimulation sequence.
 - Break Alert: The "If Break Alert" event pops up a dialog with a title (can be specified by the user) and the two buttons *Break* and *Continue*. A click on *Continue* proceeds with the first event after the *If... Then* loop. *Break* executes the events within the *If... Then* loop.
 - Batch Command: Checks if the last received batch control string contains a user-defined phrase (see *Controlling PATCHMASTER* in the *PATCHMASTER Tutorial*).
- Note: The Title strings must not include double quotes ("), since these double quotes are used as separators for splitting the event text.*
- Serial Command:
 - Series Name: Checks if the last name of a Series contains the user-defined phrase.
 - Overflow: Checks if an FIFO overflow occurred ("True") on the AD/DA board.
 - TRUE: Choose if the IF loop shall be used (True)
 - FALSE: Choose if the IF loop shall be not used (False).

Right Source:

- ADC: AD channel to be read

- Digital In: Dig-0...15. The operators for the Digital Input set the voltage.
- Parameter: Choose from a list of parameters, e.g., *Imon*, *C-Slow*... Additionally, the *Timer* parameter allows to time processes, e.g., reset a timer with the *Display Properties* event and measure time lapse since this event.
- Analysis: Function 1...16
- Value: One of the global variables (*Value-1* to *Value-n*) can be specified.
- Number: User-defined values are entered manually.
- TRUE: Choose if the IF loop shall be used (True)
- FALSE: Choose if the IF loop shall be not used (False).

Operators: Select the appropriate logical operator for the *If...Then* operation.

- >: Greater than
- <: Smaller than
- ABS >: Absolute value greater than
- ABS <: Absolute value smaller than
- =: Equal to
- <>: Between the ranges of
- MOD: A Modulo operation; it is an integer operation. The modulo operator will return TRUE, if the operation parameter value of *MOD* equals zero.
- invalid: Condition of a channel, value etc. has to be *invalid*.

8.3.1.7 Else...Then

Allows a secondary conditional event, e.g. a check after an *If...Then* step.

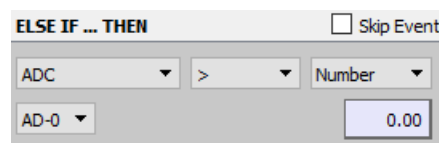


Figure 8.15: "ElseIf...Then" event of the Event Pool.

The available options are identical to the *If...Then* event (see chapter 8.3.1.6 on page 139).

8.3.1.8 Else

There are no parameters for this event. It indicates the final step of the "If" condition.

8.3.1.9 Launch

The *Launch* event allows to start another application. It is possible to enter an absolute path or a relative path to the application. The relative path to the application is defined relative to PATCHMASTER NEXT’s home path.

Note: Please be aware that the path should not contain any empty spaces inside.

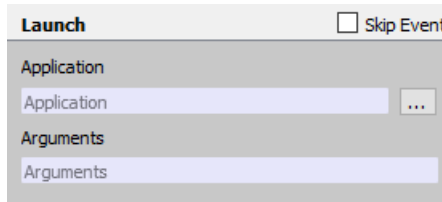


Figure 8.16: Launch event of the Event Pool.

- Application name: Enter path where the application is located either manually or use the “Edit” button to select the path.
- Arguments: Enter command line arguments if necessary.

8.3.1.10 Macro Command

Many controls that can not be accessed via the pre-defined events in the `Event Pool` can be targeted by using *Macro Commands*.



Figure 8.17: Macro Command of the Event Pool.

Command Name: Enter the name of the *Macro Command* you wish to include, e.g., "Amplifier AutoCSlow". Once you start writing a command a proposal list gets visible to enhance the *Macro Command* selection.

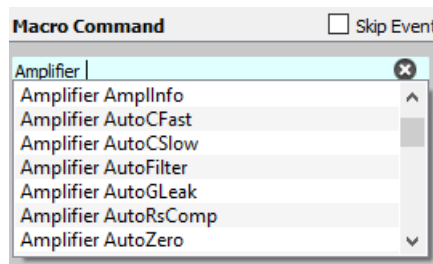


Figure 8.18: Macro Command selection list.

8.3.1.11 Repeat

Inserts a *Repeat* event which can contain other events.

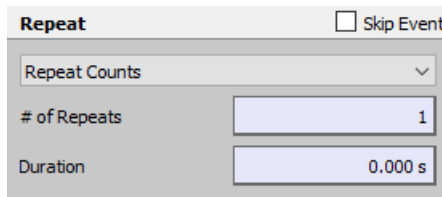


Figure 8.19: Repeat event of the Event Pool.

There are two types of repeat loops:

- Repeat Counts: Repeats for a given number of repeats.
- Repeat Indefinite: Repeats in an indefinite loop.

of Repeats: Defines the number of repeats.

Duration: Defines the time between each repeat. Please be aware that this time needs to match the time you need to go through your repeat loop.

8.3.1.12 Show Dialog

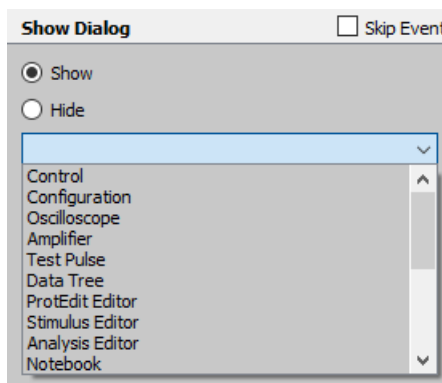


Figure 8.20: Show Dialog event of the Event Pool.

- Show: Brings the selected dialog to the front and makes it active.
- Hide: Closes the selected dialog.

8.3.1.13 Wait

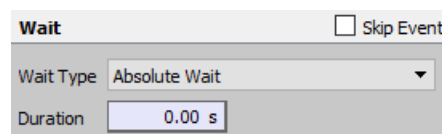


Figure 8.21: Wait event of the Event Pool.

Wait type:

- Absolute Wait: Waits exactly for the given delay time after the previous event.

- **Resume Icon:** Waits until the *Resume* button is clicked (i.e. sets the *Pause* button).
- **Key:** Waits until the specified key is entered. You can either enter a key or mark the *Any Key* option.
- **Comment Alert:** Will display a standard alert window where the user has to enter a new comment text. This comment will be used as long as no other is given.

8.3.2 Acquisition

The events of the *Acquisition* group are related to data acquisition processes.

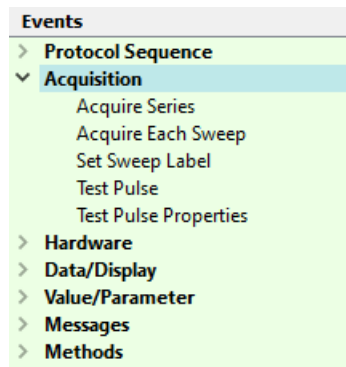


Figure 8.22: Acquisition event group of the Event Pool.

8.3.2.1 Acquire Series

Use this event to acquire an entire stimulation sequence.

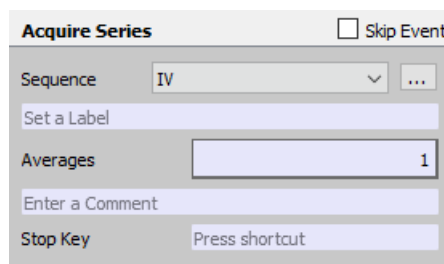


Figure 8.23: Acquire Series event of the Event Pool.

Sequence: Select the stimulation sequence for the acquisition from your currently loaded Stimulus Pool in this drop-down menu.

Edit Stimulation Sequence: Opens the stimulation sequence in the Stimulus Editor window.

Label: Enter a label for the Series. The label is taken over during data acquisition and visible in the Data Tree.

Averages: A stimulation sequence can be repeated several times. Only the average is stored to disk. Please enter the number of averages acquired for one Sweep.

Comment: Enter a comment to the Series. The entry is stored with the Series and therefore available in the *Comment* field for the Series in the Data Parameter window.

Stop Keys: Define a key to stop the acquisition of the running Series.

8.3.2.2 Acquire Each Sweep

This event allows executing other protocol events between the acquisition of Sweeps. E.g. use the *Amplifier* event to execute an *AutoCSlow* update before acquisition of each Sweep in a Series. A repeat loop is inserted with an *Acquire Each Sweep* event inside.

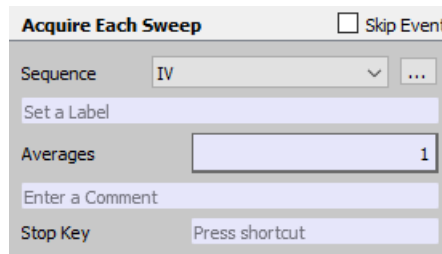


Figure 8.24: Acquire Each Sweep event of the Event Pool.

Sequence: Select the stimulation sequence for the acquisition from your currently loaded Stimulus Pool in this drop-down menu.

Edit Stimulation Sequence: Opens the stimulation sequence in the Stimulus Editor window.

Label: Enter a label for the Series. The label is taken over during data acquisition and visible in the Data Tree.

Averages: A stimulation sequence can be repeated several times. Only the average is stored to disk. Please enter the number of averages acquired for one Sweep.

Comment: Enter a comment to the Series. The entry is stored with the Series and therefore available in the *Comment* field for the Series in the Data Parameter window.

Stop Keys: Define a key to stop the acquisition of the running Series.

See chapter 8.3.1.11 on page 142 for the repeat loop options.

8.3.2.3 Set Sweep Label

Allows to put a label for the Sweep in the Data Tree to be acquired next.

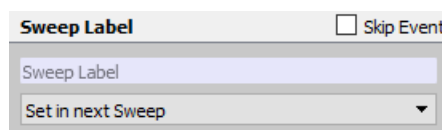


Figure 8.25: Set Sweep Label event of the Event Pool.

Sweep Label: Enter the name of the label into this entry field.

Sweep Label Target:

- Set in next Sweep: The *Sweep Label* is acquired in the next Sweep.
- Set in selected Sweep: The *Sweep Label* is acquired already in the selected Sweep.

The *Sweep Label* can be edited after the acquisition in the Data Tree.

8.3.2.4 Test Pulse Execution

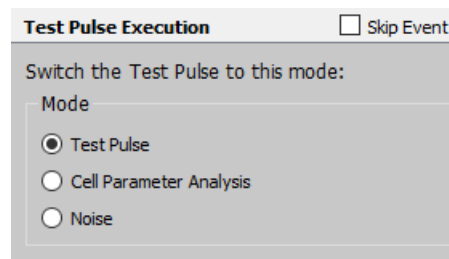


Figure 8.26: Test Pulse Execution event of the Event Pool.

- Test Pulse: Conventional Test Pulse mode
- Cell Parameter Analysis: Mode to calculate cell parameters
- Noise: For RMS noise calculation

Details about the *Test Pulse Modes* are available in chapter 5.1.2.1 on page 61.

8.3.2.5 Test Pulse Properties

The user can modify the Test Pulse Properties of the Test Pulse running in the Test Pulse Window.

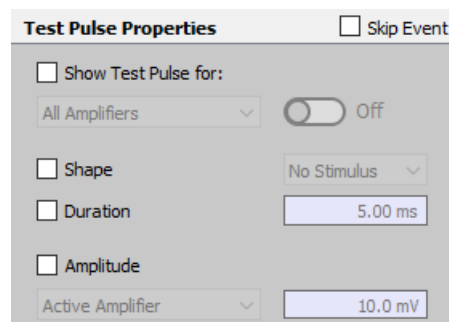


Figure 8.27: Test Pulse Properties event of the Event Pool.

Show Test Pulse for: If the checkbox is enabled, one can specify the amplifiers where the test pulse should be applied. With the slider beneath one can also activate or deactivate the test pulse for the specified amplifiers.

Shape: Enable the checkbox to activate the drop-down menu for the selection of:

- No Stimulus: No test pulse is applied
- Single: A monopolar square pulse is applied
- Double: A bipolar square pulse is applied
- Ramp: A ramp pulse is applied

Duration: Enable the checkbox to activate the entry field for setting the duration of the test pulse.

Amplitude: Enable the checkbox to activate the entry field for setting the amplitude of the test pulse. Further, one can specify if the set amplitude is applied to the active amplifier or to a selected amplifier.

8.3.3 Hardware

The events of the `Hardware` group allow to interact directly with the amplifier.

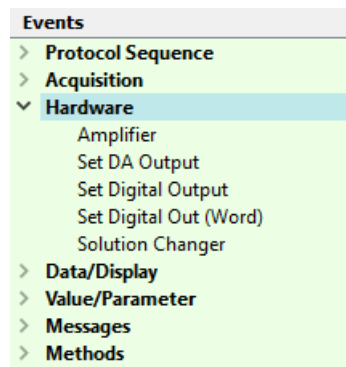


Figure 8.28: Hardware event group of the Event Pool.

8.3.3.1 Amplifier

This event is for controlling the main amplifier settings.

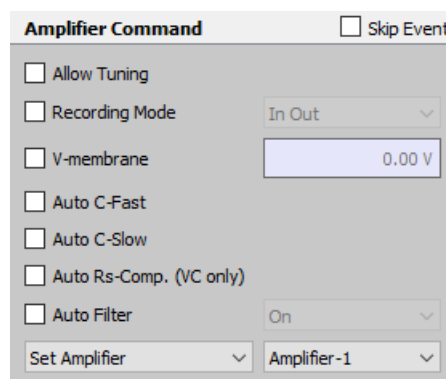


Figure 8.29: Amplifier event of the Event Pool.

Allow Tuning: The user can manually switch to the Amplifier window and adjust settings manually or just turn the *Test Pulse* on.

Recording Mode: Change the *Recording Mode* of the EPC 10 USB.

- In Out: Inside-out configuration (voltage clamp)
- On Cell: On-Cell configuration (voltage clamp)
- Out Out: Outside-Out configuration (voltage clamp)
- Whole Cell: Whole-Cell configuration (voltage clamp)
- C-Clamp: Current Clamp mode

V-membrane: Sets the desired holding voltage in voltage clamp mode and the holding current in current clamp mode.

Auto C-Fast: Perform an *Auto C-Fast* compensation.

Auto C-Slow: Perform an *Auto C-Slow* compensation.

Auto Filter: This option automatically sets a hardware filter according to the chosen sample interval.

- On: The *Auto Filter* option is enabled.
- Off: The *Auto Filter* option is disabled.

Amplifier Selection: Choose which amplifier should be selected. In general there are two options:

- Keep Amplifier: The currently active and selected amplifier board in the Amplifier window is kept.
- Set Amplifier: Defines the active amplifier board in the Amplifier window.

Note: This option is only available when using an EPC 10 USB Double, Triple or Quadro amplifier.

8.3.3.2 Set DA Output

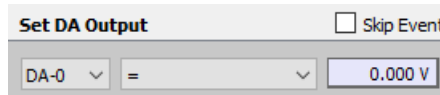


Figure 8.30: Set DA Output event of the Event Pool.

DA output: Select a channel for analog output.

Voltage: Enter a voltage that will be output via the given channel.

Note: The Set DA Output event does not consider any scaling applied by equation or lookup table. Only entered scale factors are considered.

8.3.3.3 Set Digital Output

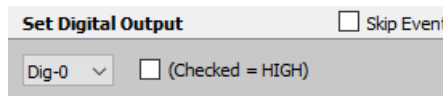


Figure 8.31: Set Digital Output event of the Event Pool.

Channel: Select a channel for digital output from the drop-down menu.

High/Low: Activate the checkbox in front of the drop-down menu to set the selected channel to “high” (5 V). If the checkbox is deactivated the selected channel will be set to “low” (0 V).

8.3.3.4 Set Digital Out (Word)

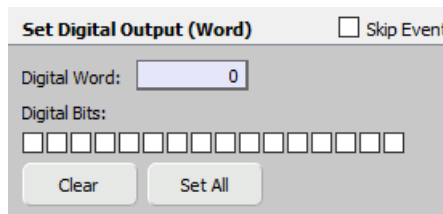


Figure 8.32: Set Digital Out (Word) event of the Event Pool.

Digital Word: The entered number will be automatically translated into the digital bits.

Digital Bits: You can manually activate or deactivate the digital bits by clicking into the checkboxes.

Clear: Deactivates all active digital bits.

Set All: Activates all digital bits.

8.3.3.5 Set Solution Changer

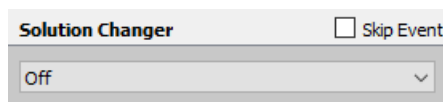


Figure 8.33: Set Solution Changer event of the Event Pool.

Solution: Select a *Solution* (Valve 1-8) at the connected *Perfusion System*.

Note: The output channels and their aligned solutions have to be defined first in the Perfusion Control dialog (chapter 15 on page 229).

8.3.4 Data/Display

The events of the `Data/Display` group allow data interaction processes and control display properties of the data.

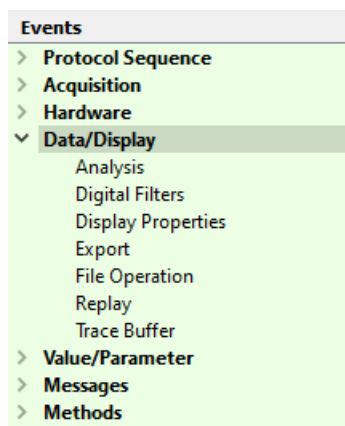


Figure 8.34: Data/Display event group of the Event Pool.

8.3.4.1 Analysis

The *Analysis* event allows to set the proper Analysis Methods in front of the subsequent data acquisition.

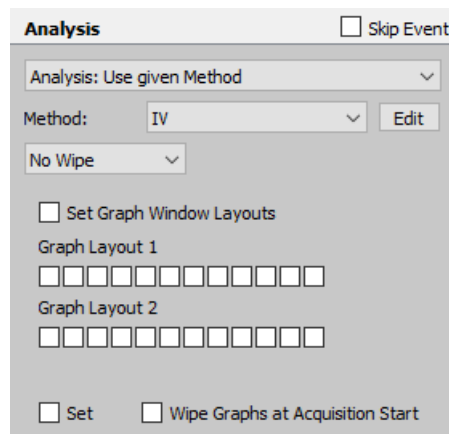


Figure 8.35: Analysis event of the Event Pool.

Analysis Mode: The following *Analysis Modes* are available:

- **Analysis: Keep:** Preserves the current Analysis Mode setting of the Analysis window.
- **Analysis: Use given Method:** This Analysis Mode selects one of the specified Analysis Methods in the drop-down menu.
- **Analysis: Use selected Method:** This Analysis Mode uses the currently active Analysis Method.
- **Analysis: Automatic Stimulus Control:** In this mode the Analysis Method specified in the stimulation sequence is used.
- **Analysis: No Analysis:** The analysis is switched off.

Method: Select the appropriate Analysis Method stored in your loaded analysis file (*.on1) from the drop-down menu.

Edit: Opens the Analysis window with the given method for direct editing.

Wipe: Can be used to wipe individual graphs or all graphs at once.

- No Wipe
- Wipe All Graphs
- Wipe Graph 1...12

Set Graph Layouts: Activate the checkbox in front to apply the set graphs to the selected Analysis Method.

Set: Activate the checkbox in front to enable the *Wipe Graphs at Acquisition Start*.

Wipe Graphs at Acquisition Start: If this option is activated the Analysis Window and the Oscilloscope Window will be wiped before data acquisition.

8.3.4.2 Digital Filters

This event defines the cut-off frequencies of digital filters in Hz for Trace 1...16 (1 – 16) and Buffer 1...4 (B1 – B4). The checkbox is used to mark which of the filters are to be set.

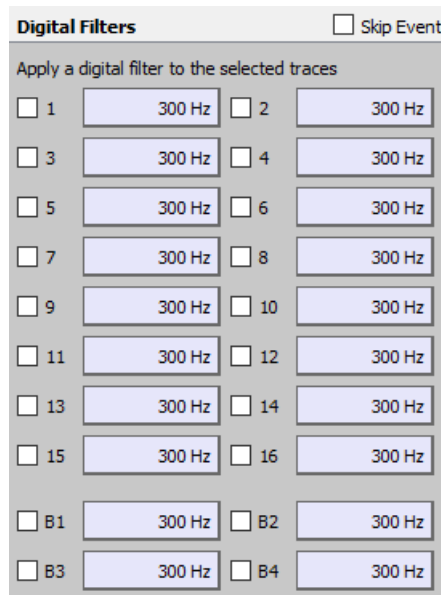


Figure 8.36: Digital Filter event of the Event Pool.

8.3.4.3 Display Properties

Use this event to control the display settings and behavior of the Oscilloscope window.

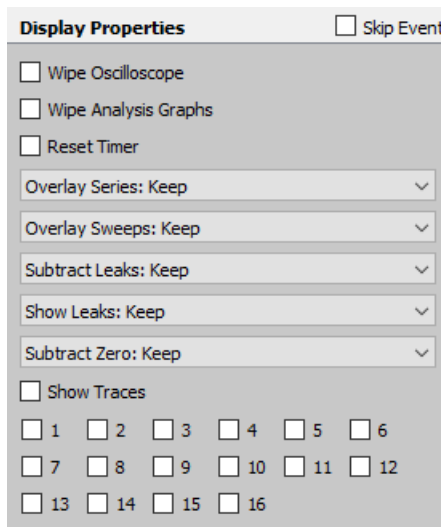


Figure 8.37: Display Properties event of the Event Pool.

Wipe Oscilloscope: Deletes all displayed data in the Oscilloscope window.

Wipe Analysis Graphs: Deletes all displayed data in the Graph Window 1 & 2.

Reset Timer: Resets the *Timer* in the Control window.

Overlay Series:

- Keep: Keep the state that was set before this event.
- On: *Overlay Series* on.
- Off: *Overlay Series* off.

Overlay Sweeps:

- **Keep:** Keep the state that was set before this event.
- **On:** *Overlay Sweeps* on.
- **Off:** *Overlay Sweeps* off.

Subtract Leaks:

- **Keep:** Keep the state that was set before this event.
- **On:** *Subtract Leaks* on.
- **Off:** *Subtract Leaks* off.

Show Leaks:

- **Keep:** Keep the state that was set before this event.
- **On:** *Show Leaks* on.
- **Off:** *Show Leaks* off.

Subtract Zero:

- **Keep:** Keep the state that was set before this event.
- **On:** *Subtract Zero* offset on.
- **Off:** *Subtract Zero* offset off.

Show Traces: Changes the show flag of all shown Traces. This function can be used to show only a subset of acquired Traces in the Oscilloscope window. Note that you have to mark *Show Traces* for the changes to take effect.

Note: The number of Traces shown in the software is defined in the Configuration.

8.3.4.4 Export

Exports the acquired data according to the export settings made in the `Data` menu of the `Data Tree` window (see chapter 11.1.2 on page 197). Unless you activate the checkbox *Export Full Sweep* the data is exported as displayed in the Oscilloscope window.

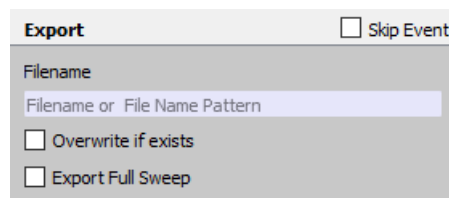


Figure 8.38: Export event of the Event Pool.

Filename: Define a name for exported file according to the auto filename components described in the Configuration (see chapter 3.5.4 on page 34).

Overwrite if exists: Enables to overwrite already existing output files.

Export Full Sweep: The full *Sweep* is exported independent of what is shown in the Oscilloscope.

8.3.4.5 File Operation

The *File Operation* event allows to manage data and file handling. With this event you are able to e.g. open or close data files, store or non-store acquired data or defining the labels of new data sets.

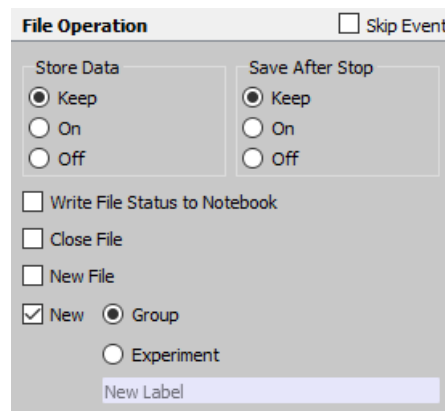


Figure 8.39: File Operation event of the Event Pool.

Store Data:

- **Keep:** Keeps the status that was set before this event (either “On” or “Off” of the *Store* button).
- **On:** Acquired data will be saved to disk. This corresponds to the active *Store* button.
- **Off:** Data will not be saved to disk. This corresponds to the deactivated *Store* button.

Save After Stop:

- **Keep:** Keeps the status that was set before this event (either “On” or “Off” of the *Save After Stop* parameter in the Configuration).
- **On:** Acquired Sweep data will be stored if the Sweep was terminated with the *Stop* button.
- **Off:** Sweep data will not be saved to disk if the Sweep was terminated with the *Stop* button.

Write File Status to Notebook: Prints the file status to the Notebook. The file status provides information e.g. about your free hard disk space, the file size, the filename and the file path.

Close File: The data file will be closed.

New File: A new file will be created. The ability to be able to create a new data file is very convenient when the auto filename generation is used.

New: This function provides the following options:

- **Group:** Creates a new *Group*.
- **Experiment:** Creates a new *Experiment*.
- **Label:** It is active when a new *Group* or a new *Experiment* is created and it specifies the *Group* label.

8.3.4.6 Replay

This event provides the options to scroll through the Data Tree and selecting or replaying your data.

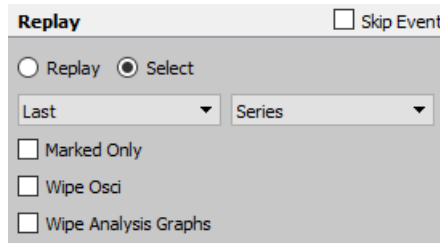


Figure 8.40: Replay event of the Event Pool.

There are two purposes for operating in the *Replay* event:

- **Replay:** Replays the selected data in the Data Tree.
- **Select:** Selects the data in the Data Tree without replaying it.

Tree Level: Selects the level of the data:

- Next
- Present
- Last

Tree Target: Selects the target level of your data:

- **Group:** Select the Group.
- **Series:** Select the Series.
- **Sweep:** Select the Sweep.
- **Trace:** Select the Trace.

Marked Only: Only marked data in the Data Tree will be affected.

Wipe Osci: Deletes all displayed data in the Oscilloscope window.

Wipe Analysis Graphs: Deletes all displayed data in the Analysis Graphs.

8.3.4.7 Trace Buffer

The *Trace Buffer* event provides the options to operate with the Trace Buffers of PATCHMASTER NEXT.

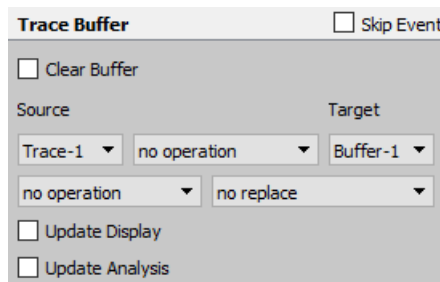


Figure 8.41: Trace Buffer event of the Event Pool.

Clear Buffer: Clears the Buffer selected in the *Target* selection.

Source: Define the data source for the buffer operation from the drop-down menu. Valid source data are:

- Trace-1 to Trace-32
- Buffer-1 to Buffer-4

Operation: Define the operation which should be applied to the data before storing it in a Buffer:

- no operation
- add to
- subtract from
- accumulate in
- deaccumulate from

Target: Select the target for the buffer operation. Valid targets are:

- Buffer-1 to Buffer-4

Scaling:

- no operation: No scaling operation is performed.
- scale trace: Use this scaling option to scale the Trace which is used for the Source.
- scale buffer: Use this scaling option to scale the Buffer content which is used for the Source.

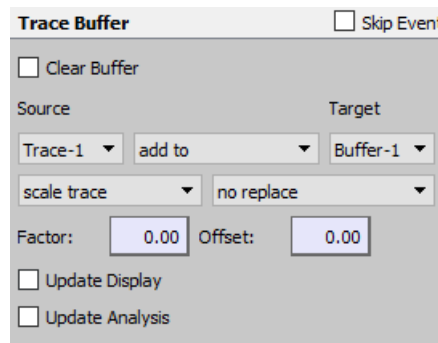


Figure 8.42: Scaling option of the Trace Buffer event.

Further options are available when either *scale trace* or *scale buffer* is selected:

- Factor: Enter a scaling factor which is used for the scaling process.
- Offset: Enter an offset value which is used for the scaling process.
- equation: Calculates the scaling of the Source via an equation. The equation can be directly entered into the text field or click the *Edit* button to open the Equation Editor. For details on the equation syntax please refer to chapter 17 on page 235.

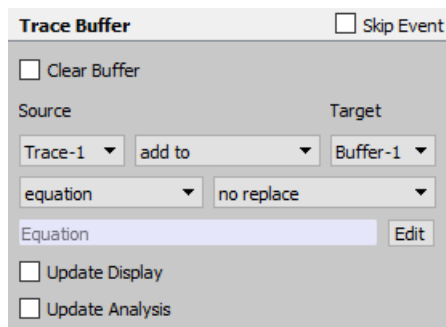


Figure 8.43: Equation option of the Trace Buffer event.

Replace: Replaces the selected Trace with the Buffer content.

Update Display: Updates the display of the Buffer in the Oscilloscope window.

Update Analysis: Updates the display of the Buffer in the Analysis Graphs.

8.3.5 Value/Parameter

The events of the Value/Parameter group enable to operate with PGF Parameters and Values.

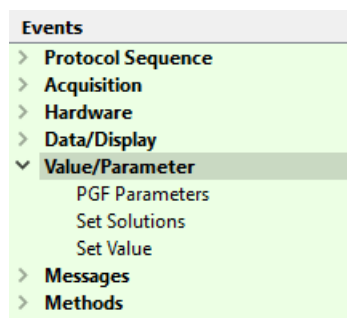


Figure 8.44: Value/Parameter event group of the Event Pool.

8.3.5.1 PGF Parameters

PGF parameters can be set or modified during the protocol to automatically adapt the acquisition sequences. They are used as variables in the Stimulus Editor (see chapter 7.10 on page 131).

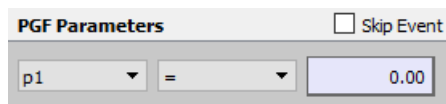


Figure 8.45: PGF Parameters event of the Event Pool.

PGF Parameters 1...10: Specify a PGF parameter from the list for the forthcoming operation.

Operation: To modify a PGF parameter the following operations are available:

- =
- increase by
- decrease by

- multiply by
- divide by
- = value
- inc by value
- dec by value
- mul by value
- div by value

Value: Either enter a value to be used for the operation with the *PGF Parameter* or select a *Value* from the drop-down menu.

Note: Please keep in mind that the stimulus amplitude is given relative to 1 V or 1 nA in voltage clamp and current clamp mode, respectively. I.e., to obtain a stimulus amplitude of 100 pA in current clamp mode you have to enter a value of "100m" (= 0.1 * 1 nA).

8.3.5.2 Set Solutions

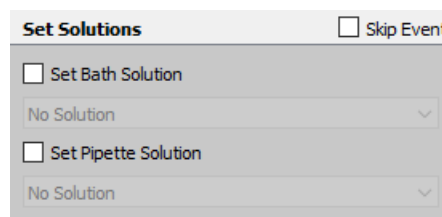


Figure 8.46: Set Solution event of the Event Pool.

Set Bath Solution: User-defined index number for the bath solution. Activate the checkbox to apply the setting.

Set Pipette Solution: User-defined index number for the pipette solution. Activate the checkbox to apply the setting.

8.3.5.3 Set Value

The *Set Value* event allows you to freely define and store values that can be used in an *If...Then* event such that the *If* event can make decisions based on these stored values. In addition, results from the *Analysis* can be stored in these values (see chapter 9.3.1.3 on page 171). The values, therefore, constitute an important interface between the Protocol Editor and the Analysis.

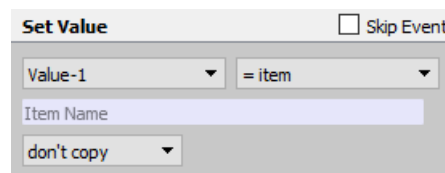


Figure 8.47: Set Value event of the Event Pool.

Source Value: Define the User Value 1 or 2 or one of the Values (1-32) for the forthcoming operation.

Operation: The following *Operations* are available:

- manually entered values:
 - =
 - increase by
 - multiply by
 - divide by
 - modulo
 - keep (no change)
- referring to an *Item*:
 - = item
 - inc by item
 - dec by item
 - mul by item
 - div by item
- referring to a *Parameter*:
 - = param
 - inc by param
 - dec by param
 - mul by param
 - div by param
- referring to a *Value*:
 - = value
 - inc by value
 - dec by value
 - mul by value
 - div by value
- referring to an *Analysis* results:
 - = analysis
 - inc by analysis
 - dec by analysis
 - mul by analysis
 - div by analysis
- = equation: An equation can be entered to compute a value. For details on the equation syntax please refer to chapter 17 on page 235.
- = input: A dialog opens for user input of a new numeric value.
- = batch comm. value: A value received via the batch communication interface is assigned to the respective value.

Copy:

- don't copy: No further operation.
- copy to item: Copies the value to a specified item.
- add to item: Adds the value to a specified item.
- multiply to item: Multiplies specified item with the value.

8.3.6 Messages

The events of the Messages group allow to create sound events, annotations or writing e.g. values to the Notebook.

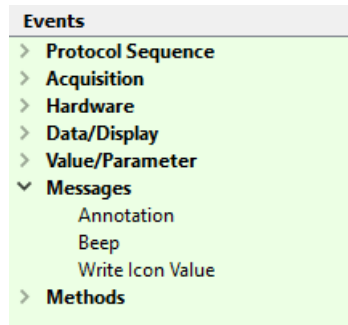


Figure 8.48: Messages event group of the Event Pool.

8.3.6.1 Annotation

Use this function to add comments to your protocol and improve the readability.

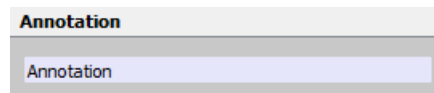


Figure 8.49: Annotation event of the Event Pool.

The annotation text can be entered into the entry field.

8.3.6.2 Beep

Use this event to create an sound output via your PC. This can e.g. be used to indicate a finished protocol sequence.

8.3.6.3 Write Icon Value

Outputs the value of the specified icon into the Notebook like this:

[Title] [ItemID]: [value]

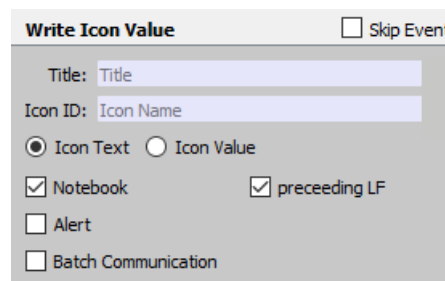


Figure 8.50: Write Icon Value event of the Event Pool.

Title: Title for the *Item ID*, leads to an improved readability of the output but is not mandatory.

Icon ID: Unique identifier of icons, text field or buttons. Start writing in the entry text field to see the auto-suggestion list (e.g. "Amplifier" to see all the items addressable from the Amplifier window).

Icon Text: Writes the text from a field. For example from the icon *Amplifier Gain* would return "1.0 mV/pA".

Icon Value: Writes the value of a field. For example from the icon *Amplifier Gain* would return "8.0", meaning the 8th item of the *Gain* list corresponding to "1.0 mV/pA".

Notebook: Writes the *Icon Text* or the *Icon Value* to the Notebook Window.

preceding LF: When active, a linefeed signal is used before the value gets e.g. printed into the Notebook.

Alert: Opens an *Alertbox* containing the *Icon Text* or the *Icon Value*.

Batch Communication: Writes the Batch Communication commands to the Notebook Window

8.3.7 Methods

The `Protocol Editor Methods` are a special feature of PATCHMASTER NEXT. They differ from other operations in the Protocol Editor because they automatically create a transient stimulation sequence and an *Analysis Method* upon execution of the protocol. Transient means that they will not be saved with other changes made in the Stimulus Editor and Analysis Editor, but instead created or overwritten when executing the protocol. Therefore, if changes should be made by the user to both the stimulation sequence and analysis method a copy with a new name of the created stimulation sequence and analysis method has to be made.

The idea of these `Protocol Editor Methods` for standard electrophysiological and electrochemical techniques is that the user does not have to create a complex stimulation sequence with several segments and an analysis method from scratch, but instead a standardized method can be executed immediately where only measurement parameters have to be entered in the Protocol Editor.

8.3.7.1 Cyclic Voltammetry

Cyclic Voltammetry Skip Event

Start / End Potential

Use V-membrane

Keep at End

Auto Ranging

Reversal Potential 1

Increment

Reversal Potential 2

Increment

Scan Rate

Increment

Cycles

+ End Cycle

Anodic Peak between and

Cathodic Peak between and

Baseline: Zero Cursor Intersections

Total Length

Figure 8.51: *Cyclic Voltammetry* event of the Event Pool.

Start/End Potential: Potential at which the start phase begins and the end phase stops. At the start of the CV the potential steps from the initial potential to the *Start/End Potential*.

Use V-membr: If checked, the initial potential is used as *Start/End Potential*.

Keep at End: If checked, the *Start/End Potential* is set as new initial potential.

Auto Ranging: If checked, the complete CV method will be executed multiple times until it has reached the optimal current range. Only the last CV will be stored.

Reversal Pot. 1: By definition, the CV should start with the more negative reversal potential.

Increment: If multiple cycles are executed the first reversal potential can be altered by a fixed increment from Sweep to Sweep.

Reversal Pot. 2: By definition, the second reversal potential should be more positive than the first one.

Increment: If multiple cycles are executed the second reversal potential can be altered by a fixed increment from Sweep to Sweep.

Scan Rate: Anodic and cathodic Sweeps use the same *Scan Rate* (see also 7.3 on page 88).

Increment: If multiple cycles are executed the scan rate can be altered by a fixed increment from Sweep to Sweep.

Cycles: If the number of cycles is larger than one, multiple Sweeps are executed in *Gap Free Mode*.

+End cycle: This option ensures a symmetric amount of anodic and cathodic peaks. Therefore the last cycle will be prolonged until the last cycle is completed.

Anodic Peak between: The *Anodic Peak* will be analyzed as *Maximum* in the segment scanning from *Reversal Pot. 1* to *Reversal Pot. 2*. Scanning towards positive potentials is required. The voltage range for analyzing the maximum current can be defined.

Cathodic Peak between: The *Cathodic Peak* will be analyzed as *Minimum* in the segment scanning from *Reversal Pot. 2* back to *Reversal Pot. 1*. Scanning towards negative potentials is required. The voltage range for analyzing the minimum current can be defined.

Baseline: For the peak analysis you can choose if the *Baseline* should be zero or a line drawn through the intersections between current and cursor lines.

Total Length: This display field shows the total length of the CV protocol (1 Sweep).

8.4 Protocol Sequence Section

The `Protocol Sequence Section` contains the list of added events of the currently selected protocol.

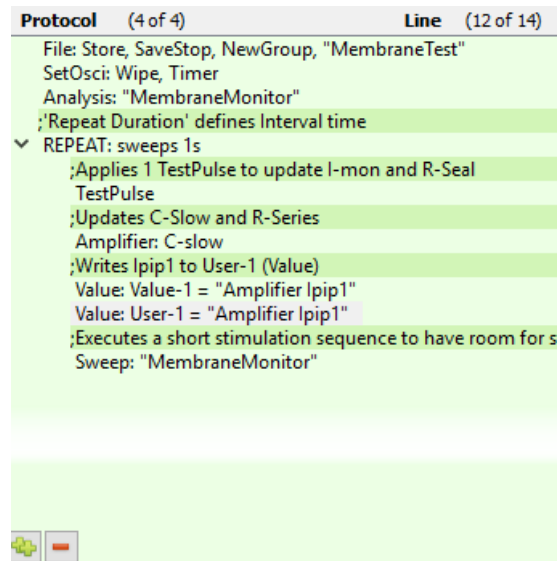


Figure 8.52: Write Icon Value event of the Event Pool.

A right-click onto one of the inserted events open a context menu providing the following options:

- Skip Event: Activates the *Skip Event* checkbox for this event to exclude it from execution.
- Duplicate Event: Duplicates the selected event line and inserts it after the selected event line.
- Delete Event: Deletes the currently selected event line.
- Step: Executes the selected event line.
- Run: Executes all event lines starting from the selected event line until the last event line of the protocol sequence.

Further, one can select an event line and keep the left-mouse button pressed. This provides the option to drag and drop the event in the protocol sequence.

For better orientation when either having several protocols in the Protocol Pool or working on a lengthy protocol sequence the number of the selected protocol in the protocol pool (here: 4 of 4) and the event line of the currently selected event in the protocol sequence is highlighted (here: 12 of 14) at the very top of this section..

Add the bottom of this section two small buttons to either duplicate a selected event line or to delete a selected event line are provided.

9 Analysis Editor

The Analysis Editor allows you to immediately calculate (online) and display data based on the acquired Traces, thus giving you a fast overview of your results. Further, you can also use it to analyze your data after the experiment (offline).

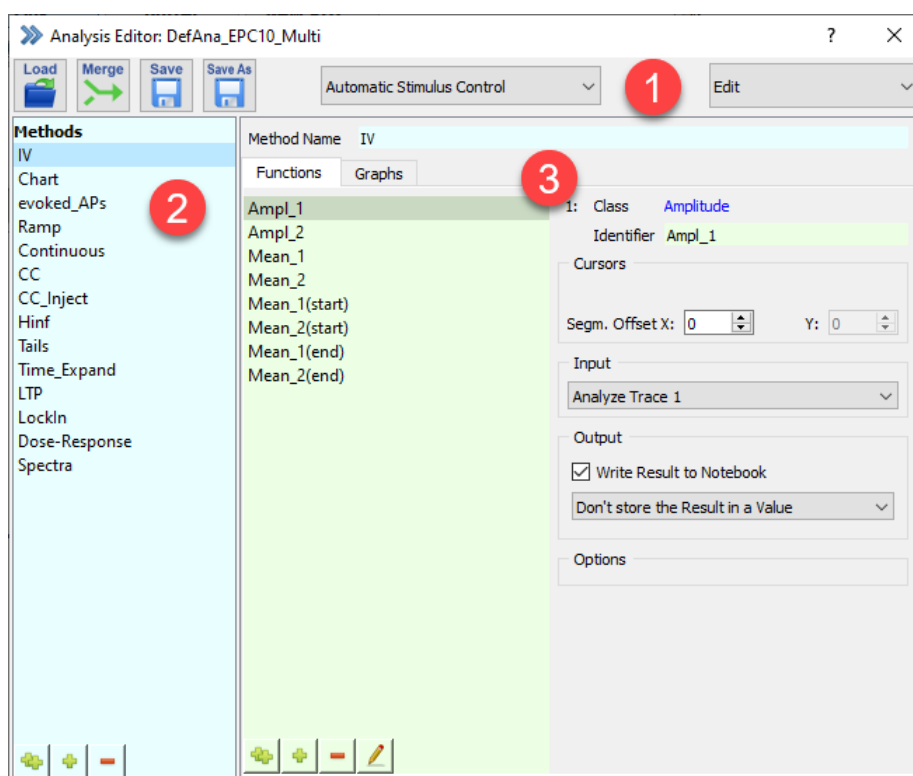


Figure 9.1: Analysis Editor of PATCHMASTER NEXT.

The Analysis Editor window consists of three sections. Each of these sections is described in detail below:

- Global Section
- Analysis Pool
- Analysis Parameters

An Analysis file (*.onl) can hold a huge number of Analysis Methods. Each of these Analysis Methods contain an assembly of Analysis Functions. Based on the type of incoming data, the user can specify a number of Analysis Functions. These functions produce analysis results which may be displayed in the Notebook window and/or shown in a graph inside either Graph Window 1 or Graph Window 2. The elements of a graph are *Graph Entries*, i.e. couples of analysis results to be used as X- and Y-references. Up to 4 *Graph Entries* can fit into one graph and multiple graphs can fit into one Analysis Window.

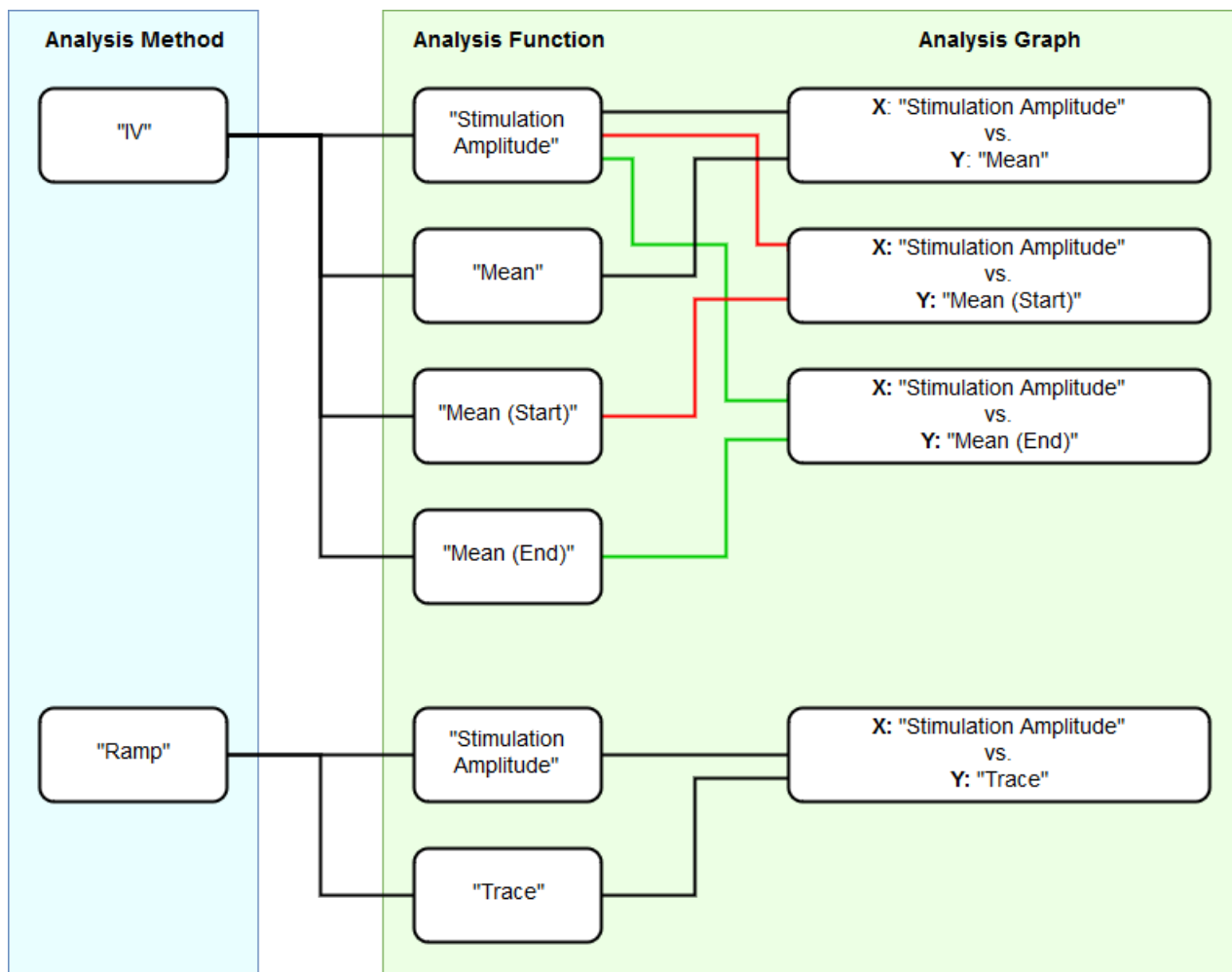


Figure 9.2: Structure of the Analysis Editor. An Analysis Method needs defined Analysis Functions for data output into the Notebook Window or into Analysis Graphs.

Thus, based on the hierarchy of the components involved in constructing an Analysis Method, Analysis Functions must first be defined because only then the respective analysis results can be placed as Graph Entries in graphs and windows.

The Analysis Editor does also have two hierarchy levels:

1. Analysis Method Level: Indicated by blue background colors
2. Analysis Parameters Level: Indicated by green background colors

The hierarchy level colors provides orientation when defining parameters in a protocol sequence.

9.1 Global Section

The upper area of the Analysis Editor window which we call here “Global Section” provides several buttons to organize the Analysis Editor.

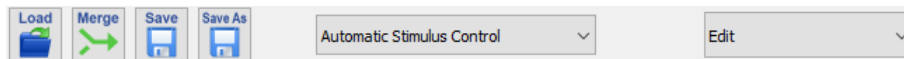


Figure 9.3: Global Section of the Analysis Editor.

Filename: The name of the currently loaded analysis file is provided in the frame of the Analysis Editor window.

Load: Via this button you can load another analysis file.

Merge: Via this function you can add all *Analysis Methods* from another analysis file (*.onl) to the currently loaded analysis file.

Save: Saves all changes made in the actual analysis file.

Save As: Saves the actual analysis file under a different name.

Stimulus Control: There are four possible options for controlling the particular *Analysis Method* of choice:

- Use Selected Method: Uses the active (selected) *Analysis Method*.
- Automatic Stimulus Control: Uses the *Analysis Method* which was defined by the stimulus sequence (see chapter 7.6.2 on page 101). Uses the *Analysis Method* stored in and loaded from the Analysis file, e.g. 'DefAnal.onl'. With this kind of Analysis one defines one *Analysis Method* for all Series until the *Analysis Method* is changed.
- No Analysis: No analysis will be performed.

Analysis Editor Mode: There are two options how to use the Analysis Editor:

- Edit: This is the default operating mode of the Analysis Editor. It allows you to edit your *Analysis Method*.
- Review: This mode is for review purposes only and allows to check the *Analysis Method* which was used for the selected Series of the Data Tree. It can only be enabled if there is a data file opened in the Data Tree.

9.2 Analysis Pool

On the left side of the Analysis Editor the Analysis Pool is shown. It is a list of *Analysis Methods* stored in the currently loaded Analysis Editor file. An *Analysis Method* is selected by clicking on it.

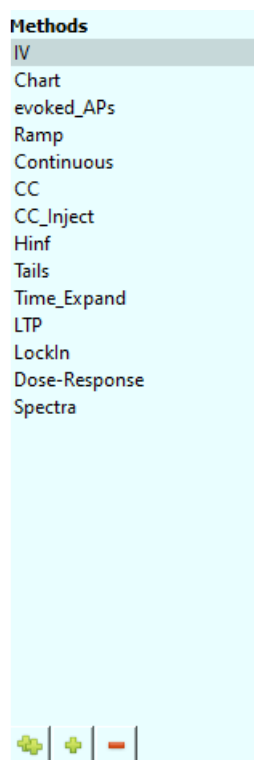


Figure 9.4: Analysis Pool of the Analysis Editor.

There are four options available to modify the `Analysis Pool` content:

- **Edit Name:** Double-click inside the *Analysis method* name to change its name.
- **Duplicate Method:** Use the small button at the bottom with the two green plus signs to copy the selected *Analysis Method*. The name is taken over but an index number is added.
- **Add new Method:** Use the small button at the bottom with one green plus sign to create a new and empty *Analysis Method*. The new protocol sequence will be label “New Method”.
- **Delete Method:** Use the small button at the bottom with the red minus sign to delete the selected *Analysis Method* from the `Analysis Pool`.

These options are also available via a right-click context menu.

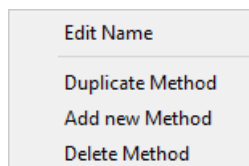


Figure 9.5: Right-click context menu of the Analysis Pool.

Moving Analysis Methods: Via drag-and-drop it is easily possible to change the position of the selected *Analysis Method* in the `Analysis Pool`.

9.3 Analysis Parameters

The Analysis Parameters section is divided into two parts by tabs:

- Analysis Functions: Defines the *Analysis Functions* and their parameters.
- Graphs: Assign *Analysis Functions* to a graph entry.

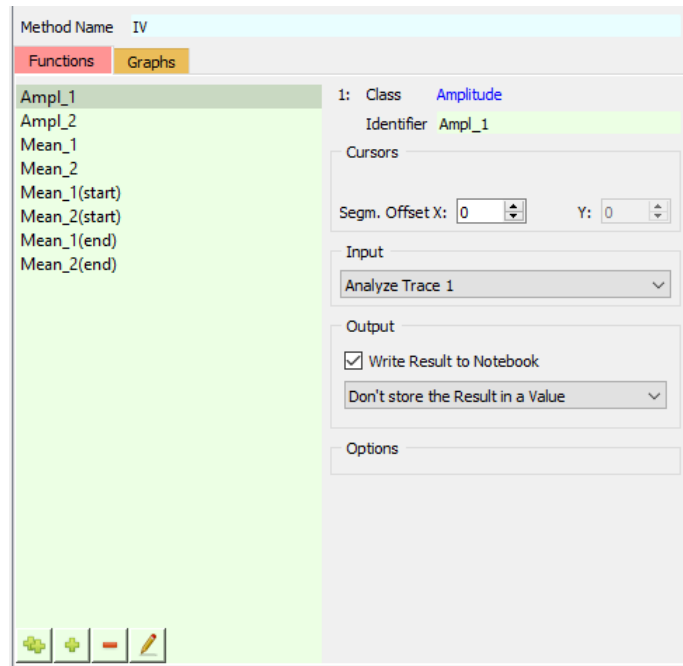


Figure 9.6: The Analysis Parameters section is separated into the two tabs Functions and Graphs.

At the top of this window there is always the name of the currently selected *Analysis Method* listed. If desired one can also change the name of the *Analysis Method* via this entry field.

9.3.1 Analysis Functions

In this section of the Analysis Editor, *Analysis Functions* can be added to the *Analysis Method* and parameters of already existing *Analysis Functions* can be modified.

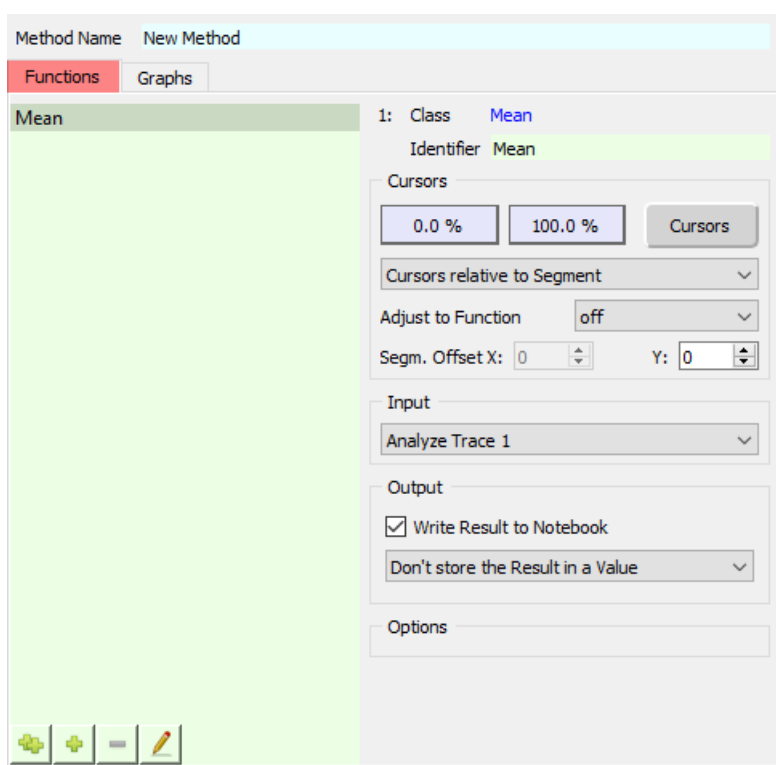


Figure 9.7: The Analysis Functions tab contains the Analysis Functions of the selected Analysis Method and allows to modify them.

By default there is always one *Analysis Function* in the *Function Pool*. There are four options available to modify the `Function Pool` content:

- **Edit Function Type:** Open the `Analysis Function Type` dialog to change the *Analysis Function Type*.
- **Duplicate Function:** Use the small button at the bottom with the two green plus signs to copy the selected *Analysis Function*.
- **Add new Function:** Use the small button at the bottom with one green plus sign to add another *Analysis Function*.
- **Delete Function:** Use the small button at the bottom with the red minus sign to delete the selected *Analysis Function* from the `Function Pool`.

These options are also available via a right-click context menu.

On the right side of the `Analysis Function Pool` one can define the analysis parameters. In the following pages we will describe all possible parameters of all *Analysis Functions*.

At the top of the `Analysis Functions Parameters` section the following options are provided:

Function Index: Indicates the *Analysis Function* index number of the active *Analysis Function*.

Function Class: Displays the *Function Class* as defined by the *Analysis Function Type* dialog.

Function Identifier: Entry field to modify the name of the *Analysis Function*.

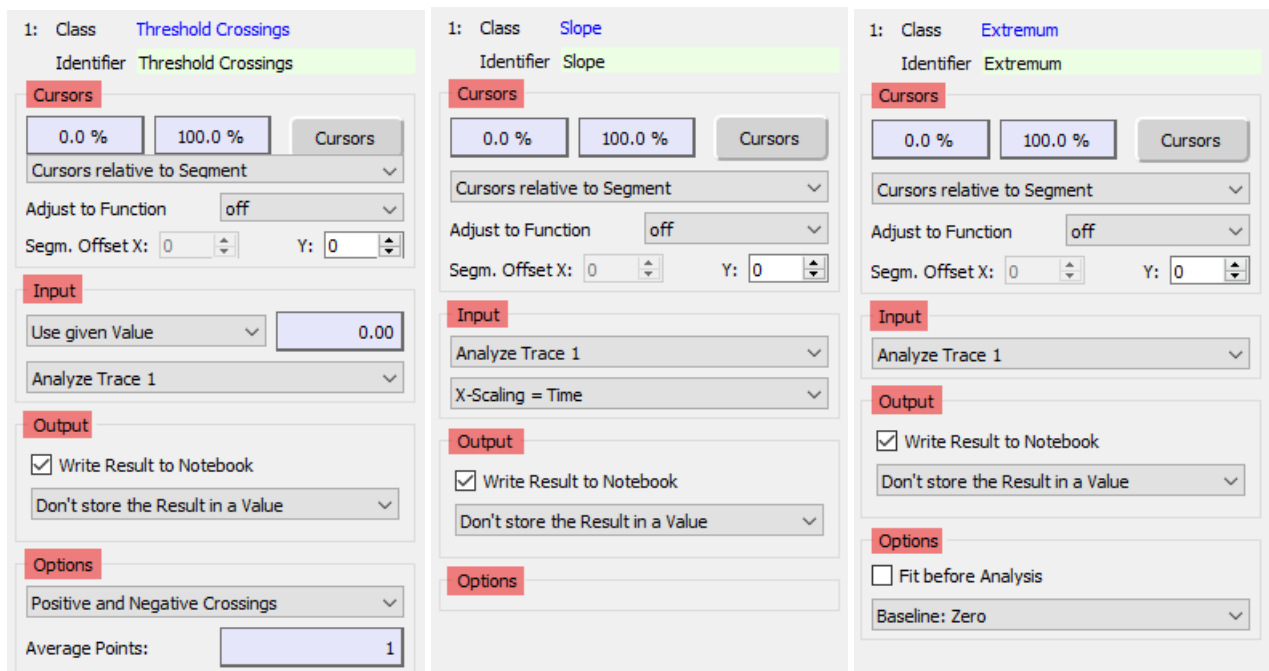


Figure 9.8: The Analysis Function parameters section varies depending on the selected Analysis Function.

Further *Analysis Function* parameters are structured according to their purpose. The following areas are defined:

- **Cursors:** Area to define the cursor ranges and settings.
- **Input:** Defines the data input or the Traces to work on.
- **Output:** Defines the output options of the analysis results.
- **Options:** Further parameters for the selected *Analysis Function*.

9.3.1.1 Cursors

In the `Cursors` section all Cursor-relevant settings can be defined.

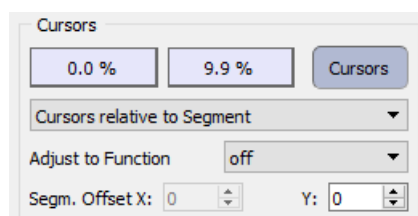


Figure 9.9: Cursors section of the Analysis Functions.

Cursor Bounds: Two entry fields for the Cursor settings [in %] are provided (right / left Cursor). Pressing the button lets pop-up the cursors in the Oscilloscope window. When moving the cursors in the Oscilloscope window, the cursors position is automatically transferred to the entry fields. The cursor limits can be outside the limits of the selected segment, i.e., they can be smaller than 0 % and greater than 100 %.

Cursor Type: Sets the definition of the *Cursor Type*. The following options are available:

- Cursors relative to Segment: The Cursor range is relative to the specified X or Y segment.
- Cursors relative to Trace: The Cursor range is relative to the whole Trace.
- Copy Cursors from: The active function gets the cursor bounds and *Cursor Type* from the selected function.

Adjust to Function: Specify an existing *Analysis Function* to set the cursor bounds relative to the found position of a peak or threshold. E.g. if one function analyzes the *Time to Maximum* and the second function analyzes the *Tau*, one can use *Adjust to Function* in the second function to set the cursor settings in respect to the found *Time to Maximum* results. This means every time the *Time to Maximum* value changes the cursors settings (here: 1-15%) for the calculation of *Tau* will be adapted.

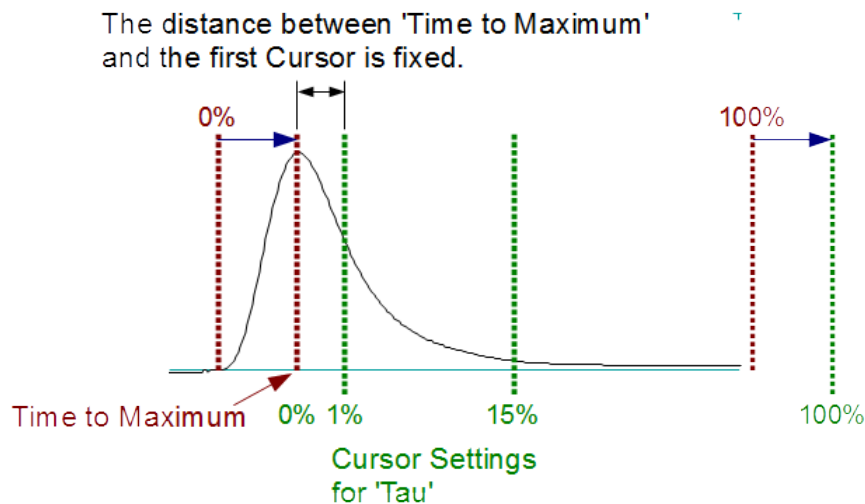


Figure 9.10: Graphical illustration showing the working principle of the Adjust to Function mode.

Note: Be aware that in this example the cursor range for the *Tau* function gets shifted to the right by *Time to Maximum* because the new 0% value is now the value of *Time to Maximum*.

Segm. Offset: The X- and Y-Segment offsets are set relative to the *Relevant X- and Y-Segments* as defined in the Stimulus Editor window (see chapter 7.6.2 on page 101).

9.3.1.2 Input

In the Input section one defines the working basis of the *Analysis Function*.

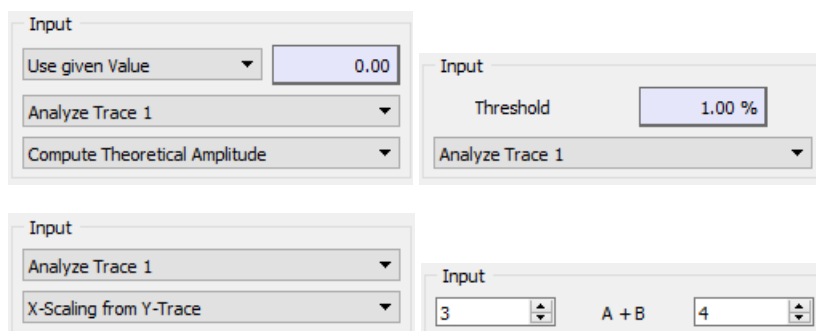


Figure 9.11: Different Input sections of the Analysis Functions.

Value: One can enter a value into the entry field for "Use Given Value" select "Get Value-1...32" from the selection list which will take over a value stored in *Value-1...32*.

Y-Trace: Select which Trace (1...32) or Buffer (1...4) shall be analyzed using the *Analysis Functions*. The Trace index usually corresponds with the channel number in the stimulation sequence (if the Trace number is not set to another value in the build instruction of the Trace (see 7.6.1 on page 98)).

X-Trace: The voltage can either be calculated from a theoretical stimulus (*Compute Theoretical Amplitude*), e.g. when there is no voltage Trace available, or it can be taken from a recorded Trace (*Amplitude from Trace 1...32*, *Amplitude from Buffer 1...4*).

Threshold: Sets the *Threshold* value for one of the *AP Analysis* functions.

X-Scaling =: Defines the X-Scaling of the *Analysis Function*. The default setting would be "X-Scaling = Time".

To illustrate the function of this option we assume that we have two input traces available: Trace 1 - Current vs. Time, Trace 2 - Voltage vs. Time. If the input Trace (*Analyze Trace*) is set to "1" the default setting would take the time for the X-scaling (*X-Scaling = Time*) because it is the "native" X-scaling basis of the Trace. If we would select "X-Scaling = Trace 1" the current information of Trace 1 would be used for the X-scaling. Further, using "X-Scaling = Trace 2" would define the voltage information of Trace 2 for the X-scaling.

Operation: Refer to the result of an already existing *Analysis Method*. In this example the *Math* operation 'a + b' refers to the *Analysis Method* index '3' and '4'.

9.3.1.3 Output

In the *Output* section the output options for the analysis results is defined.

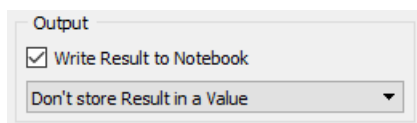


Figure 9.12: Output options of the Analysis Functions.

Write Result to Notebook: If selected, the analysis result of this function will be written into the *Notebook* window.

Don't store Result in Values / Store in Value-1...32: Analysis results can be stored in one of the *Values-1...32*. They can then be retrieved and used as a constant math function. The *Values-1...32* are used to exchange information between different *Analysis Methods* and the *Analysis Editor* or the *Protocol Editor*.

9.3.1.4 Options

Further analysis parameters are listed in the *Options* section.

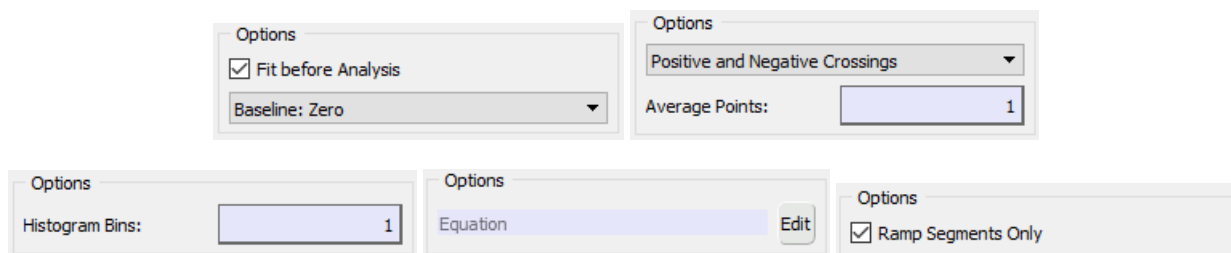


Figure 9.13: Different Input sections of the Analysis Functions.

Fit before Analysis: If selected, the peak value is determined by a polynomial fit around the first numeric estimate of the peak. With this function, the influence of noise can be reduced in the determination of peak amplitudes.

The fit function must not be used when non uniformly continuous changes are expected around the peak value. E.g. if you want to analyze the peak value of a saw-tooth signal, the Fit before Analysis function easily lead to erroneous results, because of the quick jump from the peak level to base line at the end of the saw-tooth.

Baseline: Defines the *Baseline* setting for the selected *Analysis Function*. There are three options available:

- **Baseline: Zero:** Calculates the integral between the two intersection points of the cursors and the Trace, starting at the zero line.
- **Baseline: Cursor Intersections:** Calculates the integral, starting at a virtual baseline between the two intersections points of the cursors and the Trace.
- **Auto Bounds:** Makes a derivation of the Trace and sets the cursors to the minimum and maximum value of the derivation. This option can be helpful to find the proper peak if some other global maximum (or minimum) exists.

Crossings: Sets the direction of the *Crossing*. There are three options available:

- **Positive and Negative Crossing:** Every threshold crossing is detected.
- **Positive Crossing:** A crossing is only detected if the signal runs through the threshold from negative to positive values.
- **Negative Crossing:** A crossing is only detected if the signal runs through the threshold from positive to negative values.

Average Points: Enter the number of points to be used for a running average to apply before extracting the value. The function applies to the source Trace as well as to the result Trace.

Histogram Bins: Enter the number of bins for the histogram.

Equation: Enter an equation into the text field or modify an already existing one by clicking on the *Edit* button. Further information about equation editing can be found in chapter 17 on page 235.

Ramps Segments Only: The *Analysis Function* does only work on *Ramp* segments if the checkbox is selected.

9.3.2 Graphs

In the `Graphs` section the properties of the graph display for the `Graph Window` are defined.

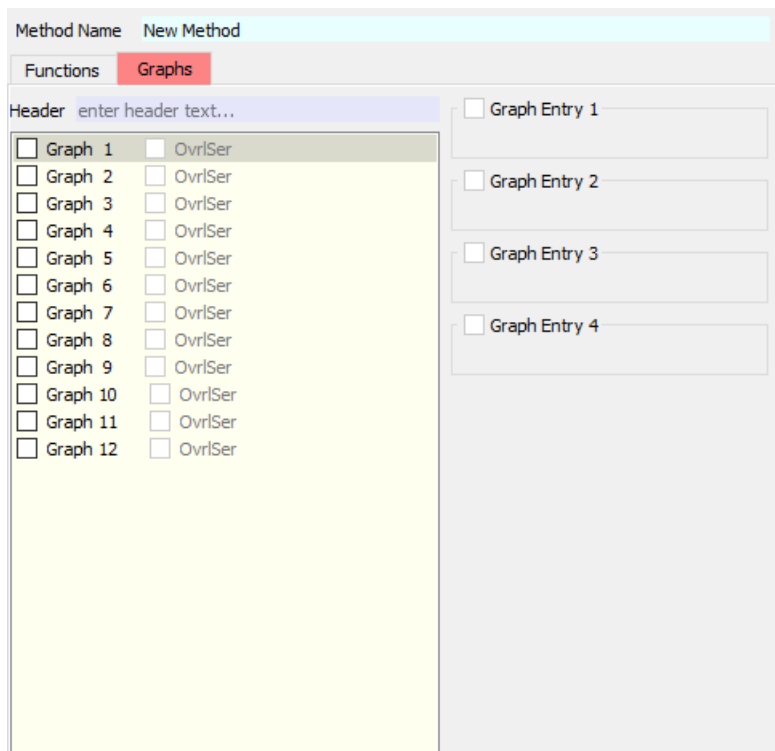


Figure 9.14: Settings of the `Graphs` tab of the `Analysis Parameters`.

Header: Via this entry field one can define a *Header* for the selected analysis graph.

Graph 1...12 By default the `Graph Pool` does not contain any preset graphs but has room for 12 analysis graphs whereby each graph can be filled with up to four *Graph Entries*.

OvrlSer: By default the graph data is wiped before a new *Series* is started. If you mark the *OvrlSer* option, the graph plot will not be cleared when performing a new analysis (new *Series* acquisition).

Note: This setting is overruled by the setting `Wipe Analysis Graphs` (see chapter 8.3.4.3 on page 151).

Graph Entries: On the right side of the `Graphs` section one defines the *Graph Entries* of the selected graph. It is possible to assign up to four *Graph Entries* for each graph.

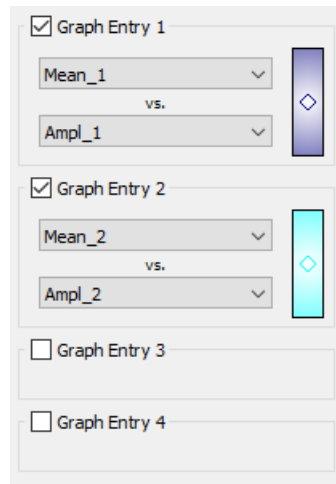


Figure 9.15: Graph Entry section of the Graphs tab.

Via the checkboxes one activates or deactivates the *Graph Entries*. An active *Graph Entry* provides the following options:

Y Axis: Choose an *Analysis Function* from the selection whose results are assigned to the Y axis.

X Axis: Choose an *Analysis Function* from the selection whose results are assigned to the X axis.

Clicking on the colored square on the right side opens the *Modify Entry* dialog:

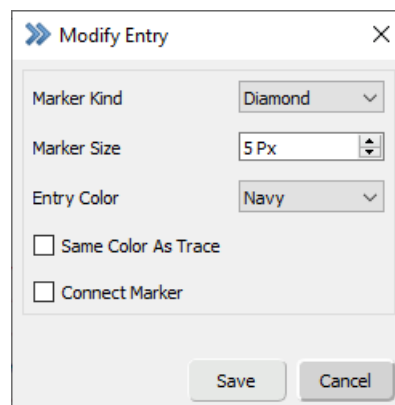


Figure 9.16: Modify Entry dialog of the Graph Entries.

Marker Kind: Choose from a huge bunch of available data marker symbols (point, plus, star, diamond, cross, square...).

Marker Size: Define the size of the data marker for the analysis graph.

Entry Color: Choose a color for the data marker (symbols) and the connection lines.

Same Color As Trace: Use the Trace color definition for the symbols and the lines (see chapter 3.5.5 on page 36). The *Marker Color* setting is ignored.

Connect Marker: If selected, the symbols will be connected with a line.

9.4 Analysis Function Types

When selecting or changing an *Analysis Function* the *Function Type* dialog pops up. Here, you can select an *Analysis Function*. The functions are organized according to their types via different tabs.

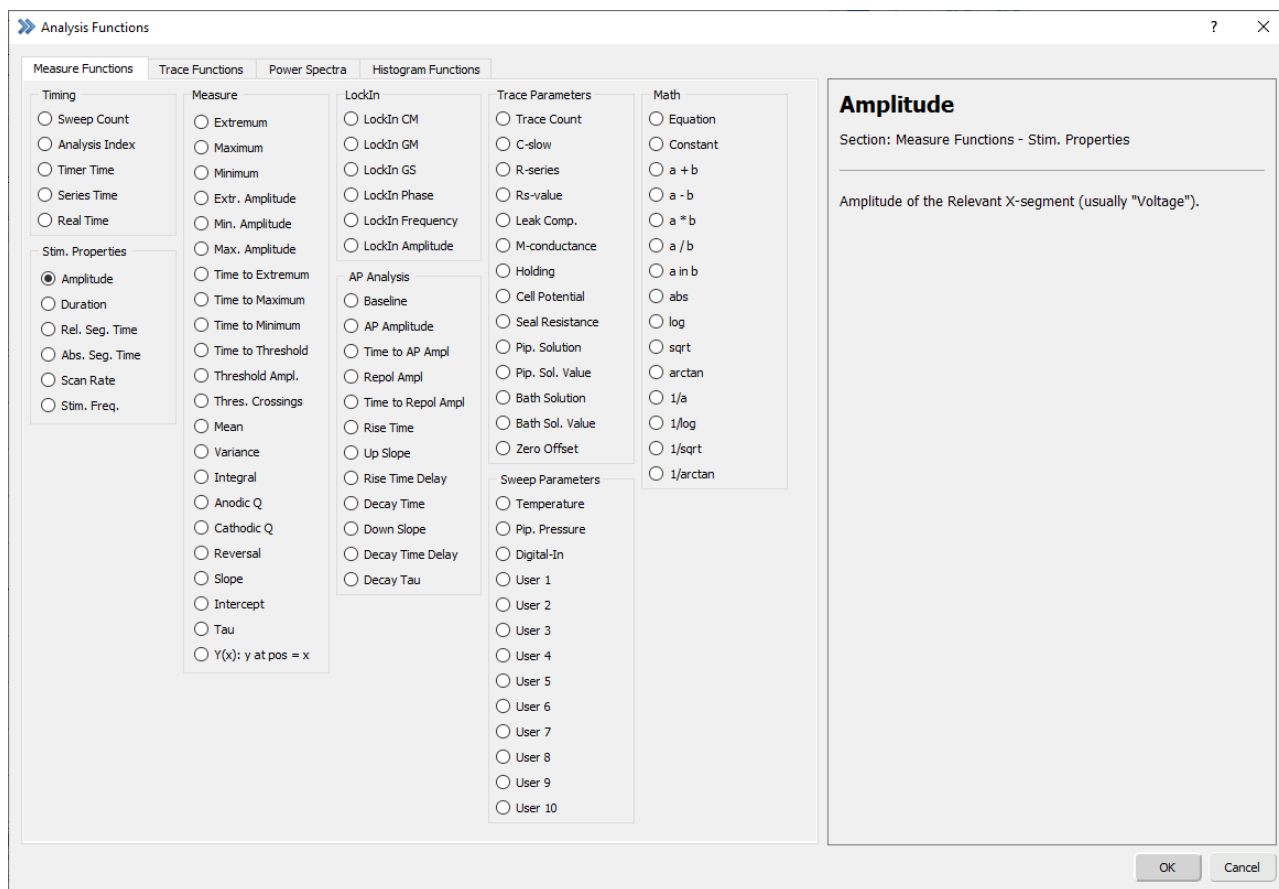


Figure 9.17: Analysis Functions dialog of the Analysis Editor.

9.4.1 Measure Functions

The **Measure Functions** tab includes all functions which are related to an analysis to mostly parts of a data Trace.

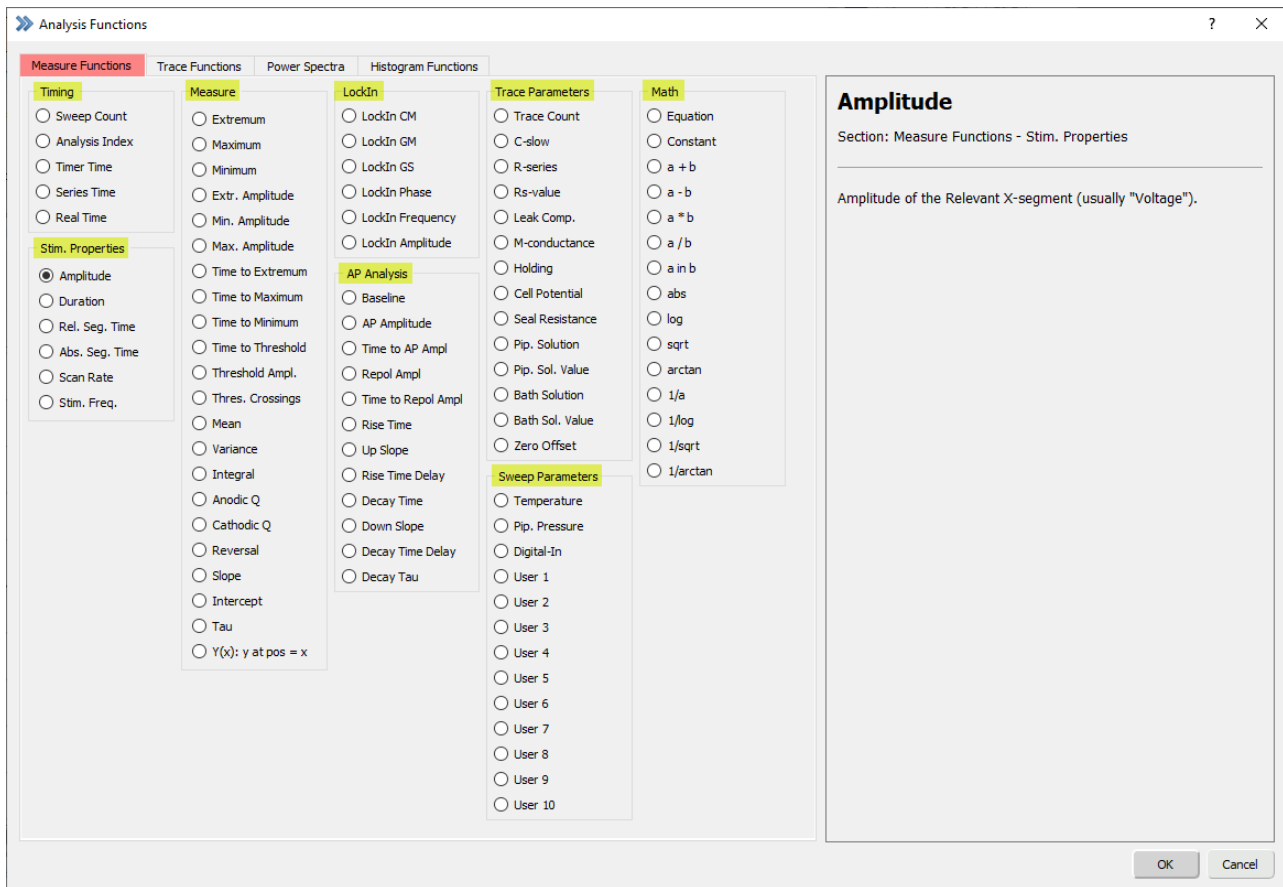


Figure 9.18: Measure Functions section of the Analysis Editor.

9.4.1.1 Timing

These functions are used as an X-reference in a graph and they are all related to some Sweep index or recording time parameters.

Sweep Count: Index of the Sweep within a Series.

Analysis Index: Index of the Analysis during the experiment. The *Analysis Index* is initialized with a *Wipe* command.

Timer Time: Time of the *Timer* in the Oscilloscope at the start of Sweep acquisition.

Series Time: Time in respect to the Sweep acquisition of the corresponding Series.

Real Time: Time elapsed since midnight at the start of Sweep acquisition. This format should be used if you want to perform mathematical operations of different time values.

9.4.1.2 Stimulation Properties

These are functions that are usually used as an X-reference in a graph. They are based on the *Relevant X-segment*, i.e. they are also sensitive to the X-segment offset (see chapter 7.6.2 on page 101).

Amplitude: Amplitude of the *Relevant X-segment* (usually "Voltage").

Duration: Duration of the *Relevant X-segment*. This may be useful if the length of the *Relevant X-segment* is changing during sweep acquisition.

Rel. Seg. Time: The *Relative Segment Time* function returns the start time of the *Relevant X-segment* with respect to the first stored point of the stimulus. The duration of non-stored segments is not taken into account.

Abs. Seg. Time: The *Absolute Segment Time* function returns the start time of the *Relevant X-segment* with respect to the first point of the stimulus. In opposite to *Relative Segment Time* the duration of non-stored segments is taken into account.

Scan Rate: Scan rate of the *Relevant X-segment*. Can be used e.g. to analyze the slope of a ramp.

Note: Do not mix up the Scan Rate with the Slope analysis function! While the Slope is calculated from the recorded Trace, the Scan Rate is taken from the stimulus.

Stim. Freq.: Use this function to read out the frequency of your relevant X-segment if you used sine or square wave segments.

9.4.1.3 Measurements

These are functions that are usually used as an Y-reference in a graph. They are based on the *Relevant Y-segment*, i.e. they are also sensitive to the Y-segment offset.

Extremum: Extreme value of data in the cursor region; either a maximum or a minimum.

Maximum: Maximum value of data in the cursor region.

Minimum: Minimum value of data in the cursor region.

Extr. Amplitude: Calculates the voltage applied at the position of the peak current (either minimum or maximum).

Min. Amplitude: Calculates the voltage applied at the position of the minimum peak current.

Max. Amplitude: Calculates the voltage applied at the position of the maximum peak current.

Time to Extremum: Time from the beginning of the Trace or the relevant segment to the extreme value in the cursor range.

Time to Maximum: Time from the beginning of the Trace or the relevant segment to the maximum value in the cursor range.

Time to Minimum: Time from the beginning of the Trace or the relevant segment to the minimum value in the cursor range.

Time to Threshold: Time from the beginning of the Trace or the relevant segment to the specified threshold value in the cursor range.

Threshold Ampl.: This function returns the corresponding X-axis value (often called amplitude) of the threshold crossing point. Use this function e.g. if you want to know the voltage value for a specific (recorded) current value.

Thres. Crossings: Counts the number of threshold crossings between the two cursors.

Mean: Mean value of the data in the cursor region.

Variance: Variance value of data in the cursor region (square of standard deviation).

Integral: Integral value of data in the cursor region.

Anodic Q: Integral of positive current in the cursor range.

Cathodic Q: Integral of negative current in the cursor range.

Reversal: This function searches for the zero crossing of the target Trace and computes the corresponding value of a second Trace (i.e. voltage).

Slope: Calculates the slope (b) in the defined region, using a linear regression ($y = a + bx$).

Intercept: Calculates the intercept (a) in the defined region, using a linear regression ($y = a + bx$).

Tau: Calculates the time constant (τ) from an exponential curve. Computes τ in two steps:

1. semi-logarithmic regression of x vs $\ln(y)$
2. $\text{abs}(1/\text{slope})$

Y(x) : y at pos = x: Calculates the Y-value at a defined X-position (e.g. time, frequency...). The X-position value can be entered into the field beneath the function.

9.4.1.4 LockIn

The following parameters can be retrieved from the `LockIn` Extension. Please note that the `LockIn` returns a mean value of all data of the Trace. In case a mean value in the cursor range is desired, then please acquire/store the parameter as individual Trace and use the `Mean` function of the `Measurements` section.

LockIn_CM: Capacitance value of the `LockIn` Extension.

LockIn_GM: Membrane conductance of the `LockIn` Extension.

LockIn_GS: Series conductance of the `LockIn` Extension.

LockIn_Phase: Returns the phase setting of the software `LockIn`.

LockIn_Freq: Returns the frequency of the sinusoidal wave used for `LockIn` measurements.

LockIn_Ampl: Returns the amplitude of the sinusoidal wave used for `LockIn` measurements.

9.4.1.5 AP Analysis

The `AP Analysis` is designed for analysis of **evoked** action potentials. The following prerequisites are essential:

- A `Sweep` consists of at least three segments:
 1. Baseline Segment
 2. Stimulus Segment
 3. Waiting Segment
- The second segment (Stimulus Segment) is marked as *Relevant X- and Y-segment*.

Note: Please switch off `Subtract Zero Offset` in the `Oscilloscope` menu of the `Oscilloscope` window (see chapter 6.1.1 on page 74).

Baseline: Defines the baseline value or resting membrane potential. It is measured as the mean amplitude of the first segment (Baseline Segment).

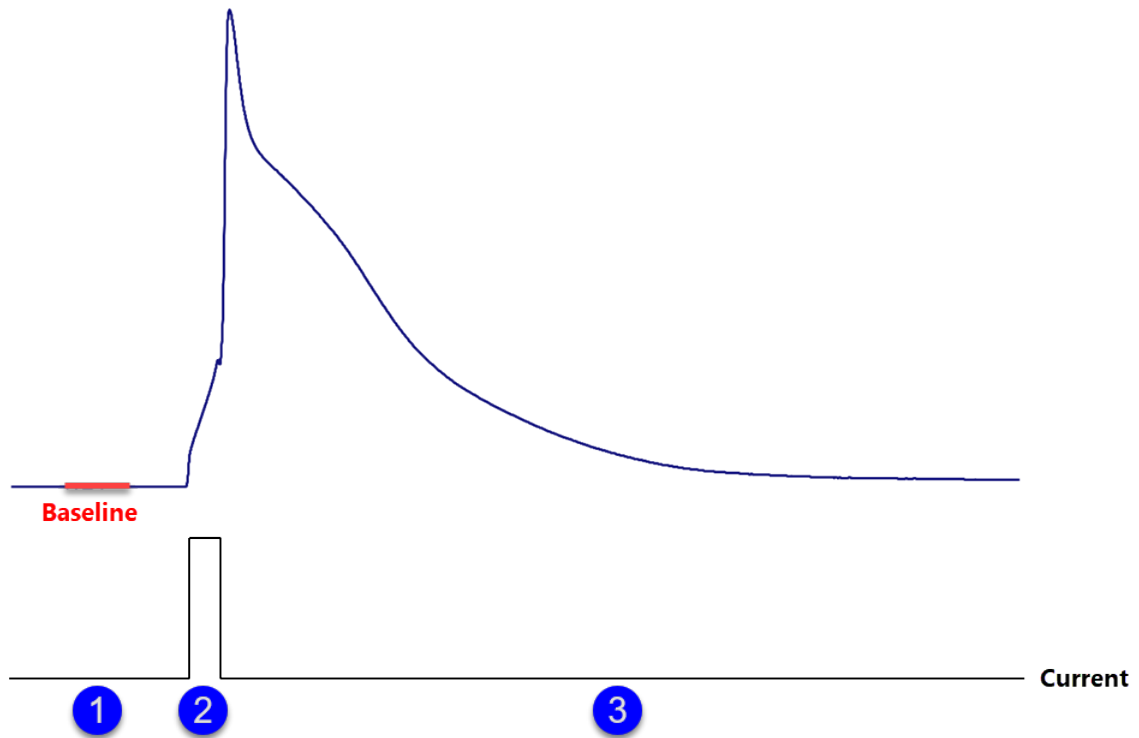


Figure 9.19: The Baseline parameter is taken from the first segment.

AP Amplitude: Amplitude of the action potential.

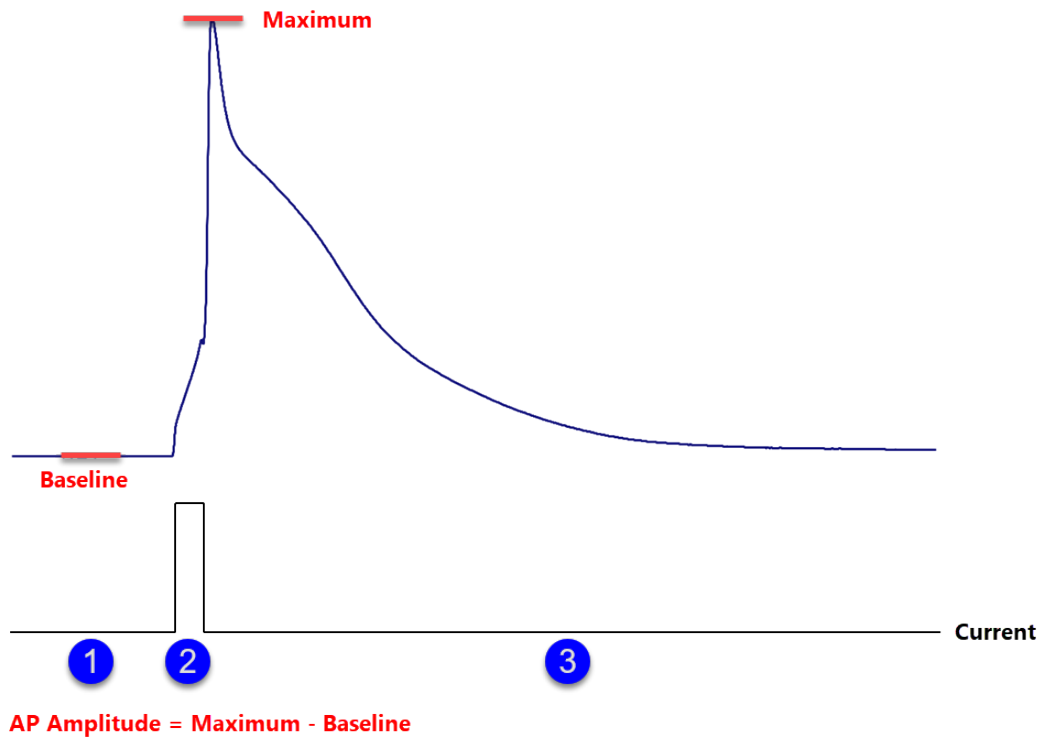


Figure 9.20: The Action Potential Amplitude is the calculated value from Baseline value and Maximum value.

Time to AP Ampl: Time from start of the stimulation segment to the maximum of the action potential.

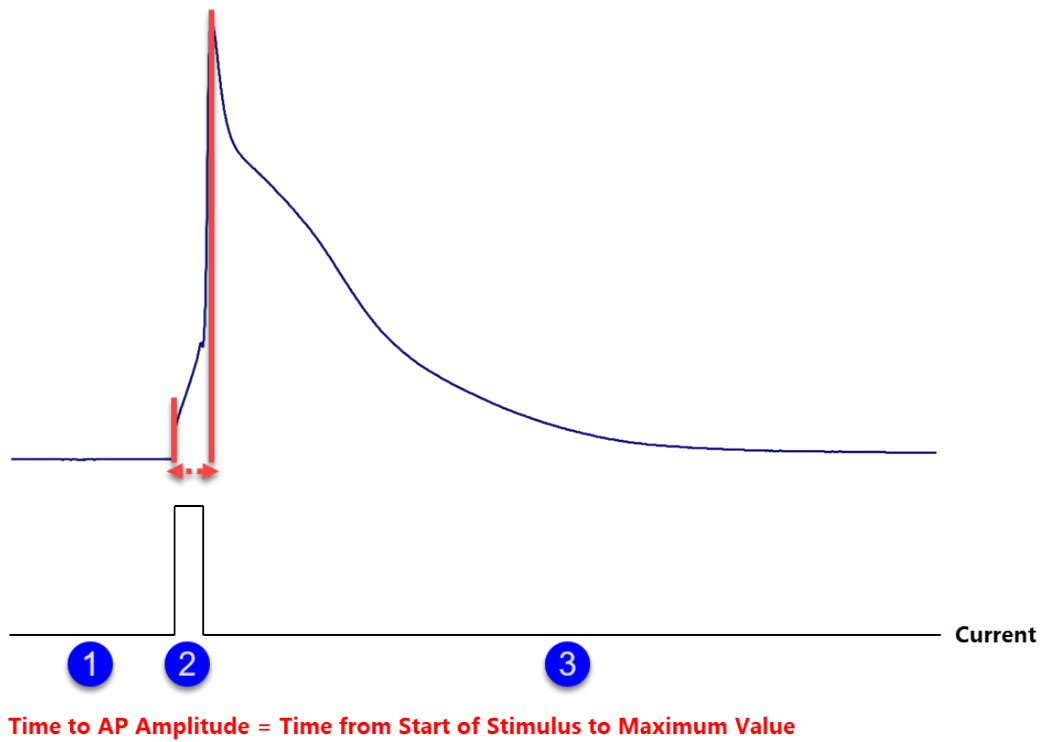


Figure 9.21: The Time to AP Amplitude is the calculated time from start of stimulus to Maximum.

Repol Ampl: Repolarization Amplitude is the difference between the *Minimum* amplitude after the peak and the *Baseline* value.

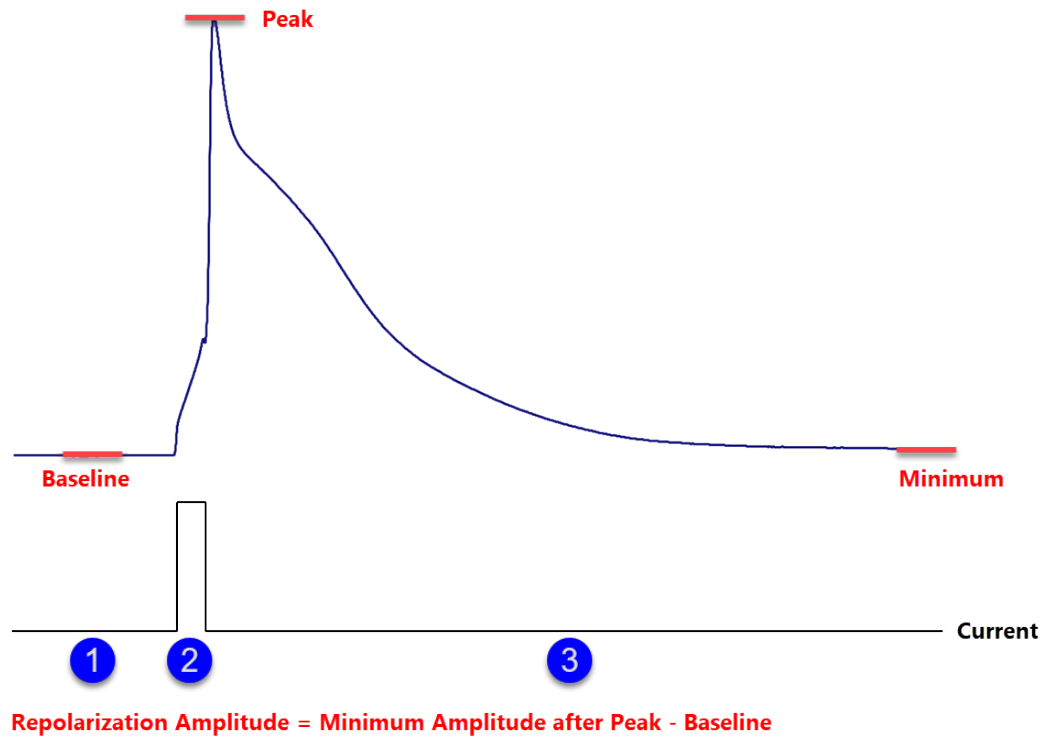


Figure 9.22: Repolarization Amplitude is calculated from Baseline to Minimum after the action potential peak.

Time to Repol Ampl: Time from start of the stimulation segment to the *Minimum* value of the action potential.

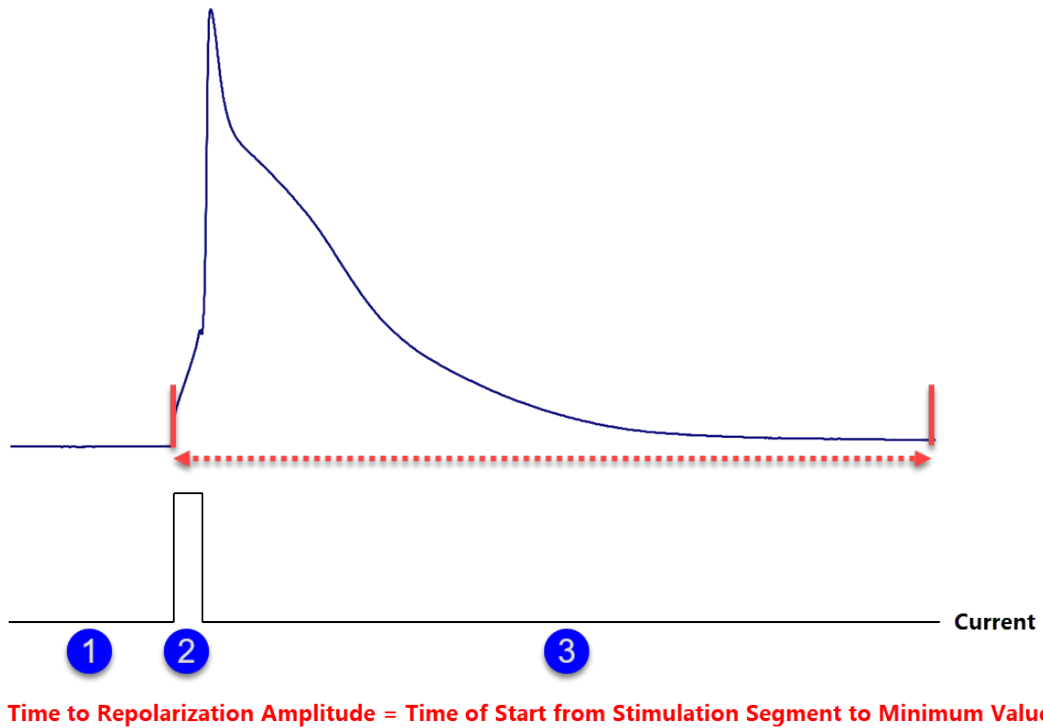


Figure 9.23: The Time to Repolarization Amplitude is calculated from the start of the stimulation segment (segment "2") to the Minimum value of the action potential.

Rise Time: Time between the low level and up level on the rising edge of the action potential. The low level can be entered as parameter (*threshold*). The upper level is then given by 100 % - *Threshold*.

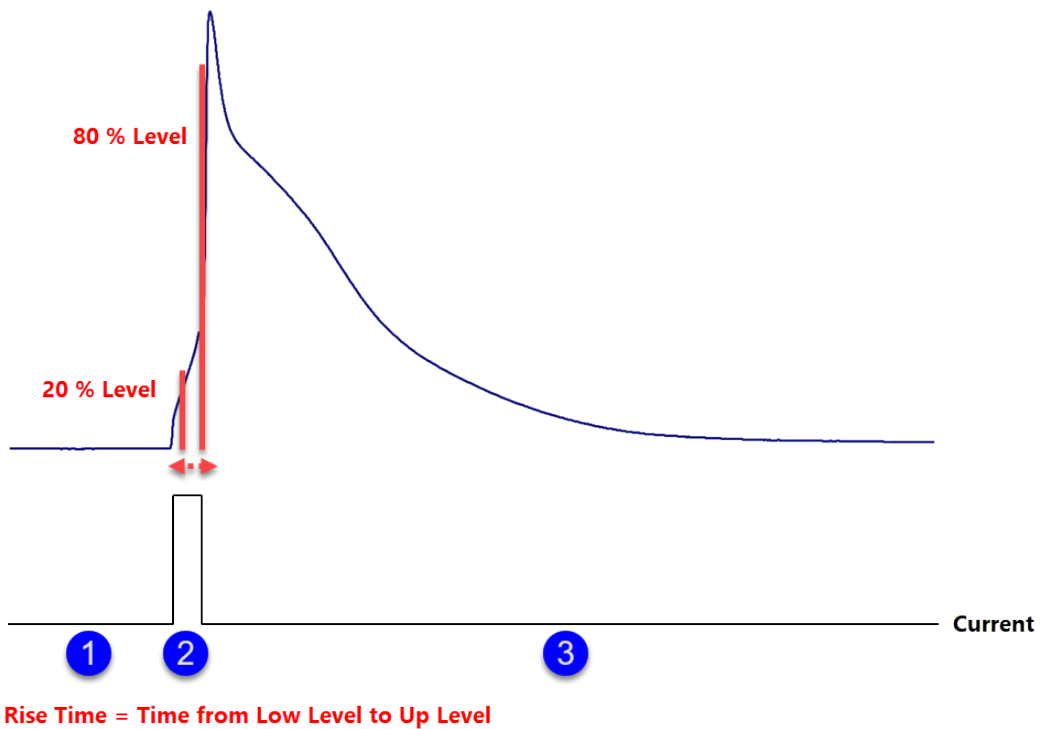


Figure 9.24: The Rise Time of the action potential is the calculated time between the set threshold levels.

Decay Time: Time between the up level and low level on the falling edge of the action potential. The low level can be entered as parameter (*Threshold*). The upper level is then given by 100 % - *Threshold*.

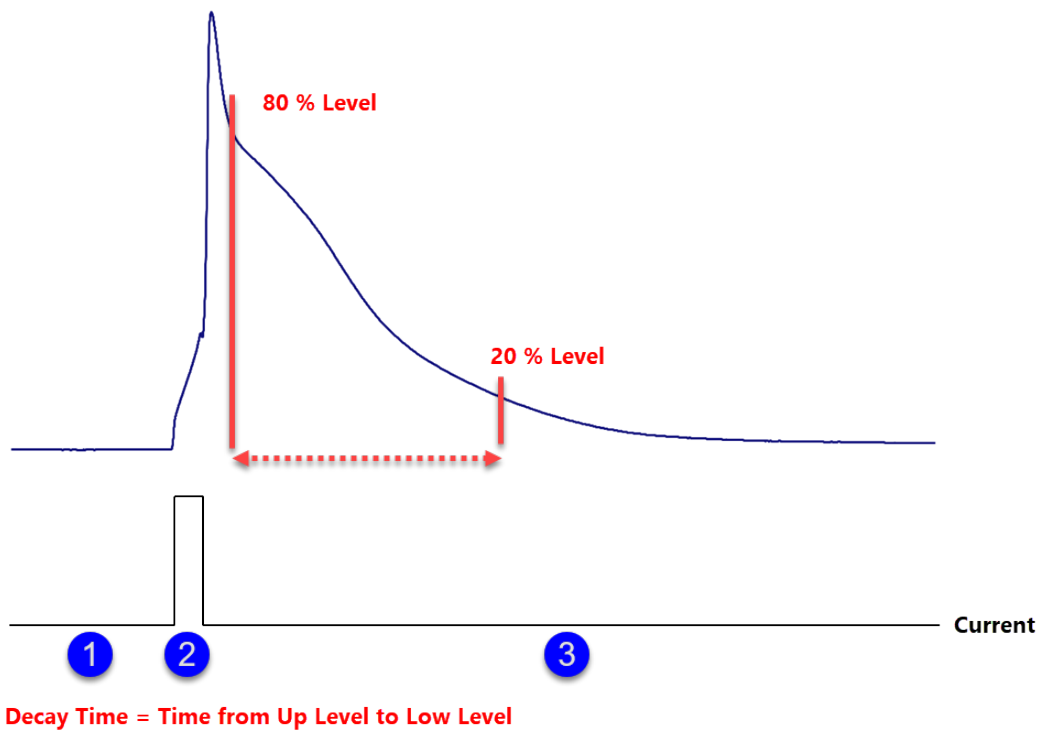


Figure 9.25: The Decay Time of the action potential is the calculated time between the set threshold levels.

Up Slope: Slope between the two levels on the rising edge of the action potential. The low level can be entered as parameter (*Threshold*). The upper level is then given by 100 % - *Threshold*.

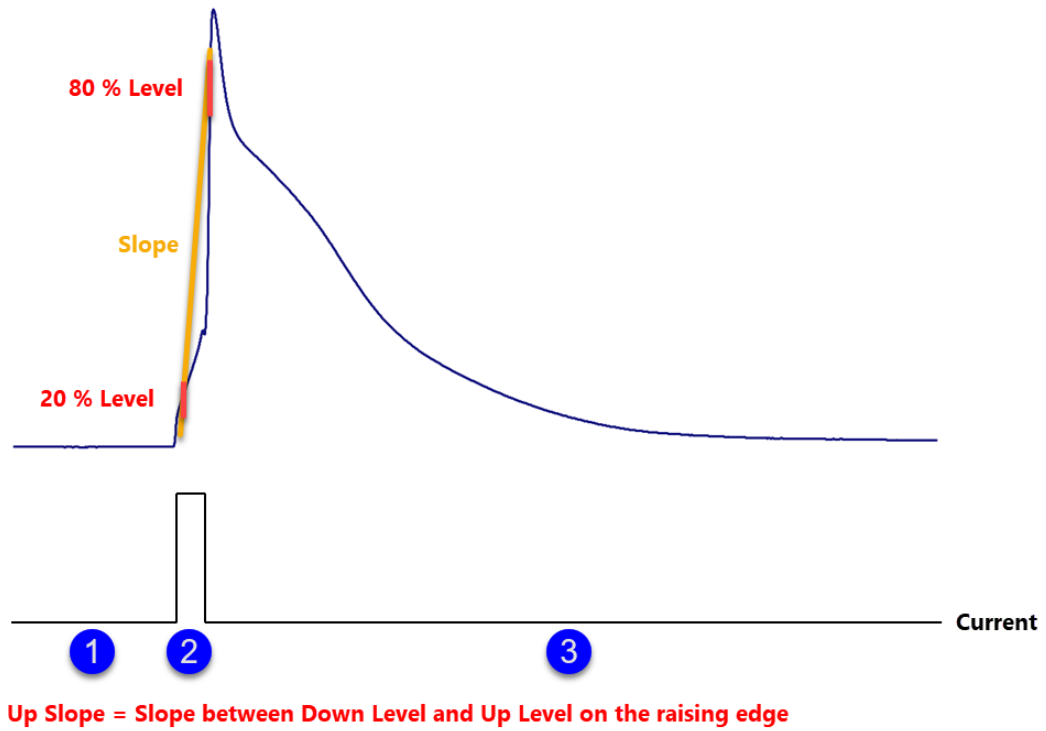


Figure 9.26: The Up Slope of the action potential is the calculated slope between the set threshold levels.

Down Slope: Slope between the two levels on the falling edge of the action potential. The low level can be entered as parameter (*Threshold*). The upper level is then given by $100\% - \text{Threshold}$.

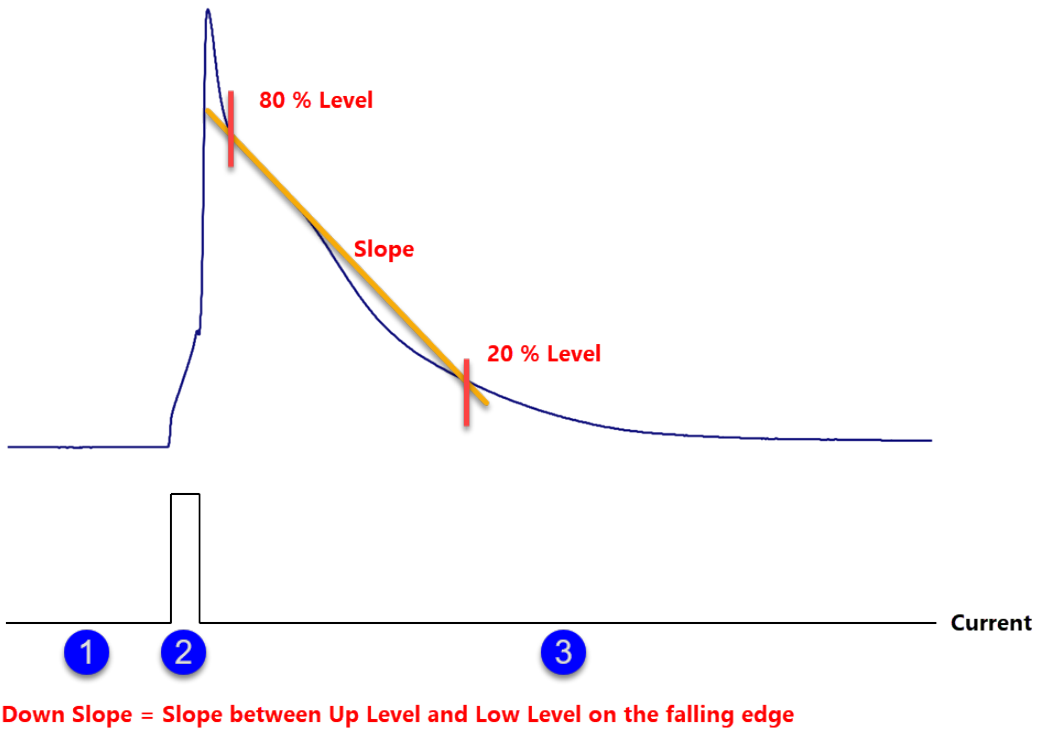


Figure 9.27: The Down Slope of the action potential is the calculated slope between the set threshold levels.

Rise Time Delay: Time between the start of the stimulation and start of the rise of the AP.

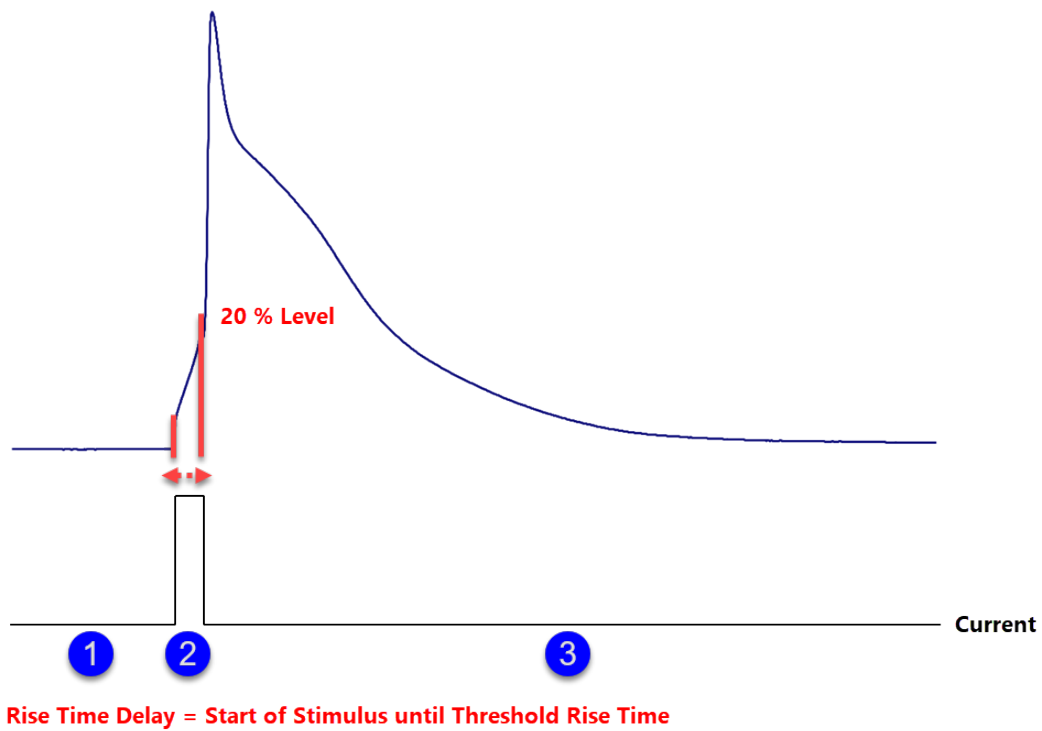


Figure 9.28: The Rise Time Delay of the action potential is the calculated time between the start of the stimulus and the set Rise Time threshold level.

Decay Time Delay: Time between start of the stimulation and begin of the AP decay.

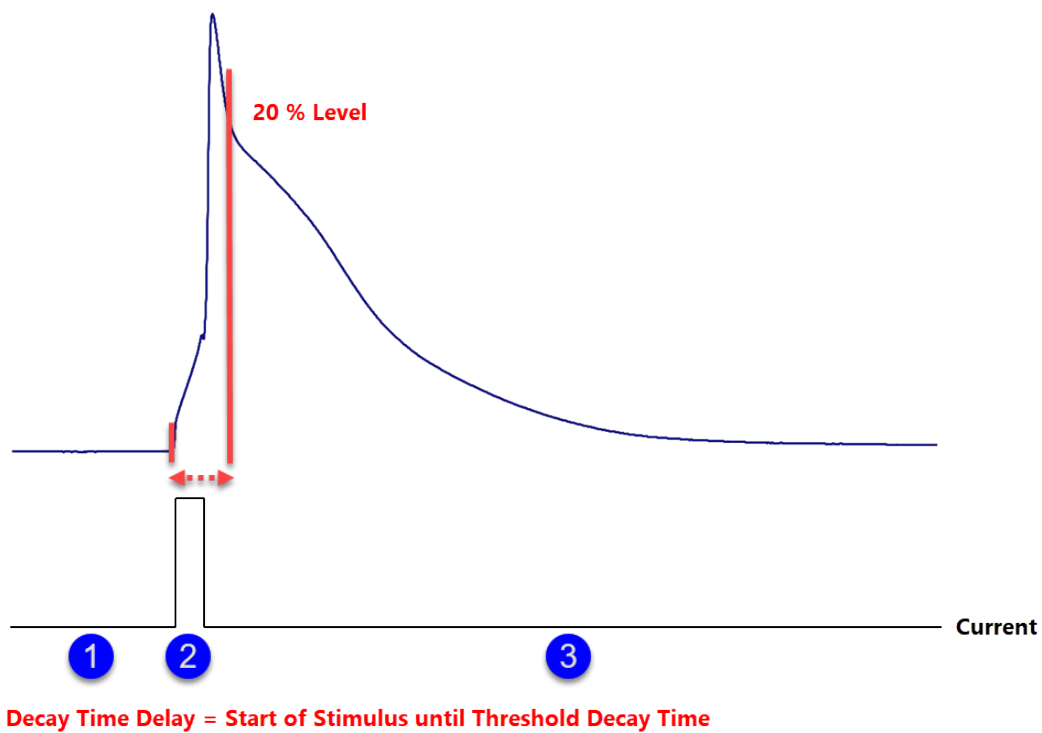


Figure 9.29: The Decay Time Delay of the action potential is the calculated time between the start of the stimulus and the set Decay Time threshold level.

Decay Tau: Calculates the exponential decay time constant (τ) between the defined threshold and 50 % decay time level of the amplitude.

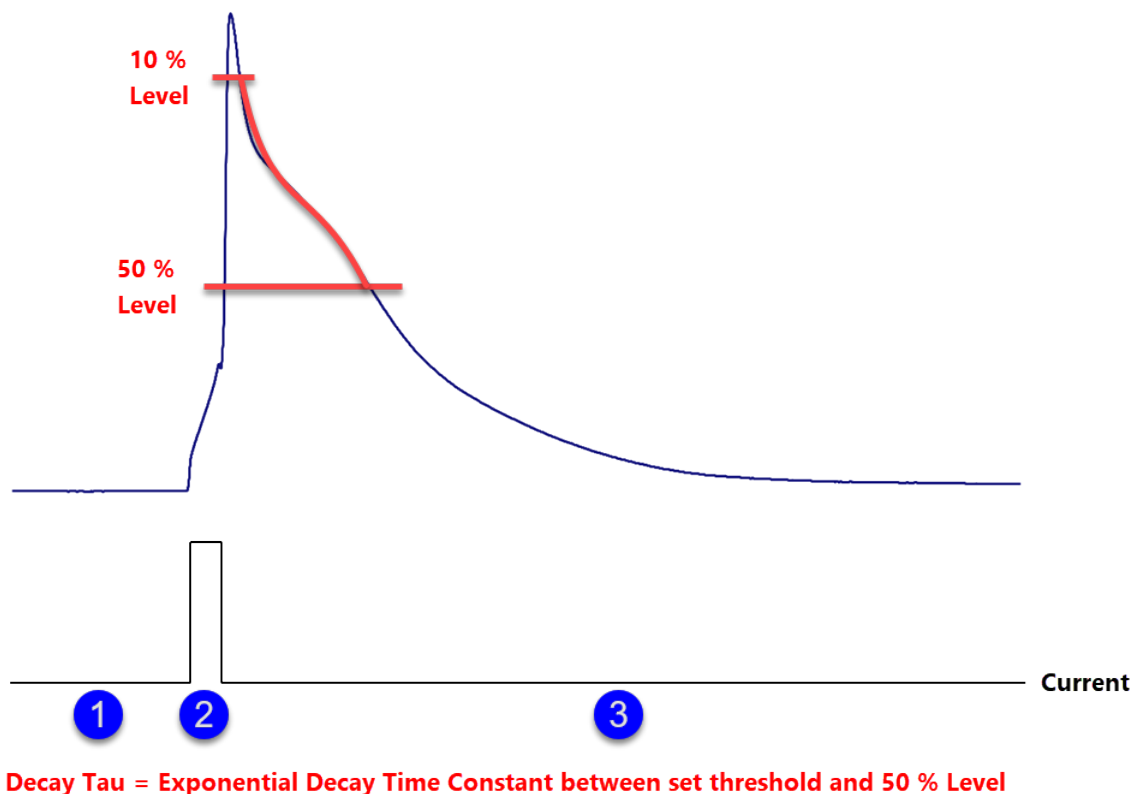


Figure 9.30: The Decay Tau is calculated via an exponential fit between the set Decay Time threshold level and the 50 % decay time level of the action potential amplitude.

9.4.1.6 Trace Parameters

The following parameters can be retrieved for analysis from the parameter set that is stored with the data (see Parameter window, 12 on page 209).

Trace Count: Gives back the index number of the Trace which is set in Trace #. If no Trace with the index number exists a "NAN" result will be reported.

C-slow: Slow capacitance value taken from each Trace.

R-series: Series resistance value taken from each Trace.

Rs-value: Compensated Series resistance value.

Leak Comp.: Leak compensation value.

M-conductance: The membrane conductance value as determined by the last *Auto C-Slow* compensation. It is only updated after an *Auto C-slow* update.

Note: This value depends very much on the Auto C-Slow Settings in the Amplifier window (see chapter 4.3 on page 54). Therefore this value might only be a rough estimate of the real membrane conductance.

Cell Potential: Cell potential read out from an AD input channel.

Seal Resistance: Seal resistance value (*R-membrane*) from the Amplifier dialog.

Int. Solution: Index number of the internal solution.

Int. Sol. Value: Relevant concentration value of the internal solution.

Ext. Solution: Index number of the external solution.

Ext. Sol. Value: Relevant concentration value of the external solution.

Zero Offset: Returns the calculated *Zero Offset* value.

9.4.1.7 Sweep Parameters

The following parameters can be retrieved for analysis from the parameter set that is stored with the data (see *Parameter window*, 12 on page 209).

Temperature: Temperature value e.g. read in by an external device.

Pip. Pressure: Pipette pressure (from an application device).

Digital-In: State of the Digital-In ports of EPC 10 USB amplifiers.

User_Param1...10: Parameter as set by the user in the *Hardware* section of the *Configuration* window (see chapter 3.5.3.6 on page 33).

9.4.1.8 Math

Math supplies various calculations on one or two function results. By combining several *Math* functions, complex expressions can be generated.

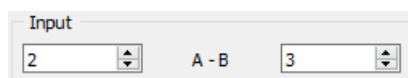


Figure 9.31: Example for mathematical operation of the kind “a-b” which calculates the difference of two analysis results.

Equation: An equation can be entered to compute a value. For details on the equation syntax please refer to the section *Equation Syntax*, 17 on page 235.

Constant: Sets a constant value if *Use Given Value* is selected. Alternatively, one can select any *Value* stored before. The constant value may be determined either by entering a value in the input field beneath the function name or by using a value (see also function $Y(x)$: *y at pos = x* above.)

a + b: Calculates the sum of two analysis results.

Note: Depending on the Math function one or two results are available for the calculation.

a - b: Calculates the difference of two analysis results.

a * b: Calculates the product of two analysis results.

a / b: Calculates the quotient of two analysis results.

a in b: Calculates the amount of one analysis result that is present within another analysis result.

abs: Calculates the absolute value of an analysis result.

log: Calculates the logarithmic value of an analysis result.

sqrt: Calculates the square root of an analysis result.

arctan: Calculates the arc tangent of an analysis result.

1 / a: Calculates the reciprocal value of an analysis result.

1 / log: Calculates the reciprocal logarithmic value of an analysis result.

1 / sqrt: Calculates the reciprocal square root of an analysis result.

1 / arctan: Calculates the reciprocal arc tangent of an analysis result.

9.4.2 Trace

This analysis is applied to a selected Trace. The result is again a Trace.

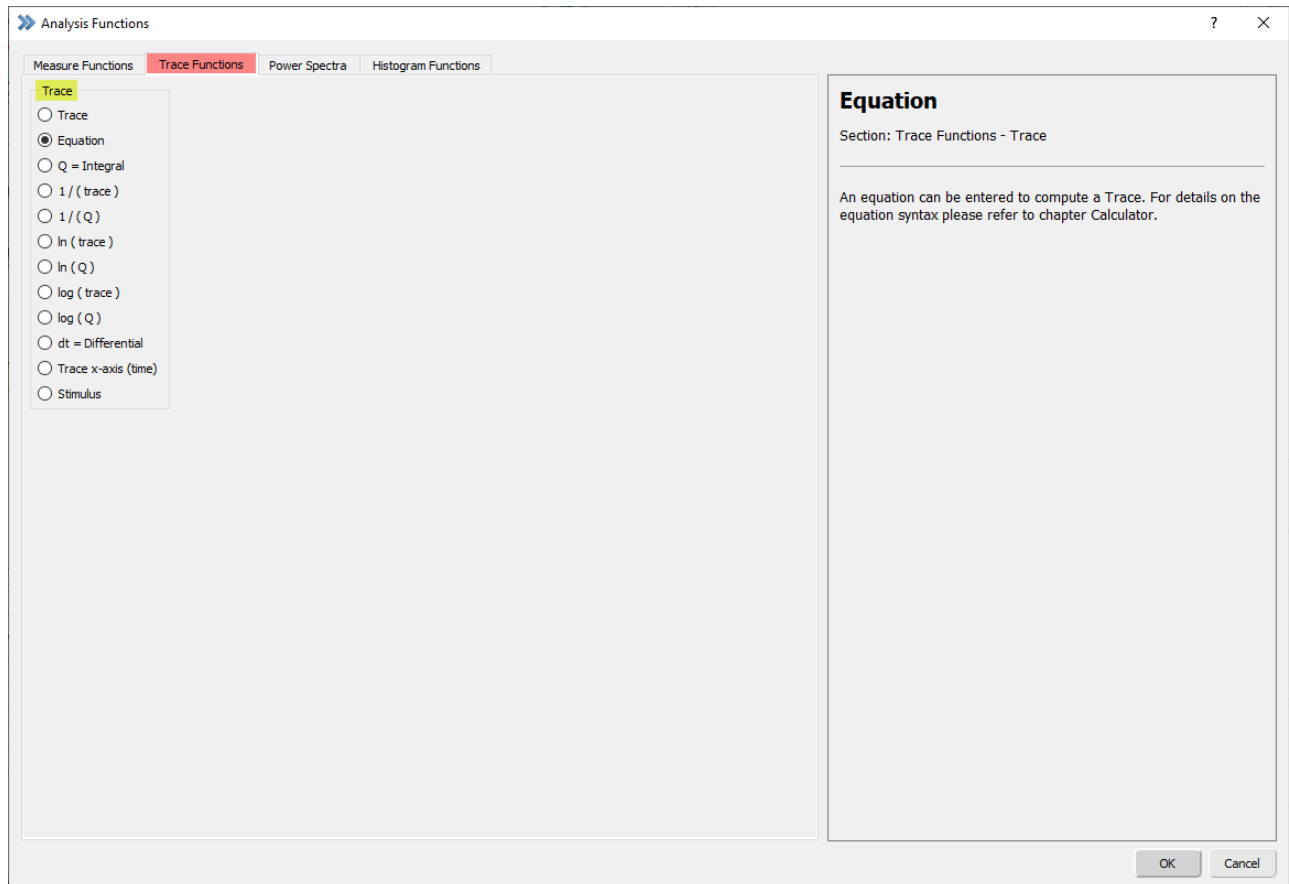


Figure 9.32: Trace Functions section of the Analysis Editor.

Trace: Sets a Trace as an analysis result.

Equation: An equation can be entered to compute a Trace. For details on the equation syntax please refer to chapter 17 on page 235.

Q=integral: Calculates the integral over time of the Trace.

1 / (trace): Calculates the reciprocal of a Trace.

1 / (Q): Calculates the reciprocal of the integrated Trace.

ln (trace): Calculates the natural logarithm of the Trace.

ln (Q): Calculates the natural logarithm of the integrated Trace.

log (trace): Calculates the logarithm of the Trace.

log (Q): Calculates the logarithm of the integrated Trace.

dt = Differential: Calculates the differential over time of the recorded Trace.

Trace x-axis (time): Returns the Trace time (first data point of Trace has time = 0). You may use this as X-reference when plotting a Trace versus time in the Graph Window.

Stimulus: The stimulus that is linked to the Trace is returned.

9.4.3 Power Spectra

For calculation of the power spectrum. Useful for measurement of the noise performance. Please note, that at least 1024 data points are required for compiling such a power spectrum.

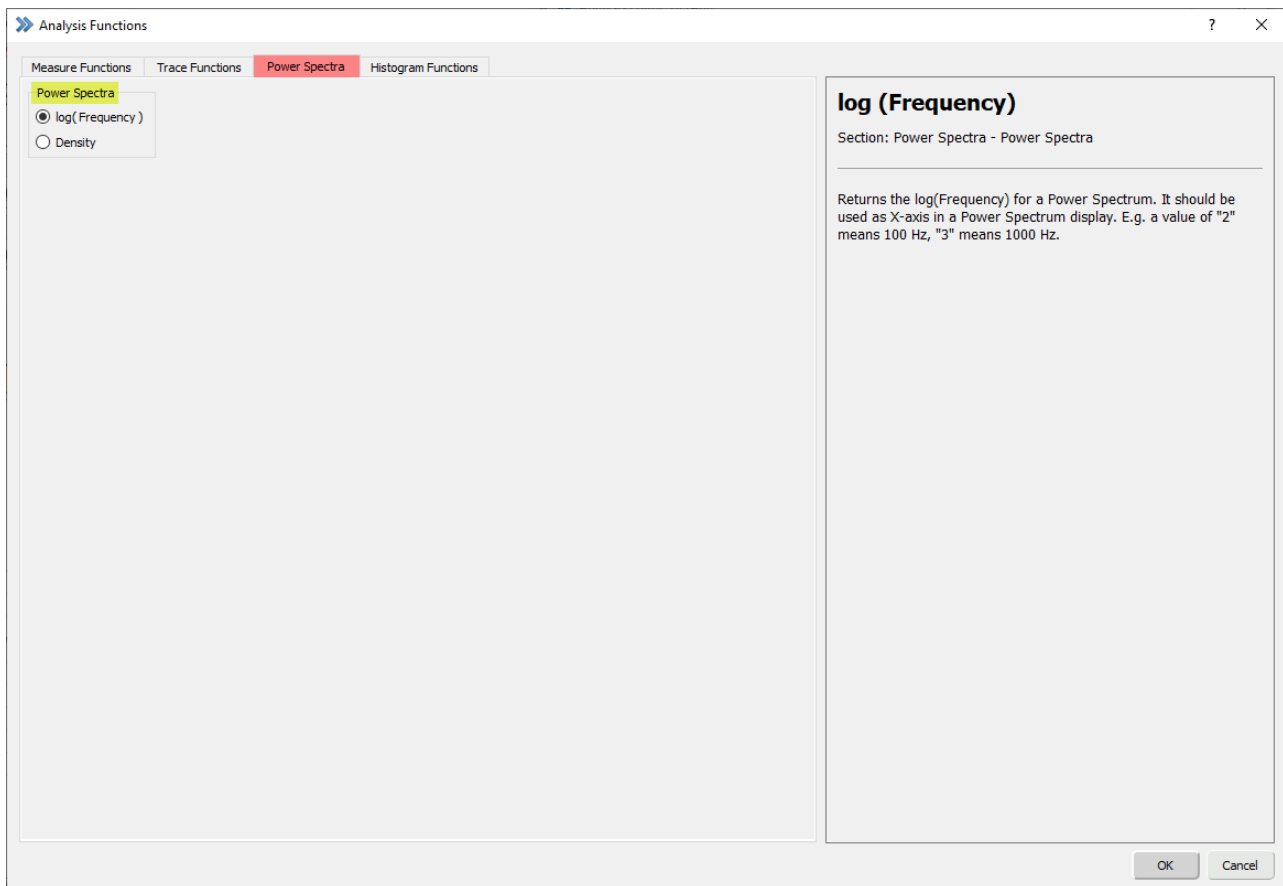


Figure 9.33: Power Spectra section of the Analysis Editor.

log(Frequency): Returns the $\log(\text{Frequency})$ for a *Power Spectrum*. It should be used as X-axis in a *Power Spectrum* display. E.g. a value of '2' means 100 Hz, '3' means 1000 Hz.

Density: Returns the $\log(\text{Distribution})$ of a *Power Spectrum*. The distribution is also called spectral density. It should be used as Y-axis in a *Power Spectrum* display. The displayed values are the linear exponents, e.g. '-25' means $1 * 10^{-25}$. The unit is in A^2/Hz .

Note: Please check chapter 3.5.1 on page 24 in the *Configuration* window for further details in the configuration for this analysis.

9.4.4 Histogram Functions

The histogram functions can be used e.g. for a fast online detection of different amplitude levels.

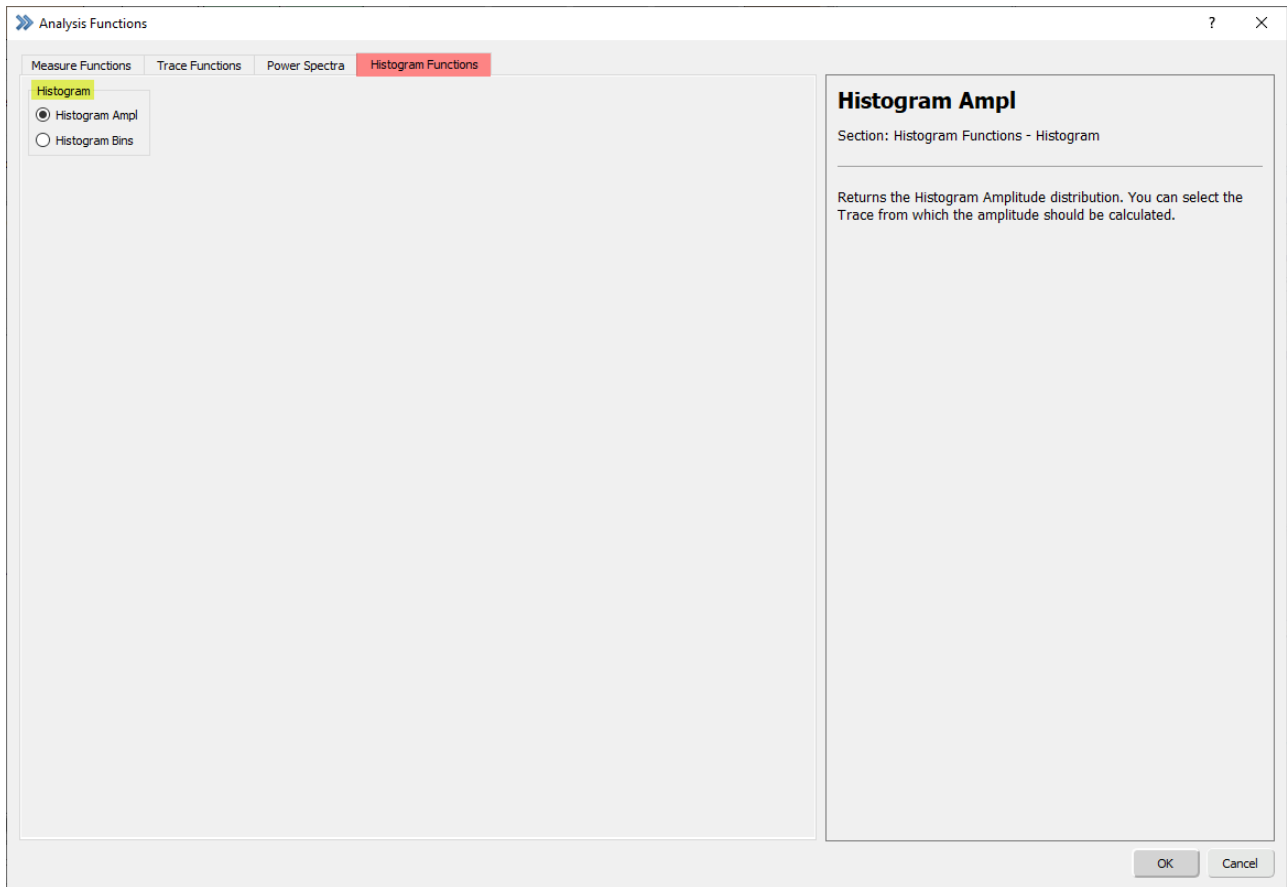


Figure 9.34: Histogram section of the Analysis Editor.

Histogram Ampl: Returns the *Histogram Amplitude* distribution. You can select the Trace from which the amplitude should be calculated.

Histogram Bins: Defines the number of *Histogram Bins* for the analysis of *Histogram Amp*. The number of *Histogram Bins* is entered manually in the `Option` section of this function.

The width of a bin is computed as:

$$\text{bin width} = \text{Y-range} / \text{number of bins}$$

Note: A fixed Y-range is used to analyze all Traces of one Series. In Fixed mode it is the Y-range of the first analyzed Trace, in Auto Scale mode it is the largest Y-range of all analyzed Traces of the parent Series.

10 Graph Layout

The Graph Layout 1 and 2 are used to plot the analysis results in analysis graphs. Each graph can contain up to four graph entries which is defined in the Analysis Editor (see 9.3.2 on page 173). The maximum number of graphs which can be used in each Graph Layout is limited to “12”.

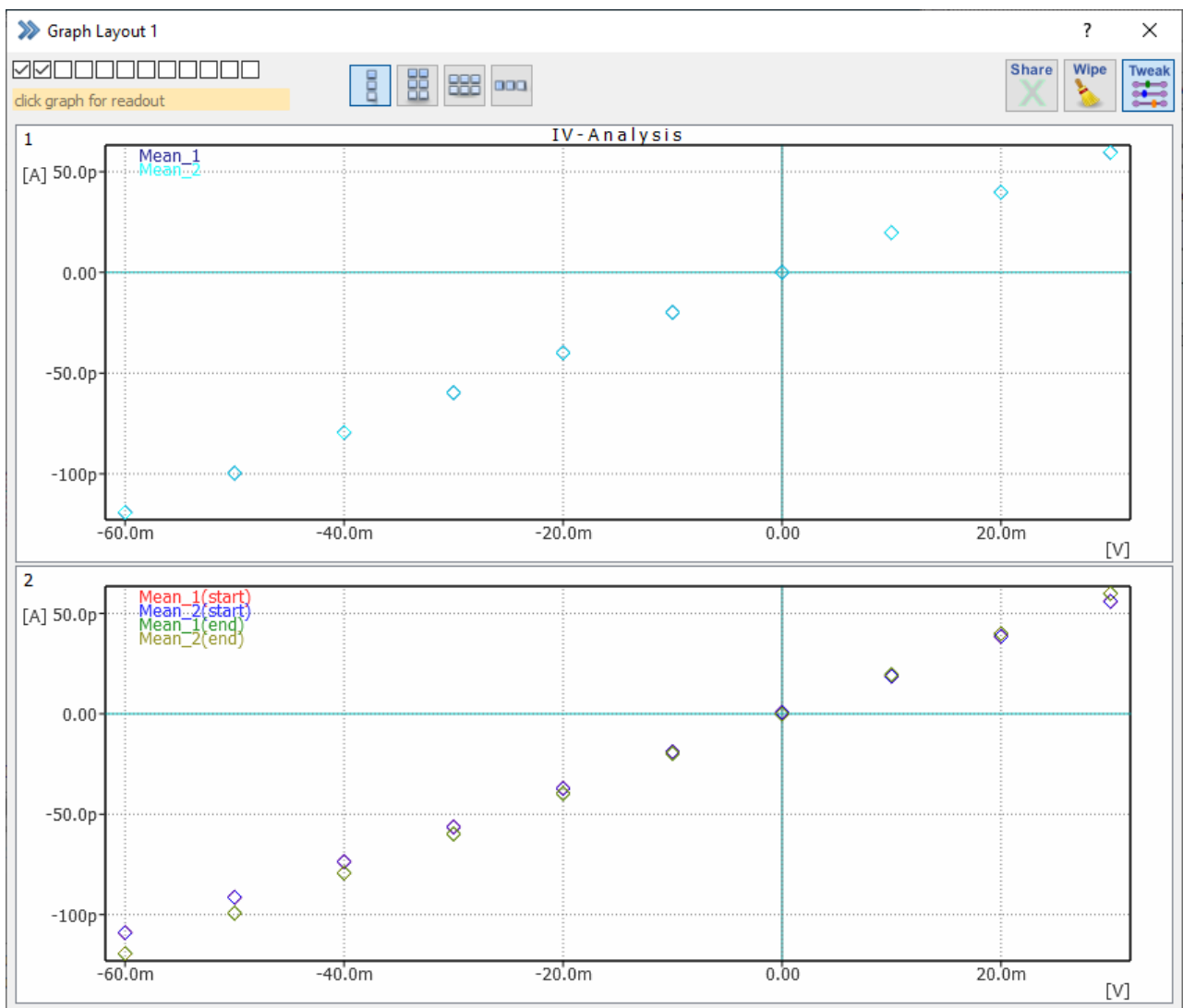


Figure 10.1: The Graph Layout window of PATCHMASTER NEXT depicts the analysis results.

On top of the Graph Layout one can manage the number of graphs displayed by activating the checkbox. The numbering starts on the left side with graph “1” and ends with the graph number “12” on the right side. Below, there is a yellow read-out text field (display only). Here, whenever you click with the mouse on data in a graph, the data value is depicted. Via the paste and copy option you can extract the data.

Note: If a checkbox of a graph is not active, the graph is not displayed in the Graph Layout.

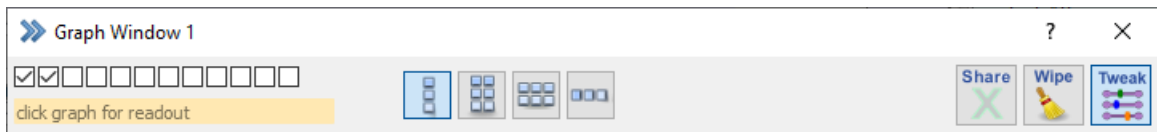


Figure 10.2: The top section of the Graph Layout window.

In the middle of the top section there are several buttons to define the settings of the Graph Layout.

On the right side of the top section there are three more icon buttons:

Share: If active, the same X-axis parameters are used for all graphs (e.g. "Min", "Max", "Scale"...).

Wipe: This function wipes all the graph content.

Tweak: Hides or displays the graph layout options.

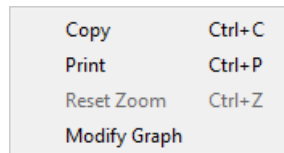


Figure 10.3: Right-Click Context Menu options of the Graphs.

All Graph Layout have a right-click context menu offering the following options:

Copy: Copies the graph to the clipboard. Use the paste option to insert it into any other appropriate software.

Print: Opens the printer settings on your PC to print the graph.

Reset Zoom: Resets the Y- and X-settings of the graph. This option does only get active if any zooming of the data was done before.

Modify Graph: If one clicks on the *Modify Graph* option the *Modify Graph* dialog opens. All the settings available there are explained in the next chapter (10.1 on the next page).

10.1 Modify Graph

The `Modify Graph` dialog provides all parameters to customize the displayed graphs of the Graph Layout.

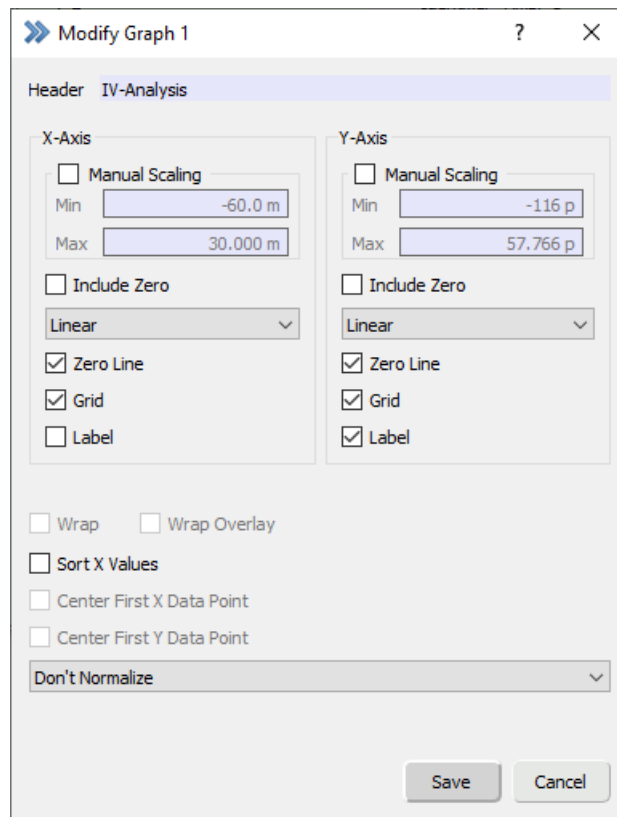


Figure 10.4: Modify Graph dialog of the Graph Layout.

Header: Via this entry field one can define a *Header* for the selected analysis graph.

Manual Scaling: By default, the scaling option for the graphs are set to auto scaling. Here, the software checks for the maximal and minimal values to calculate the best scaling of the graph.

If the scaling of the data is already known or only a small range should be plotted in the graphs one can activate the *Manual Scaling* option. The user has to specify the maximum and minimum X- and Y-axis value for the selected graph.

Include Zero: The Y and X axis always contains "0" at their origin independent of wrapping events.

Axis Mode: This option defines the scaling type of the X/Y axis. Several *Axis Modes* are available:

- Linear: Linear axis scaling
- Log10: Common logarithmic axis scaling
- Log2: Natural logarithmic axis scaling

Zero Line: If selected, a horizontal/vertical line (X/Y) is drawn through the zero value of the graph.

Grid: If selected, a grid is drawn in the graph.

Label: If selected, the name of the *Analysis Function* is used as label for X- and/or Y-axis.

Wrap: Selecting *Wrap* leads to a wrapping of the data at the end of the display, e.g. the display will start again at the left edge.

Note: *The Wrap option is limited to Fixed Scaling of the X axis and Y axis and the X axis mode has to be linear.*

Wrap Overlay: If *Wrap Overlay* is selected, the data will wrap around at the end of the graph without clearing the graph.

Sort X Values: If selected, the data will be sort according to ascending X-values.

Center First X Data Point: If selected, the first X data point will be set in the center of the graph. This allows the monitoring of ongoing experiments. The displayed scaling defined by the values entered in the *Fixed Scaling* is not considered as an absolute value range but defines a relative one.

Center First Y Data Point: If selected, the first Y data point will be set in the center of the graph. This allows the monitoring of ongoing experiments. The displayed scaling defined by the values entered in the *Fixed Scaling* is not considered as an absolute value range but defines a relative one.

Normalization: Select the type of normalization of the data:

- Don't Normalize
- $y' = y / \max$
- $y' = (y - \min) / (\max - \min)$

Note: *This option is only available if Fixed Scaling is on.*

11 Data Tree

Acquired and stored data can be reviewed and edited in the Data Tree window.

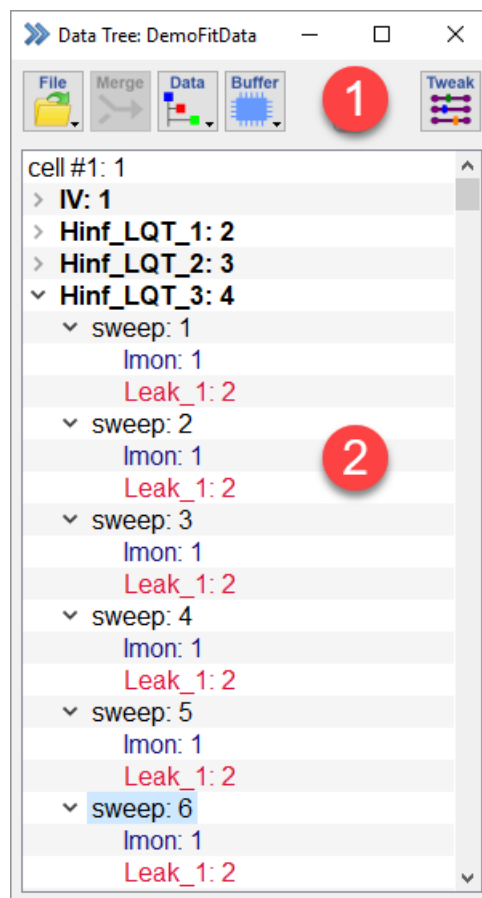


Figure 11.1: Data Tree window of PATCHMASTER NEXT.

The Data Tree window consists of two main sections which are explained in the next chapters:

- Global Section
- Tree Display

11.1 Global Section

File Menu: The File Menu has all options to handle the data files of PATCHMASTER NEXT. The options available are explained in chapter 11.1.1 on the following page.

Merge: Appends the data tree from an existing data file to the data tree of the actually opened file.

Data Menu: The `Data` Menu contains all options to edit, check or export the data files of PATCHMASTER NEXT. The options available are explained in chapter 11.1.2 on the next page.

Buffer Menu: The `Buffer` Menu provides the options for storing and processing intermediate data in up to four independent Trace Buffers. All available options are explained in chapter 11.1.3 on page 200.

Write Protection Icon: The *Write Protection Icon* allows to easily check if the currently opened data file was opened in read-only mode or in modify mode. Whenever the icon is displayed the file was opened in read-only mode and cannot be modified.

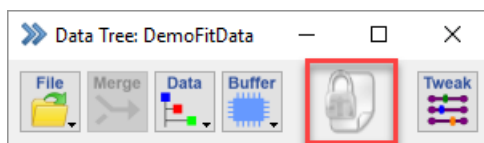


Figure 11.2: The Write Protection Icon indicates that the data file is opened in read-only mode.

Tweak: By activation of the `Tweak` function more `Data Tree` options are displayed below the `Global Section` of the `Data Tree` window. The available options are explained in detail in chapter 11.1.4 on page 202.

11.1.1 File Menu

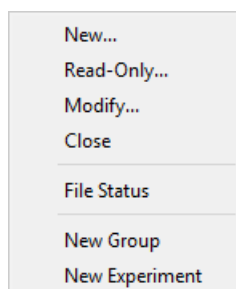


Figure 11.3: File Menu of the Data Tree.

New...: Creates a new, empty data file that is ready for data acquisition. The file has read and write permission.

Read-Only...: Loads an existing file in *Read-Only* mode. Modification of the file is not allowed. Use this option when you want to analyze data and to make sure not to change or delete anything.

Modify...: Loads an existing file with read and write permission. Modification of the file such as appending or deleting data is allowed.

Deleting entries in the data file is not reversible. Make sure to always have a backup of the original files when modifying an experiment. The exception is, of course, when you really want to delete a part of the stored data.

Close: Closes the actual file.

File Status: Prints information about the status of the currently opened file such as the path, length, etc. to the Notebook window.

New Group: Generates a new Group in the output data tree of the `Data Tree` window if the file is opened without write protection. After the addition of a new Group a file update is automatically performed.

New Experiment: Generates a new experiment and a new Group (see above) and increments the experiment number.

11.1.2 Data Menu

The `Data` Menu provides all options for data editing, checking or exporting.

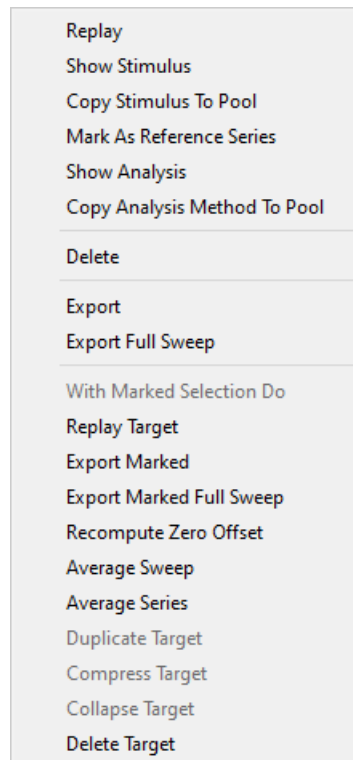


Figure 11.4: Data Menu of the Data Tree.

Replay: Displays the content of the selected target. Traces are displayed according to the settings specified in the Oscilloscope window. If the Experiment/Group is selected for *Show*, all Traces are displayed one after another. This can be aborted by mouse click on the *Break* or *Stop* buttons in the Control window.

Show Stimulus: This option opens the stimulation file of the corresponding selected Series in the Data Tree (or the first Series, if a Group is the target). Leave the window open and scroll through your acquired data in the Data Tree to get an overview of the stimulation settings of your recordings (Review Mode).

Copy Stimulus to Pool: Copies the stimulus protocol of a selected Trace, Sweep or Series into the current Stimulus Editor file.

Mark As Reference Series: Selects a target as reference with the *Set* function. The *Reference Series* is subtracted from all replayed data, if *Subtract Ref. Series* in the Oscilloscope menu is active (6.1.1 on page 74). The subtraction of the *Reference Series* not only affects the display of the data in the Oscilloscope window, but also data analysis and export. A previously selected *Reference Series* can be turned off with the *Off* option.

Show Analysis: This option opens the Analysis Editor showing the corresponding *Analysis Method* of the selected Series in the Data Tree. Leave the window open and scroll through your acquired data in the Data Tree to get an overview of the *Analysis Methods* of your recordings (Review Mode).

Copy Analysis Method to Pool: With this option allows you to copy the *Analysis Method* of the currently selected data to your analysis file.

Delete: Deletes the selected target in the Data Tree.

Export: Exports the selected target. Display gain, leak subtraction or zero line subtraction will be applied.

Note: The Export option will try to keep a "what-you-see-is-what-you-get" behavior. This means that the display options define the export options; e.g., only the displayed data are exported; or when Show Leak Traces is on, the leak Traces are also exported. For full Sweep export please use Export Full Sweep (see below) or make sure that the display is set to full time range.

Export Full Sweep: Exports the full Sweep. This function works independent from the time range settings in the Oscilloscope window. Display gain, leak subtraction or zero line subtraction will be applied.

Note: Export formats and settings are defined in the Export settings (see chapter 11.1.4.2 on page 204).

With Marked Selection Do: Actions executed from this section of the Data menu are performed on all marked children of the selected target in the data tree:

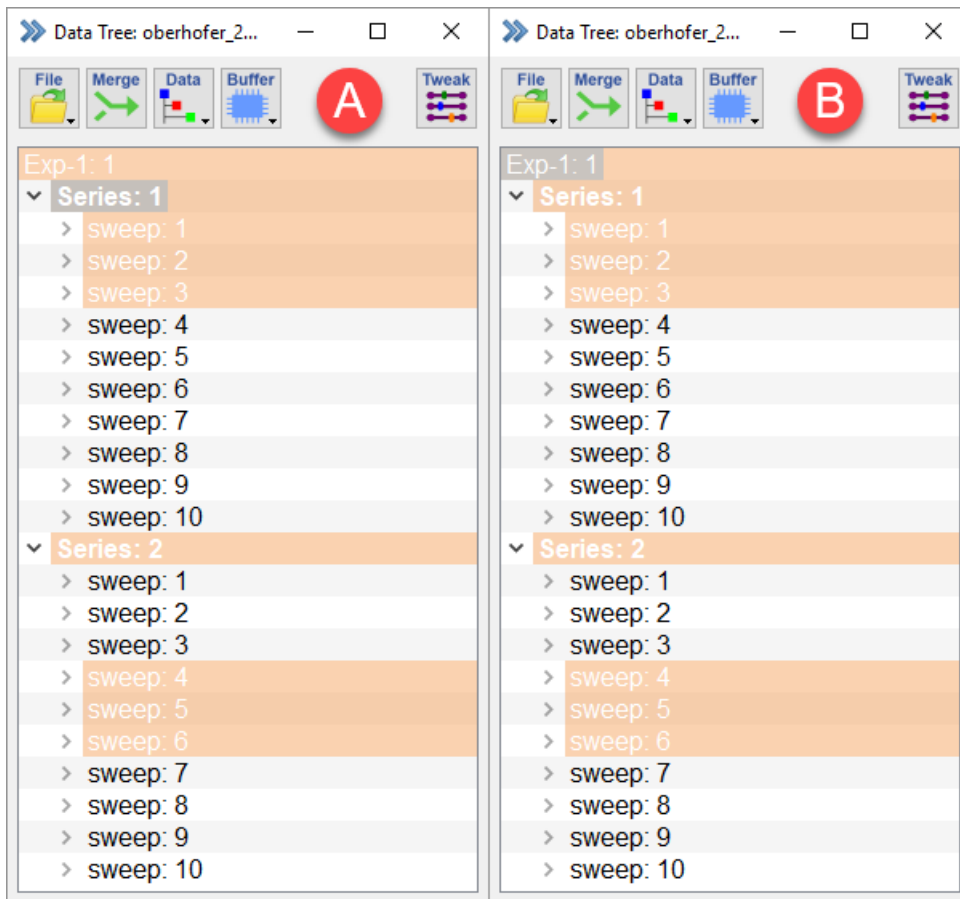


Figure 11.5: Different targets effect the export of marked structures in the Data Tree.

In example "A" the With Marked Target and Children → export command would export only Sweep 1 to 3 of the first Series, while in example "B" all marked Sweeps of Series 1 and 2 would be exported.

- Replay: Displays marked targets in the selected tree branch.
- Export Marked: Exports marked targets and children of the selected tree branch. The display options in the Oscilloscope window define the export options.

- **Export Marked Full Sweep:** Exports the full marked targets and children independent from the time range settings in the Oscilloscope window.
- **Recompute Zero Offset:** The zero offset value of all Traces in the selected target is recomputed. The zero offset value is used by the *Subtract Zero Offset* function (see chapter 7.6.1 on page 98).

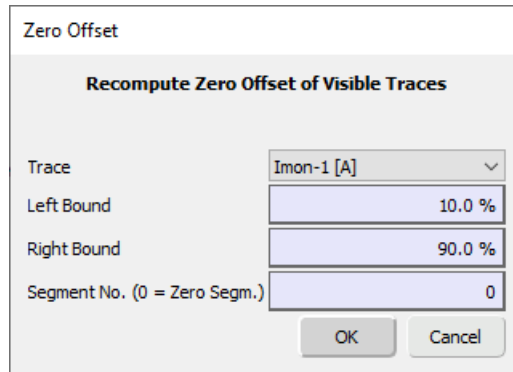


Figure 11.6: Zero Offset recompute options of the Data Menu.

- **Trace:** Selects the Trace for the zero offset recomputing (available selection depends on the target).
- **Left Bound:** Defines the left bound of the selected segment for zero offset computation.
- **Right Bound:** Defines the right bound of the selected segment for zero offset computation.
- **Segment No. (0 = Zero Segm.):** Defines the segment (of the stimulus) for the zero offset calculation.

Note: Only visible Traces are recomputed.

Note: Only Traces originating from an AD-channel are recomputed; virtual Traces are not affected.

Note: The zero offset of leak Traces is only recomputed if the "Show Leak Traces" is selected from the Oscilloscope menu.

- **Average Sweep:** Averages all Sweeps of a Series and stores them as Series with one Sweep. The Sweeps have to have the same length.
- **Average Series:** Generates one new Series in which each Sweep is the average of all marked Series with the same Sweep index and each Trace with the same Trace index.
- **Duplicate Target:** Creates a new Group containing all marked targets. This is e.g. convenient when one needs to compress a Trace for export.
- **Compress Target:** Compresses all marked Traces by a given compression factor. Optionally one can supply a maximal compression factor. This can be used e.g., if one has Traces which are already compressed and having much fewer points per second, e.g., a FURA trace. The Traces cannot reside in a read-only file. To compress a Trace in a read-only file, duplicate the required targets.
- **Collapse Target:** The Sweeps of all marked Series are copied into a new Series. This is typically used when one acquired many Series with one single Sweep and one wants to combine them into one Series for easier analysis.
- **Delete Target:** Deletes the marked targets and children of the selected tree branch.

Note: Delete and Delete Target are disabled when a data file is opened in read-only mode.

11.1.3 Buffer Menu

When a Buffer is filled with data for the first time (after a *Clear* command) all parameters of the target Trace are transferred to the Buffer data structure. When adding further Traces to the Buffer these parameters are not changed. It is assumed that all Traces that are added to the Buffer are of same type. When replacing a target Trace with the data of a Buffer, data only are transferred. The parameters of the target Trace stay unchanged.

It is the responsibility of the user that the key parameters such as Gain and Sampling Interval are chosen adequately when adding importing data to a Buffer or processing different Traces in a Buffer. Once a Trace is replaced by a Buffer, most parameters i.e. Zero Offset or C-Slow become meaningless.

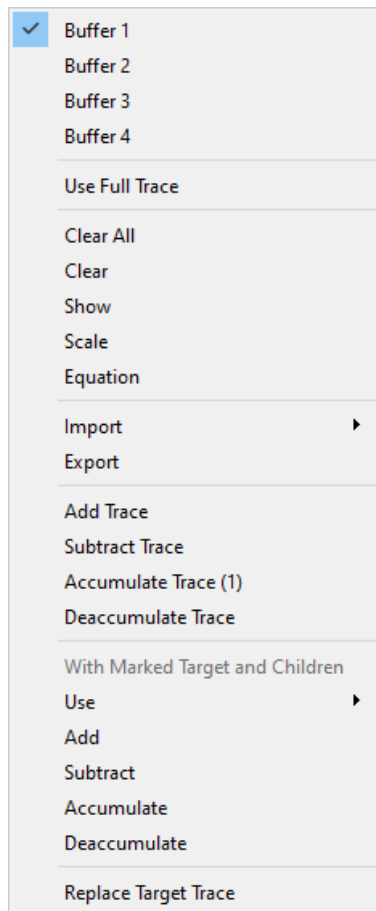


Figure 11.7: Buffer Menu of the Data Tree.

Buffer 1... Buffer 4: Select the Buffer you want to work with.

Use Full Trace: If this option is selected the functions *Add Trace*, *Subtract Trace*, and *Accumulate Trace* work on the complete Trace. If this option is not selected the above mentioned functions work on the part of the Trace which is displayed in the Oscilloscope window only.

Clear All: Clears all Buffers.

Clear: Clears the selected Buffer.

Show: Shows the selected Buffer content in the Oscilloscope window.

Scale: Scales the selected Buffer with *Scale* and *Offset*.

Equation: Allows to perform a Buffer calculation based on an equation. For details see chapter 17 on page 235.

Import: Imports data into a selected Buffer.

Please make sure that the key parameters *Gain* and *Sampling Interval* of the imported data match the parameters of the Buffer. In case a Buffer is filled with data for the first time, the parameters are retrieved from the Trace currently selected in the **Data Tree** window.

You can import the following three formats:

- from ASCII file: Imports data which were saved as CSV (=Comma-Separated Values).
- from Binary file: Imports data which were saved in the IGOR binary format.
- from Igor Wave: Imports data which were saved in the IGOR Wave format.

Export: Exports data from a selected Buffer. The export format is defined in the **Export Settings** (see chapter 11.1.4.2 on page 204).

Add Trace: Adds the Trace selected in the **Data Tree** to the active Buffer.

Subtract Trace: Subtracts the Trace selected in the **Data Tree** from the active Buffer (Trace).

Accumulate Trace: Accumulates the Trace selected in the **Data Tree** to the active Buffer:

$$\text{Buffer}(n+1) = (\text{Buffer}(n) * n + \text{Trace})/n+1$$

with n = number of Traces in the Buffer.

Deaccumulate Trace: Deaccumulates the Trace selected in the **Data Tree** window from the active Buffer:

$$\text{Buffer}(n-1) = (\text{Buffer}(n) * n - \text{Trace})/n-1$$

with n = number of Traces in the Buffer.

With Marked Target and Children: Multiple Traces can be marked in the **Data Tree** and processed simultaneously.

- Use: Specify which Trace of the marked Sweeps should be processed.
- Add, Subtract, Accumulate, Deaccumulate: Work on all marked targets.

Replace Target Trace: The Trace selected in the **Data Tree** (target Trace) will be replaced with the active Buffer.

11.1.4 Tweak

By activating the *Tweak* button a bunch of options are displayed between the Global Section and the Tree Display section of the Data Tree window.

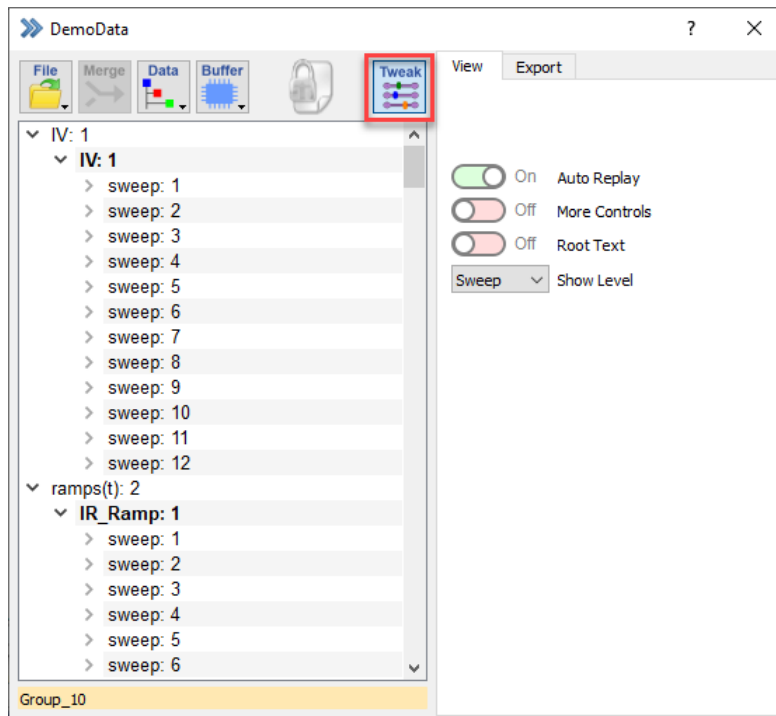


Figure 11.8: Tweak options of the Data Tree.

11.1.4.1 View

In the *View* tab of the *Tweak* section you can define the options which should be hidden or displayed in the Data Tree window.

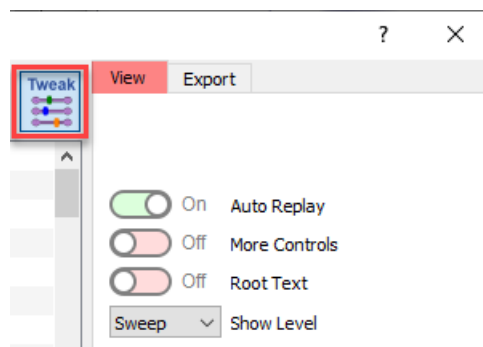


Figure 11.9: View options of the Data Tree.

Auto Replay

If this option is active the data selected in the Data Tree window is automatically replayed.

More Controls

When activating this slider a new sections with parameters and settings appears above the data structure of the Data Tree.

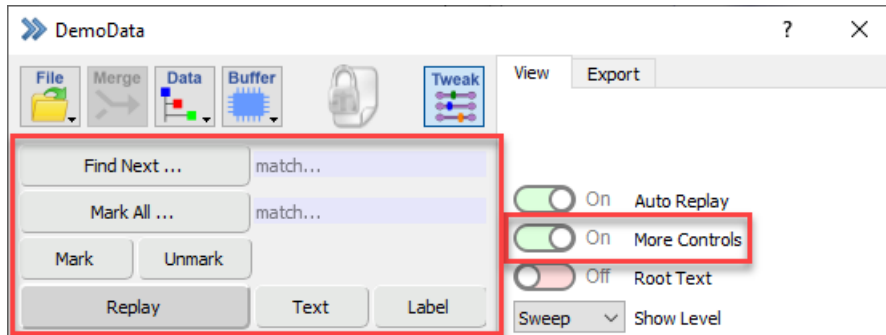


Figure 11.10: Control options of the Data Tree.

Find Next: Enter a search string into the text entry field and click this button to find any data in the Data Tree with the specified name.

Mark All...: Enter a text string into the text entry field and click this button to mark all tree structures in between the selected target with the specified name.

Mark [M]: Marks the selected target in the Data Tree. It is possible to mark more than one tree entry. Several functions in the Data menu apply for the marked entries (see chapter 11.1.2 on page 197).

Note: Set marks will be written into the data file of the data when you save the data (modify mode only). That means that marks will also be reloaded when you open such a marked data file.

Unmark [U]: Unmarks the selected marked target in the Data Tree.

Note: Use "" to mark all data in the opened data file.*

Text: At the Series or Group level it is possible to enter a comment which is saved with the data. The entered text gets visible at the bottom of the Data Tree window if the corresponding Series or Group is selected.

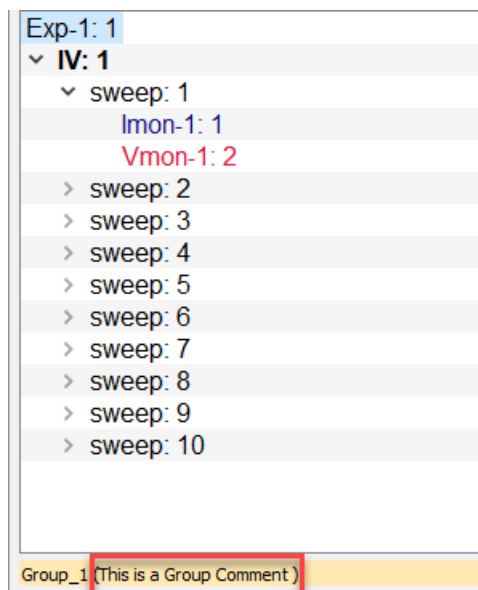


Figure 11.11: Entered text comments are displayed at the lower border of the Data Tree.

Label: Use this option to change the labels for a Trace, Sweep, Series or Group.

Root Text

If *Root Text* is enabled and you have a data file opened in modify mode you are able to enter free text into the appearing text box above the data structure of the Data Tree. This is especially useful to store information about the data of this file.

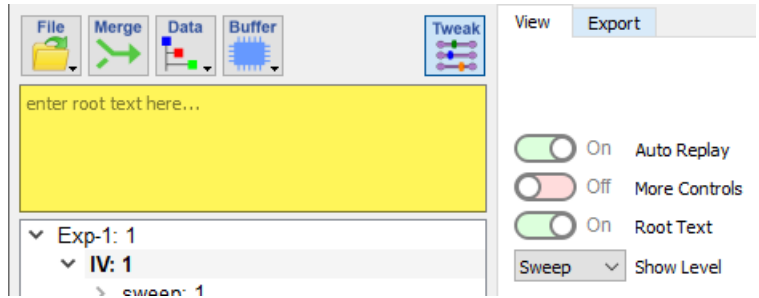


Figure 11.12: Enter information about the whole data file into the Root Text box of the Data Tree.

Show Level

Defines which level of the Data Tree will be displayed. Available parts of the data tree are Root, Group, Series, Sweep, and Trace.

11.1.4.2 Export

In the *Export* tab of the *Tweak* section you can define the data export settings.

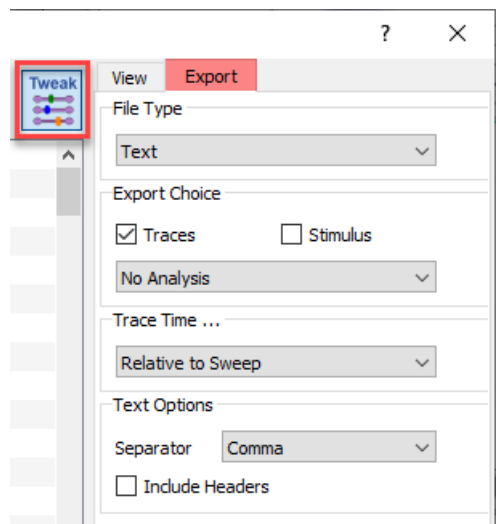


Figure 11.13: Export options of the Data Tree.

File Type: This determines the type of output to be created. The following export formats are implemented:

- Text: Sweeps are output as columns of ASCII numbers representing the data Traces (all in scientific format). Each Sweep and Series starts with an identifier. The separator can be modified (space, comma, or tab separators) by using the ASCII-Text format option in the *Data* menu.
- Igor Pro: Exports into the Igor Pro format.

- MatLab: Sweeps are exported into a MatLab file.

Export Choice: There are several export options:

- Traces: Exports the selected Traces.
- Stimulus: Exports information on the used stimulation pattern (segment duration and amplitude).

Note: This option can only be used when either Traces or Analysis - Graphs is selected.

- No Analysis: No analysis results are exported.
- Analysis Graphs: The graphs of the Graph Layout get exported.
- Analysis Notebook: The Analysis Editor function results which are displayed in the Notebook Window get exported. Please note that the option *Write Result to Notebook* in the Analysis Editor has to be selected.

Note: This option is not available when using the Igor Pro Export Format.

Trace Time: The following *Trace Time* parameters can be set:

- Relative to Sweep: Individual Sweeps will be exported in an overlaid fashion. Time of the first data point of each Sweep starts with "0".
- Relative to Series: Individual Sweeps will be exported in an overlaid fashion. Time of the first data point of each Sweep starts with the difference between the start time of the Series and the start time of the 1. Sweep of that Series.
- Relative to Timer: Individual Sweeps will be exported in a concatenated fashion. The Trace time is offset by the *TimerTime* at Sweep start.
- Relative: Individual Sweeps will be exported in a concatenated fashion. Same as above, but the time is subtracted by the *TimerTime* at the start time of the 1. Sweep. Resulting that the time of the first starts with "0".

Depending on the selected *File Type* there are a number of options to adjust the export format.

Text Options: For *Text* there are the following options:

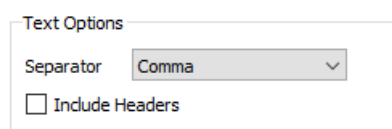


Figure 11.14: Text options of the data export settings.

- Space: Analysis results are separated by a SPACE.
- Comma: Analysis results are separated by a COMMA.
- Tab: Analysis results are separated by a TAB.
- Include Headers: If checked, a header that specifies various parameters of the exported data will precede the actual values.

Igor Pro Options: For *Igor Pro* there are the following options:

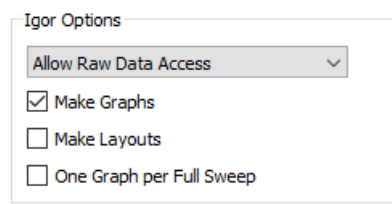


Figure 11.15: Igor Pro options of the data export settings.

- **Allow Raw Data Access:** Whenever possible an Igor Info file is generated allowing access to raw PATCHMASTER NEXT data which should be stored in the same folder. Using this output option assures fastest output and the smallest output files.
- **Create Binary Wave:** Export of Sweeps to Igor Pro as binary data. This function generates an Igor macro which contains the instructions for Igor on how the data are to be loaded, scaled, and displayed. It has the extension `*.itx`. A double-click on it will make Igor Pro load that macro file and execute the instructions in it, importing, scaling, and displaying the data. The actual data are not really exported when using the *Allow Raw Data Access* option. That option will make use of the "GBLoadWave" Igor extension to read the data directly from the PATCHMASTER NEXT raw data file, i.e., the `*.dat` file. The data are converted to Igor binary waves when the simple export option is used. However, even in that case a macro file is generated and you should load the data via that file. When you want to import data from within Igor Pro, use the option *Load...Igor Text* to load the macro file. Use the option *Load...Igor Binary* only when you want to explicitly load one of the generated Igor binary waves (file extensions `*.ibw` or `*.wav`).

Note: It is much faster to work with "Create Binary Wave" than with "Create Text Wave", and the created files are considerably smaller.

- **Create Text Wave:** Export of Sweeps as ASCII waves in "Igor Text" format for the analysis and display program Igor. In Igor Pro, each wave is identified by indices "Group_Series_Sweep_Trace" (e.g., "Name2_4_3_1"). If the file name starts with a number, a "W" is placed in front of it, because in Igor Pro, waves are not allowed to start with a number. The created file has the extension `*.itx` and is recognized by Igor Pro, i.e., double-click on this file will start Igor Pro, load and display the file content (not for Sweeps). The waves will immediately be displayed in Igor Pro only when the *Igor: Make Graphs* option was selected. Otherwise, the Sweeps will be loaded, but must be displayed by Igor Pro's "Display Wave" or "Make Graph" command. When loading Igor Text output files, do not use the "General Text" import option in Igor; always use the option "Load...Igor Text".
- **Make Graphs:** The Sweeps are exported as Igor graphs as they appear in PATCHMASTER NEXT in the Oscilloscope window. The created files have the extension `*.itx` and are recognized by Igor Pro, i.e., double-click on this file will start Igor Pro, load and display the file content.
- **Make Layouts:** This option is useful if several Series shall be exported to Igor Pro. The exported Traces are arranged on an Igor Pro layout page. The created files have the extension `*.itx` and are recognized by Igor Pro, i.e., double-click on this file will start Igor Pro, load and display the file content.
- **One Graph per Full Sweep:** With this option, a Trace that consists of several pages (e.g. continuous data) is exported as one single Igor Pro wave. Without this option, one wave per page is exported.

11.2 Tree Display

The Data Tree has four hierarchy levels:

1. Experiment/Group: A Group can be a section of an Experiment e.g. working on a specific cell type defines the *Experiment* and the usage of different drugs define the individual Groups.
2. Series: A Series contains the whole defined stimulation sequence including all iterations (Sweeps).
3. Sweep: A Sweep is an iteration of the executed stimulus pattern. The minimum Sweep number is “1”.
4. Trace: Lowest level, showing containing the recorded signals from the selected input sources (e.g. AD channels containing a voltage or current signal).

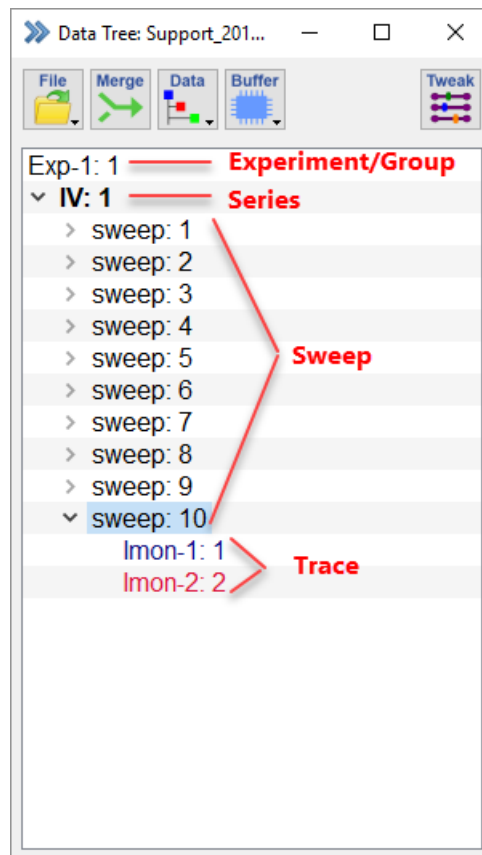


Figure 11.16: Hierarchy levels of the Data Tree.

By clicking on the branch arrows you can individually collapse or extend tree levels in the `Tree Display` of the `Data Tree` window.

A right-click inside the `Tree Display` section opens a context menu offering the following options:

- **Replay:** Replays the targeted data of the Data Tree.
- **Delete:** Deletes the targeted data of the Data Tree (modify mode only).
- **Show Stimulus:** Opens the Stimulus Editor in the *Review* mode.
- **Show Analysis:** Opens the Analysis Editor in the *Review* mode.
- **Data Parameter:** Opens the Data Parameters window.

- **Mark:** Marks the selected data of the Data Tree.
- **Unmark:** Unmarks the selected data of the Data Tree.
- **Comment:** Allows to enter a comment for the selected data of the Data Tree (Series and Group level only).
- **Label:** Allows to change the label of the selected data of the Data Tree.

12 Data Parameter

Data Parameters are additional values describing the experimental and measurement conditions. The *Data Parameters* are required to interpret the data correctly. In PATCHMASTER NEXT we distinguish different types of *Data Parameters* depending to which level of the data structure they relate.

The screenshot shows the 'Data Parameters' window with the 'Amplifier Items' tab selected. The window contains a list of parameters, each with a checkbox and an input field. The parameters are organized into two columns.

Parameter	Value
<input type="checkbox"/> Amplifier State	
<input type="checkbox"/> Amplifier	EPC10_USB 1/2
<input type="checkbox"/> Clamp Mode	V-Clamp
<input type="checkbox"/> V-Pipette	0.00 V
<input checked="" type="checkbox"/> Gain	1.00 mV/pA
<input type="checkbox"/> Ext.Stimulation	0.00
<input type="checkbox"/> Filter 1	Bessel-10kHz
<input checked="" type="checkbox"/> Filter 2	2.87 kHz
<input type="checkbox"/> Filter 2 Type	Bessel
<input type="checkbox"/> Stimulus Filter	10.0 kHz
<input checked="" type="checkbox"/> C-fast Total	0.00F
<input checked="" type="checkbox"/> C-fast Amp1	0.00F
<input checked="" type="checkbox"/> C-fast Amp2	0.00F
<input checked="" type="checkbox"/> C-fast Tau	500 ns
<input type="checkbox"/> C-fast Range	Normal
<input checked="" type="checkbox"/> C-slow	1.00 pF
<input type="checkbox"/> C-slow Range	CSlowOff
<input type="checkbox"/> R-series	5.00 MΩ
<input type="checkbox"/> Rs-comp.	0.00%
<input type="checkbox"/> Rs-comp. Tau	0.00s
<input type="checkbox"/> Leak Comp.	0.00S
<input type="checkbox"/> Liquid Junction	0.00V
<input type="checkbox"/> V-offset	0.00V
<input type="checkbox"/> CC-Gain	1 pA/mV
<input type="checkbox"/> Low Freq VC	LFVC_Off
<input type="checkbox"/> LFVC V-memb	0.00
<input type="checkbox"/> CC-fast	CC-fast
<input type="checkbox"/> Auto C-fast	undefined
<input type="checkbox"/> Auto C-slow	undefined
<input type="checkbox"/> V-mon Scale	10x
<input type="checkbox"/> Stim Scale	10x
<input type="checkbox"/> Electrode Mode	2
<input type="checkbox"/> Calibration Date	09-Nov-2022
<input type="checkbox"/> Serial Number	00550582-T
<input type="checkbox"/> LockIn Phase	0.00°
<input type="checkbox"/> LockIn Attenuation	1.00
<input type="checkbox"/> LockIn Mode	0
<input type="checkbox"/> LockIn Calibration	0

Figure 12.1: Data Parameter window of PATCHMASTER NEXT.

Note: If possible PATCHMASTER NEXT will use internal values to set the Data Parameters. E.g. C-Slow values will be automatically read from the amplifier and stored with the Data Parameters. For some Data Parameters it might be necessary to assign a source (e.g. AD channel or manual input) in the I/O Control tab of the Configuration. For other amplifiers than EPC 10 USB most of the external Data Parameters have to be entered manually via the I/O Control window.

In the *Data Parameter* window either the *Data Parameters* that are saved with already acquired data, or the *Data*

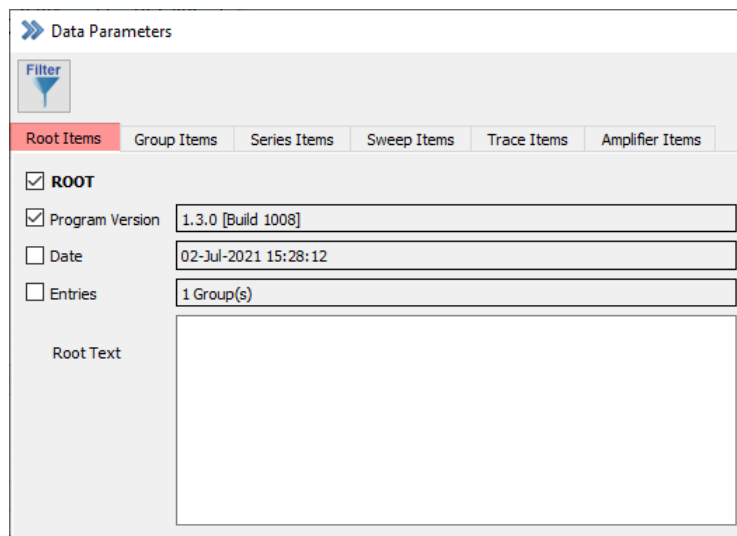
Parameters of the last acquisition (stored or not stored) are displayed.

12.1 Parameter Tabs

The Data Parameter window contains several tabs wherein the *Data Parameters* are grouped according to the hierarchy level of the data tree.

12.1.1 Root

The “Root” level, or level “0”, is the top level of the data tree. The *Data Parameters* of the “Root” level are:



The screenshot shows the 'Data Parameters' window with the 'Root Items' tab selected. The window has a 'Filter' button and a tabbed interface with 'Root Items', 'Group Items', 'Series Items', 'Sweep Items', 'Trace Items', and 'Amplifier Items'. Under the 'Root Items' tab, there are several parameters:

- ROOT**
- Program Version: 1.3.0 [Build 1008]
- Date: 02-Jul-2021 15:28:12
- Entries: 1 Group(s)
- Root Text: (empty text area)

Figure 12.2: Root tab of the Data Parameter window.

- Date: Date at the time of acquisition.
- Entries: Number of Groups in the file.
- Program Version: Version number of PATCHMASTER NEXT.
- Root Text: A descriptive text can be entered and stored if the data file is opened in the *Open Modify...* mode.

12.1.2 Group

Level “1” of the data tree. The *Data Parameters* of the “Group” level are:

Parameter	Value
<input checked="" type="checkbox"/> GROUP	
<input type="checkbox"/> Header	GROUP_1
<input type="checkbox"/> Label	Exp-1
<input type="checkbox"/> Count	1
<input type="checkbox"/> Entries	1 Series
<input checked="" type="checkbox"/> Exp. Number	1
<input checked="" type="checkbox"/> Text	

Figure 12.3: Group tab of the Data Parameter window.

- Header: Index number of the Group (e.g. GROUP_1).
- Label: Text identifier; default label is the experiment number.
- Count: Number of Group entries.
- Entries: Number of Series in the Group.
- Exp. Number: Number of the experiment. The *Exp. Number* can be incremented by creating a new *Experiment*. An *Experiment* might contain a collection of different Groups with the same *Exp. Number*.
- Text: A descriptive text can be entered and stored if the data file is opened in the *Open Modify...* mode.

12.1.3 Series

Level “2” of the data tree. The *Data Parameters* of the “Series” level are:

Parameter	Value
<input checked="" type="checkbox"/> SERIES	
<input type="checkbox"/> Header	SERIES_1
<input checked="" type="checkbox"/> Label	IV
<input type="checkbox"/> Count	1
<input type="checkbox"/> Entries	10 Sweep(s)
<input type="checkbox"/> Number Sweeps	10
<input checked="" type="checkbox"/> User Name	
<input checked="" type="checkbox"/> Date	01-Dec-2022
<input type="checkbox"/> Time	09:22:17.624
<input type="checkbox"/> Timer	00:00:10.914
<input type="checkbox"/> Method	
<input type="checkbox"/> Comment	
<input type="checkbox"/> Stim Reference	1

Figure 12.4: Series tab of the Data Parameter window.

- Header: Index number of the Group and the Series (e.g. SERIES_1_2).
- Label: Text identifier; default label is the name of the Series.
- Count: Index number of the Series.
- Entries: Number of Series in the Group.
- Number of Sweeps: Original number of Sweeps in the Series. The number of *Entries* gets lower after deleting individual Sweeps.
- User Name: Name of the user which was entered in the Configuration (3.5.1 on page 24).
- Date: Date at the time of acquisition.
- Time: Time of the acquisition.
- Timer: Timer time of the acquisition.
- Method: Name of the used method (see chapter 8.3.7 on page 160)
- Comment: Text identifier (see Control window, 3.2 on page 21).
- Stim Reference: Index of the stimulation sequence used in the data file.

12.1.4 Sweep

Level “3” of the data tree. The *Parameters* of the “Sweep” level are:

The screenshot shows the 'Data Parameters' window with the 'Sweep Items' tab selected. The parameters are organized into two columns. The left column contains a list of parameters with checkboxes and text input fields. The right column contains four parameters with checkboxes and numeric input fields.

Parameter	Value
<input checked="" type="checkbox"/> SWEEP	
<input type="checkbox"/> Header	SWEEP_1
<input checked="" type="checkbox"/> Label	
<input type="checkbox"/> Count	1
<input type="checkbox"/> Entries	2 Trace(s)
<input checked="" type="checkbox"/> Time	15:33:03.300
<input type="checkbox"/> Timer	04:21:02.948
<input type="checkbox"/> Temperature	20
<input type="checkbox"/> User1	0
<input type="checkbox"/> User2	0
<input type="checkbox"/> User3	0
<input type="checkbox"/> User4	0
<input type="checkbox"/> User5	0
<input type="checkbox"/> User6	0
<input type="checkbox"/> User7	0
<input type="checkbox"/> User8	0
<input type="checkbox"/> User9	0
<input type="checkbox"/> User10	0
<input type="checkbox"/> RMS-Noise	0
<input type="checkbox"/> Pipette Pressure	0
<input type="checkbox"/> Digital In	0
<input type="checkbox"/> Digital Out	0

Figure 12.5: Sweep tab of the Data Parameter window.

- Header: Index number of the Group, the Series and the Sweep (e.g. SWEEP_1_4_1).
- Label: Text identifier; label of the Sweep (not the index number).
- Count: Index number of the Sweep.

- Entries: Number of Traces in the Sweep.
- Stim. Count: Total number of Series in the data tree.
- Time: Time of the acquisition.
- Timer: Timer time of the acquisition.
- Temperature: Temperature value from an external device (see Configuration, 3.5.3.6 on page 33).
- User 1...10: User-defined external values (see Configuration, 3.5.3.6 on page 33).
- RMS-Noise: Value of RMS (=Root Mean Square; see Test Pulse, 13.1.2.3 on page 222).
- Pipette Pressure: Pressure from an external device (see Configuration, 3.5.3.6 on page 33).
- Digital In: Status of the input bits of the digital channels (see I/O Control, 13.2 on page 222).
- Digital Out: Status of the output bits of the digital channels (see I/O Control, 13.2 on page 222).

12.1.5 Trace

Level "4" of the data tree. The *Parameters* of the Trace level are:

Parameter	Value
<input checked="" type="checkbox"/> TRACE	
<input type="checkbox"/> Header	TRACE_1
<input checked="" type="checkbox"/> Label	Imon-1
<input type="checkbox"/> Count	1
<input checked="" type="checkbox"/> Data Points	3500
<input type="checkbox"/> File Offset	0
<input checked="" type="checkbox"/> Average Count	1
<input type="checkbox"/> Leak ID	0
<input type="checkbox"/> Leak Traces	0
<input type="checkbox"/> Recording Mode	Whole-Cell
<input type="checkbox"/> Amplifier	1
<input type="checkbox"/> Data Kind	9
<input type="checkbox"/> Data Format	4-byte real
<input type="checkbox"/> Data Factor	1
<input type="checkbox"/> Time Offset	0
<input type="checkbox"/> Y-unit	A
<input type="checkbox"/> X-unit	s
<input type="checkbox"/> X-interval	20.0 µs
<input type="checkbox"/> X-start	0
<input type="checkbox"/> Y-Offset	0.00A
<input type="checkbox"/> Y-range	10.2 nA
<input type="checkbox"/> Y-min	-7.48 nA
<input type="checkbox"/> Y-max	7.30 nA
<input type="checkbox"/> Zero Offset	-38.9 fA
<input type="checkbox"/> Bandwidth	2.87 kHz
<input type="checkbox"/> DA Channel	1
<input type="checkbox"/> AD Channel	6
<input type="checkbox"/> Holding	0
<input type="checkbox"/> Pip. Resistance	0.00Ohm
<input type="checkbox"/> Cell Potential	0
<input type="checkbox"/> Seal Resistance	64.2 MOhm
<input checked="" type="checkbox"/> C-slow	0.00F
<input checked="" type="checkbox"/> R-series	5.00328e+06
<input type="checkbox"/> Rs-value	0
<input type="checkbox"/> Leak Comp.	0
<input type="checkbox"/> M-conductance	0.00S
<input checked="" type="checkbox"/> Int. Sol.	show
<input type="checkbox"/> Int. Sol. Value	0
<input checked="" type="checkbox"/> Ext. Sol.	show
<input type="checkbox"/> Ext. Sol. Value	0

Figure 12.6: Trace tab of the Data Parameter window.

- Header: Index number of the Group, the Series, the Sweep and the Trace (e.g. TRACE_1_1_1_2).
- Label: Text identifier; label of the Trace.

- Count: Index number of the Trace.
- Data Points: Number of stored data points.
- File Offset: Offset of the data in the according memory block (minimum number is 256 which is the size of the header).
- Average Count: Number of acquired Traces for the calculation of the stored average Trace.
- Leak Count: Index number of the stored *Leak Trace*.
- Leak Traces: Number of stored *Leak Traces*.
- Recording Mode: Recording mode of the amplifier during recording (e.g. "Whole-Cell").
- Amplifier: Notification field of any amplifier event e.g. for "Clipping" (examples: "NoClip-1" = Amplifier-1 has not clipped, "CLIP-2" = Amplifier-2 has clipped).
- Data Kind: AD channel index number and kind of acquired data (e.g. Adc6-Imon).
- Data Format: Format of the data (either 2-byte integer, 4-byte integer, 4-byte real or 8-byte real).
- Data Factor: Scaling factor for the conversion of interface values into e.g. current or voltage values.
- Time Offset: Time delay of DA or AD channels.
- Y-unit: Unit of the Y-axis.
- X-unit: Unit of the X-axis.
- X-interval: *Sample Interval* (see Stimulus Editor, 7.3 on page 87).
- X-start: Indicates any delay of the X-axis, mostly a time delay when using a *Start-Segment* (see Stimulus Editor, 7.3 on page 88). But it may also indicate other delays when X-axis is not time but e.g. frequency.
- Y-Offset: Y-axis offset in the Oscilloscope window.
- Y-range: Maximal range of the Y-axis (one direction only). This depends on the *Gain* settings.
- Y-min: Minimum Y-value of the Trace (e.g. current or voltage).
- Y-max: Maximum Y-value of the Trace (e.g. current or voltage).
- Zero Offset: Calculated Zero Offset (see Stimulus Editor, 7.6.1 on page 98).
- Bandwidth: Filter frequency of the acquired Trace.
- DA Channel: DA channel number (see Stimulus Editor, 7.5.1 on page 91).
- AD Channel: AD channel number (see Stimulus Editor, 7.5.2 on page 96).
- Holding: Holding potential/current value.
- Pipette Resistance: Value of *Pipette Resistance* when the button R-memb → R-pip in the Amplifier Window was pressed before the Trace was acquired.
- Cell Potential: *Cell Potential* acquired from an external device.
- Seal Resistance: Resistance value of the *R-memb* field of the Amplifier window (see Amplifier Window, 4.2 on page 50).
- C-slow: Last *C-slow* value (see Amplifier window, 4.3 on page 54).
- R-series: Last *R-series* value (see Amplifier window, 4.3 on page 55).

- Rs-value: Absolute fraction of the compensated *R-series* value. The value depends on the % of *R-series* compensation.
- Leak Comp.: Leak conductance value set in the Amplifier window (see 4.3 on page 56).
- M-conductance: Membrane conductance (see Analysis Editor, 9.4.1.6 on page 185).
- Int. Sol.: Name of the used internal solution. Click on the *View* button to get the solution content displayed.
- Int. Sol. Value: Value entered in the selected solution (see Solution Editor, 14 on page 225).
- Ext. Sol.: Name of the used external solution. Click on the *View* button to get the solution content displayed.
- Ext. Sol. Value: Value entered in the selected solution (see Solution Editor, 14 on page 225).

12.1.6 Amplifier

Describes the amplifier settings at the beginning of the Series (Amplifier state record). This is only available for fully software controllable amplifiers like EPC 10 USB.

The screenshot shows the 'Data Parameters' window with the 'Amplifier Items' tab selected. The parameters are organized into two columns. The left column contains parameters with checkboxes, and the right column contains parameters without checkboxes. Each parameter has a text input field with its current value.

Parameter	Value
<input type="checkbox"/> Amplifier State	
<input type="checkbox"/> Amplifier	EPC10_USB 1/2
<input type="checkbox"/> Clamp Mode	V-Clamp
<input type="checkbox"/> V-Pipette	0.00 V
<input checked="" type="checkbox"/> Gain	1.00 mV/pA
<input type="checkbox"/> Ext. Stimulation	0.00
<input type="checkbox"/> Filter 1	Bessel-10kHz
<input checked="" type="checkbox"/> Filter 2	2.87 kHz
<input type="checkbox"/> Filter 2 Type	Bessel
<input type="checkbox"/> Stimulus Filter	10.0 kHz
<input checked="" type="checkbox"/> C-fast Total	0.00F
<input checked="" type="checkbox"/> C-fast Amp1	0.00F
<input checked="" type="checkbox"/> C-fast Amp2	0.00F
<input checked="" type="checkbox"/> C-fast Tau	500 ns
<input type="checkbox"/> C-fast Range	Normal
<input checked="" type="checkbox"/> C-slow	1.00 pF
<input type="checkbox"/> C-slow Range	CSlowOff
<input type="checkbox"/> R-series	5.00 MΩ
<input type="checkbox"/> Rs-comp.	0.00%
<input type="checkbox"/> Rs-comp. Tau	0.00s
<input type="checkbox"/> Leak Comp.	0.00S
<input type="checkbox"/> Liquid Junction	0.00V
<input type="checkbox"/> V-offset	0.00V
<input type="checkbox"/> CC-Gain	1 pA/mV
<input type="checkbox"/> Low Freq VC	LFVC_Off
<input type="checkbox"/> LFVC V-memb	0.00
<input type="checkbox"/> CC-fast	CC-fast
<input type="checkbox"/> Auto C-fast	undefined
<input type="checkbox"/> Auto C-slow	undefined
<input type="checkbox"/> V-mon Scale	10x
<input type="checkbox"/> Stim Scale	10x
<input type="checkbox"/> Electrode Mode	2
<input type="checkbox"/> Calibration Date	09-Nov-2022
<input type="checkbox"/> Serial Number	00550582-T
<input type="checkbox"/> LockIn Phase	289°
<input type="checkbox"/> LockIn Attenuation	956 m
<input type="checkbox"/> LockIn Mode	0
<input type="checkbox"/> LockIn Calibration	0

Figure 12.7: Amplifier tab of the Data Parameter window.

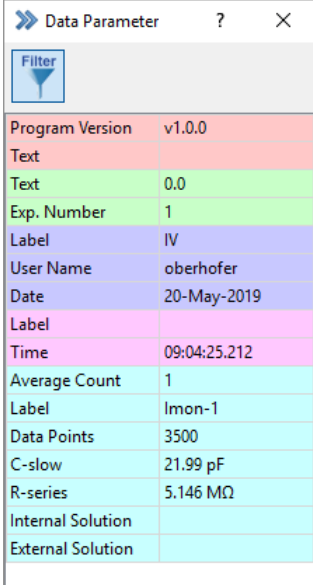
The parameters of the *Amplifier* level are:

- Amplifier: Type of amplifier used for data acquisition (e.g. "EPC10 USB/2-1").
- Clamp Mode: Recording mode of the amplifier during recording (e.g. "Whole-Cell").
- V-pipette: Measured pipette voltage (see Amplifier window, 4.2 on page 50).

- Gain: Current Gain setting (see Amplifier window, 4.3 on page 52).
- Ext. Stimulation: Scaling factor when the *External Stimulation Input* was used (see Amplifier window, 4.1.1.2 on page 46).
- Filter 1: Bandwidth and type of Filter 1 (see Amplifier window, 4.3 on page 56).
- Filter 2: Bandwidth of Filter 2 (see Amplifier window, 4.3 on page 56).
- Filter 2 Type: Filter type of the Filter 2 (see Amplifier window, 4.3 on page 56).
- Stimulus Filter: Filter frequency of the stimulus (see Amplifier window, 4.1.1.2 on page 46).
- C-fast Total: The total value of the C-Fast amplitude (see C-fast, 4.3 on page 53).
- C-fast Amp1: The unfiltered C-Fast amplitude (see C-fast, 4.3 on page 53).
- C-fast Amp2: The filtered C-Fast amplitude (see C-fast, 4.3 on page 53).
- C-fast Tau: τ value of the C-fast compensation (see C-fast, 4.3 on page 53).
- C-fast Range: Range of the C-fast compensation either "normal" or "Extended" (see Amplifier window, 4.1.1.2 on page 45).
- C-slow: C-slow value (see Amplifier window, 4.3 on page 54).
- C-slow Range: Range of the C-slow compensation (see Amplifier window, 4.3 on page 54).
- R-series: R-series value (see Amplifier window, 4.3 on page 55).
- Rs-comp.: % of R-series compensation (see Amplifier window, 4.3 on page 55).
- Rs-comp. Tau: Speed of feedback compensation (see Amplifier window, 4.3 on page 55).
- Leak Comp.: Applied leak conductance value (see Amplifier window, 4.3 on page 56).
- Liquid Junction: *Liquid Junction Potential* value (see Amplifier window, 4.3 on page 53).
- V-offset: *V-offset* value (see Amplifier window, 4.3 on page 53).
- CC-gain: *CC-gain* setting (see Amplifier window, 4.1.1.2 on page 45).
- Low Freq VC: LFVC mode indicator (see Amplifier window, 4.1.1.2 on page 47).
- LFVC V-memb: Membrane potential used for LFVC (see Amplifier window, 4.1.1.2 on page 47).
- CC-fast: Selected Current-Clamp speed circuitry (either *CC-fast* or *CC-slow*).
- Auto C-fast: Status indicator of last *Auto C-fast* compensation ("success" or "failed").
- Auto C-slow: Status indicator of last *Auto C-slow* compensation ("success" or "failed").
- V-mon Scale: *V-mon* scaling factor (see Amplifier window, 4.1.1.2 on page 46).
- Stim Scale: *Stimulus Scale* factor (see Amplifier window, 4.1.1.2 on page 46).
- Electrode Mode: Indicates if the *2- or 3-Electrode Mode* was used (see Amplifier window, 4.1.1.2 on page 45).
- Calibration Date: Date of the last calibration.
- Serial Number: Serial number of the amplifier and revision state.
- LockIn Phase: Phase of the LockIn (see Lock-In, 19.1 on page 253).
- LockIn Attenuation: Attenuation value of the LockIn (see Lock-In, 19.1 on page 253).
- LockIn Mode: "Sine+DC", "Piecewise Linear" or "On Cell" (see Lock-In, 19.1 on page 253).
- LockIn Calibration: Measured, computed or manual *LockIn Calibration* (see Lock-In, 19.1 on page 253).

12.1.7 Marked Items

If one enables the “Filter” function on top of the Data Parameter window a reduced view of all marked *Data Parameters* (Root, Group, Series, Sweep, Trace or Amplifier) is provided. This is especially helpful if one is interested in certain *Data Parameters* and wants them to monitor e.g. during an experiment.



Data Parameter	
Program Version	v1.0.0
Text	
Text	0.0
Exp. Number	1
Label	IV
User Name	oberhofer
Date	20-May-2019
Label	
Time	09:04:25.212
Average Count	1
Label	Imon-1
Data Points	3500
C-slow	21.99 pF
R-series	5.146 MΩ
Internal Solution	
External Solution	

Figure 12.8: Selection of marked data parameters of the Data Parameter window.

13 I/O Control

The I/O Control window is providing a lot of technical parameters. Hence, the main purpose of this window is to control and edit specified values and parameters during data acquisition or for testing purposes.

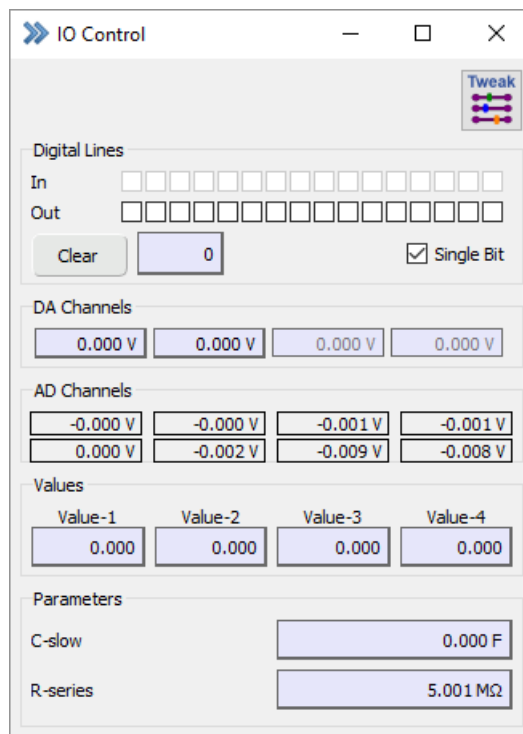


Figure 13.1: IO Control window of PATCHMASTER NEXT.

The I/O Control window is structured into several sections:

- Tweak
- Digital Lines
- DA Channels
- AD Channels
- Values
- Parameters

All options, functions and parameters are explained in the following chapters.

13.1 Tweak

The *Tweak* section of the I/O Control window opens an extension bay providing two more tabs which allow to configure the I/O Control window according to the users convenience.

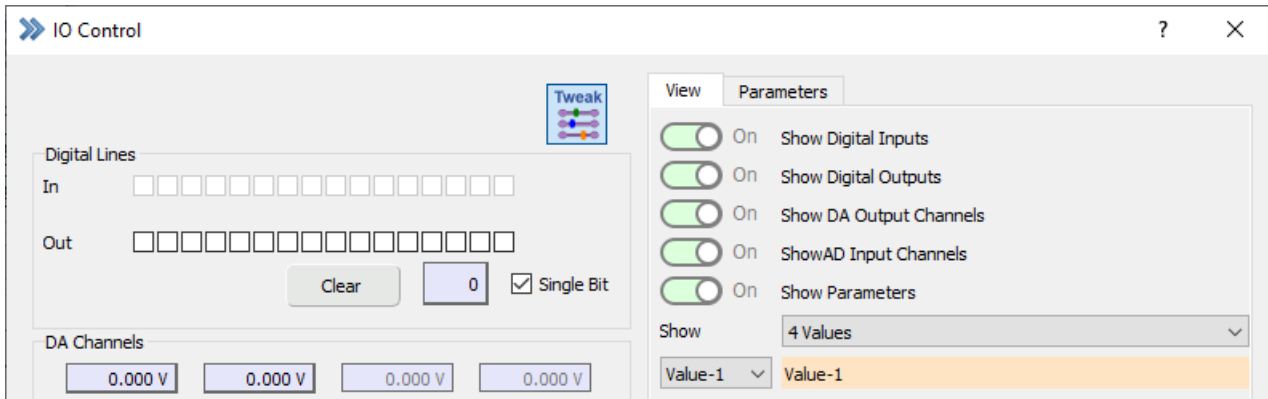


Figure 13.2: Tweak section of the I/O Control window.

13.1.1 View

The *View* options allow to configure the displayed parameters in the I/O Control window.

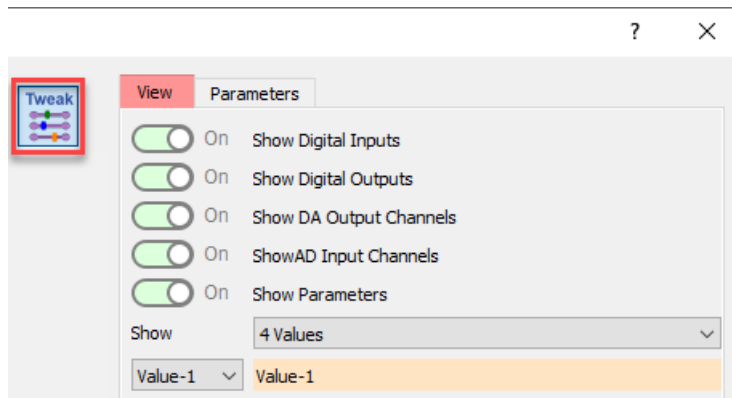


Figure 13.3: View area of the Tweak section.

Show Digital Inputs: Show or hide the digital input lines (see chapter 13.2 on page 222).

Show Digital Outputs: Show or hide the digital output lines (see chapter 13.2 on page 222).

Show DA Output Channels: Show or hide the DA output channels (see chapter 13.3 on page 223).

Show AD Input Channels: Show or hide the AD input channels (see chapter 13.4 on page 223).

Show Parameters: Show or hide the Parameters (see chapter 13.1.2 on the next page).

Show Values: Show or hide *Values* in the main section of the I/O Control window (see chapter 13.5 on page 223). Use the selection list to define how many *Values* should be displayed. Further, it is possible to change the name for each *Value* by entering a new name in the text entry field.

13.1.2 Parameters

Various *Parameters* can be shown (if activated) in the main section of the I/O Control window. Some of them are editable, some are just for display purposes.

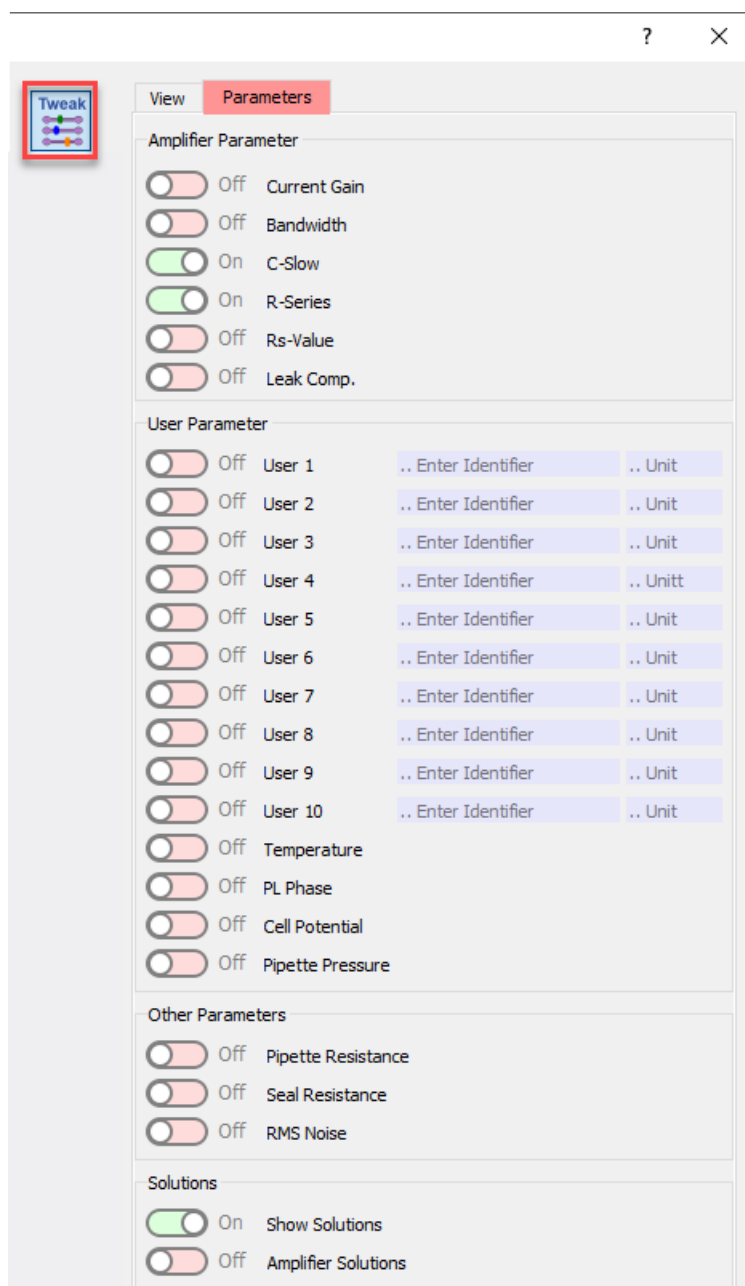


Figure 13.4: Parameters area of the Tweak section.

13.1.2.1 Amplifier Parameter

Current Gain: Gain of the Current Monitor input.

Bandwidth: Record the bandwidth of an external (telegraphing) amplifier.

C-Slow: Membrane capacitance.

R-Series: Series resistance.

Rs-Value: Series resistance compensation.

Leak Comp.: Leak conductance for leak compensation.

13.1.2.2 User Parameter

User 1...10: There are ten parameter fields reserved for user-specific assignments. These fields act like other parameter fields with the addition that parameter name and unit can be specified (e.g., "pH" and "U"). Name and unit are also stored in the data file on the level of each Sweep, so they can be changed during an experiment, if required.

Temperature: Read an external voltage signal (from an external device) and convert it into a temperature value.

PL-Phase: Record the *Phase Angle* of an external LockIn amplifier.

Cell Potential: Cell potential (from an external device).

Pipette Pressure: Pressure (from an external device).

13.1.2.3 Other Parameters

Pipette Resistance: Value of R-membrane after pressing W (this writes the value of R-membrane into Pipette Resistance).

Seal Resistance: Value of R-membrane.

RMS Noise: Value of the RMS (=Root Mean Square) noise.

13.1.2.4 Solutions

Show Solutions This options enables two selection lists:

- Bath Solution
- Pipette Solution

Here, it is possible either to monitor the presently active solution or to manually change the solution index (see Solution Editor, 14 on page 225). The information is stored together with the data file and can be reviewed in the Parameters window (see 12 on page 209).

Note: When changing the solution in the I/O Control window there will be no active setting of external solutions. If you want to do that please use the Solution Changer event in the Protocol Editor (chapter 8.3.5.2 on page 157) and the Perfusion Control dialog (chapter 15 on page 229).

Amplifier Solutions: In case an EPC 10 USB Double, Triple or Quadro amplifier is used you can assign used Bath and Pipette solutions for each amplifier board.

13.2 Digital Lines

Control field of the digital output and input channels. The digital input bits are only read-only fields whereas the digital output bits can be set by selecting or deselecting the checkboxes.

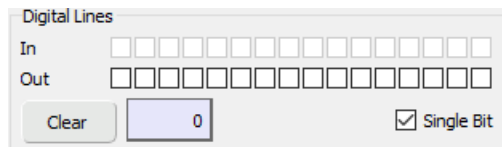


Figure 13.5: Digital Lines section of the I/O Control window.

Clear: Clear all settings for the digital output, e.g., deselect all bits. Clear actions are written to the Notebook.

Single Bit: If selected, only one bit can be set for *Dig-Out*. The output digital value is set to the highest bit.

13.3 DA Channels

This section allows you to set the output of the free DA-channels. The currently sampled voltages at the used DA-channels are read-only fields.

Note: When using these DA-channels to control external devices from the I/O Control window, make sure that the same DA-channels are not used by other parts of PATCHMASTER NEXT, i.e. as a stimulation channel in the Stimulus Editor.

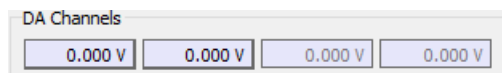


Figure 13.6: DA Channels section of the I/O Control window.

13.4 AD Channels

This section allows you to monitor the AD-Channels of the amplifier.

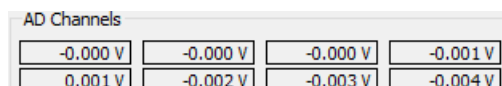


Figure 13.7: AD Channels section of the I/O Control window.

13.5 Values

Values (1-32) are global variables which can be used for calculations in the Protocol Editor and in the Analysis Editor. In addition, they are used to exchange information between these two program modules. In the I/O Control window the *Values* are displayed and they can be manually changed. The number of displayed *Values* (4, 8, 12, 16, 20, 24, 28 or 32) and the name of the value can be specified in the *View* section of the I/O Control window (see 13.1.1 on page 220).

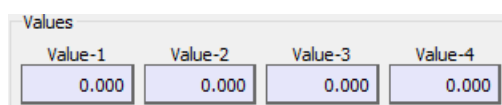


Figure 13.8: Values section of the I/O Control window.

13.6 Parameters

In the `Parameters` section of the `I/O Control` window the *Parameters* activated in the `Parameters` section of the `Tweak` menu are displayed.

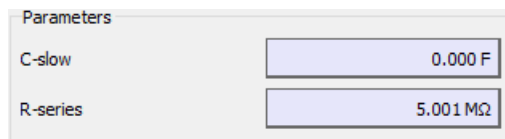


Figure 13.9: Parameters section of the I/O Control window.

14 Solution Editor

In most electrophysiological experiments bathing solutions are changed during the experiment. Thus, it is of great importance to keep track, which solutions were on both sides of the membrane.

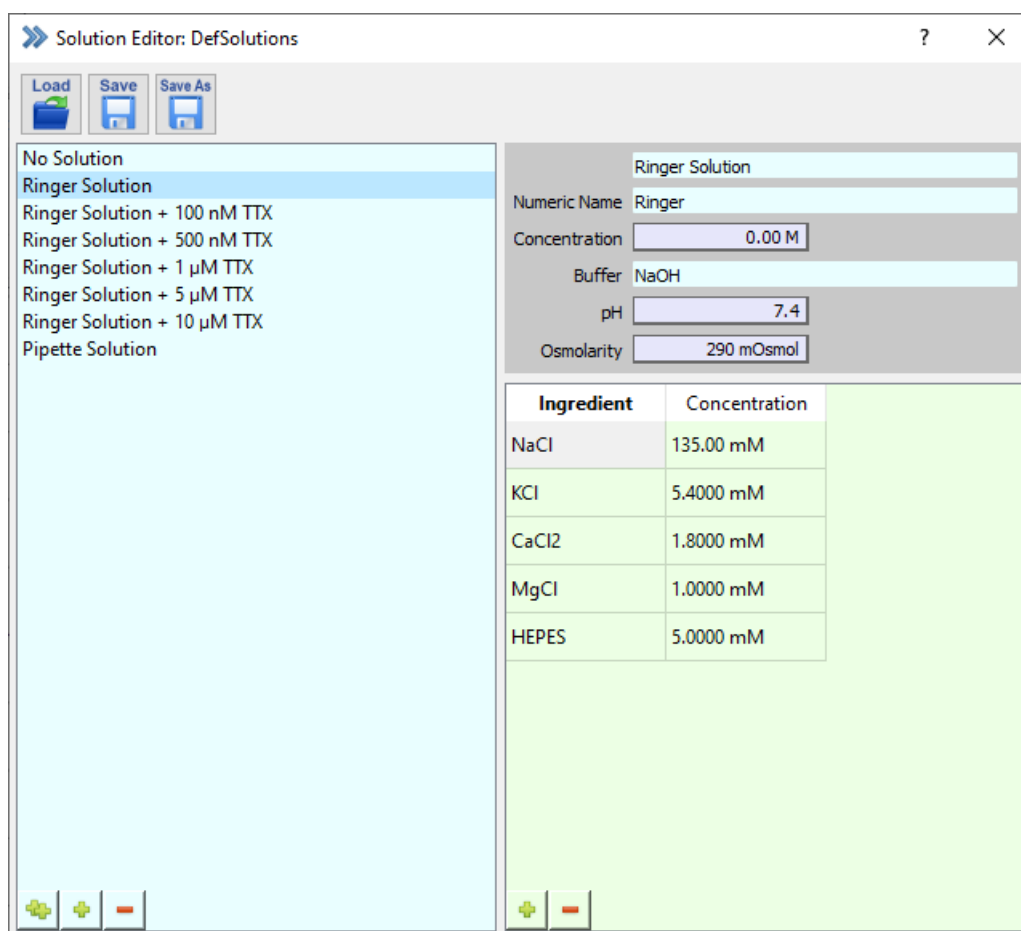


Figure 14.1: Solution Editor window of PATCHMASTER NEXT.

Further, the Solution Editor is a great tool to manage and organize the used solution of your electrophysiological experiment.

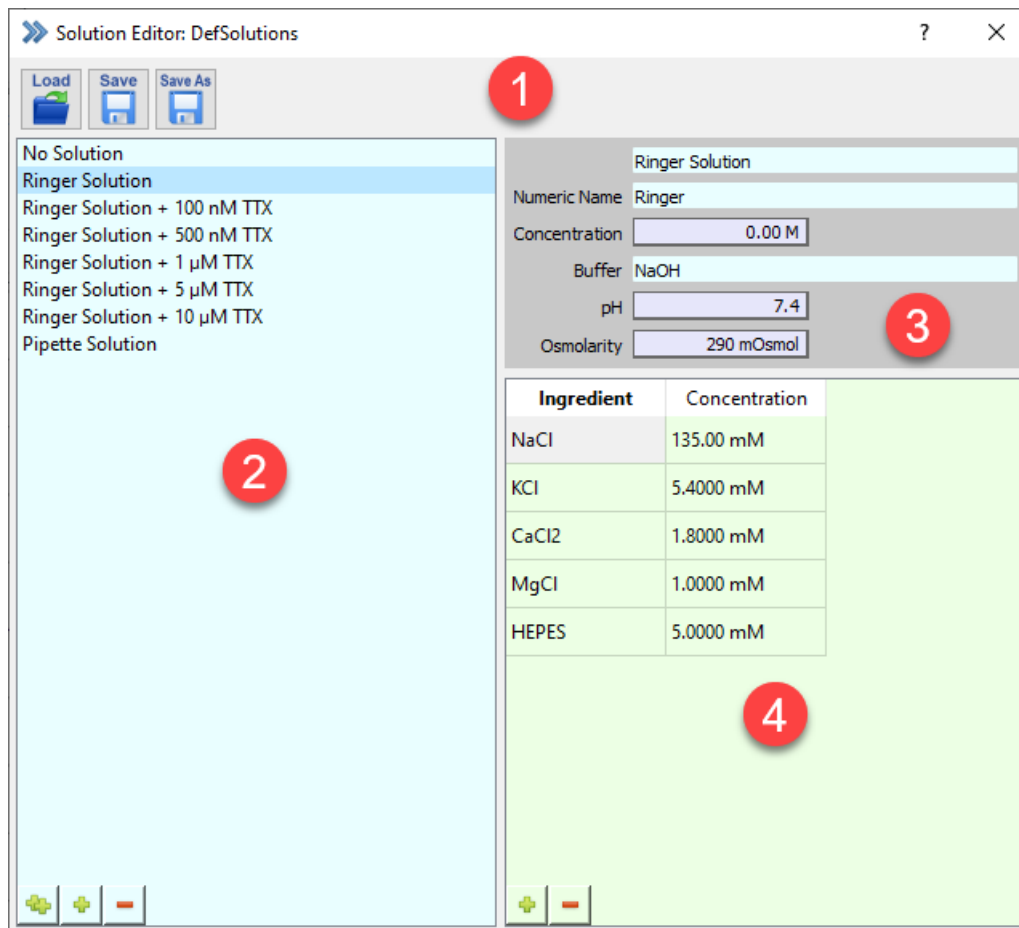


Figure 14.2: The Solution Editor window consists of four different sections.

The Solution Editor can be divided into four different sections:

1. Global Section
2. Solution Pool
3. Solution Information
4. Solution Ingredients

14.1 Global Section

The icon buttons in the Global Section of the Solution Editor are used to organize the solution files.

Load: Opens the finder and enables to load another solution file (*.sol).

Save: Saves the currently loaded solution file.

Save As: Saves the currently loaded solution file under a different name.

14.2 Solution Pool

Here, all solutions of the currently loaded solution file are listed. Like in other Editors (Stimulus Editor, Protocol Editor, Analysis Editor) the list of solutions can be modified by drag and drop the solutions to different positions.

Further, there are icon buttons to duplicate, add or delete solutions. One can also use the right-click context menu which offer the following options:

- **Edit Name:** Edit the name of the currently selected solution.
- **Duplicate Solution:** Creates a copy of the selected solution.
- **Add new Solution:** Creates a new empty solution.
- **Delete Solution:** Deletes the selected solution from the list.

14.3 Solution Information

Solution Name: Enter a name for the selected solution.

Numeric Name: An editable field that may hold a feature of the solution that is not easily determined from its ingredients (e.g., free calcium concentration, etc.).

Concentration: An editable field containing a number of value (like Osmolarity or pH) which is in relation to the name entered in *Numeric Name*.

Buffer: Enter the name of the solution used to set the pH value.

pH: Holds the value of the pH of the solution.

Osmolarity: Holds the value of osmolarity of the solution.

14.4 Solution Ingredients

To enter new ingredients for a solution you either click on the green “plus” sign button (use the red “minus” sign button to remove one ingredient) or use the right-click context menu which would offer you the same selection (Add new Chemical - Delete Chemical).

Ingredient: Enter the name of the ingredient.

Concentration: Enter the concentration of the ingredient.

14.5 Using Solutions in Your Experiment

PATCHMASTER NEXT provides several ways how to handle solution changes during the experiment.

In case you are using a multiple amplifier and you selected *Amplifier Solutions* in the I/O Control window (13.1.2.4 on page 222) you have to specify the used solutions for every amplifier.

The solution names are stored together with the recorded data and can be reviewed in the *Data Parameter* window (12.1.5 on page 213).

14.5.1 I/O Control

An easy way is to set the entries *Pipette Solution* and *Bath Solution* in the I/O Control window (13.1.2.4 on page 222) manually. To activate this option please go to the I/O Control window and active the *Show Solution* parameter in the *Tweak* tab.

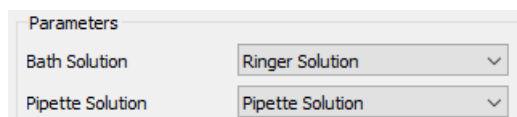


Figure 14.3: In the I/O Control Window the used solutions can be defined.

14.5.2 Protocol Editor

One can also set the entries *Pipette Solution* and *Bath Solution* via the Protocol Editor automatically. Therefore, one needs to set the *Set Solutions* event (8.3.5.2 on page 157).

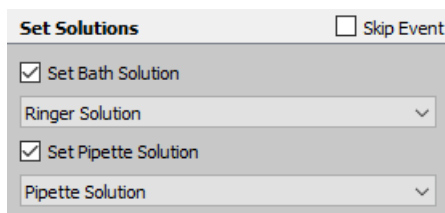


Figure 14.4: The Set Solutions event of the Protocol Editor.

14.5.3 Perfusion Control

If a solution device known to PATCHMASTER NEXT is used one can activate the *Perfusion Device* extension in the Configuration (see chapter 15 on the facing page).

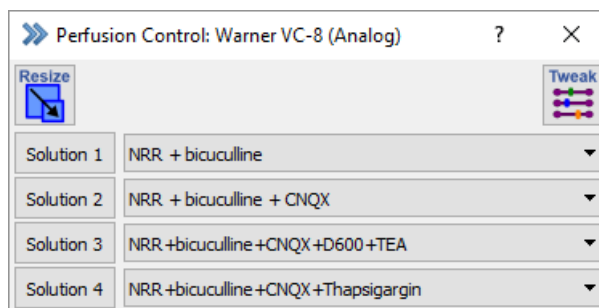


Figure 14.5: Perfusion Control window of PATCHMASTER NEXT.

Once it is activated you can use the *Solution Changer* event of the Protocol Editor to automatically trigger solution changes during an ongoing experiment. The solution changes are consistently tracked in the Data Parameters (see chapter 12.1.5 on page 213).

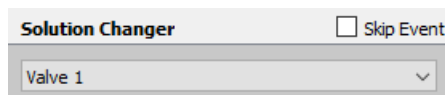


Figure 14.6: Solution Changer event of the Protocol Editor.

15 Perfusion Control

The Perfusion Control dialog has two main purposes:

1. Manage analog or digital outputs of your perfusion system
2. Align external or internal solutions to the perfusion system

Thus, using the Perfusion Control helps to keep track with the solutions used during an perfusion experiment. The solution information is stored in the data file of PATCHMASTER NEXT. To look up this information use the Data Parameter dialog (see chapter 12.1.5 on page 213).

The Perfusion Control is handled as an extension of PATCHMASTER NEXT and therefore, needs to be activated in the Configuration of PATCHMASTER NEXT (see chapter 3.5.2.4 on page 26).

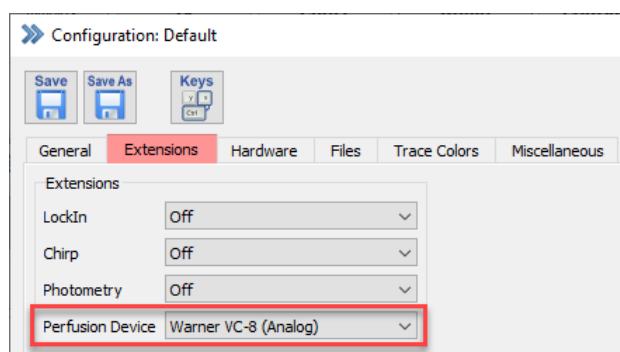


Figure 15.1: Activation of the Perfusion Control extension in the Configuration of PATCHMASTER NEXT.

Currently, there are two supported solution changer devices supported offering four options:

- ALA VC-3 4 Channel (analog)
- ALA VC-3 8 Channel (analog)
- Warner VC-8 (analog)
- Warner VC-8 (digital)

The correct hardware settings are automatically applied in the software once a selection is made.

Next, go to the Windows Menu of the Control Window and open the Perfusion Control dialog.

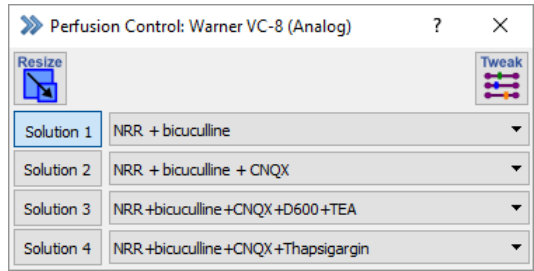


Figure 15.2: Perfusion Control dialog of PATCHMASTER NEXT.

For better orientation we separated the Perfusion Control window into two sections:

1. Global Section
2. Solution Definitions

15.1 Global Section

The global section does contain two icon buttons which define the layout of the dialog.

15.1.1 Resize

The *Resize* button changes the arrangement of the solution buttons (“Solution 1”, “Solution 2” etc.). It does also hide the solution selection menu (chapter 14 on page 225) leaving the solution buttons in the dialog.

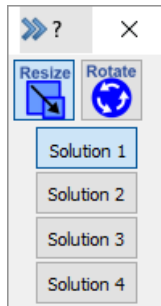


Figure 15.3: Resizing the Perfusion Control window.

Further, another button appears beneath the *Resize* button, called “Rotate”. By pressing this button the arrangement of the solution buttons are changed from vertical to horizontal alignment or vice versa.

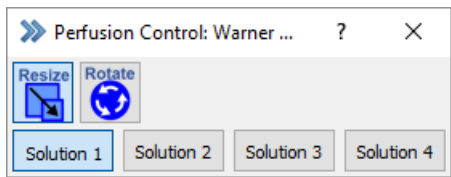


Figure 15.4: Rotate the solution arrangement.

15.1.2 Tweak

The **Tweak** menu extension offers settings to either configure the selected perfusion device or to arrange the layout of the **Perfusion Control** dialog.

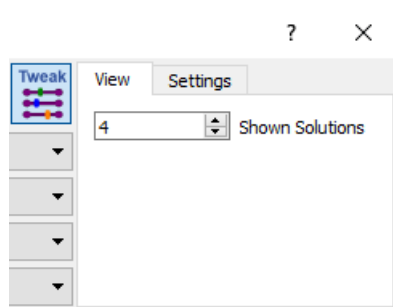


Figure 15.5: Tweak option of the Perfusion Control window.

15.1.2.1 View

Define the number of shown solutions in the *Solution Definitions* section of the **Perfusion Control** window. The number of solutions depend on the selected perfusion device.

15.1.2.2 Settings

Max Opened Valves: Defines the number of valves which can be opened simultaneously. If the maximum number allowed by the device is "1" this setting cannot be modified.

Analog Out Channel: Defines the Analog Output Channel of the EPC 10 USB amplifier for the perfusion device control. If the set device is operating with digital output channels this option will be deactivated.

15.2 Solution Definitions

Solution 1	NRR + bicuculline
Solution 2	NRR + bicuculline + CNQX
Solution 3	NRR +bicuculline +CNQX +D600+TEA
Solution 4	NRR +bicuculline +CNQX +Thapsigargin

Figure 15.6: Solution Definitions section of the Perfusion Control window.

There are two columns with buttons:

- **Solution:** The *Solution* buttons indicate the currently set solution, either set by pressing the button or set set elsewhere. These buttons can also be used to manually switch on or off certain solutions.
- **Solution Name:** For each solution there is a selection list listing all solutions of the currently loaded solution file. Thus, this allows to link the used solution channels with a solution managed and stored in the **Solution Editor** and the **Data Parameters**.

16 Notebook

The Notebook window is used to display messages and warnings of the program, such as error messages, analysis results, and information about displayed data. In many of the PATCHMASTER NEXT windows there are checkboxes or options that can be selected to target settings and results to be written into the Notebook window. The content of the Notebook can be stored on disk. The Notebook window does contain the normal "Notebook" view and a special "Log" view (see below). By default, the "Notebook" view is active.

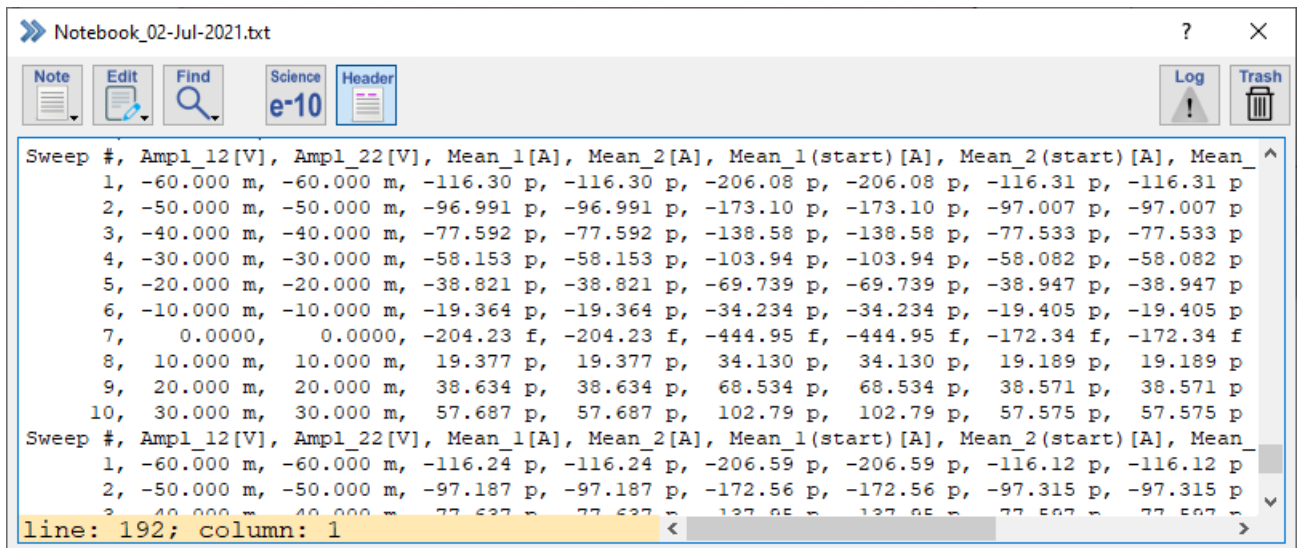


Figure 16.1: The Notebook window is used to plot analysis results or to indicate error messages.

When the Notebook window is activated (by clicking with the mouse pointer into it), the text editing functions are activated and applicable. Thus, the Notebook window is an editor window of the memory-resident text file *.txt. Therefore, one can modify text in the Notebook window just as in any other text file. This option can be used to add further information to the text file, or to get rid of messages that should not be stored to the disk file.

Note: The Note icon button contains the following menu:

- Save: Saves the Notebook window content under its default name: "Notebook_Date".
- Save As...: Asks for a file name before saving.
- Merge...: Merges a text file to the content of the Notebook.
- Print...: Output the content of the Notebook to a printer.
- Clear when Saved: Automatically clears the Notebook after the present content is saved to disk.
- Auto Store: This option will automatically store the Notebook together with the data file ([data file name]*.txt). Upon opening a data file, its Notebook file will automatically be loaded as well.

-
- **Set Length...:** Specifies the maximal number of text lines in the Notebook. The maximal number of lines is given in parentheses.
 - **Line Numbers:** Shows line and column numbers when moving with the cursors inside the Notebook.
 - **Font Size...:** Allows to select the font size of the Notebook text.

Edit: The `Edit` icon button contains the following menu:

- **Undo:** Undo the last modification made in the Notebook window.
- **Redo:** If one used at least once the “Undo” option one can also use the “Redo” option to undo the last “Undo” step.
- **Copy:** Copies the selected text passage.
- **Cut:** Cuts out the selected text passage.
- **Paste:** Pastes clipboard content to the Notebook window.
- **Clear:** Clears the Notebook.
- **Select All:** Selects the whole content of the Notebook window.

Find: The `Find` icon button contains the following menu:

- **Find...:** Enter a search string which should be looked up in the Notebook window.
- **Find Same:** Uses the previously entered search string for the search.
- **Find Selection...:** Searches for the currently selected string in the Notebook window.
- **Replace:** Enter a search string to be found and a replacement string.
- **Replace Same:** Uses the search and replacement string previously entered.

Science: If set, the results of the analysis are written to the Notebook in scientific notation (e.g., 1.23e-12). The default is engineering format (e.g., 1.23p). The scientific notation is mostly used when the user wants to copy results from the Notebook to a spread sheet program by copying to the clipboard.

*Note: This setting also applies to the ASCII-export format entry in the **Data Tree** (see 11.1.2 on page 197).*

Include Headers: If this button is selected, the column header of the Analysis results written into the Notebook is displayed. If you deactivate the checkbox the headers will not be shown.

Log: Switches between the “Notebook” and the “Log” view of PATCHMASTER NEXT. Once an error occurs the “Notebook” view does automatically switch to the “Log” view to provide further information. The “Log” content is not stored.

Trash: Deletes the “Notebook” view content but not the “Log” view content.

17 Equation Editor

Equations are a very flexible way to perform complex calculations within PATCHMASTER NEXT. Equations can be used at various places, such as Analysis Editor, Protocol Editor or for *DA-Scaling*.

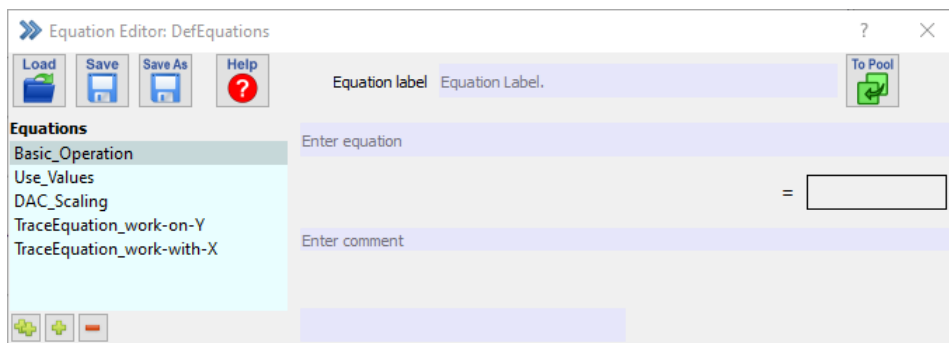


Figure 17.1: Equation Editor window of PATCHMASTER NEXT.

The Equations Editor can be separated into three sections:

- Global Section
- Equations Pool
- Equation Edit

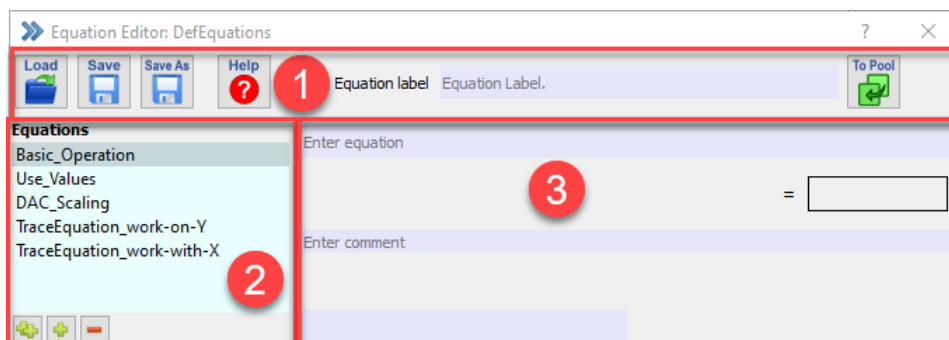


Figure 17.2: Sections of the Equation Editor window.

17.1 Global Section

Load: Brings up the file selector to load an equation file (e.g. `DefEquations.txt`).

Save: Saves all equations in the active equation file.

Save As: Brings up the file selector to save all equation in an equation file.

Help: Pressing the *Help* button opens a new dialog showing the available syntax for editing equations:

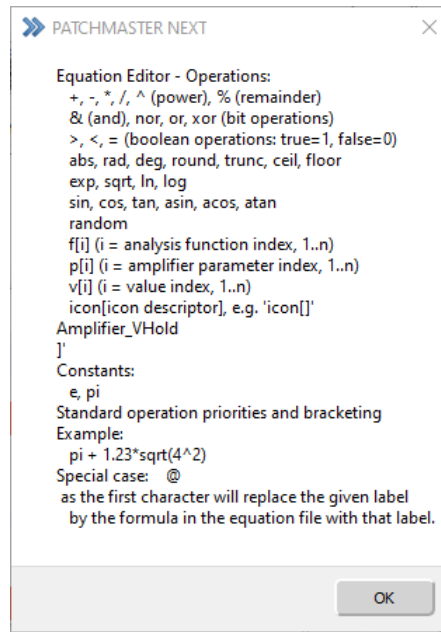


Figure 17.3: Equation syntax of the Equation Editor.

To Pool: Copies the currently active equation to the `Equation Pool`. Please make sure that the *Equation label* is not already existing in the `Equation Pool`.

17.2 Equations Pool

On the left side of the Equation Editor the `Equations Pool` is shown. It is a list of *Equations* stored in the currently loaded `Equations Editor` file. By selecting an equation its content is shown in the `Equation Edit` on the right side of the Equation Editor.

There are four options available to modify the `Equations Pool` content:

- Edit Label: Double-click inside the *Equation* name to change its name.
- Duplicate Equation: Use the small button at the bottom with the two green plus signs to copy the selected *Equation*. The name is taken over but an index number is added.
- Add new Equation: Use the small button at the bottom with one green plus sign to create a new and empty *Equation*. The new protocol sequence will be label "New Equation".
- Delete Equation: Use the small button at the bottom with the red minus sign to delete the selected *Equation* from the `Equations Pool`.

These options are also available via a right-click context menu.

Moving Equations: Via drag-and-drop it is easily possible to change the position of the selected *Equation* in the *Equations Pool*.

17.3 Equation Edit

Equation Label: You can enter an equation name here. Alternatively, if an equation was selected in the *Equations Pool* the equation name will be displayed in this field.

Equation: Enter the equation string into this field.

Results: The result of the equation will is shown here.

Comment: Write a comment or description for the active equation.

*New equations and their content are only stored and transferred into the *Equations Pool* when the *To Pool* function is used. If one wants to modify an existing equation one needs to overwrite it via the *Too Pool* function.*

18 Event Detection

The Event Detection represents a small and modified part of the FITMASTER NEXT functionality. Here, the Event Detection dialog offers the option to analyze spontaneous action potentials online during a running acquisition.

Events in the terminology of PATCHMASTER NEXT refers to a data structure in the analysis file. These are values appended to the results. I.e. for each Sweep in a data file, we can have many *Parameters* and *Results*. These structures are then extended by an arbitrary number of *Events*. *Events* in a physical sense are certain structures in a stream of data (e.g. current versus time) that have a certain property. A typical application is a single-channel event, i.e. an jump in a single-channel current signal from one level to another. Such a physical *event* can, for example, be described by two numbers, the time when the transition happens and a current level. In PATCHMASTER NEXT we would then define two data structures holding this information. For other applications, e.g. detection and characterization of an action potential, two parameters may not be sufficient. In such cases more data are stored simultaneously (in form of *Events*) to describe the physical *event* (e.g. by specifying time, duration, amplitude, slope, after-potential, etc.). Thus, the PATCHMASTER NEXT data structure provides full flexibility for defining *events* of arbitrary complexity. Therefore, the first thing to be done in *Event Detection* is to select the type of detection and then to specify by which parameters the detected event is to be characterized.

The main conceptual features and the workflow of the *Event Detection* are explained in the corresponding user manual (FITMASTER NEXT).

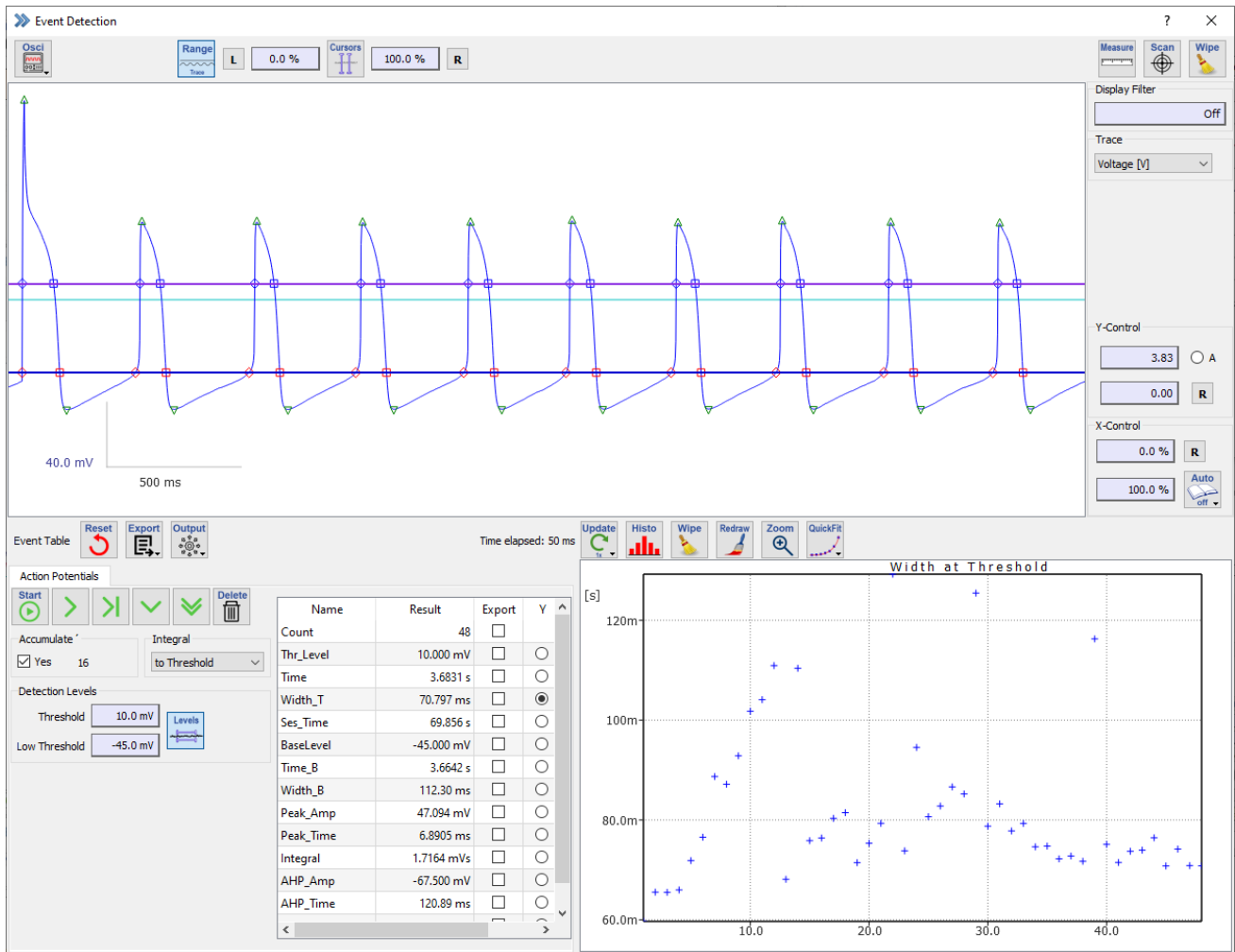


Figure 18.1: Event Detection window of Patchmaster Next.

The Event Detection dialog is only available if a valid FITMASTER NEXT license is detected by PATCHMASTER NEXT. Further event detection methods and more versatile analysis tools are available in the standalone FITMASTER NEXT software.

If one opens the Event Detection window, one can identify four different sections:

- Global Section
- Trace Viewport
- Event Detection Settings
- Event Graph

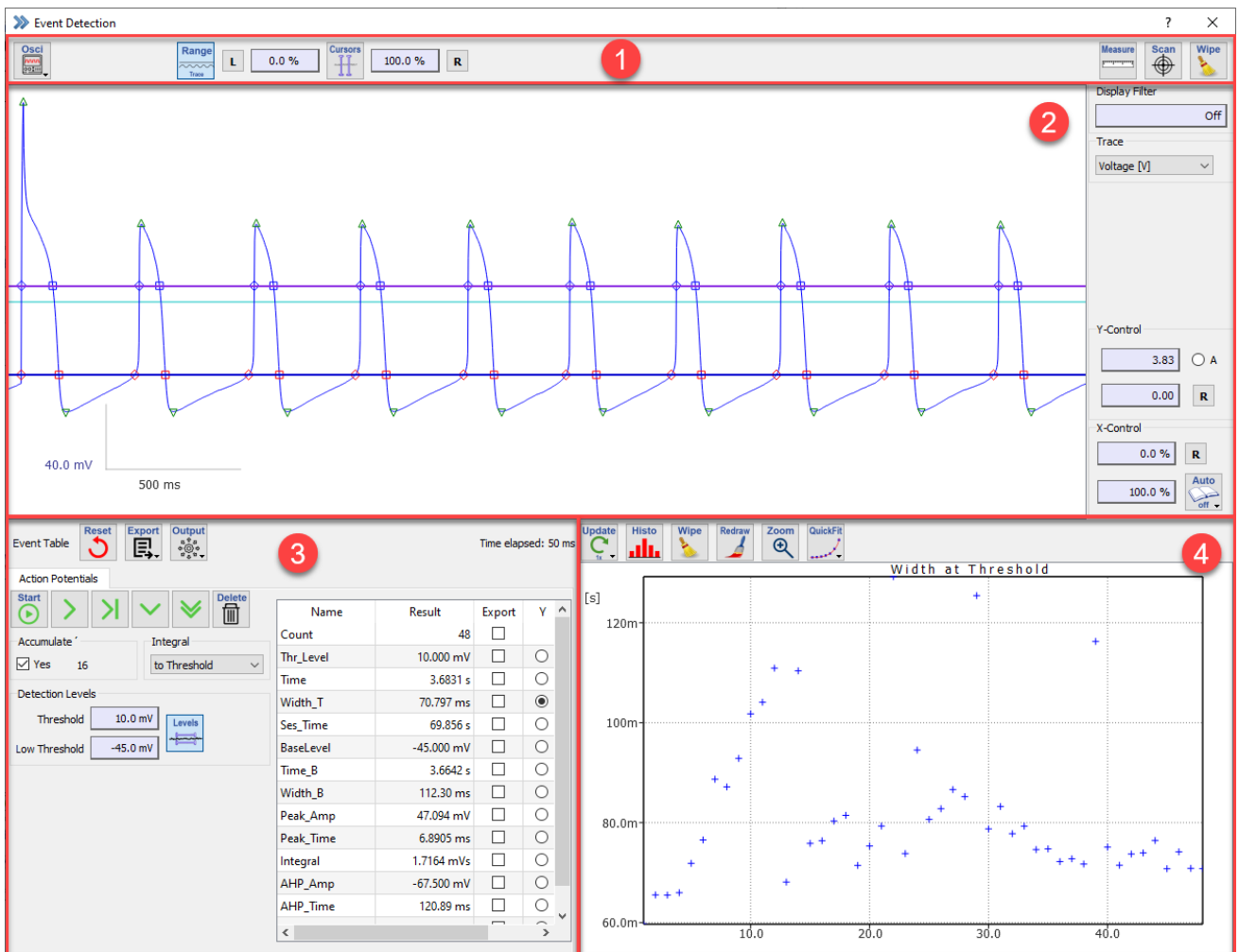


Figure 18.2: The sections of the Event Detection window.

In the following chapters we explain all functions of each section.

18.1 Global Section

Oscilloscope: The Oscilloscope menu contains settings for the Trace display in the Trace Viewport.

The available options are:

- Marker: Shows the Trace data points as markers.
- Subtract Leak Trace: Subtracts the P/n leak responses from the measurement signals.
- Subtract Zero Offset: Subtracts the zero offset from the measured signals (see chapter 7.6.1 on page 98).
- Subtract Ref. Series: If this option is checked, then the *Reference Series* is subtracted from all replayed data (see chapter 6.1.2 on page 75).
- Subtract Trace Buffer: If this item is checked, then the Trace Buffer is subtracted from all replayed Traces (see chapter 6.1.2 on page 75).
- Freeze Zero Line: This button freezes the present content of the data displayed in the Trace Viewport window. Either press the *Freeze* button again to unfreeze the display or acquire new data.

- Auto Y-Range Series: Calculates the optimal display range of the data for the whole Series. If this option is active, the Y-Control options are not available.

Range: By default the *Cursor Settings* work on the segment range. By clicking this toggle button the cursor range operation is changed to the full Trace.

Cursor Settings: This button turns a pair of cursor lines either on or off. They can be set in % of the selected range, i.e., either “Segment” or “Trace”. For “Segment”, a relevant Y segment offset can be provided. “L” and “R” reset the left and right cursor position to 0 and 100%, respectively.

Measure: This function allows to measure amplitudes and duration of the displayed data. If the function is enabled an extension bay of the Event Detection window gets visible at the bottom:

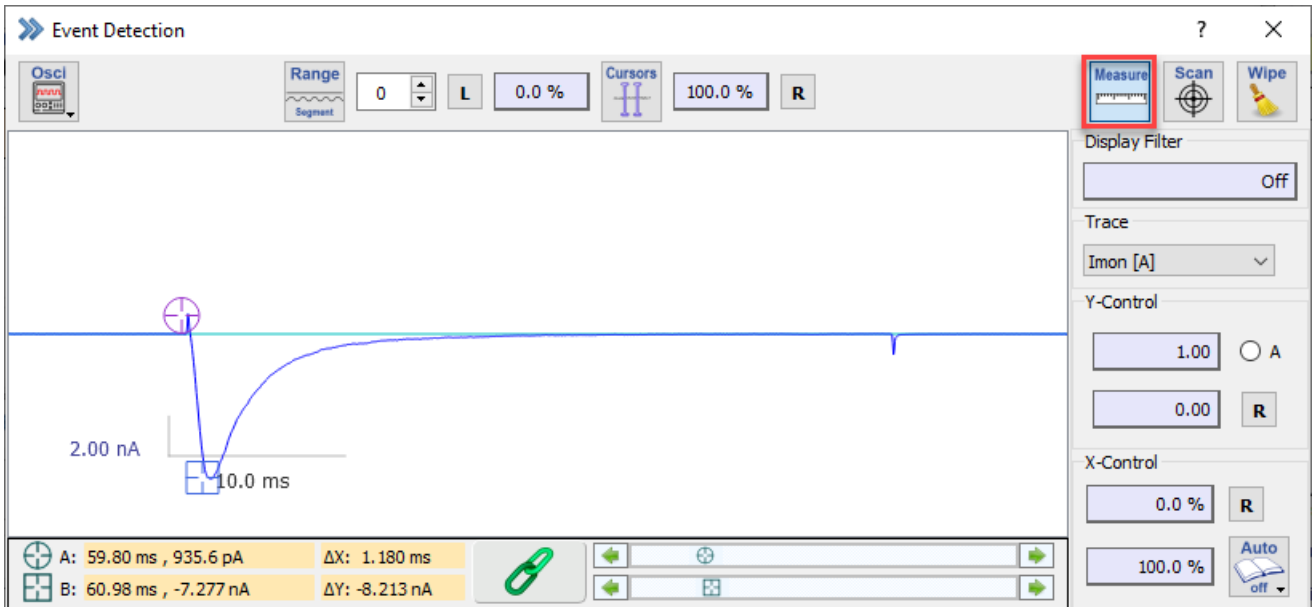


Figure 18.3: The Measure functionality of the Event Detection window.

Two cursors (Cursor A & Cursor B) can be used to manually measure relative or absolute amplitudes. Relative changes indicated by ΔX or ΔY are calculated between both cursor positions. The *Chain Link* button allows to fix the distance of both cursors to each other. The corresponding values of the cursor positions are displayed in the text fields with the orange background. Once you right-click on it a context menu opens offering two options to extract the values:

- Copy to Clipboard
- Write to Notebook

Note: Please be aware that each of these orange text fields offers this functionality to extract the values.

Instead of moving the cursors with the mouse you can also use the corresponding sliders beside the *Chain Link* button.

Scan: The *Scan* function does also provide two cursors (Cursor A & B) but in opposition to the *Measure* function the cursors are sticking to the selected Traces and cannot freely moved inside the Trace Viewport. This means the cursors can be moved along the selected Traces point by point.

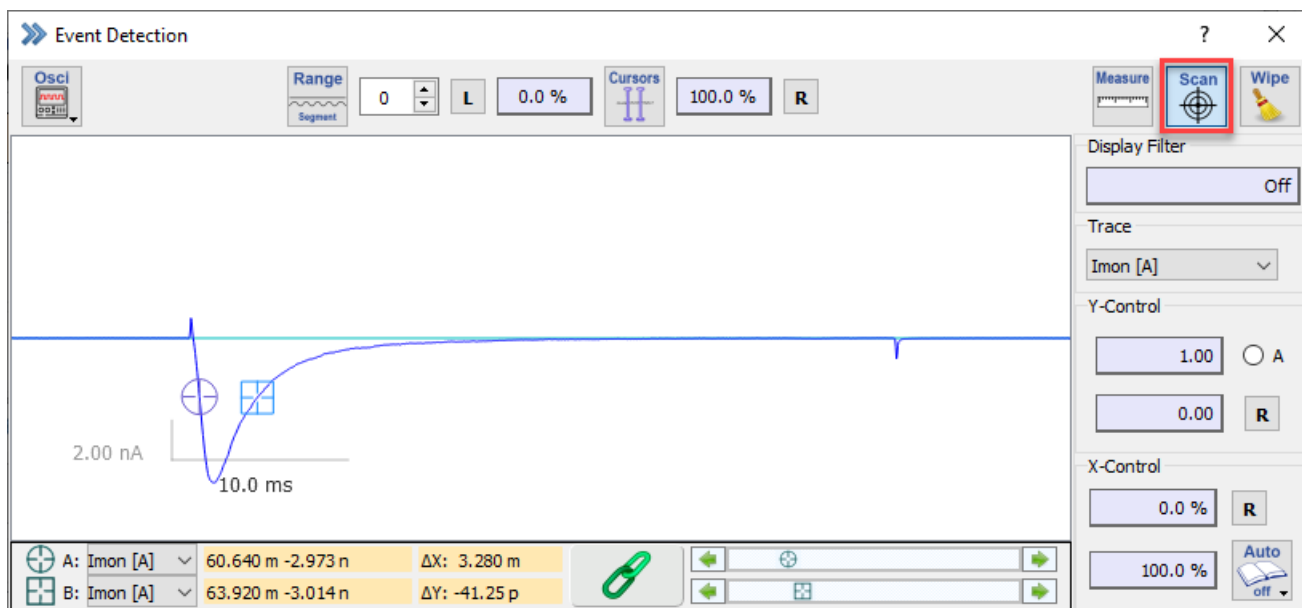


Figure 18.4: The Scan functionality of the Event Detection window.

The Trace selection option for each cursor is indicated in the lower left corner of the extension bay. All other functionality of the *Scan* option is identical to the *Measure* option (see above).

Wipe: Clears the viewport of the Event Detection window.

18.2 Trace Viewport

Viewport: The selected data (Data Tree) is shown in this viewport. Please consider that only one Trace per Sweep is displayed in the Trace Viewport.

Display Filter: A digital filter is applied to the shown data in the Trace Viewport. The filter is applied for analysis purposes without affecting the original data. The *Display Filter* sets the bandwidth of a digital non-lagging Gaussian low-pass filter. The -3dB cutoff frequency is specified in Hertz. This allows to reduce the original bandwidth by the factor 0.0025 (1/400).

Trace: Allows the selection of one Trace from the currently selected Sweep in Data Tree.

Y-Control: For the selected Trace you can control the display scaling (upper entry field) or the offset of the zero line (lower entry field). It is also possible to auto-adjust the displayed data either by (*Auto Y-Range Series*) (see chapter 18.1 on page 241) or by using *Automatic Y-Scaling based on the Trace* with the *A* checkbox. Via the *Reset* option you can reset the scaling and the offset of the Trace.

X-Control: Via the *X-Control* options one can define the display range of the X-axis of the selected Trace. This can be extremely helpful if one wants to focus with the analysis on a defined part of the X-axis. The *R* button resets the display range to the default values.

There are also three *Auto Paging* options available:

- **Paging Off:** No paging, i.e., there is no automatic change of the X range of the graphs.
- **Full Page:** X range moves to a new page whenever the desired focus does not fit into the currently selected page.
- **Center Page:** A new page is generated such that the focus is in the center.

The *Paging* defines how the analysis functions set aspects of the Trace in focus of the viewport. It is applied in event detection (e.g., action potentials).

18.3 Event Detection Settings

In the Event Detection Settings section one can define all parameters of the *Action Potentials* analysis and handle the *Event Table*.

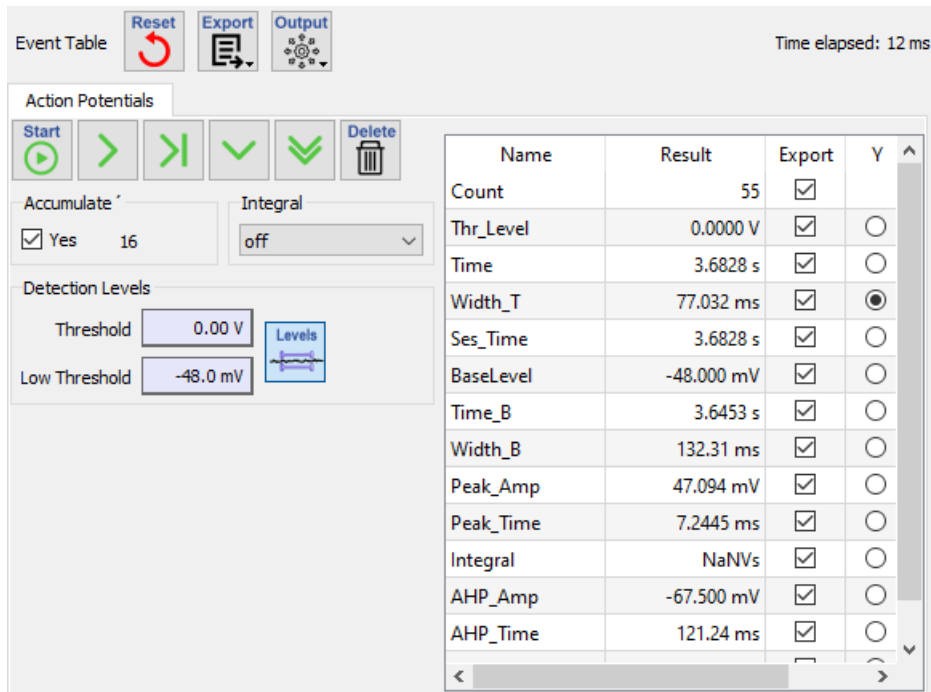


Figure 18.5: Event Detection Settings of the Event Detection dialog.

18.3.1 Event Table

Reset: Resets the *Event Table* by deleting all content.

Export: In the *Export* menu one defines the output of the *Event Table* data:

- Notebook: Prints the event table data into the Notebook window.
- Clipboard: Copies the event table data into the clipboard of the operating system.
- Clipboard+: Copies the event table data into the clipboard and appends it if there is already previous event data existing.
- ASCII File: Exports the event data table into an ASCII text file.
- Igor Waves: Exports the event data table into an Igor Wave file.
- Table: Creates an event table which can be saved as text file.

Output:

- to Notebook: Events are directly written into the Notebook window during the analysis.

- to GUI: Option to update the fit values in the event table during a running analysis.
- to Viewport: The fitting parameters are displayed with the Trace data in the Trace Viewport.

18.3.2 Action Potentials

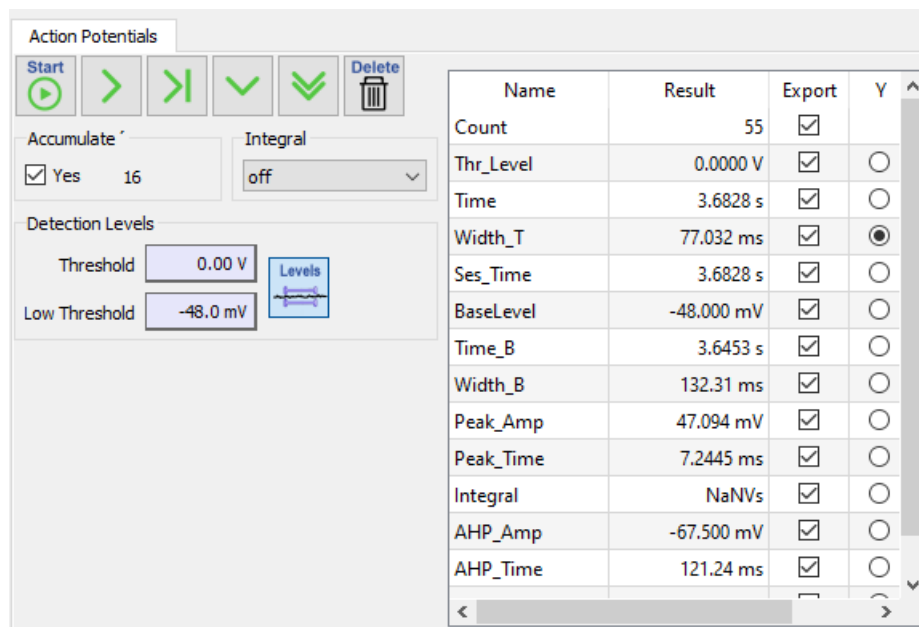


Figure 18.6: Action Potentials settings of the Event Detection dialog.

Start: Initializes the event detection for the selected Sweep in the Data Tree.

Next event: Search and analyze the next event in the Sweep.

All events of Sweep: Search and analyze all events of the active Sweep.

All events of next Sweep: Selects the next Sweep in the Data Tree and analyzes all events of it.

Analyze all Sweeps of Series: Analyzes all events of all Sweeps of the active Series.

Delete: Deletes the last event of the event table.

Accumulate: If selected, all events of different Sweeps will be accumulated. If not active the options *All events of next Sweep* and *Analyze all Sweeps of Series* are not displayed. The number of analyzed Sweeps is displayed on the right side of the checkbox.

Integral: Defines the integration method for the action potentials.

- to Base: Uses the calculated *Base* level for the integral calculation.
- to Threshold: Uses the *Threshold* level for the integral calculation.

Detection Levels: Here, one sets *Threshold* and *Low Threshold* for the action potential analysis. *Threshold* is used for the action potential detection and *Low Threshold* is used as a base parameter for integral calculation and action potential parameters.

Action Potentials Parameters Table: In this table all the parameters of the action potential analysis are displayed. The table does only show the latest set of parameters from the last event. For exporting individual parameters the corresponding checkbox needs to be selected. Further, via the "Y" and "X" selection one can define the parameters for the Event Graph plot.

18.4 Event Graph

In the Event Graph the two selected X and Y parameters of the event analysis are plotted.

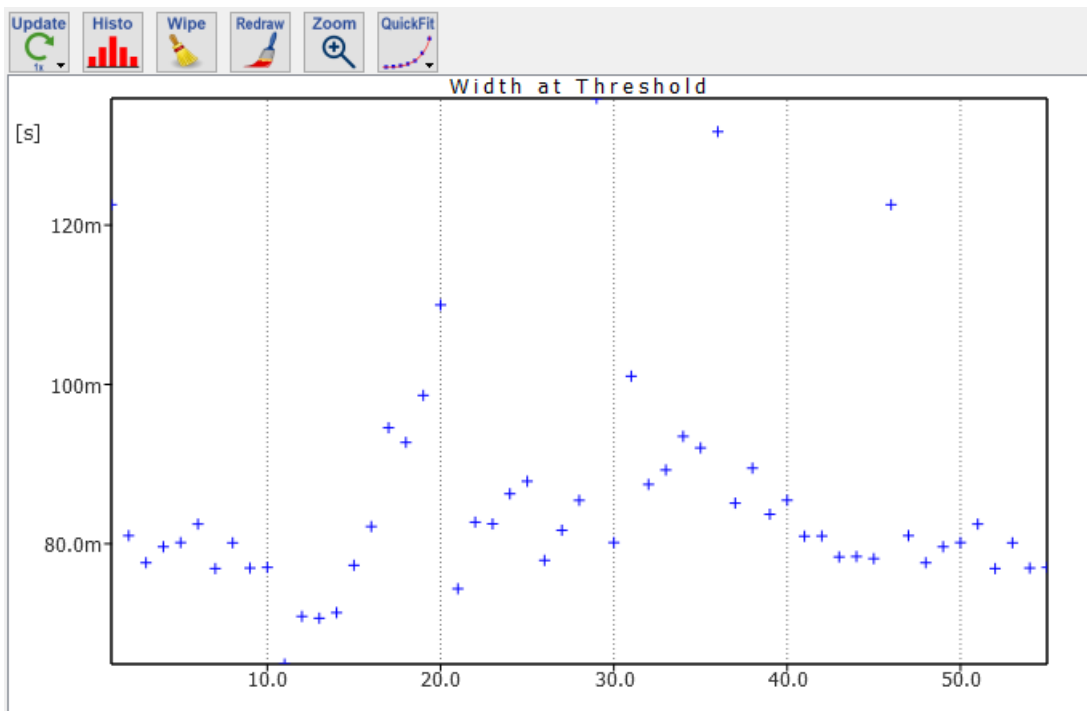


Figure 18.7: Event Graph of the Event Detection dialog.

With a right-click on the graph a context menu opens:

Copy	Ctrl+C
Print	Ctrl+P
Graph Properties	Ctrl+G
Reset>Auto	
Reset>Linear	

Figure 18.8: Graph properties of the Event Graph.

The following options for the graph settings are available:

- **Copy:** Copies the graph to the clipboard. Use the paste option to insert it into any other appropriate software
- **Print:** Opens the printer settings on your PC to print the graph.
- **Graph Properties:** Opens another dialog for more settings of the Event Graph. The settings are described in chapter 18.4.1 on the facing page.
- **Reset>Auto:** Resets the graph settings to auto scaling.
- **Reset>Linear:** Resets the graph axis proportionality to linear scaling.

Update: Defines how often data in the Event Graph are updated. The following options are available:

- **Off:** The graph is not updated.

- 1x: The graph is updated after every new event.
- 10x: The graph is updated after ten new events.
- 100x: The graph is updated after 100 new events.
- Sweep: The graph is updated after every Sweep.

Histogram: Determines whether the graph shows parametric (Y vs X) data or compiles a histogram out of the Y data. In case of *Histogram*, there will be an info line on top of the graph featuring the number of points that are too small and too large, as well as mean and standard error of mean (SEM) of the data falling into the histogram. Parameters for setting up a histogram are edited in the Event Graph Properties dialog.

Wipe: Clears the graph.

Redraw: Redraws the graph.

Zoom: A click activates this field; in the activated mode, the graph can be zoomed with the lassoing function (drag mouse). Clicking on "Zoom" again resets the scaling to automatic values.

QuickFit: Fits a selected function to the data in the graph. The *QuickFit* feature is meant to give a very simple overview by fitting a selected function. The starting parameters are estimated from the data. Depending on the data, the results may not always be what one expects. When the fit is done, result parameters are printed in the Notebook window (when selected).

The following functions are provided:

- Table Info: Print statistics information on the selected Y/X couple of the event table data into the Notebook window.
- Constant: Mean value of the data, $y = a$.
- Linear: $y = bx + a$
- Parabola: $y = cx^2 + bx + a$
- Cubic: $y = dx^3 + cx^2 + bx + a$
- 2 Lines: Two linear functions intersecting at time0.
- Exponential: $a * \exp(-x/tau)$
- 1-Exponential: $a * (1 - \exp(-x/tau))$
- Onrate: $x < time0 : y = offset$; else $> : y = offset + a1 * (1 - \exp(-x/tau1))$
- Gaussian: A single Gaussian.
- Boltzmann: A single Boltzmann (sigmoid) function.
- Lorentzian: A single Lorentzian (spectral) function.

18.4.1 Event Graph Properties

Header:

- Off: No *Header* is created.
- Auto: A *Header* is automatically created, depending on the selected X and Y parameter in the event table.
- Manual: Allows the user to enter its own *Header* definition.

The Event Graph Properties dialog consists of four tabs which are described below.

18.4.1.1 X Axis

The X Axis tab shows all settings available for the X-axis graph settings.

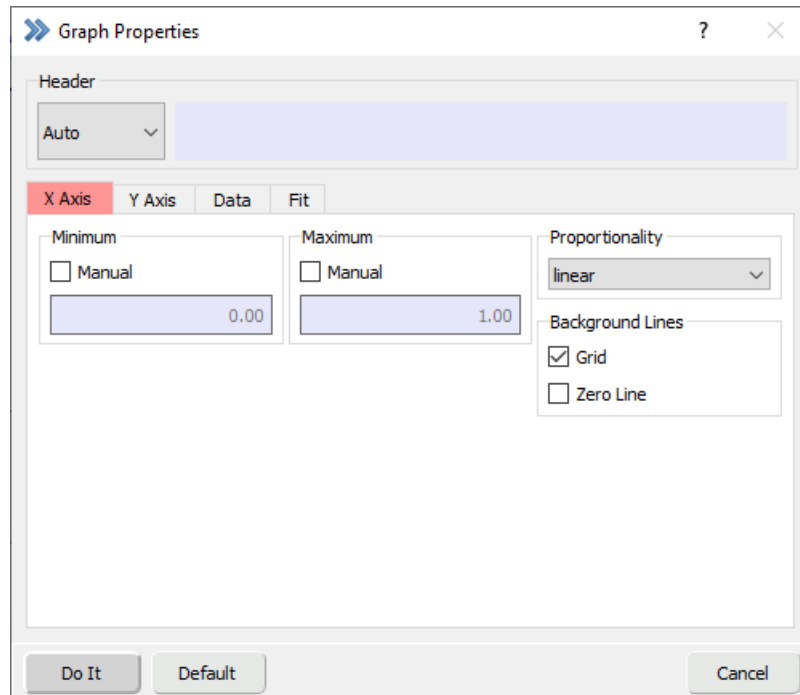


Figure 18.9: X-axis properties of the Event Graph.

Minimum: By default, the scaling option for the graphs are set to auto scaling. The software checks for the maximal and minimal values to calculate the best scaling of the graph. If the scaling of the data is already known or only a small range should be plotted in the graphs one can activate the *Manual* option. The user has to specify the minimum X-axis value for the selected graph.

Maximum: By default, the scaling option for the graphs are set to auto scaling. The software checks for the maximal and minimal values to calculate the best scaling of the graph. If the scaling of the data is already known or only a small range should be plotted in the graphs one can activate the *Manual* option. The user has to specify the maximum X-axis value for the selected graph.

Proportionality:

The following options are available:

- linear: Linear scaling of the X axis.
- log10: Decimal logarithm scaling of the X axis.
- log2: Binary logarithm scaling of the X axis.

Background Lines:

The following options are available:

- Grid: Shows the vertical grid lines for the X-axis.
- Zero Line: Shows the X-axis zero line.

18.4.1.2 Y Axis

The `Y Axis` tab shows all settings available for the Y-axis graph settings.

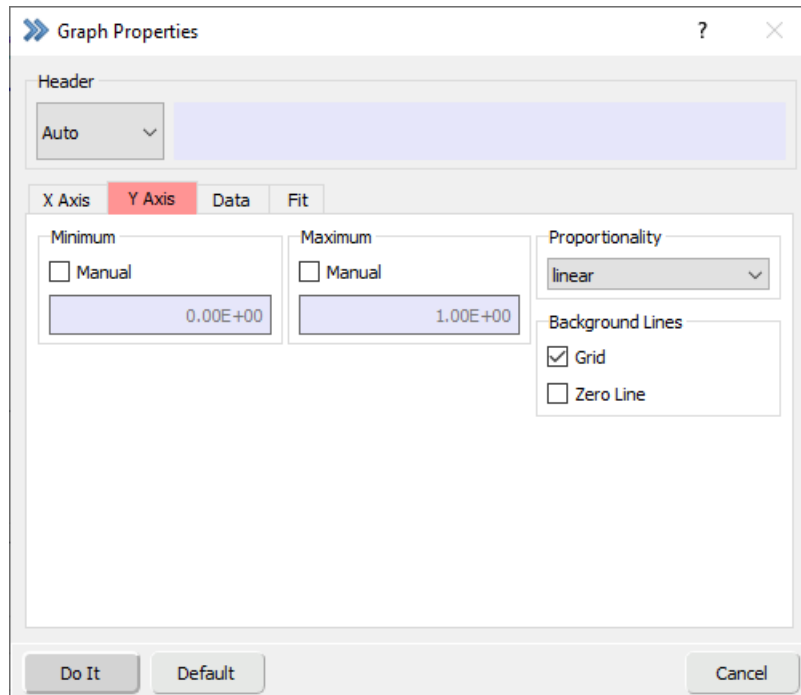


Figure 18.10: Y-axis properties of the Event Graph.

Minimum: By default, the scaling option for the graphs are set to auto scaling. The software checks for the maximal and minimal values to calculate the best scaling of the graph. If the scaling of the data is already known or only a small range should be plotted in the graphs one can activate the *Manual* option. The user has to specify the minimum Y-axis value for the selected graph.

Maximum: By default, the scaling option for the graphs are set to auto scaling. The software checks for the maximal and minimal values to calculate the best scaling of the graph. If the scaling of the data is already known or only a small range should be plotted in the graphs one can activate the *Manual* option. The user has to specify the maximum Y-axis value for the selected graph.

Proportionality:

The following options are available:

- linear: Linear scaling of the Y axis.
- log10: Decimal logarithm scaling of the Y axis.
- log2: Binary logarithm scaling of the Y axis.

Background Lines:

The following options are available:

- Grid: Shows the vertical grid lines for the Y-axis
- Zero Line: Shows the Y-axis zero line.

18.4.1.3 Data

The `Data` tab shows all settings available for the data of the Event Graph.

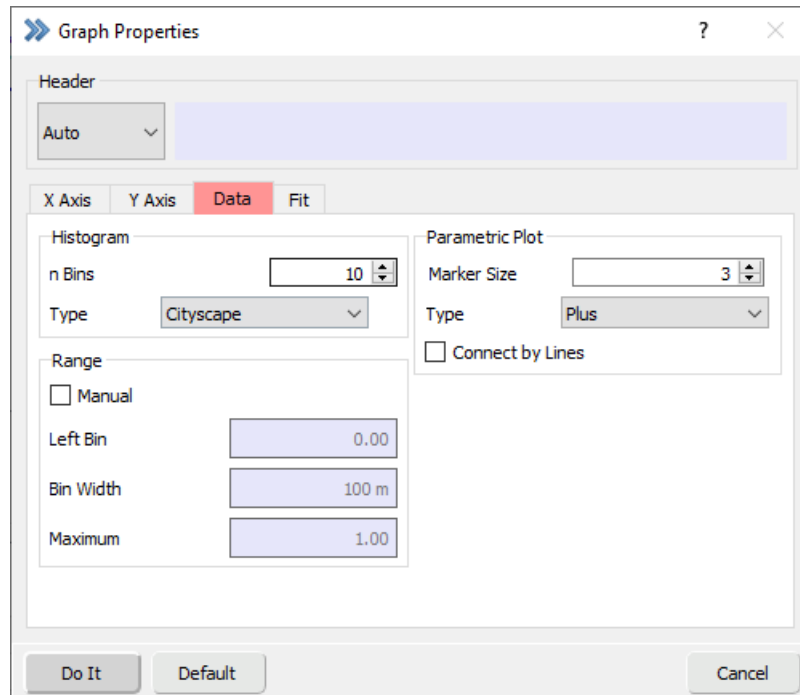


Figure 18.11: Data properties of the Event Graph.

Histogram: Define the number of bins for a histogram. Further, one can select the type of histogram presentation:

- Lines
- Sticks
- Cityscape
- Bars

If the *Range* is off, the bounds of the histogram are calculated automatically based on the data. If *Manual* is active the three options for *Left Bin* (value of left edge of smallest bin), *Bin Width* and *Maximum* (automatically calculated high end of the histogram) are available.

Parametric Plot: Define the size of the markers (*Marker Size*) and one of the available *Marker Types*. Select *Connect by Lines* if you want to connect the markers by lines.

18.4.1.4 Fit

The `Fit` tab shows all settings available for the fit of data in the Event Graph

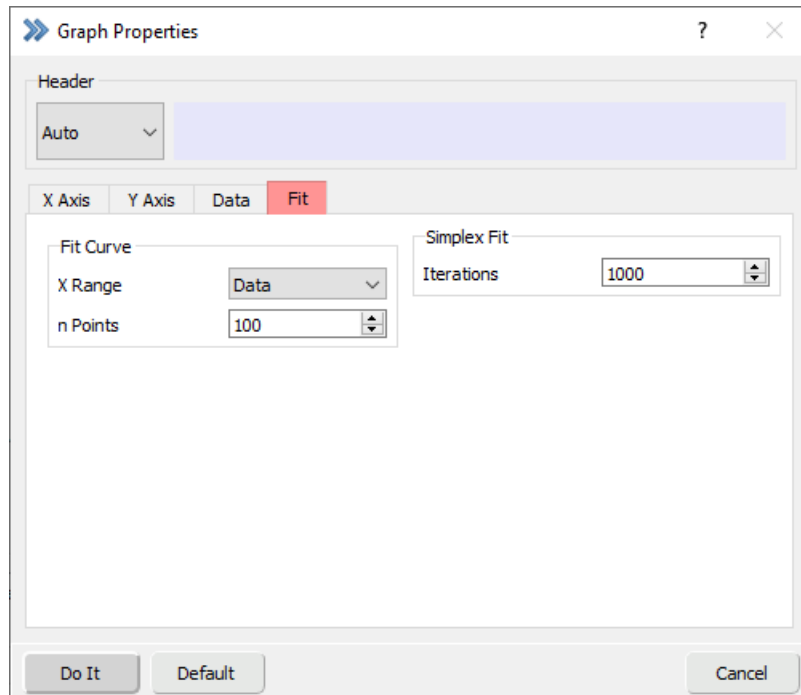


Figure 18.12: Fit properties of the Event Graph.

Fit Curve: Draw a fit curve in the X-range according to *Full Graph*, *Data* or *Fitted Data*. The number of data points considered for the fit can be specified by the *n Points* option.

Simplex Fit: Define the number of maximal iterations for a *Simplex Fit*.

19 LockIn Extension

For the activation and the configuration of the LockIn Extension you have to enable the software lock-in extension in the Configuration (see chapter 3.5.2.1 on page 25).

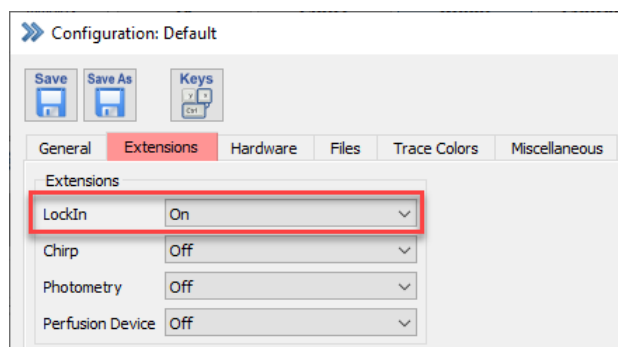


Figure 19.1: Enable the LockIn extension in the Configuration.

In this manual only the main user functions are explained. If you are interested in a detailed description how the LockIn Extension works or what might be the best approach to determine membrane capacitances in different recording modes we refer to the tutorial: "Capacitances Measurements using the LockIn Extension".

19.1 LockIn Configuration

After switching on the LockIn extension in the configuration the LockIn Configuration window opens. It allows to define the used *LockIn Mode* and other settings. Once closed, the window can be re-opened in the Windows menu (see 3.1 on page 18).

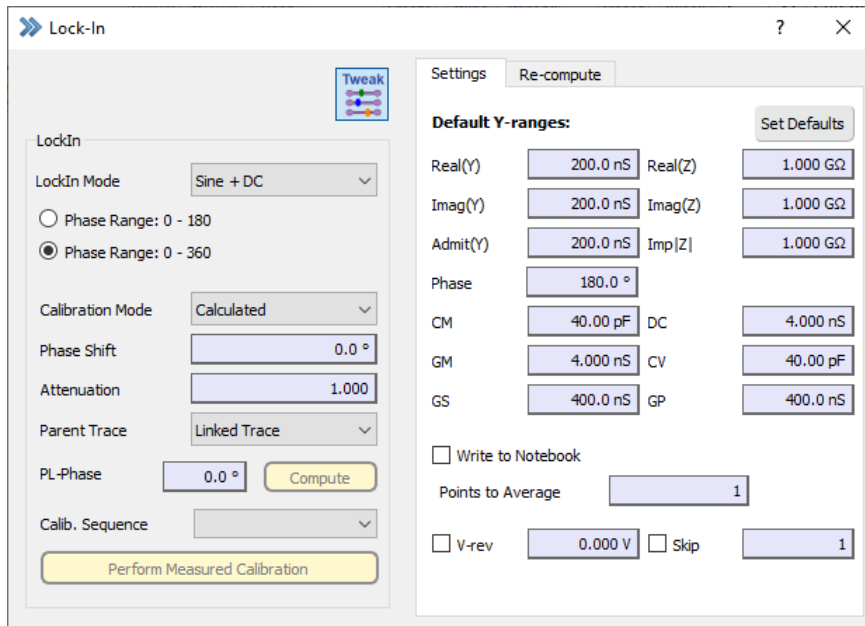


Figure 19.2: Lock-In configuration dialog of PATCHMASTER NEXT.

The following options and parameters available are described below.

Tweak: The *Tweak* menu opens further options for the *LockIn* extension. These settings are explained in detail in chapter 19.1.1 on page 256 and chapter 19.1.2 on page 257.

LockIn Mode: Here, you can set the LockIn Mode. You have these possibilities:

- Off: No LockIn mode is set.
- Sine + DC: Calculates absolute values of all three LockIn parameters; it is therefore the preferred method. A disadvantage is that the software has to know all hardware settings, which is the case when PATCHMASTER NEXT operates an EPC 10 USB.
- Piecewise Linear: It is an older, more simplified method which only calculates *relative* changes of C_M and G_S or G_M .
- On Cell: Alternative method that has to be used in case of the On Cell recording mode. The reason being that R_S , which is used as denominator in the usual formula, is near zero or zero, so that an alternative calculation method is necessary.
- Basic Spectroscopy: Limits the LockIn to basic computations (CM, GM, GS, DC, CP and GP are disabled). When this *LockIn Mode* is set two other options are available:
 - Phase Range: 0..180: Limits the phase to a range of 0° to 180°.
 - Phase Range: 0..360: Allows to use the full phase range.

Calibration Mode: The *Sine + DC* method calculates 4 values: a real part, a imaginary part, a DC value and a phase shift. It needs two calibration parameters: phase shift ("y") and attenuation ("a") of the amplifier. The calibration can be done with measured or calculated values.

- Measured ("Hardware" calibration): It is more accurate but has to be repeated every time if any critical parameter like filter, gain, frequency, etc. changes. This method should be used if sine wave frequencies exceeding ~2 kHz.
- Calculated: It is easy to handle and accurate enough, thus the recommended method for sine wave frequencies up to ~2 kHz.

- **Manual:** It can be used to set the *Phase* and *Attenuation* of the software lock-in manually. This mode is used:
 - If the user estimates the *Phase* and *Attenuation* settings with special methods, i.e. *C-Fast* dithering in *On Cell* mode. *C-Fast* dithering methods are most accurate when working with sine wave frequencies in the range between 10 and 40 kHz.
 - When hardware other than EPC 10 USB is used the *Manual Calibration* mode is an alternative to the *Measured Calibration* mode.

Note: If you are intending to perform LockIn measurements on multiple Traces, only Calculated Calibration is supported. This warning is displayed if you try to select a Measured Calibration.

Phase Shift: The user can specify a *Phase Shift* to be applied to the residual admittance before the equivalent circuit parameters are calculated. This *Phase Shift* is only applied to the actual current that is measured and not the component of admittance which is "zeroed" by *C-Slow* compensation.

This feature can be used, for example, to "tweak" the phase determined by *Measured* or *Calculated Calibration*. Another example where this would be useful is if data are recorded with a set of "critical parameters" which differs from those that were used when a *Measured Calibration* was performed (this is prevented by the software if an EPC 10 USB is used, but can occur for other amplifiers). The phase difference can be determined by performing a new *Measured Calibration* and can be specified as the *Phase Shift* for replay of the recorded data.

When *Manual Calibration* is used, the *Phase Shift* sets the absolute phase of the software lock-in.

Note: Generally the Phase Shift should be left at 0.0°.

Attenuation: It is an amplitude scaling factor for the current signal that can be used to correct the transfer function of the complete system. This attenuation factor is applied to the residual admittance before the equivalent circuit parameters are calculated.

Note: Generally the Attenuation should be left at 1.0°.

When *Manual Calibration* is used the *Attenuation* should be set to the value estimated by the *Measured Calibration*.

Generally, the *Attenuation* might be set to a value unequal to 1 when the transfer function of the system is not ideal, i.e. the sine wave frequency is higher than the current filter frequency or the sine wave frequency approaches the overall bandwidth of the amplifier.

Parent Trace: This option is only available if *Manual Calibration* is selected. Select the corresponding Trace (e.g. current or voltage Trace) for the calibration, either the *Linked Trace* assigned in the PGF (*Link*) or any other Trace (Trace 1...16).

PL-Phase: These settings are only applicable if *Piecewise Linear* mode is used. *PL-Phase* is the setting of the phase sensitive detector (LockIn amplifier) after correction for shifts introduced in the instrumentation (see *Calibration Mode*, 19.1 on the preceding page). Ideally, this phase setting results in a C_M output which is maximally sensitive to changes in capacitance while insensitive to changes in G_S or G_M . The value can be entered manually or is *calculated* from the current *C-Slow* and *G-Series* values by pressing the *Compute* button. This calculated method of determining the *PL-Phase* differs fundamentally from (and avoids errors associated with, see Gillis 1995) empirical determinations using series resistance dithering ("phase tracking", Fidler and Fernandez, 1989). It is more closely related to finding the phase empirically via "C-Slow dithering" (Neher and Marty, 1981) but, just like the *Sine + DC* mode, requires the *Phase Shift* introduced by the instrumentation to be known. The *PL-Phase* is stored with each Sweep that is acquired in *Piecewise Linear* mode.

Compute: Calculates the *PL-Phase* from the current *C-Slow* and *G-Series* values.

Calib. Sequence: Here, the user specifies a sequence as calibration stimulus to be used to perform *Measured Calibration* or to compute the *PL-Phase*.

Note: Adjacent stimulation protocols require the same settings as used in the Calib. Sequence, otherwise the results may be faulty.

Perform Measured Calibration: Starts the Measured Calibration.

Note: How to perform a Measured Calibration is explicitly described in the PATCHMASTER NEXT Tutorial: "Capacitance Measurements using the LockIn Extension".

19.1.1 Settings

The user has to define *Default Y-ranges* for the resulting LockIn Traces. PATCHMASTER NEXT does not know the range of the forthcoming data because this depends on cell size. Thus, the user has to provide a default range (e.g. 0 to 40 pF for the C_M Trace would be a reasonable estimate).

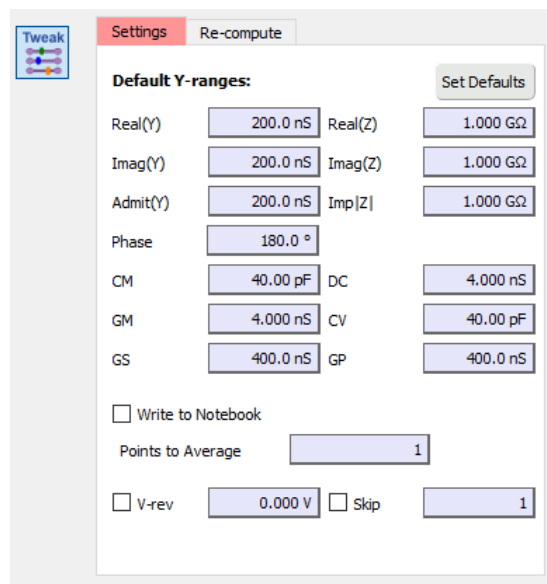


Figure 19.3: Settings of the LockIn Extension.

The following lock-in Traces are available:

- Real(Y): Real part of the admittance
- Real(Z): Real part of the impedance
- Imag(Y): Imaginary part of the admittance
- Imag(Z): Imaginary part of the impedance
- Admit(Y): The admittance
- Imp | Z | : The impedance
- Phase: Phase angle
- CM: Membrane capacitance
- GM: Membrane conductance
- GS: Series conductance

- DC: DC conductance
- CV: Vesicle capacitance
- GP: Pore conductance

Set Defaults: Resets the *Y*-ranges to the default values.

Write to Notebook: Enabling this option will print out the LockIn results to the Notebook window.

Note: The Write to Notebook option does only work if you acquire at least one LockIn Trace in your stimulation sequence.

Points to Average: A global number of points to average can be entered.

V-reversal: A global reversal potential used for computation and re-computation can be entered.

Skip: A global number of cycles to skip can be entered.

The global settings V-rev and Skip, when activated, overrule the settings made in the Wave Parameters of the stimulation sequence.

19.1.2 Re-compute

This section of the LockIn Configuration window is used for calculating LockIn data from pre-recorded Traces (for further details see 19.3 on page 259).

Generate Traces for: Due to the full implementation of the LockIn software for C_M measurements, if a Double, Triple or Quadro EPC 10 USB amplifier is used, membrane capacitance measurements can be performed simultaneously with two, three or four amplifiers. Alternatively, multiple amplifiers can be connected to a single computer and controlled by PATCHMASTER NEXT. Thus, when a multiple amplifier is used there is an additional control with the option to generate *Traces* for only the active amplifier, all amplifiers or only user-selected amplifiers. By default, all available amplifiers are selected.

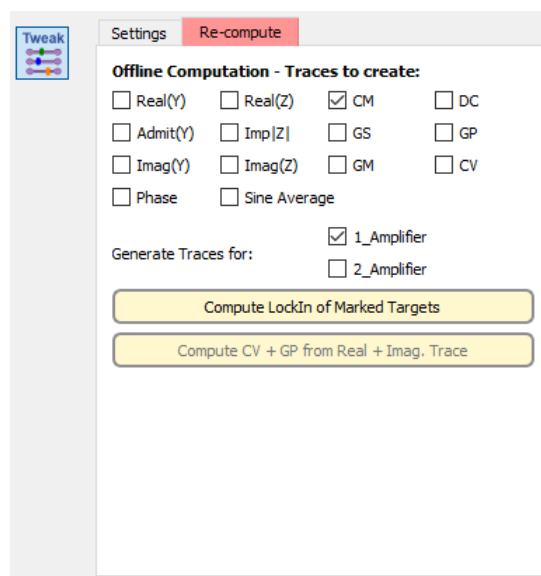


Figure 19.4: Re-compute settings of the LockIn Extension.

Compute LockIn of Marked Targets: Computes the LockIn Traces selected in *Offline Computation - Trace to create* of the marked targets in the in the Data Tree.

Compute CV + GP from Real + Imag. Trace: Computes vesicle capacitance (CV) and pore conductance (GP) from the real and imaginary Traces. This might be only necessary when using the LockIn in the *On Cell* mode.

19.2 Using LockIn in Voltage or Current Clamp Mode

When using the LockIn in *Voltage Clamp* mode, the stimulus is a voltage and the recorded signal is usually a current Trace. Hence, the LockIn analysis returns admittance values.

```

Calculated LockIn Calibration:
Est. Int. Phase: 296.7   Est. Att.: 0.960
A: 61.19nS   B: 83.95nS   b: 1.966nS   Phase: 53.9°
RS: 5.550Mohm   RM: 503.2Mohm   CM: 20.455pF

```

Note: To see these values in the *Notebook* window you have to activate *Write to Notebook* in the *LockIn Configuration* window and you have to select at least one LockIn Trace in your stimulation sequence.

A short explanation of the values printed into the *Notebook* window:

- Est. Int. Phase: Estimated internal (hardware) phase angle.
- Est. Att.: Estimated attenuation caused by the hardware.
- A: Real part of the admittance (Siemens).
- B: Imaginary part of the admittance (Siemens).
- b: DC current value (Siemens).
- Phase: Phase angle determined by the LockIn Extension (degree).
- RS: Series resistance determined by the LockIn Extension (Ohm).
- RM: Membrane resistance determined by the LockIn Extension (Ohm).
- CM: Membrane capacitance determined by the LockIn Extension (Farad).

In *Current Clamp* mode the LockIn can be used in the *Manual Calibration Mode*. When doing so, the stimulus is a current and the recorded signal is usually a voltage Trace. Hence the LockIn analysis returns impedance values. Values for A and B which are printed into the *Notebook* window by the LockIn Extension are scaled in Ohms instead of Siemens.

```

A: 10.24Mohm   B: -20.78kOhm   b: 10.50Mohm   Phase: 359.9°

```

Note: The derived results such as "RS", "RM" and "CM" etc. are valid in *Voltage Clamp* mode only.

19.3 The LockIn Traces

The LockIn Extension provides several output Traces which can be selected in the AD channel section of the Stimulus Editor (see 7.5.2 on page 96).

The following Traces which are independent from the LockIn mode are available:

LockIn_Avg: An average of all sine wave cycles.

LockIn_Phase: The phase angle between stimulus and measured signal.

$$Phase = \frac{180}{\pi} * \arctan(B/A)$$

LockIn_Real(Y): The real part of the admittance.

$$\begin{aligned} \text{Voltage Clamp: } Real(Y) &= A \text{ (Siemens)} \\ \text{Current Clamp: } Real(Y) &= \frac{A}{A^2+B^2} \text{ (Ohm)} \end{aligned}$$

LockIn_Imag(Y): The imaginary part of the admittance.

$$\begin{aligned} \text{Voltage Clamp: } Imag(Y) &= B \text{ (Siemens)} \\ \text{Current Clamp: } Imag(Y) &= \frac{B}{A^2+B^2} \text{ (Ohm)} \end{aligned}$$

LockIn_Admit(Y): The admittance (Siemens).

$$\begin{aligned} \text{Voltage Clamp: } Admit(Y) &= \sqrt{A^2 + B^2} \\ \text{Current Clamp: } Admit(Y) &= \frac{1}{\sqrt{A^2+B^2}} \end{aligned}$$

LockIn_Real(Z): The real part of the impedance.

$$\begin{aligned} \text{Voltage Clamp: } Real(Z) &= \frac{A}{A^2+B^2} \text{ (Siemens)} \\ \text{Current Clamp: } Real(Z) &= A \text{ (Ohm)} \end{aligned}$$

LockIn_Imag(Z): The imaginary part of the impedance.

$$\begin{aligned} \text{Voltage Clamp: } Imag(Z) &= \frac{B}{A^2+B^2} \text{ (Siemens)} \\ \text{Current Clamp: } Imag(Z) &= B \text{ (Ohm)} \end{aligned}$$

LockIn_Imp|Z|: The impedance (Ohm).

$$\text{Voltage Clamp: } Imp(Z) = \frac{1}{\sqrt{A^2+B^2}}$$

$$\text{Current Clamp: } Imp(Z) = \sqrt{A^2 + B^2}$$

LockIn_CM: The membrane capacitance (Farad).

$$C_m = \frac{1}{\omega_c B} * \frac{(A^2+B^2-AG_{t,a})^2}{(A-G_{t,a})^2+B^2}$$

LockIn_GM: The membrane conductance (Siemens).

$$G_m = G_{t,a} * \frac{A^2+B^2-AG_{t,a}}{(A-G_{t,a})^2+B^2}$$

LockIn_GS: The series conductance (Siemens).

$$G_s = \frac{A^2+B^2-A*G_{t,a}}{A-G_{t,a}}$$

LockIn_DC: The DC conductance (Siemens).

$$G_{t,m} = \frac{I_{dc}}{V_h - E_{rev}}$$

LockIn_CV: The vesicle capacitance (Farad). Only for LockIn measurements in *On Cell* mode!

$$C_v = \frac{1}{2\pi * f_{sine}} * \frac{A^2+B^2}{B}$$

LockIn_GP: The pore conductance (Siemens). Only for LockIn measurements in *On Cell* mode!

$$G_p = \frac{A^2+B^2}{A}$$

Abbreviations:

- A = Real part of the admittance.
- B = Imaginary part of the admittance.
- I_{dc} = DC current.
- E_{rev} = Reversal potential.
- V_h = Stimulus voltage.
- f_{sine} = Stimulation frequency.

19.4 Capacitance Measurements - Step by Step

The following section was made for PATCHMASTER NEXT and HEKA EPC 10 USB amplifier. If you are using CHARTMASTER or POTMASTER several modifications may be necessary!

Here, we make our first capacitance measurement using a model circuit and the LockIn Extension. We used an EPC 10 USB Single for this tutorial, but most settings are identical for EPC 10 USB Double, Triple or Quadro amplifiers. We will explicitly mention the particular amplifiers, where it is required.

19.4.1 Activating the LockIn Extension

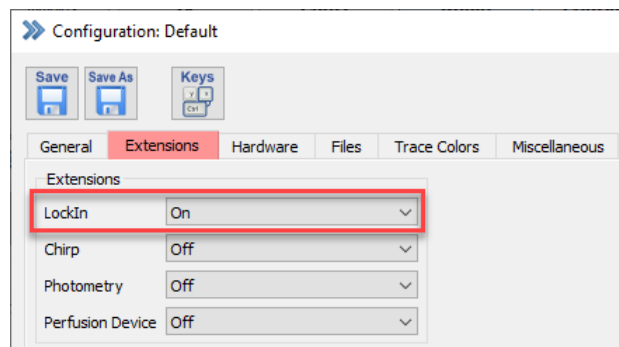


Figure 19.5: Enable the LockIn Configuration in the Control window.

By default, the LockIn Extension of PATCHMASTER NEXT is inactive. To activate the extension, we have to open the Configuration window and turn on the LockIn extension. This step only has to be done the first time one uses LockIn. The setting is stored in the PATCHMASTER NEXT configuration file.

19.4.2 LockIn Configuration

Immediately after switching on the LockIn Extension, the LockIn Configuration window comes up. Please set the LockIn mode to *Sine + DC*. This is the recommended mode, if you are using an EPC 10 USB. At the moment, no other settings need to be made. We will come back to the LockIn Configuration window later.

If you want LockIn calculations to be written to the Notebook window, Write to Notebook should be checked and at least one LockIn Trace has to be selected as an AD input channel in the stimulation sequence.

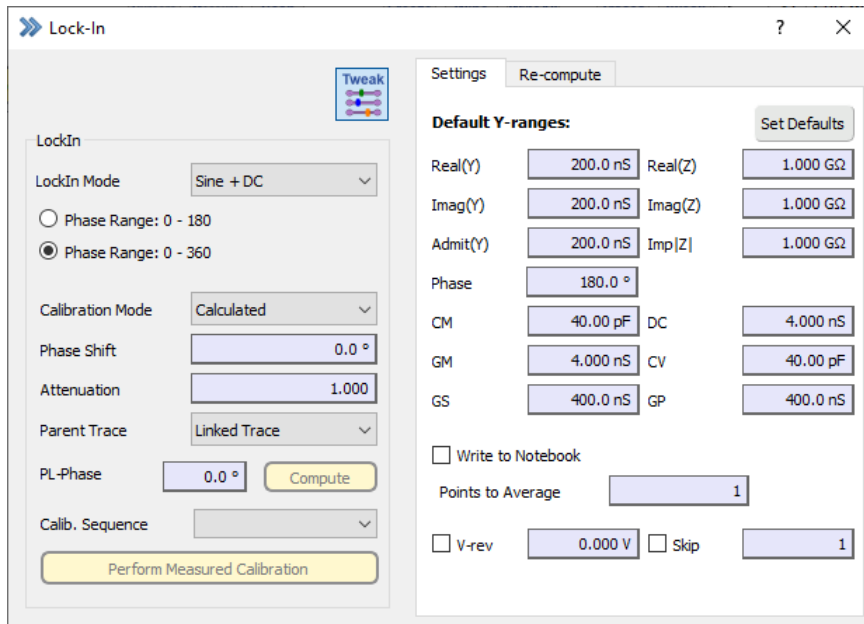


Figure 19.6: LockIn configuration dialog of PATCHMASTER NEXT.

Note: The Default Y-ranges section can be used to set reasonable display ranges according to the expected values.

19.4.3 Creating a Stimulation Sequence

The next step is to prepare a stimulation sequence, that can be used for C_M measurements. Please open the Stimulus Editor window. We could either modify an existing sequence or create a new one from the scratch. Let us create a new sequence this time. A click on the green “plus sign” button at the bottom of the stimulation sequence pool will create a new sequence (7.2 on page 86). Double-click on the new entry and enter a name for the new sequence. We name the sequence we want to create "LockIn".

PATCHMASTER NEXT automatically creates a new and very simple protocol: it just consists of three *Constant* segments each with a duration of 10 ms. We will delete two segments and change the duration of the residual segment to 100 ms. Furthermore, we have to change the *Segment Class* to *Sine*, since at least one sine wave segment is required for the LockIn. Of course, one can also use other segment classes (*Constant*, *Ramp*, *Square*) in the same sequence, for example to define a depolarizing voltage step at the beginning of the Sweep. But LockIn data can only be calculated from *Sine* segments.

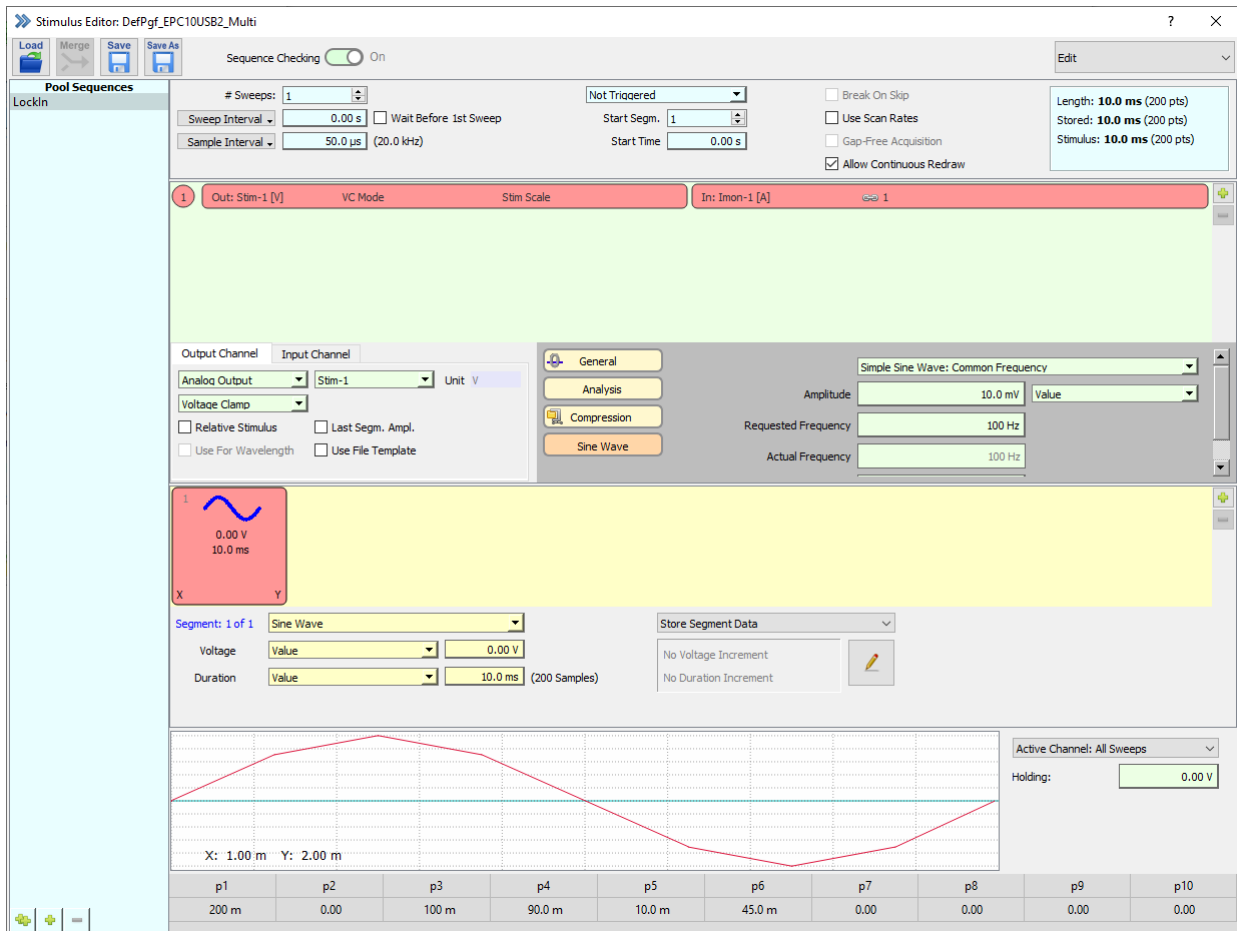


Figure 19.7: Stimulation sequence for LockIn experiments.

The *Sample Interval* is set to $50 \mu\text{s}$. This corresponds to a sampling rate of 20 kHz. Please keep that sampling rate in mind. We will need that information later on. For the calculation of the LockIn parameters, we need to know the whole-cell conductance. The conductance can only be measured, if the holding potential is not "0". Therefore, you should not forget to enter a holding potential for the *Sine* segment. One can either enter a fixed value, for example "-70 mV" or set *Voltage* to "Holding". If *Voltage* is set to "Holding", the holding potential from the Amplifier window is used.

If a *Sine* segment exists in the stimulation sequence an additional section appears in the *Parameter Section* of the Stimulus Editor (7.6.5 on page 105). There, the parameters (frequency, amplitude,...) of the sine wave segments of the stimulation sequence can be modified.

In this section, you can decide, if the *Sine* segments in the sequence should be used for LockIn measurements (*LockIn Sine Wave*) or just as a simple sine wave stimulus (*Simple Sinewave: Common/Separate Frequency*). Of course, we want to use it as *LockIn Sine Wave*.

Now, we increase the *Requested Frequency*. A frequency of 800 Hz is quite good for most approaches. All segment duration must be integral multiples of the sine wave period. Therefore the *Actual Frequency* might be slightly different from the *Requested Frequency*. An appropriate value is calculated by the software and cannot be changed.

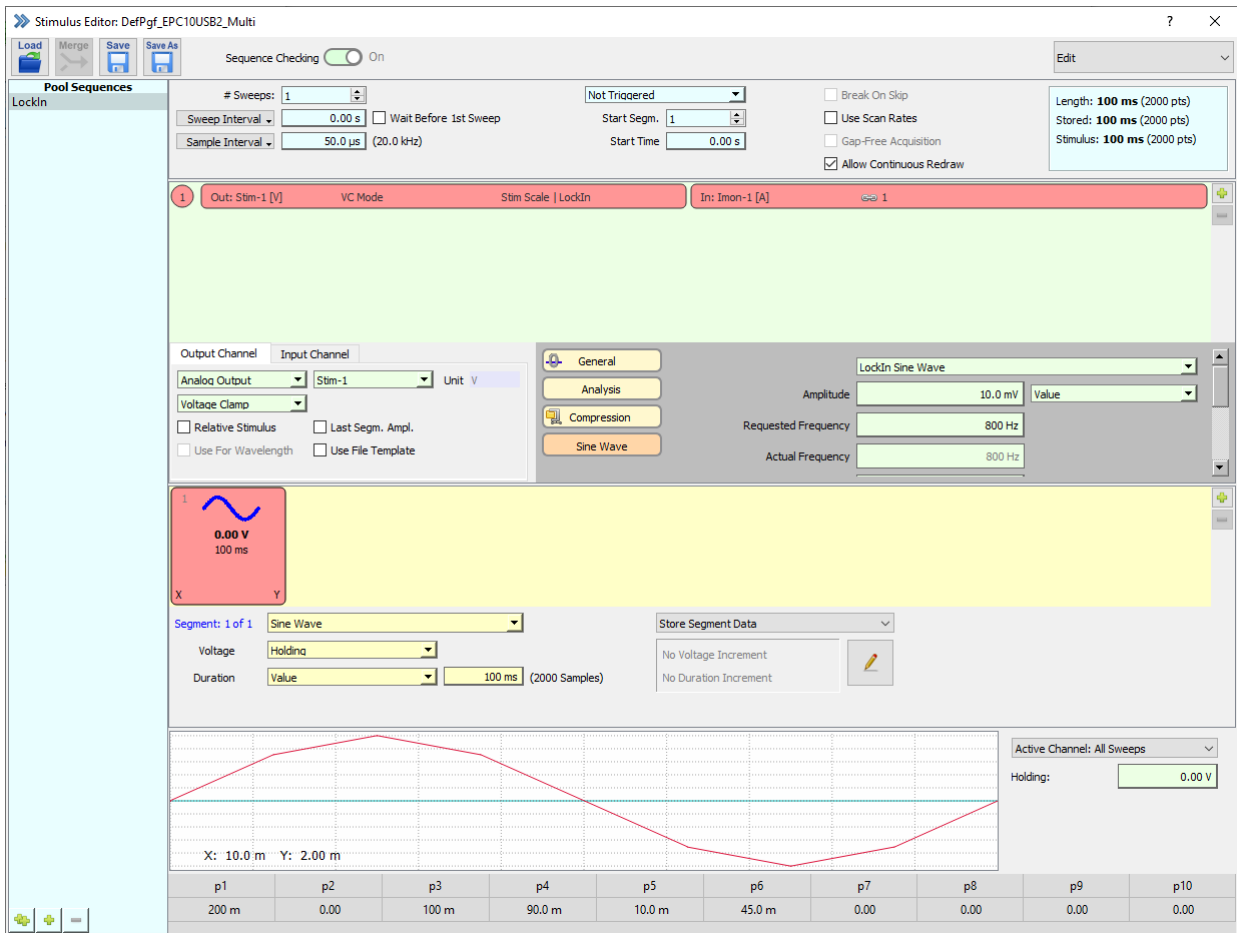


Figure 19.8: Stimulation sequence (Part 2) for LockIn experiments.

Please note, that the *Sample Interval* in the stimulation sequence is adopted, upon changing the setting for *Requested Frequency* or *Points/Cycle*. It is intuitive that for higher frequencies higher sampling rates are required. If you want to reduce the sampling rate, you can decrease the number of *Points/Cycle*. We will do that and set *Points/Cycle* to "25".

The number of Points/Cycle should never be below 10, otherwise the accuracy of the C_m measurement is dramatically reduced.

The setting of 10 mV for the sine wave amplitude is fine and a *Reversal* of 0 mV is also correct for our model circuit. The *Reversal* setting is actually not very critical if G_M is low. A value of zero is often used in the common situation where G_M is low and the actual reversal potential is unknown.

Since we decided to use the *Sine* segment in the sequence for LockIn measurements, the output channel *Stim-1* got an additional "LockIn" flag. This LockIn flag is important. Without that flag, no C_M data can be calculated.

The *AD input* is set to "Imon-1", the current input of your amplifier. This is undoubtedly a good idea, however, we need an additional Trace to store the C_M value. Click on the green "plus" sign button on the right side of the channel listing (or right-click on the section) to open the "Select Input and Output" dialog.

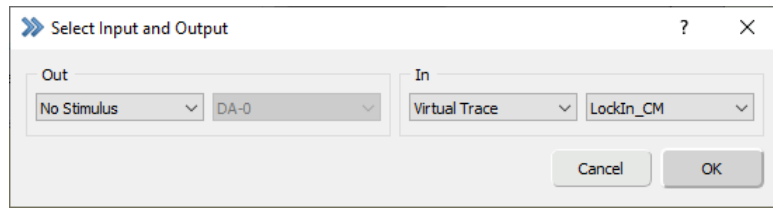


Figure 19.9: Input and Output selection dialog of the Stimulus Editor.

What we need is a *LockIn_CM* Trace. Select “Virtual Trace” in the first selection list and “LockIn_CM” in the second selection list. Confirm the selection by pressing the “OK” button.

You will see for this second AD input channel (LockIn_CM) another flag which indicates the compression factor. It is automatically set to “25” (the value, we have set for *Points/Cycle* in the sine wave parameters section dialog). That means, 25 data points are in one cycle and one C_M value is calculated from each cycle, therefore you will get 80 C_M data points in that 100 ms Sweep.

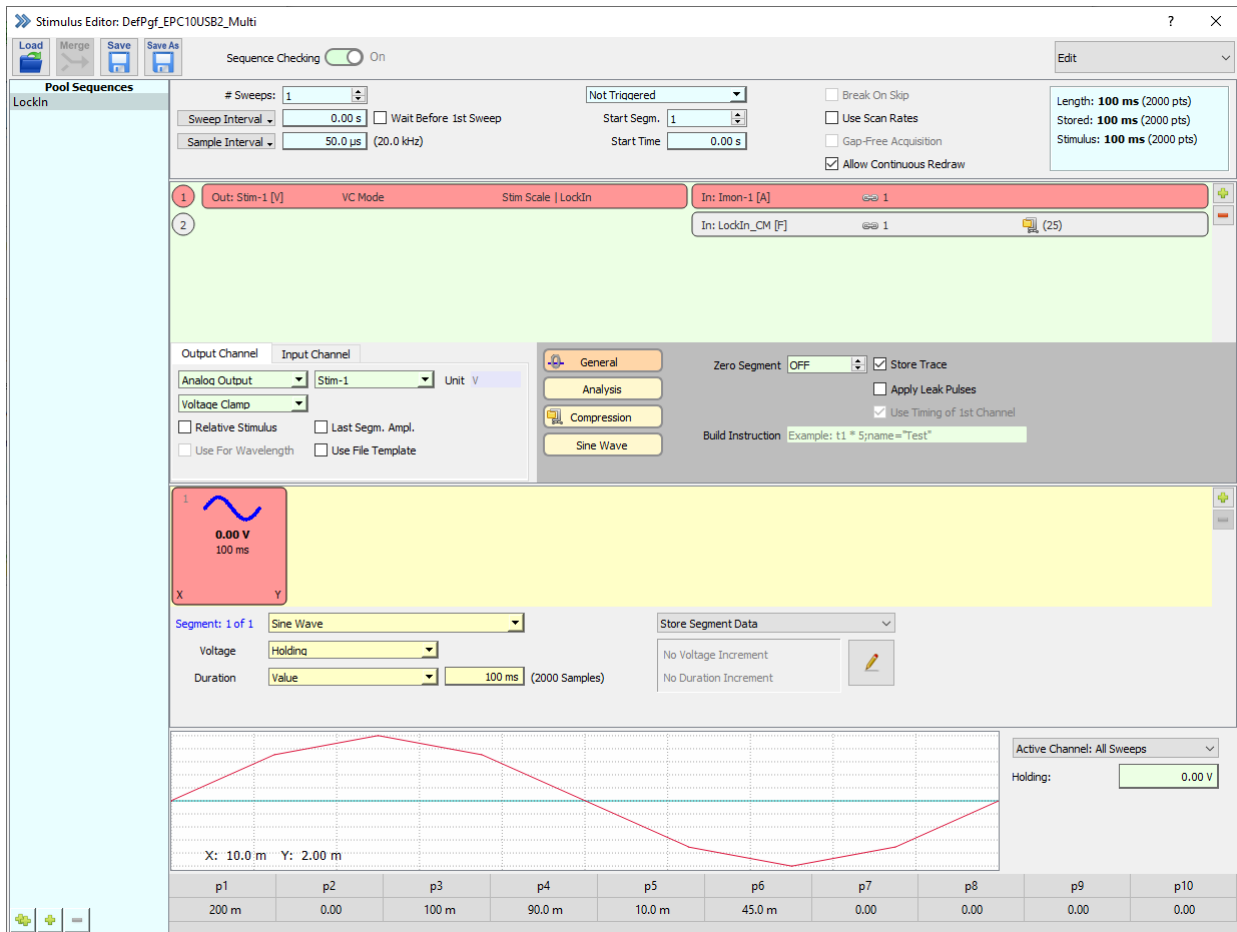


Figure 19.10: Stimulation sequence (Part 3) for LockIn experiments.

Please note, that *Link* (chain symbol) is set to “1”. This tells PATCHMASTER NEXT to use the first input Trace (the current Trace “Imon-1”) for calculating the LockIn information. This point is not crucial as long as you have only one current Trace and one LockIn Trace, however, it is important if you should have more than one current Trace (e.g. if you are using a Double or Triple amplifier).

Store Trace is active for both Traces. It would also be possible to store only the LockIn information and to discard the current data. However, in most cases, it is reasonable to store both Traces.

19.4.4 Amplifier Settings

Now it is time for the experiment and therefore we are executing the following steps.

1. Connect the model circuit and switch it to the “10 M” position to simulate a 10 M Ω pipette that is open to the bath solution.
2. Correct the pipette offset potentials in the Amplifier window by adjusting the V_{pip} Offset value. Alternatively click on the *Auto V_{pip} Offset* button to let PATCHMASTER NEXT do this correction automatically for you. The same is done by calling the protocol “SETUP” in the Test Pulse window.
3. Simulate a pipette sealed to the membrane by switching the model circuit into the middle position. Make an automatic fast capacitance cancellation by clicking on the *Auto C-Fast* or the “SEAL” protocol button in the Test Pulse window.
4. To break into the cell, set the switch of the model circuit to the “0.5 G” position. Make an automatic slow capacitance cancellation by clicking on the *Auto C-Slow* or “WHOLE-CELL” protocol button in the Test Pulse window. With the *V-membrane* control change the pipette holding potential to “-70 mV”.

Now we are ready to run the stimulation sequence we defined before.

19.4.5 Running the Stimulation Sequence

In the Control window we double-click on the “LockIn” sequence button in the *Stimulus Pool* section to execute the stimulation sequence.

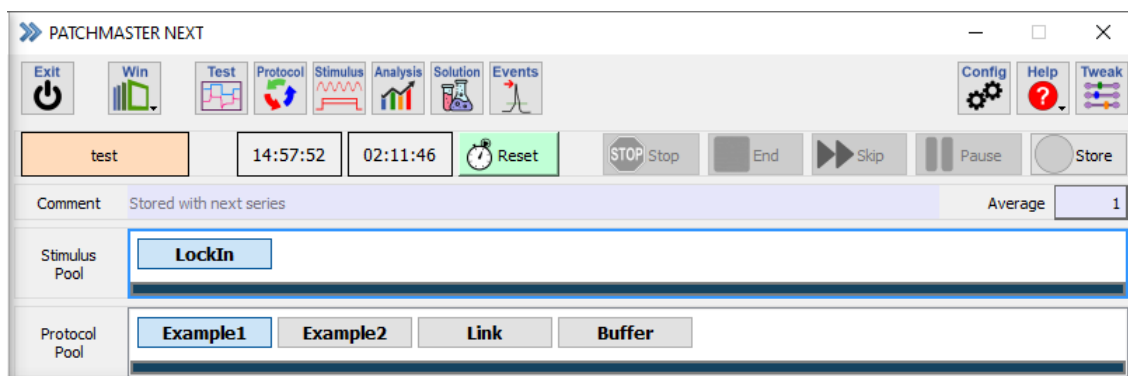


Figure 19.11: Control window of PATCHMASTER NEXT.

The results are displayed in the Oscilloscope window. As defined in the stimulation sequence, you can see the current Trace (black) and a second Trace (red) with the LockIn data.

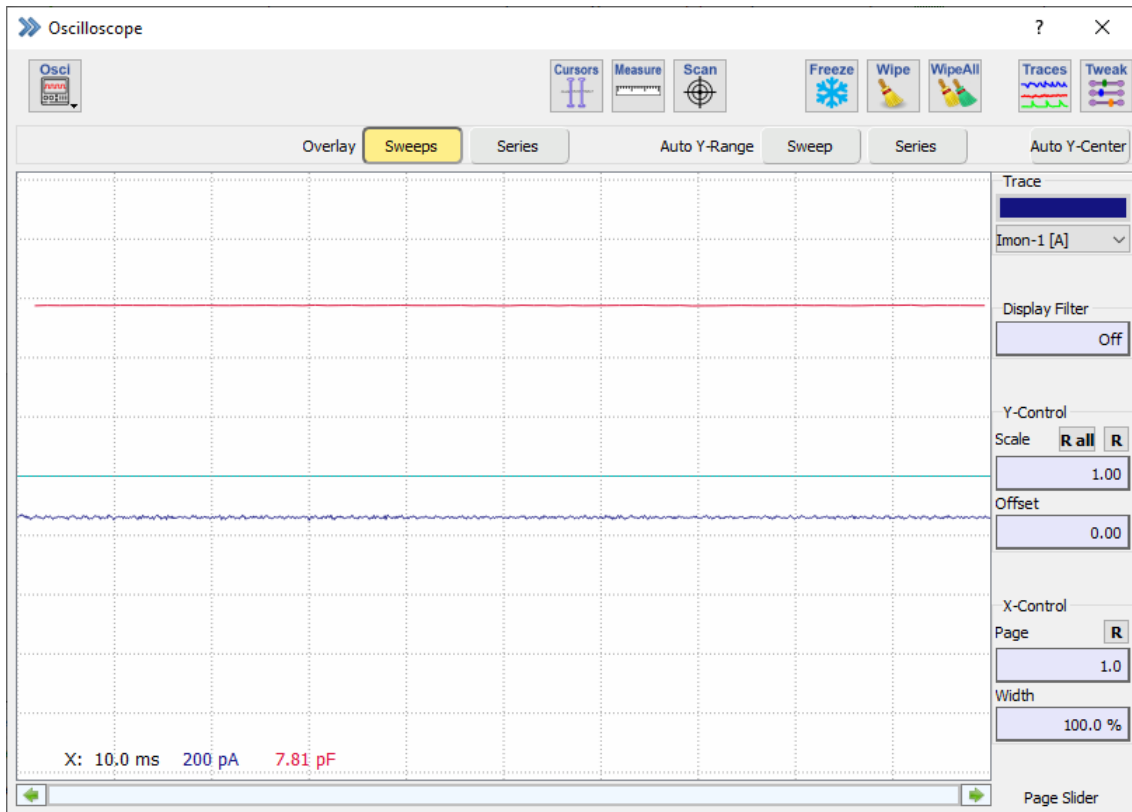


Figure 19.12: Current (blue) and LockIn_CM (red) data Trace measurement.

By default, all data points are connected by lines. If you want to see the individual data points, you can modify the *Trace Properties* in the *Oscilloscope* menu of the *Oscilloscope* window for the capacitance Traces to display marker symbols for each data point.

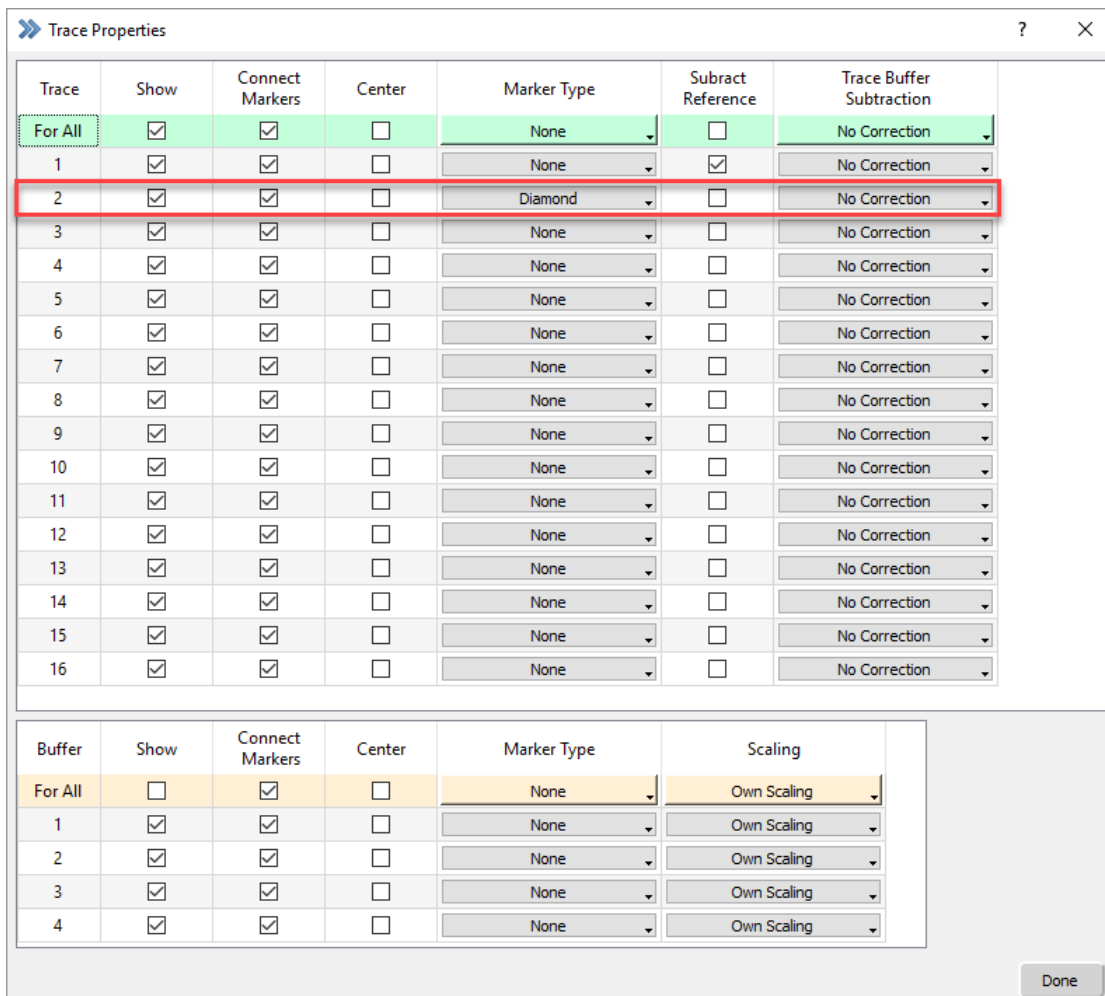


Figure 19.13: Trace Properties setting for the LockIn Trace.

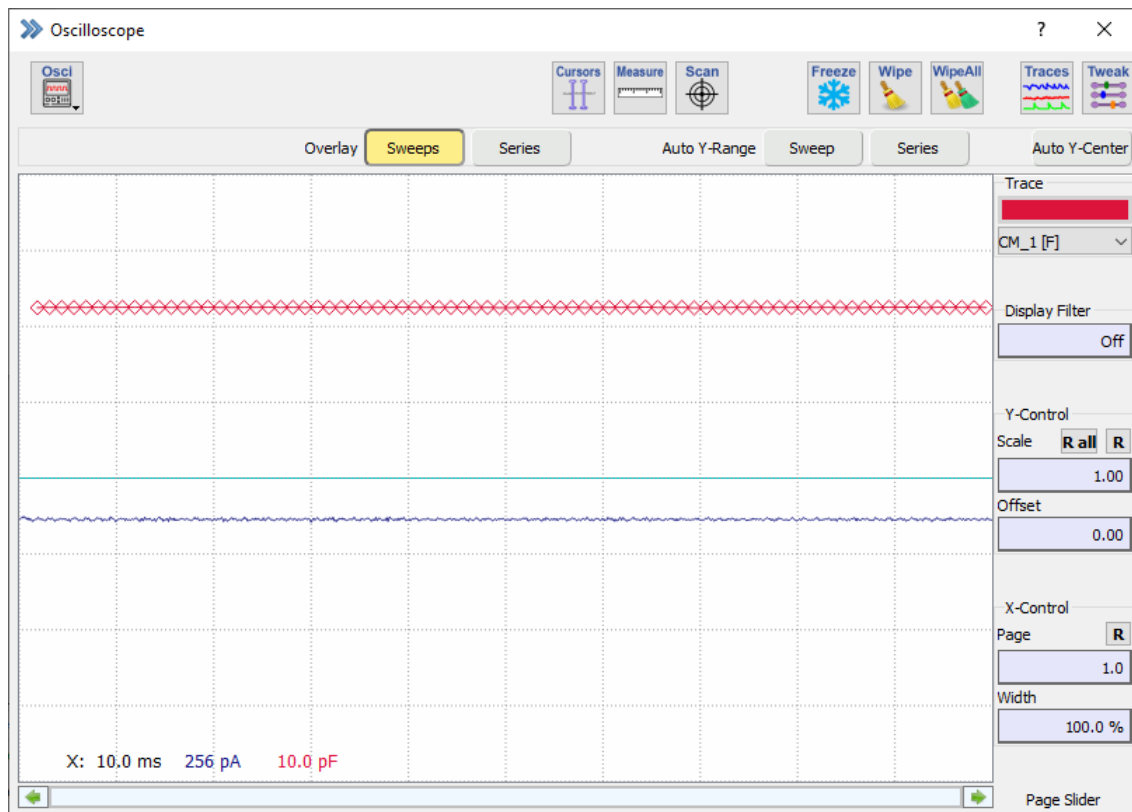


Figure 19.14: Display of the LockIn Trace (red) with markers.

The MC-10 model cell circuit in the “0.5 G” position has a capacitance of 20-22 pF. The calculated C_m values are well within this range which can be seen in the Notebook window (only if *Write to Notebook* is activated in the LockIn Configuration).

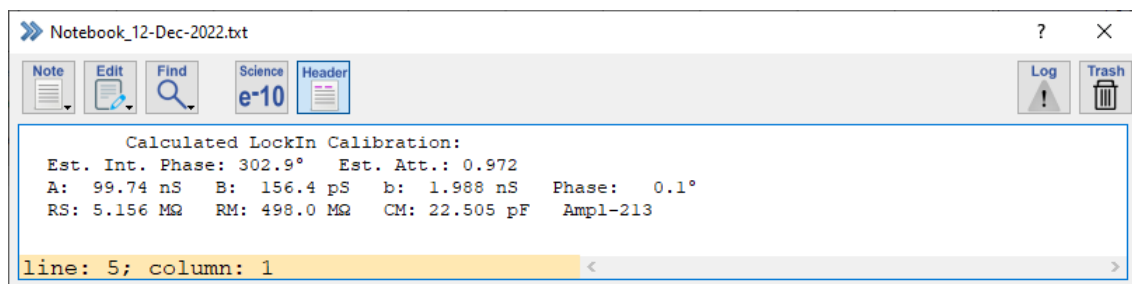


Figure 19.15: Calculated LockIn parameters of the LockIn Extension.

19.4.6 Analysis

In the Oscilloscope window, the LockIn information is displayed with a high time resolution. Even fast membrane capacitance changes can be observed in that way.

However, in some experiments, the expected C_m changes are in the range of several seconds or minutes. In that case, we should use the Analysis Editor to monitor the changes in membrane capacitance. For that purpose, it is necessary to modify the LockIn stimulation sequence. We will increase the # Sweeps from 1 to 100. That is all we have to do for the moment.

Now, we open the Analysis Editor and set the *Stimulus Control* option to “Use Selected Method”. Click on the green “+” button in the Analysis Method section on the left side to create a new *Analysis Method*. Double-click

on it to rename the new *Analysis Method* to “LockIn”. For this new *Analysis Method* we make the following settings:

1. Functions Tab: Click on the already existing *Analysis Function* and press the “Edit” button with the pen symbol to modify it. The *Analysis Functions* dialog will open. First, select *Sweep Count* in the “Timing” column for the X-axis analysis and close the dialog. Then click on the green “+” button of the *Analysis Function* section to add another *Analysis Function*. The *Analysis Function* dialog will open again. Select *LockIn_CM* in the “LockIn” column for Y-axis analysis. Please take care that *Input Trace* for the proper C_M values has to be set to “Analyze Trace 2”.

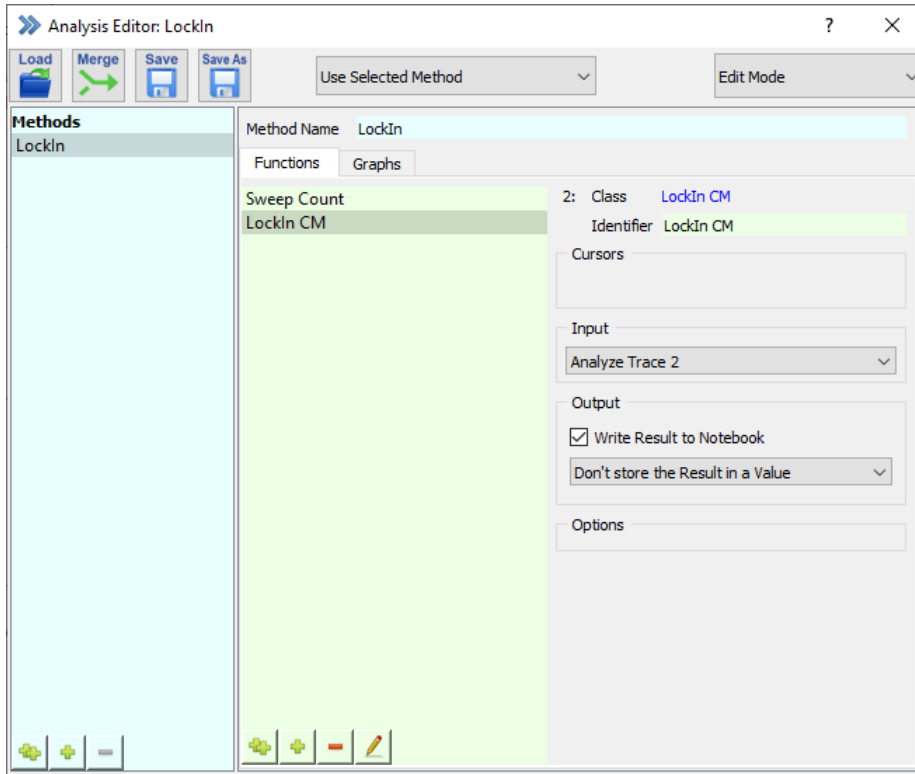


Figure 19.16: Analysis Method for the LockIn measurement.

2. Graphs Tab: Activate *Graph Entry 1* to be able to select the data plotted for the X- and the Y-axis. Select “LockIn_CM_1” vs. “Sweep Count” there.

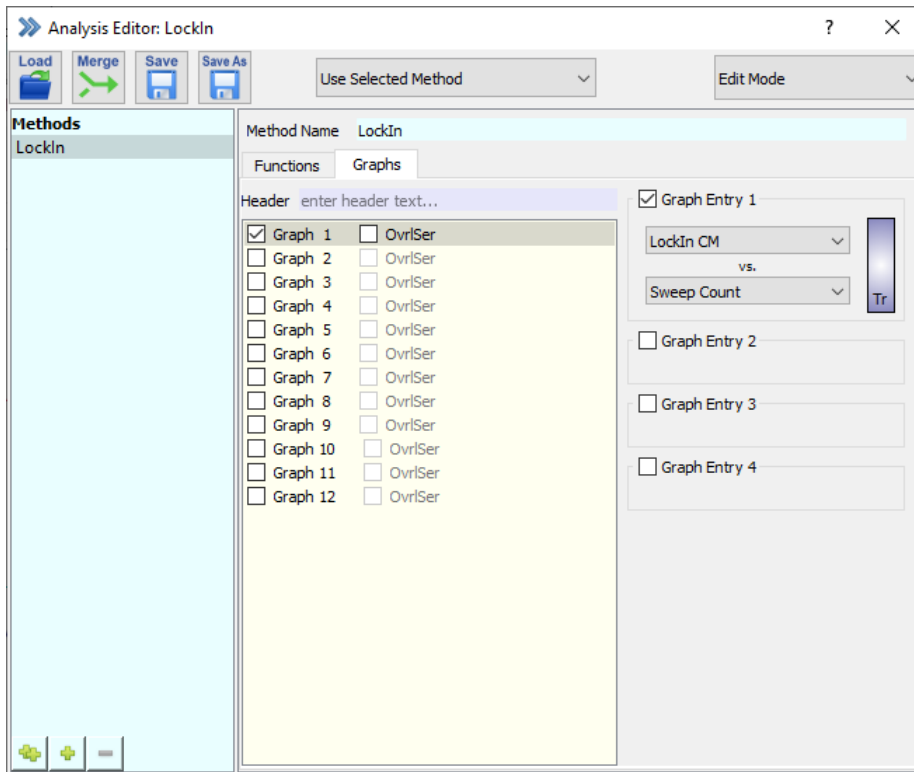


Figure 19.17: Analysis Method (Part 2) for the LockIn measurement.

- Graph Window 1: Next, open the Graph Layout 1 from the `Windows` menu of the Control window. Activate the checkbox "Graph 1" (if not active already) to get the Graph 1 displayed in the graph layout later on. Deselect all other checkboxes to only get one graph displayed in the Graph Layout 1.

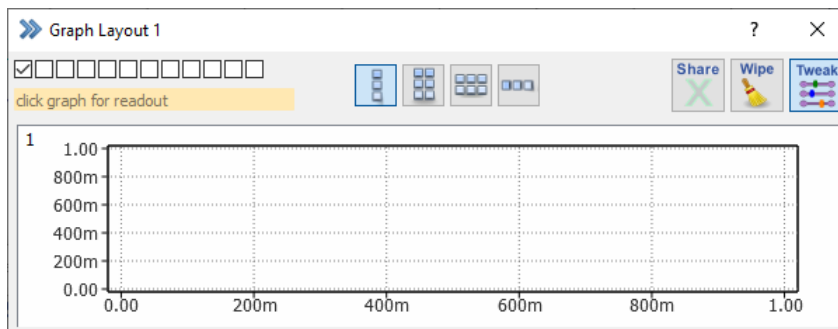


Figure 19.18: Graph Layout 1 settings for the LockIn measurement.

- Make a right-click onto the graph layout and select "Modify Graph" from the menu.

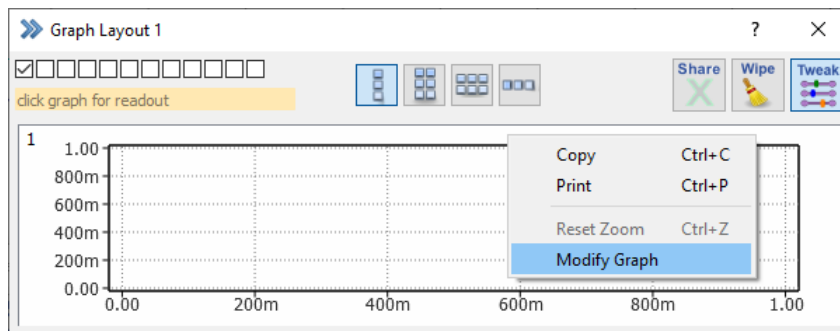


Figure 19.19: Graph Layout 1 settings (part 2) for the LockIn measurement.

5. Select *Manual Scaling* for the Y-axis and enter “10p” to “30p” (10 pF / 30 pF) which are sufficient for our model cell.

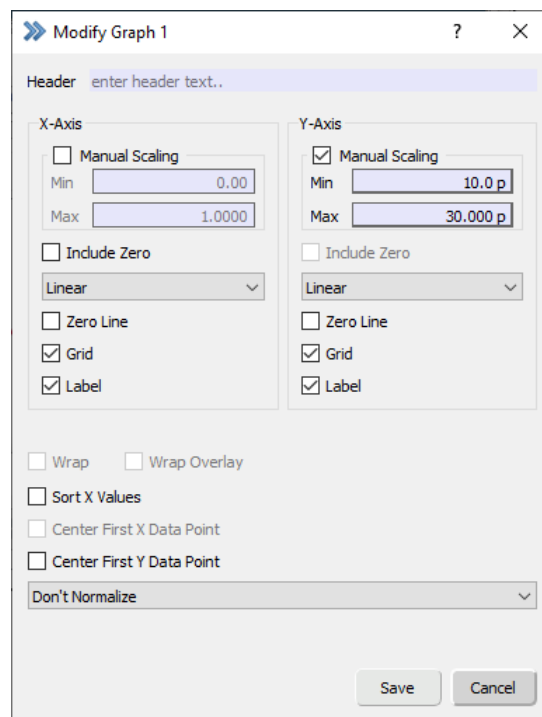


Figure 19.20: Graph Layout 1 settings (part 3) for the LockIn measurement.

If we now run the LockIn stimulation sequence, one LockIn data point per Sweep is plotted in the Graph Layout 1. This single point is the mean value from all C_m values within a Sweep.

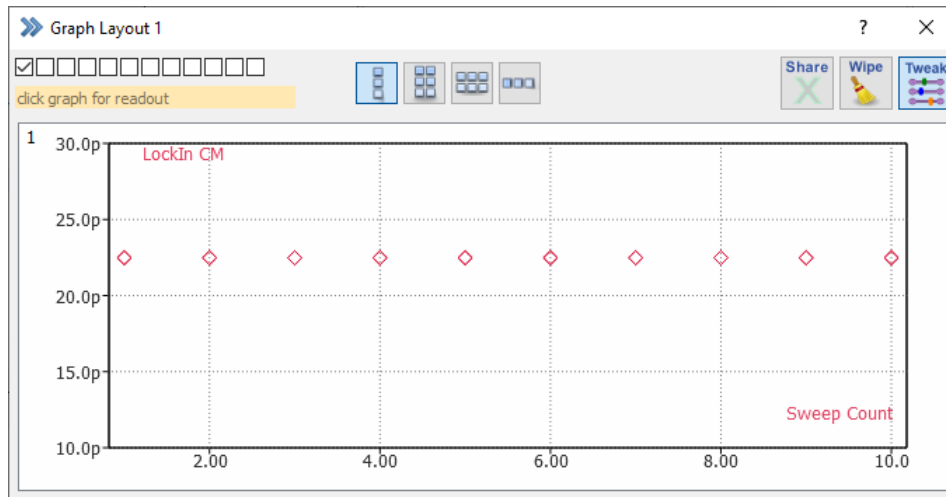


Figure 19.21: Analysis Results of the LockIn experiment.

19.5 Capacitance measurements with Double or Triple amplifiers

We recommend to read the section *Capacitance Measurements – Step by Step* (see 19.4 on page 261) before you go on with this section.

If a multiple amplifier configuration is used, you can use all amplifier boards for LockIn measurements. Go to the LockIn configuration dialog of PATCHMASTER NEXT and set *Generate Traces for:* to "Amplifier 1" and "Amplifier 2".

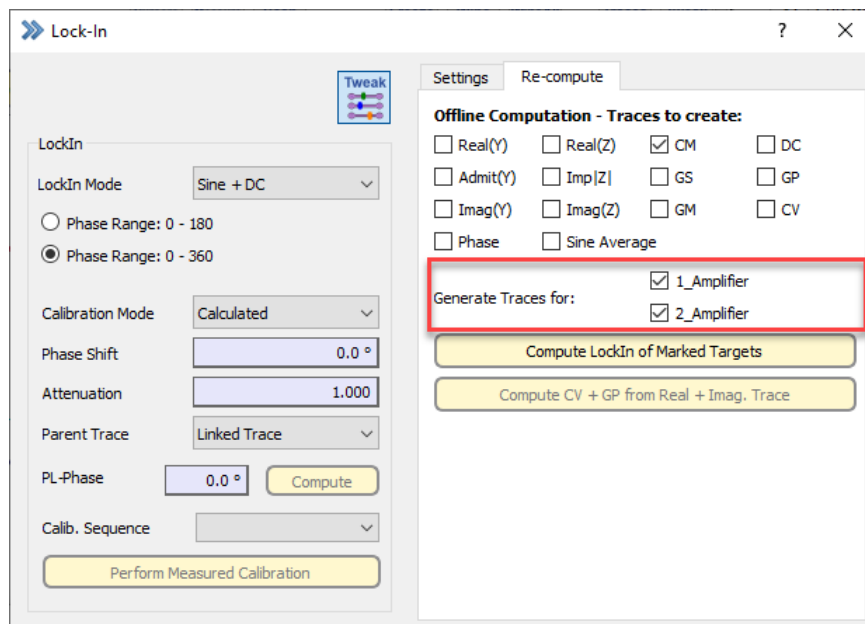


Figure 19.22: LockIn Configuration settings for multiple EPC 10 USB.

For LockIn measurements with multiple amplifiers, we have to add additional Traces to our stimulation sequence. To add the second amplifier (we use an EPC 10 USB DOUBLE here), we click again on the right “plus” sign button to add another channel. In the upcoming dialog we select the DA output channel *Stim-2*, which is the stimulus output of the second amplifier board. Furthermore, we set the AD input channels *Imon-2* (current channel of amplifier 2) and another virtual Trace (*LockIn_CM*). These additional settings are in analogy to the settings made

in chapter 19.4.3 on page 262.

Now, let's have a look on the *Link* setting in the *Analysis* section of the stimulation sequence:

1. Channel 1: AD channels are always linked to a DA output channel. Therefore *Imon-1* is linked to the corresponding *Stim-1* DA output. Set the *Link* to "1".
2. Channel 2: LockIn channels are "virtual" Traces. The data of a virtual Trace does not depend on a DA output, but is calculated from another AD channel. This *LockIn_CM* channel uses *Imon-1* as data input, therefore the *Link* is set to "1".
3. Channel 3: *Imon-2* is linked to the corresponding *Stim-2* DA output channel of the second amplifier board. Set the *Link* to "3".
4. Channel 4: This is a "virtual" channel and therefore is linked to "its" current channel (*Imon-2*). Set the *Link* to "3".

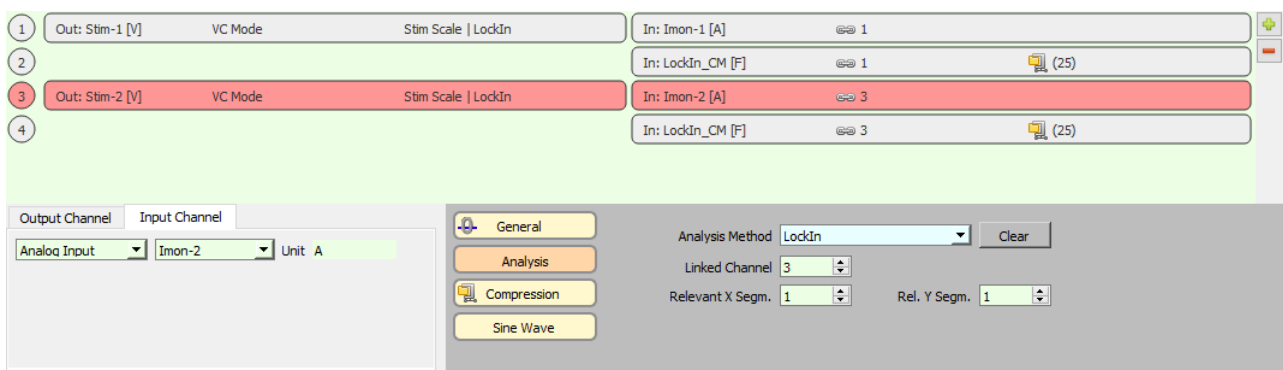


Figure 19.23: LockIn channel settings in the stimulation sequence for multiple amplifiers.

After you execute the new stimulation sequence you get the following Traces in the Data Tree:

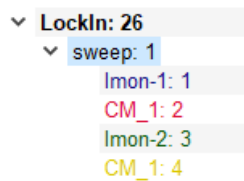


Figure 19.24: Recorded Traces of the LockIn measurement.

Each Sweep contains two current Traces (*Imon-1*, *Imon-2*) and two LockIn capacitance Traces (*CM_1*).

Of course, we want to monitor the measures C_M values in the Graph Layout 1. Therefore, we have to modify the Analysis Editor settings. The required changes are quite moderate:

The X-axis for both C_M Traces are identical which means that we use again *Sweep Count*, but for the Y-axis, we need a new *Analysis Function*. Add another *Analysis Function* and select *LockIn_CM* in the *Analysis Function Type* dialog. It is now important to set the *Input* to "Analyze Trace 4". The *Analysis Functions* 2 and 3 are now renamed (*Identifier*) to "LockIn CM_2" and "LockIn CM_4", respectively.

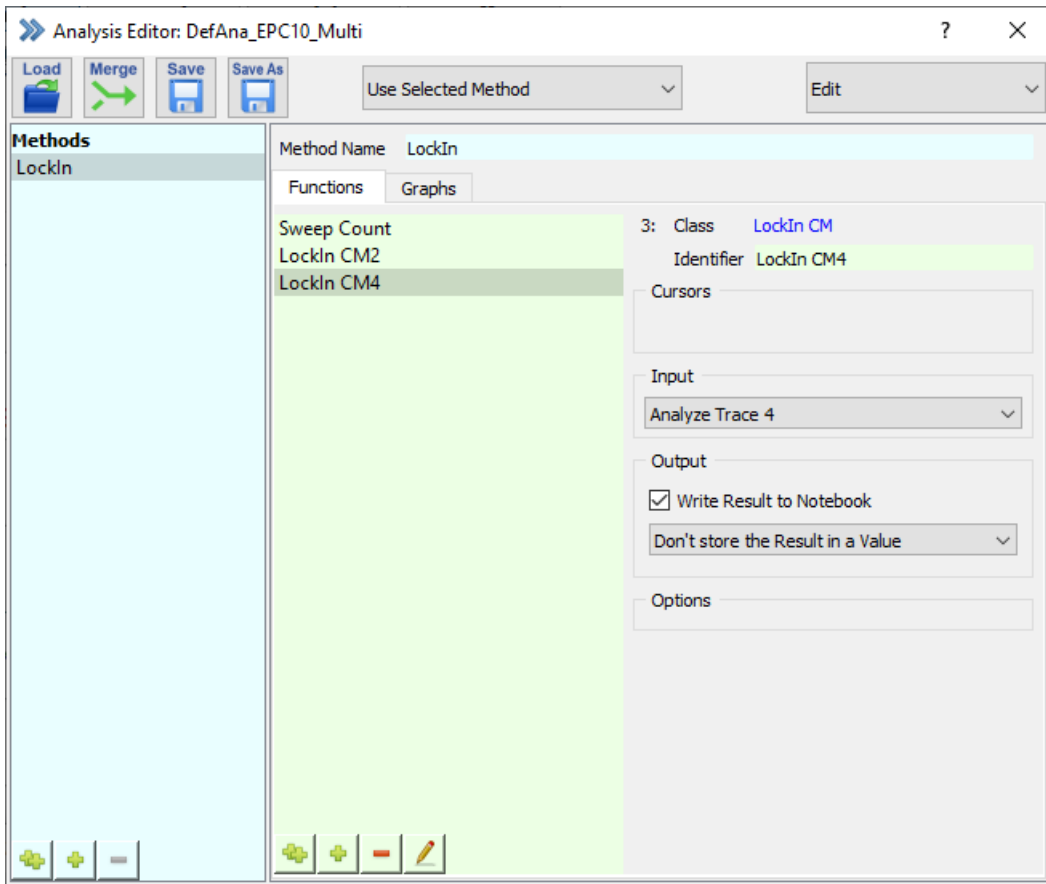


Figure 19.25: Analysis Functions settings for multiple capacitance measurements.

The C_M Trace from the second amplifier is intended to be displayed in the same window. Therefore, we activate an additional *Graph Entry* for *Graph 1* and set “LockIn CM_4” vs. “Sweep Count”.

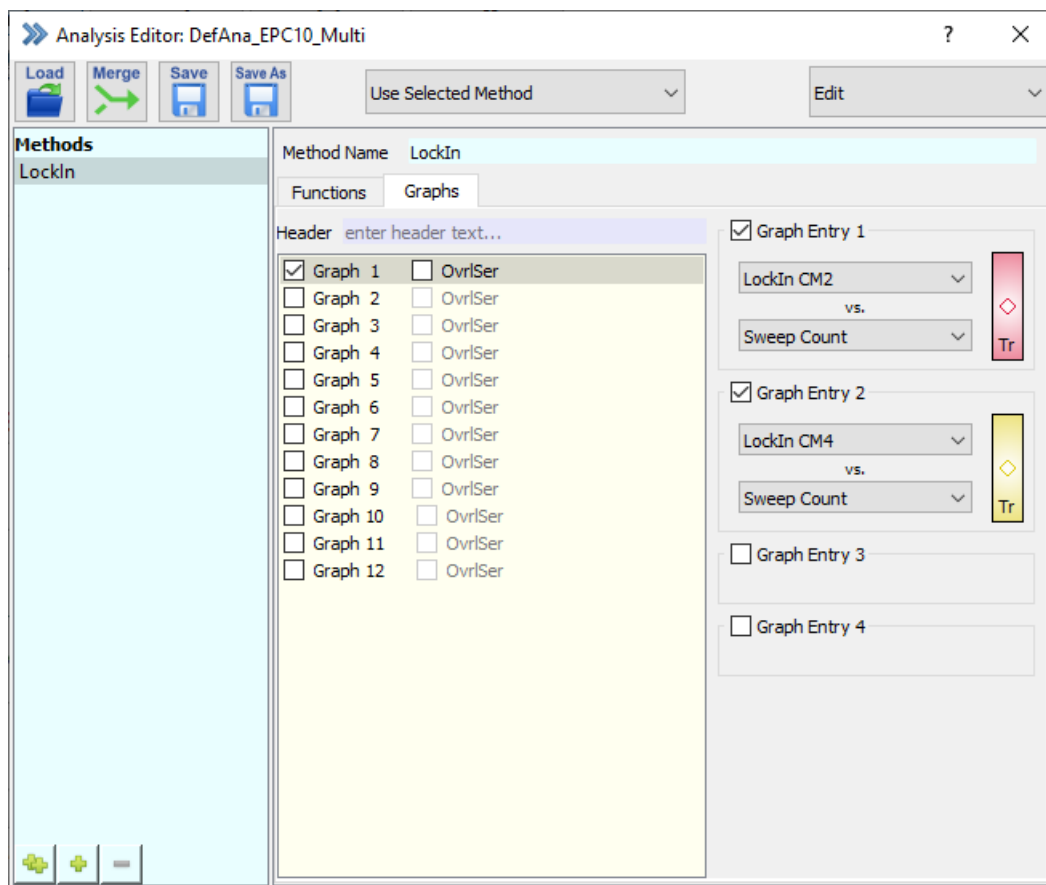


Figure 19.26: Analysis Graph settings for multiple capacitance measurements.

Before starting the PGF sequence, you should make all required settings for both amplifiers as described in chapter 19.4.4 on page 266 and 19.4.5 on page 266.

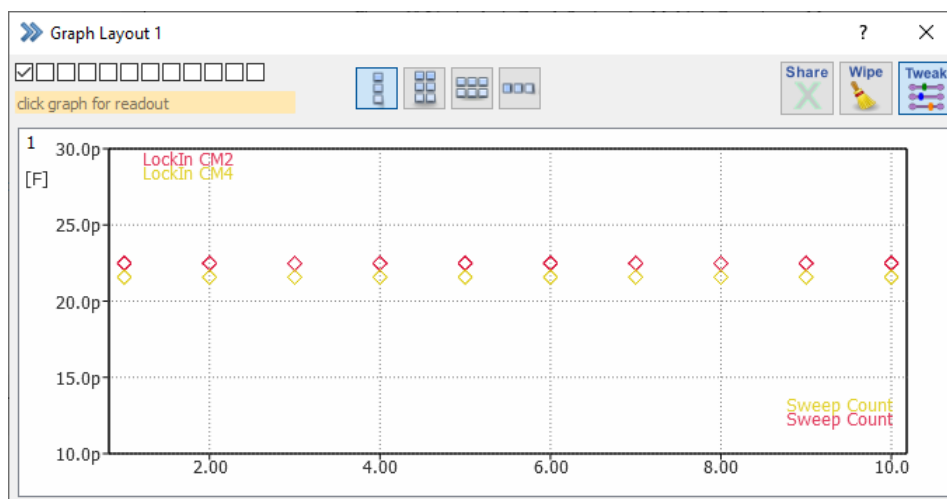


Figure 19.27: Analysis Results of the LockIn experiment with multiple amplifiers.

Note: If you want to enter fixed values for the X- or Y-scale, you should take care to enter "10 p" and not just "10", otherwise the display range will be set to 10-30 F, instead of 10-30 pF.

19.6 Using LockIn with amplifiers other than EPC 10 USB

The following are important points to keep in mind when using LockIn with an amplifier other than an EPC 10 USB.

LockIn Configuration: “Measured” must be selected as the *Calibration Mode* in the LockIn Configuration dialog. Capacitance compensation must be switched “Off” and the *C-Slow* parameter must be set to “0.0” in the I/O Control window when a *Measured Calibration* is performed.

Note: As discussed in chapter 19.4.2 on page 261, any change of a “critical parameter”, e.g. frequency of sinusoid, external filter setting, stimulus time constant, requires that a new *Measured Calibration* be made. There is no way for LockIn to “know” if you change a critical parameter, so it is up to the user to ensure that the calibration remains valid.

Use of Series Resistance Compensation: The use of the series resistance compensation is **not** supported by the software and will produce erroneous results.

Capacitance Compensation: *C-Slow* compensation is supported. Compensated values (*C-Slow*, *R-Series*) are entered in the LockIn Configuration dialog as default *Parameter* values or via the I/O Control window.

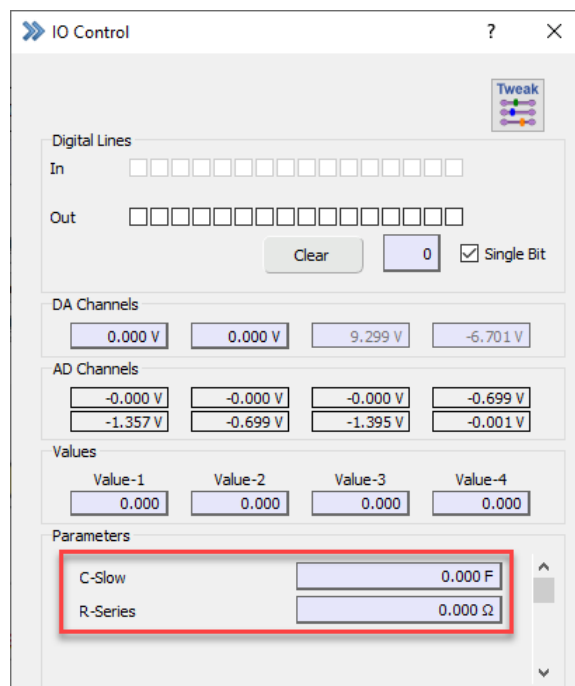


Figure 19.28: IO Control window for setting C-Slow and R-Series values of the measured calibration.

If capacitance compensation is “Off”, enter a value of “0.0” for C-slow (not for R-series). The R-series value always has to be specified.

If your patch clamp amplifier is calibrated to give *G-Series* rather than *R-Series* values (e.g. EPC 7), you must invert the value and enter it as *R-Series*.

Note: It is up to the user to ensure that *R-Series* and *C-Slow* values always reflect the current settings of the patch clamp amplifier.

20 Photometry Extension

If one wants to use the Photometry extension with an EPC 10 USB QUADRO amplifier an additional LIH 8+8 is needed to provide the excitation and emission channels.

After starting PATCHMASTER NEXT, go to the Configuration dialog in the Control window and select the appropriate light source in the *Hardware* section of the dialog to activate the Photometry Extension (see chapter 3.5.2.3 on page 25).

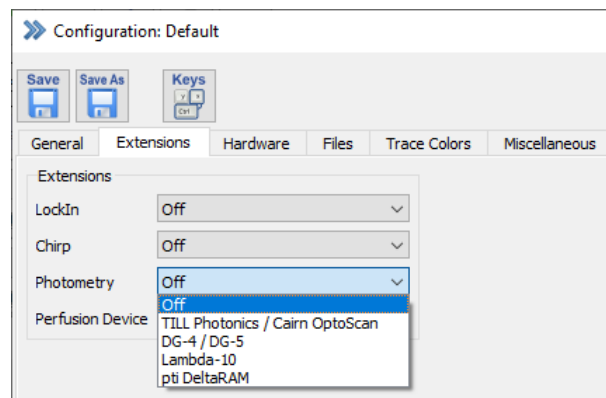


Figure 20.1: Enable the Photometry extension in the Configuration.

The options are:

- TILL Photonics / Cairn OptoScan
- DG4 / DG5
- Lambda-10
- PTI DeltaRAM

In the following sections we provide a brief overview of the settings for each provided light source.

20.1 TILL Photonics / Cairn OptoScan

This dialog can be used to define the settings for either the Polychrome devices from TILL Photonics or the Cairn OptoScan system.

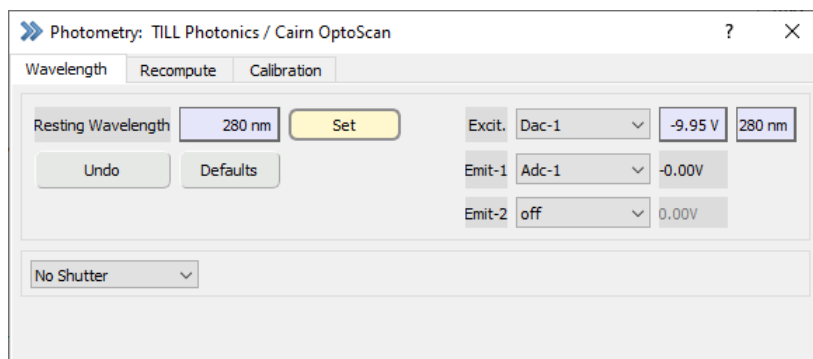


Figure 20.2: TILL Photonics/Cairn OptoScan light source setting dialog of the Photometry Extension.

20.1.1 Wavelength

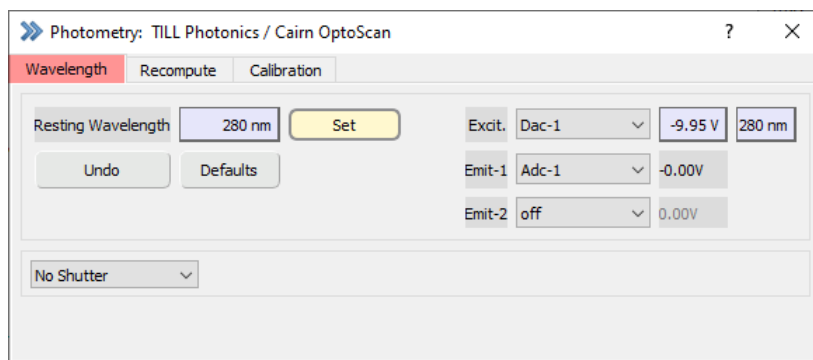


Figure 20.3: Wavelength settings of the TILL Photonics/Cairn OptoScan light source.

Resting Wavelength: Enter the wavelength which should be set during resting periods.

Set: Only after pressing the *Set* button the wavelength entered in the *Resting Wavelength* field is output.

Excit.: Select the DA channel for the voltage output. This function is used to control the monochromator. Once it is calibrated, the output can be defined either in Volts (left) or in nm (right).

Emit-1, Emit-2: Select the AD channels for sampling the photometry responses. *Emit-1* is always active whereas *Emit-2* can be turned off. It may be used for dual emission fluorescence experiments.

Shutter Selection:

There are three *Shutter Selections* available:

- No Shutter: No shutter
- Shutter Control: Shutter can be closed/opened via a button
- Digital-out 7: Shutter control via digital output

20.1.2 Recompute

Figure 20.4: Recompute settings of the TILL Photonics/Cairn OptoScan light source.

R-max: Enter the maximum ration value ($F1/F2$) here to subtract it from your measurements.

R-min: Enter the minimum ration value ($F1/F2$) here to subtract it from your measurements.

Kd*Sf: Enter the effective Kd value (dissociation constant) of the fluorescence dye.

Note: These values are required for calculating the free calcium concentration (according to the Grynkiewicz formalism). The calcium concentration can be stored as a Trace by use of the "Photo_Ca" input channel in the Stimulus Editor dialog.

Background 1...3: Enter the background fluorescence value for each of the used wavelengths to exclude any auto-fluorescence from your measurements.

*Note: The parameters R-max, R-min, Kd*Sf and Background 1...3 are stored as "Series Parameters" with the raw data file and are listed in the Data Parameter window (see figure below).*

Figure 20.5: Photometry parameters are stored in the Series record of the Data Tree and can be accessed by the Data Parameter window.

Dead Time: Time to allow for settling of the commanded wavelength before starting the image exposure.

Compute Traces of Marked Targets: This function allows to recompute stored "Photo" Traces (Photo_W1, Photo_W2, Photo_W3, Photo_R, and Photo_Ca) with modified fluorescent background values.

20.1.3 Calibration

Before starting the calibration please select the correct Polychrome device from the drop-down menu, either "Polychrome I - IV" or "Polychrom V (linear)".

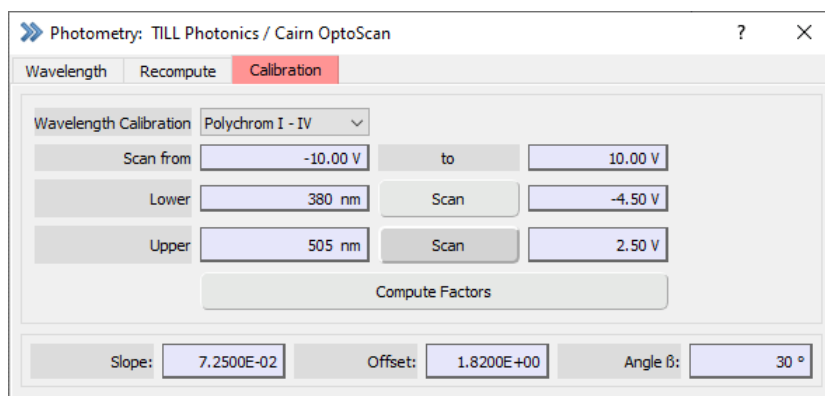


Figure 20.6: Calibration settings of the TILL Photonics/Cairn OptoScan light source.

Slope, *Offset* and *Angle* are internal calibration constants used to control the monochromator. These values are the result of the calibration procedure of the Polychrome unit.

The conversion between voltages and wavelength is done as follows:

$$F(\lambda) = \arcsin(\lambda/1666.67 * \cos(\pi/360 * \beta)) \quad (20.1)$$

$$V = (2\pi * F(\lambda) - Offset)/Slope \quad (20.2)$$

$$\lambda = (1666.67 * \cos(\pi/360 * \beta) * \sin((Slope * V + Offset)/2\pi)) \quad (20.3)$$

$$Slope = 2\pi * (F(\lambda_1) - F(\lambda_2))/(V_1 - V_2) \quad (20.4)$$

$$Offset = 2\pi * F(\lambda_1) - Slope * V_1 \quad (20.5)$$

with

- β = typically 30 degree; depends on your hardware. Please refer to the manual of the monochromator.
- λ = requested wavelength in [nm]; λ_1 = calibration wavelength 1.
- V = DA voltage in [V]; V_1 = DA voltage of calibration wavelength 1.
- arguments of trigonometry functions in [RAD].

Here an example: We calibrate our monochromator at the wavelength 380 nm and 505 nm. With the 380 nm and 505 nm filters we measure an intensity maximum at -4.5 V and 2.5 V, respectively. Now, we calculate $F(\lambda)$ for both wavelength: $F(\lambda_1) = 0.2383$, $F(\lambda_2) = 0.3191$. Then the *Slope* is 0.0725 and the *Offset* is 1.8235.

20.2 DG-4/DG-5

In this dialog the settings are made for the DG-4/DG-5 light source.

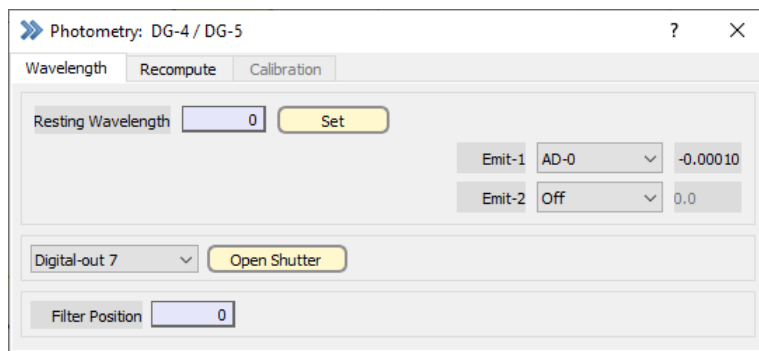


Figure 20.7: DG-4/DG-5 light source setting dialog of the Photometry Extension.

20.2.1 Wavelength

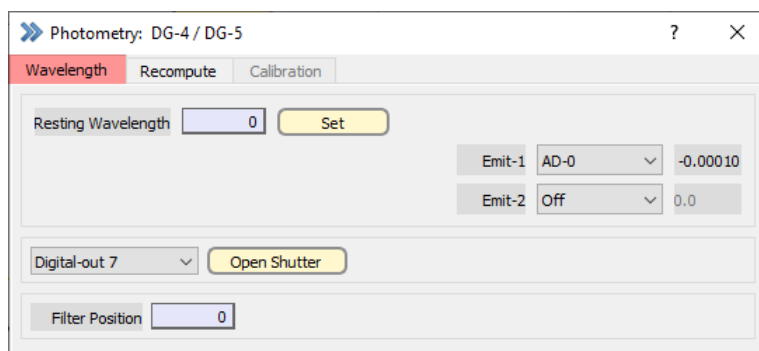


Figure 20.8: Wavelength settings of the DG-4/DG-5 light source.

Resting Wavelength: Set the *Filter Position* which should be used to set the *Resting Wavelength*.

Set: Only after pressing the *Set* button the *Filter Position* entered in the *Resting Wavelength* field is output.

Emit-1, Emit-2: Select the AD channels for sampling the photometry responses. *Emit-1* is always active whereas *Emit-2* can be turned off. It may be used for dual emission fluorescence experiments.

Shutter Open/Close: Toggles between an open and a closed shutter.

Shutter Selection:

- No Shutter: No shutter available
- Shutter Control: Shutter can be closed/opened via a button
- Digital-out 7: Shutter control via digital output

Filter Position: Enter the number of the *Filter Position* (0 - 15).

Note: To control the DG-4/DG-5 or Lambda-10 via the PATCHMASTER NEXT software you need a special cable connecting the Digital Outputs of the interface and the DG-4/DG-5 or Lambda-10 (Order Number: 895102). Please contact HEKA (sales@heka.com) if you need such a cable.

20.2.2 Recompute

Figure 20.9: Recompute settings of the DG-4/DG-5 light source.

R-max: Enter the maximum ration value ($F1/F2$) to subtract it from your measurements.

R-min: Enter the minimum ration value ($F1/F2$) to subtract it from your measurements.

Kd*Sf: Enter the effective Kd value (dissociation constant) of the fluorescence dye.

Note: These values are required for calculating the free calcium concentration (according to the Grynkiewicz formalism). The calcium concentration can be stored as a Trace by use of the "Photo_Ca" input channel in the Stimulus Editor dialog.

Background 1...3: Enter the background fluorescence value for each of the used wavelengths to exclude any auto-fluorescence from your measurements.

Note: The parameters R-max, R-min, Kd*Sf and Background 1...3 are stored as "Series Parameters" with the raw data file and are listed in the Data Parameter window (see figure below).

Figure 20.10: Photometry parameters are stored in the Series record of the Data Tree and can be accessed by the Data Parameter window.

Dead Time: Time to allow for settling of the commanded wavelength before starting the image exposure.

Compute Traces of Marked Targets: This function allows to recompute stored "Photo" Traces (Photo_W1, Photo_W2, Photo_W3, Photo_R, and Photo_Ca) with modified fluorescent background values.

20.2.3 Stimulus Editor Sequence

When using the DG-4/DG-5 or Lambda-10 you need to define the *Filter Position* in your stimulation sequence. Therefore you have to set the DA output channel to *Dig Out (word)*. Then you can enter the *Filter Position* directly in the segments (Wavelength) of the stimulation sequence.

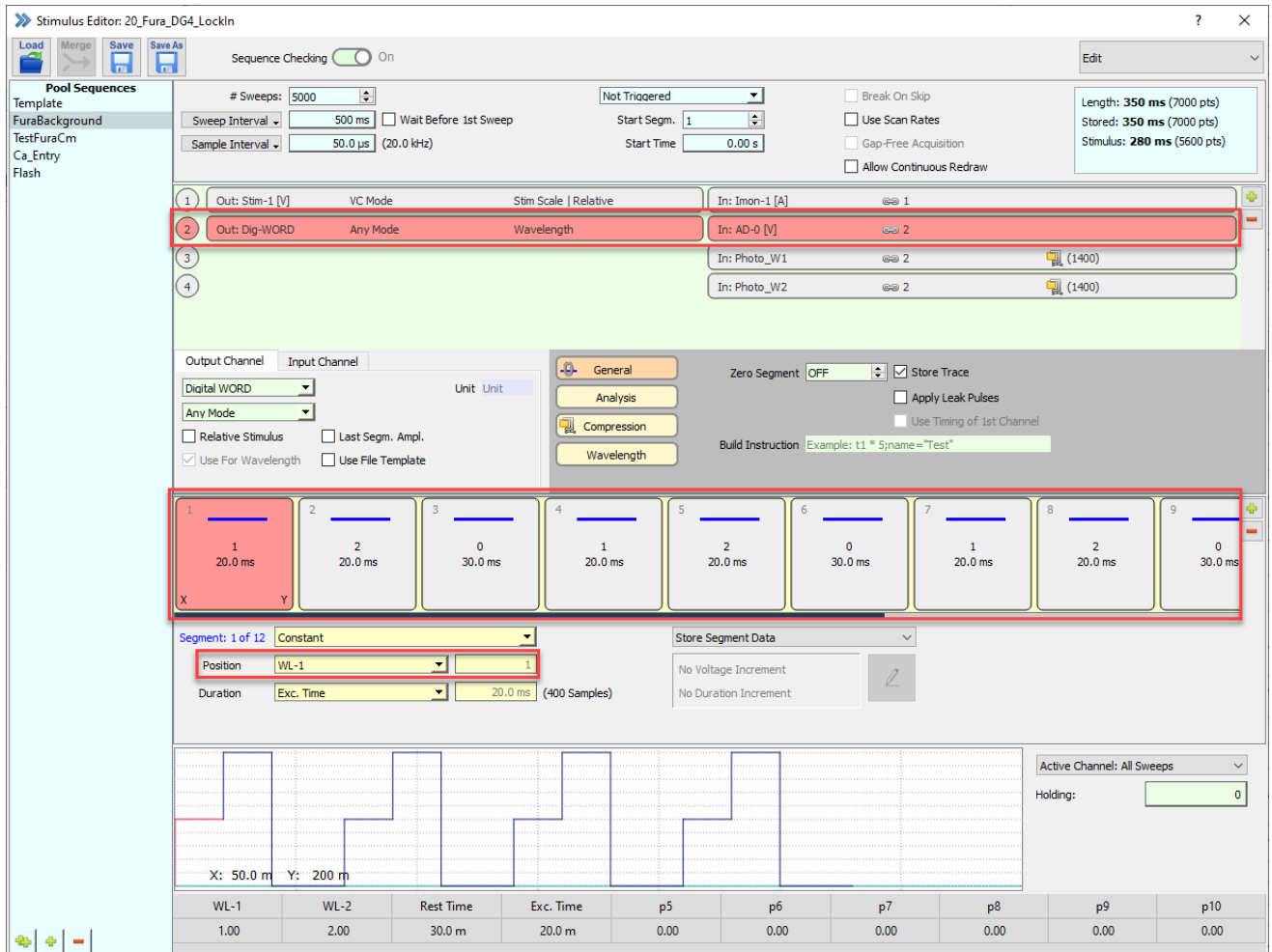


Figure 20.11: Example of a stimulation sequence for DG-4/DG-5 or Lambda-10 light source.

20.3 Lambda-10

In this dialog the settings are made for the Lambda-10 light source.

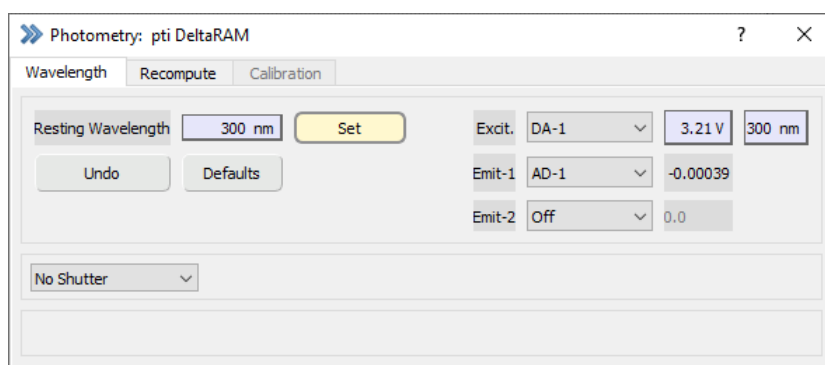


Figure 20.12: DG-4/DG-5 light source setting dialog of the Photometry Extension.

20.3.1 Wavelength

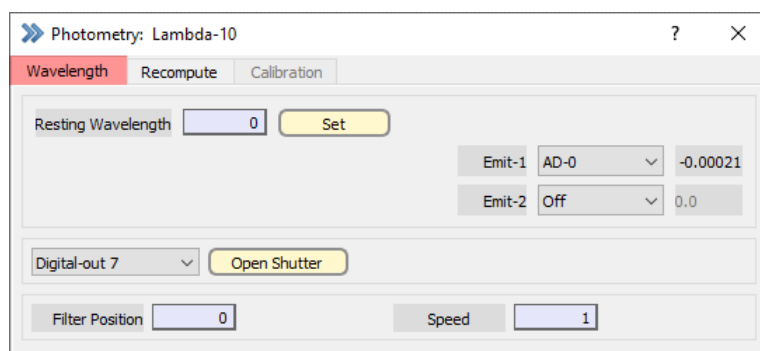


Figure 20.13: Wavelength settings of the Lambda-10 light source.

Resting Wavelength: Set the *Filter Position* which should be used to set the *Resting Wavelength*.

Set: Only after pressing the *Set* button the wavelength entered (*Filter Position*) in the *Resting Wavelength* field is output.

Emit-1, Emit-2: Select the AD channels for sampling the photometry responses. *Emit-1* is always active whereas *Emit-2* can be turned off. It may be used for dual emission fluorescence experiments.

Shutter Open/Close: Toggles between an open and a closed shutter.

Shutter Selection:

- No Shutter: No shutter available
- Shutter Control: Shutter can be closed/opened via a button
- Digital-out 7: Shutter control via digital output

Filter Position: Enter the number of the *Filter Position* (0 - 15).

Speed: Enter a number between 0 and 7 for the speed setting.

Note: To control the DG-4/DG-5 or Lambda-10 via the PATCHMASTER NEXT software you need a special cable connecting the Digital Outputs of the interface and the DG-4/DG-5 or Lambda-10 (Order Number: 895102). Please contact HEKA (eimeaorderscmt@harvardbioscience.com) if you need such a cable.

20.3.2 Recompute

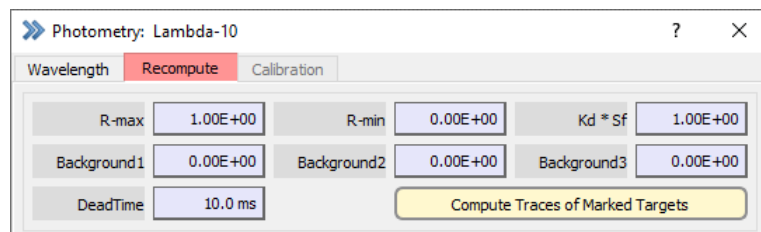


Figure 20.14: Recompute settings of the Lambda-10 light source.

R-max: Enter the maximum ration value ($F1/F2$) to subtract it from your measurements.

R-min: Enter the minimum ration value ($F1/F2$) to subtract it from your measurements.

Kd*Sf: Enter the effective Kd value (dissociation constant) of the fluorescence dye.

Note: These values are required for calculating the free calcium concentration (according to the Grynkiewicz formalism). The calcium concentration can be stored as a Trace by use of the "Photo_Ca" input channel in the *Stimulus Editor* dialog.

Background 1...3: Enter the background fluorescence value for each of the used wavelengths to exclude any auto-fluorescence from your measurements.

Note: The parameters R-max, R-min, Kd*Sf and Background 1...3 are stored as "Series Parameters" with the raw data file and are listed in the *Data Parameter* window (see figure below).

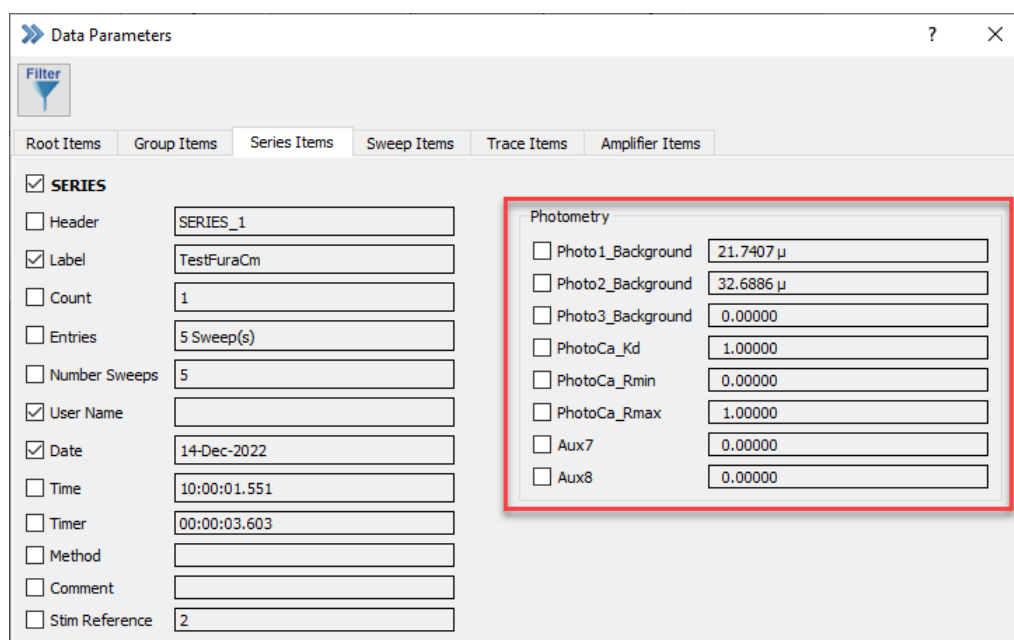


Figure 20.15: Photometry Parameters are stored in the Series record of the Data Tree and can be accessed by the Data Parameter window.

Dead Time: Time to allow for settling of the commanded wavelength before starting the image exposure.

Compute Traces of Marked Targets: This function allows to recompute stored "Photo" Traces (Photo_W1, Photo_W2, Photo_W3, Photo_R, and Photo_Ca) with modified fluorescent background values.

20.3.2.1 Lambda 10-3

To control the shutter and the filter wheel via the PATCHMASTER NEXT software (Stimulus Editor sequence or Photometry dialog) you need to enable "TTL Input" at the Lambda 10-3 controller.

If the "TTL Input" option at the Lambda 10-3 is disabled it is not possible to use the Photometry extension to control the monochromator or the shutter. In such a case there would be the option to activate the "Conditional Mode" of the Lambda 10-3. Therefore, open the I/O Control dialog and enter the bit "299" to activate it (deselect *Single Bit* first). This means the shutter closes automatically when the filter wheel is moved and opens again when the filter wheel movement has finished.

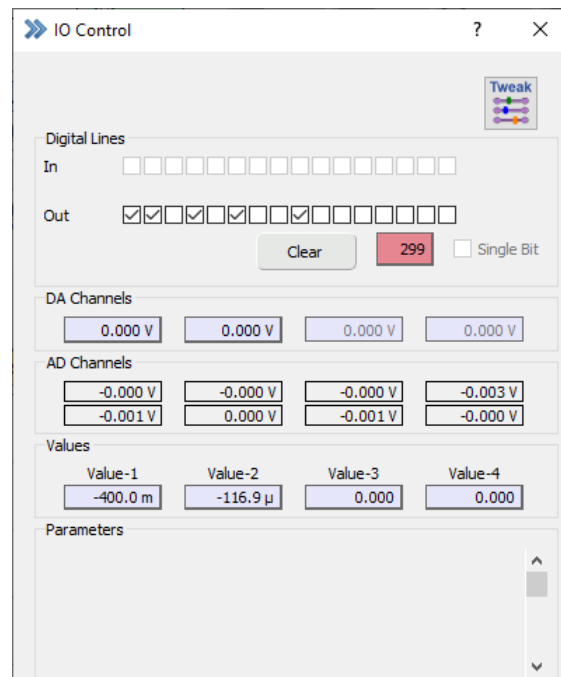


Figure 20.16: I/O Control window setting of PATCHMASTER NEXT.

To switch off the “Conditional Mode” you need to send either bit “298” or bit “300”. Bit “298” disables the “Conditional Mode” but leaves the shutter open. Bit “300” disables the “Conditional Mode” but closes the shutter.

20.3.3 Stimulus Editor Sequence

When using the DG-4/DG-5 or Lambda-10 you need to define the *Filter Position* in your stimulation sequence. Therefore you have to set the DA output channel to *Dig Out (word)*. Then you can enter the *Filter Position* directly in the segments (Wavelength) of the stimulation sequence.

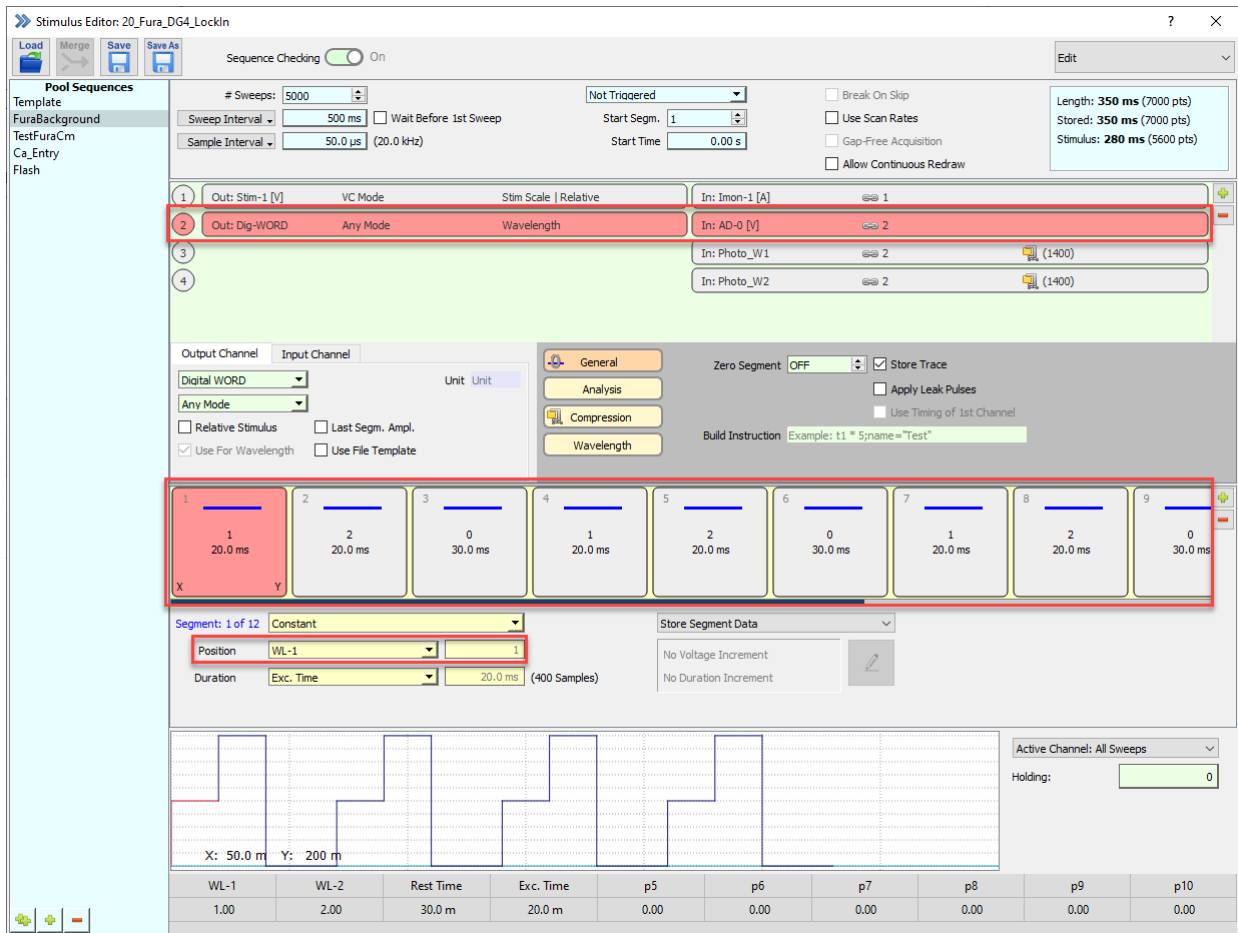


Figure 20.17: Example of a stimulation sequence for DG-4/DG-5 or Lambda-10 light source.

20.4 PTI DeltaRAM

In this dialog the settings are made for the PTI DeltaRAM light source.

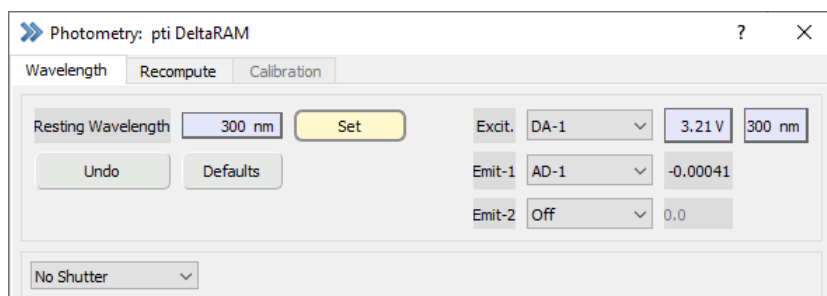


Figure 20.18: PTI DeltaRAM light source setting dialog of the Photometry Extension.

20.4.1 Wavelength

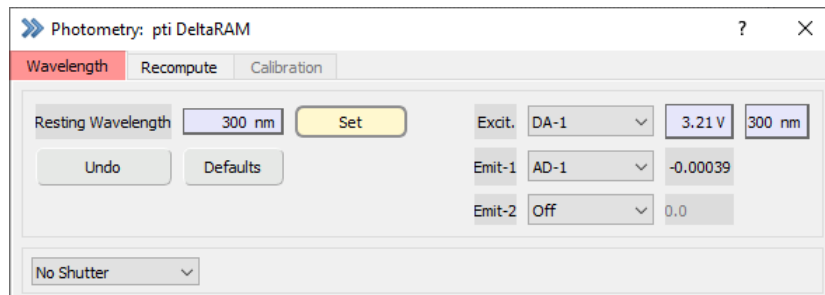


Figure 20.19: Wavelength settings of the PTI DeltaRAM light source.

Resting Wavelength: Wavelength to be set during resting periods.

Set: Only after pressing the *Set* button the wavelength entered in the *Resting Wavelength* field is output.

Excit.: Select the DA channels for the voltage output. This function is used to control the monochromator. Once it is calibrated, the output can be defined either in [V] (left) or in [nm] (right).

Emit-1, Emit-2: Select the AD channels for sampling the photometry responses. *Emit-1* is always active whereas *Emit-2* can be turned off. It may be used for dual emission fluorescence experiments.

Shutter Selection:

- No Shutter: No shutter available
- Shutter Control: Shutter can be closed/opened via buttons
- Digital-out 7: Shutter control via digital output

20.4.2 Recompute

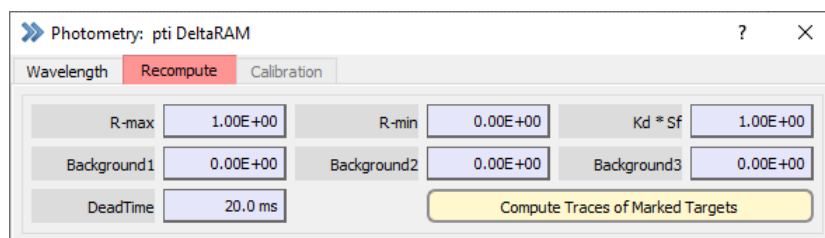


Figure 20.20: Recompute settings of the PTI DeltaRAM light source.

R-max: Enter the maximum ration value ($F1/F2$) to subtract it from your measurements.

R-min: Enter the minimum ration value ($F1/F2$) to subtract it from your measurements.

Kd*Sf: Enter the effective Kd value (dissociation constant) of the fluorescence dye.

Note: These values are required for calculating the free calcium concentration (according to the Grynkiewicz formalism). The calcium concentration can be stored as a Trace by use of the "Photo_Ca" input channel in the *Stimulus Editor* dialog.

Background 1...3: Enter the background fluorescence value for each of the used wavelengths to exclude any auto-fluorescence from your measurements.

Note: The parameters *R-max*, *R-min*, *Kd*Sf* and *Background 1...3* are stored as "Series Parameters" with the raw data file and are listed in the *Data Parameter* window (see figure below).

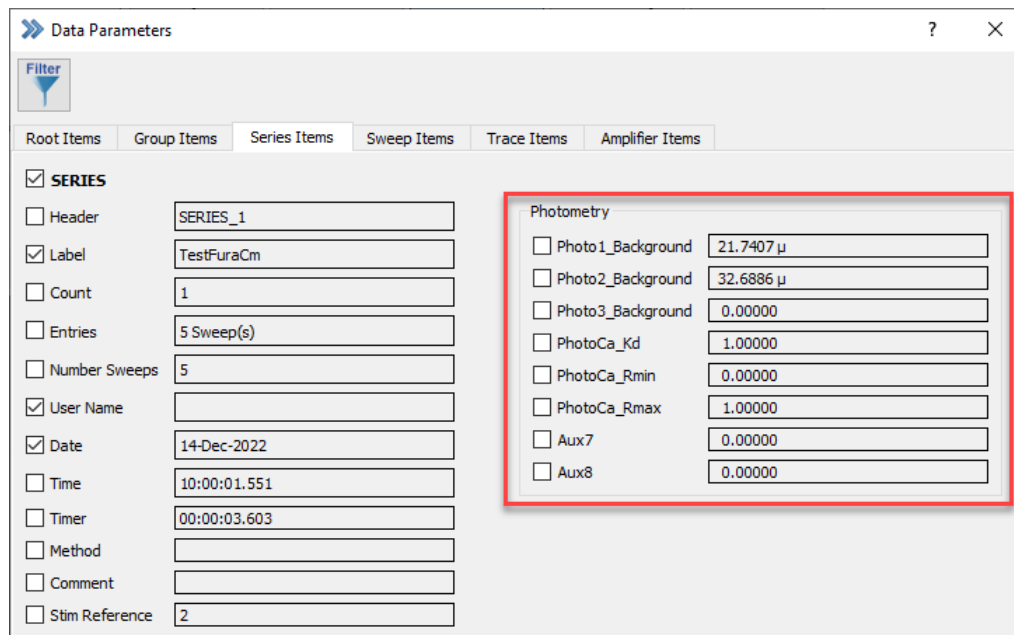


Figure 20.21: Photometry parameters are stored in the Series record of the Data Tree and can be accessed by the Data Parameter window.

Dead Time: Time to allow for settling of the commanded wavelength before starting the image exposure.

Compute Traces of Marked Targets: This function allows to recompute stored "Photo" Traces (Photo_W1, Photo_W2, Photo_W3, Photo_R, and Photo_Ca) with modified fluorescent background values.

21 Chirp Extension

For the activation and the configuration of the `Chirp Extension` you have to enable it in the `Configuration` (see 3.5.2.2 on page 25).

21.1 Introduction

The `Chirp Extension` allows to measure the transfer functions of a system. The complex transfer function can be presented as two real traces: *Magnitude* (or *Gain*) and *Phase*. E.g. if we send a sine wave stimulus of amplitude 1 into a system and we measure as response a sine wave of the same frequency but amplitude of "0.5", then we would get a *Magnitude* or *Gain* of "0.5".

In case the system converts a voltage stimulus to a current signal, we can interpret the *Magnitude* (or *Gain*) as *Admittance* (*Y*) or *Impedance* (*Z*).

The `PATCHMASTER NEXT LockIn Extension` performs this type of analysis at a given single frequency. In order to obtain a complete spectrum, repetition of the measurement at different frequencies is required. This ends up in a very elaborate procedure. The `Chirp Extension`, however, uses a stimulus containing a set of multiple frequencies, allowing to calculate a complete spectrum from a single measurement.

21.1.1 Chirp Wave Forms

The Stimulus Editor of `PATCHMASTER NEXT` provides three different chirp wave forms:

Linear Chirp

In a *Linear Chirp*, the frequency $f(t)$ varies linearly with time:

$$f(t) = f_0 + kt \quad (21.1)$$

where f_0 is the starting frequency (at time $t = 0$), and k is the rate of frequency increase or chirp rate. The corresponding time-domain function for a sinusoidal *Linear Chirp* is:

$$x(t) = \sin\left[2\pi\left(f_0 + \frac{k}{2}t\right)t\right] \quad (21.2)$$

Exponential Chirp

In an *Exponential Chirp*, the frequency of the signal varies exponentially as a function of time.

$$f(t) = f_0 k^t \quad (21.3)$$

where f_0 is the starting frequency (at $t = 0$), and k is the rate of exponential increase in frequency. Unlike the *Linear Chirp*, which has a constant chirp rate, an *Exponential Chirp* has an exponentially increasing chirp rate. The corresponding time-domain function for a sinusoidal *Exponential Chirp* is:

$$x(t) = \sin\left[\frac{2\pi f_0}{\ln(k)}(k^t - 1)\right] \quad (21.4)$$

Spectroscopy Chirp

A chirp wave form created by inverse *Fourier Transform* from a spectrum with frequencies increasing with t^2 and constant amplitude. The *Chirp Extension* uses this stimulus wave form as input only.

21.1.2 Transfer Function Ratio

The transfer function (H) of two signal processing units in series is given by:

$$H(A + B) = H(A) \otimes H(B) \quad (21.5)$$

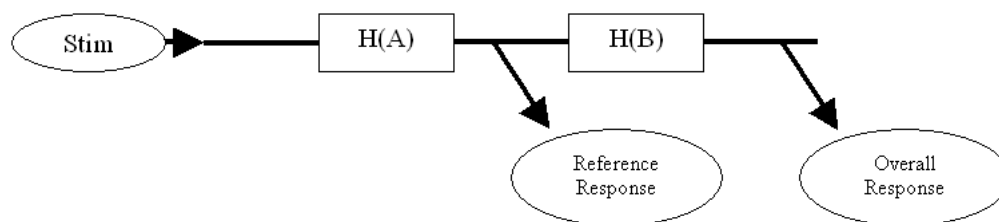


Figure 21.1: Transfer Function Scheme of two signal processing units.

In case one is interested the transfer function of an individual signal processing unit, the "ratio" of the transfer function can be calculated:

$$H(B) = \frac{H(A + B)}{H(A)} \quad (21.6)$$

The *Chirp Extension* provides a so-called *Correction Mode* to calculate the *Transfer Function Ratio* of the recorded response (nominator) and the reference response (denominator).

In some application a reference element (e.g. calibrated resistor) is used for a calibration measurement of the system. In this case the value of the reference element has to be taken into account when calculating the transfer function of the measuring system without reference element. We offer the option to use the measured resistance or a given resistance for this correction.

21.2 Activating the Chirp Extension

The Chirp Extension is activated in the Hardware tab of the Configuration dialog.

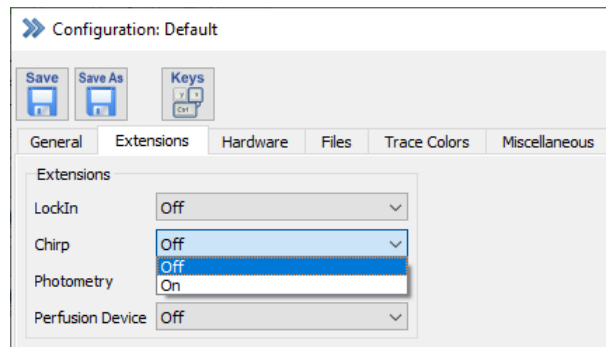


Figure 21.2: The Chirp Extension is activated in the Configuration dialog of PATCHMASTER NEXT.

Once the extension is turned *On*, the Chirp window can be opened via the Windows menu in the Control window.

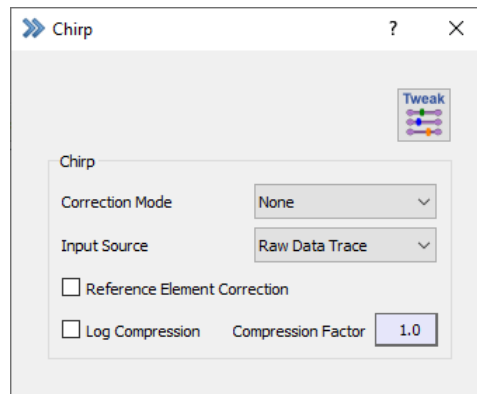


Figure 21.3: Chirp dialog of PATCHMASTER NEXT.

The following options are available:

Correction Mode: It is possible to correct the spectra during online and offline calculation with respect to another chirp response (see *Transfer Function Ratio*, 21.1.2 on the preceding page).

In case a simultaneous recording of the reference chirp is possible:

Under certain circumstances it might be possible to record the reference Trace simultaneously with the chirp response. In this case you can select the respective Trace which contains the reference chirp response in the *Correction Mode*.

In case the reference chirp is recorded in advance:

The reference chirp has to be copied in one of the four Trace Buffers and in the *Correction Mode* the respective Buffer has to be selected.

If a correction Trace is selected, the admittance of the recorded response is divided by the normalized magnitude of the reference. In addition, the phase of the reference is subtracted from the phase of the recorded response.

Input Source: Defines the data type from which the spectra are calculated. In case a *Correction Mode* is used, the type of input data for the acquired data and the reference have to be the same.

Reference Element Correction: Must be used in case the measurement system has been calibration with respect to a reference element (e.g. a 10 M Ω resistor), which is removed and replaced by the cell under study. Two options are available:

1. Measured Resistance: The resistance of the reference element is measured at the lowest frequency.
2. Given Resistance: A given resistance can be used to normalize the calibration measurement.

In case *Reference Element Correction* is not activated the raw (no scaling applied) data of the reference transfer function are used. This method usually applies only if it is possible to measure from two different locations in the signal processing path.

Log Compression: Often the results of the chirp analysis are plotted on a logarithmic frequency scale. In this case the higher frequencies contain much more measuring points than the lower frequencies. For better display and post analysis (e.g. fitting of the spectra) an equidistant scaling on the logarithmic frequency axis is of advantage. Therefore we offer the option of a logarithmic compression. The *Compression Factor* (usually a value between 1 and 2) defines the strength of the compression. When logarithmic compression is used the frequency axis is scaled in units of logarithm to basis "10".

Tweak: The *Tweak* menu opens further options for the *Chirp* extension. These settings are explained in detail in chapter 21.2.1 and chapter 21.2.2 on the next page.

21.2.1 Chirp Settings

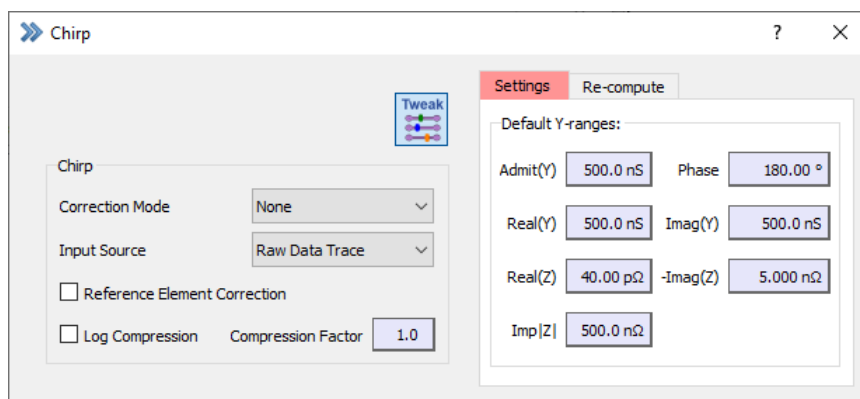


Figure 21.4: Chirp settings of PATCHMASTER NEXT.

Default Y-ranges: For the various chirp Traces the default Y-ranges for display in the Oscilloscope window are defined. E.g. a Y-range of 1 S (Siemens) defines that at scaling of 1 and offset of 0, the Oscilloscope window shows a range from -1 S to +1 S.

21.2.2 Chirp Recompute

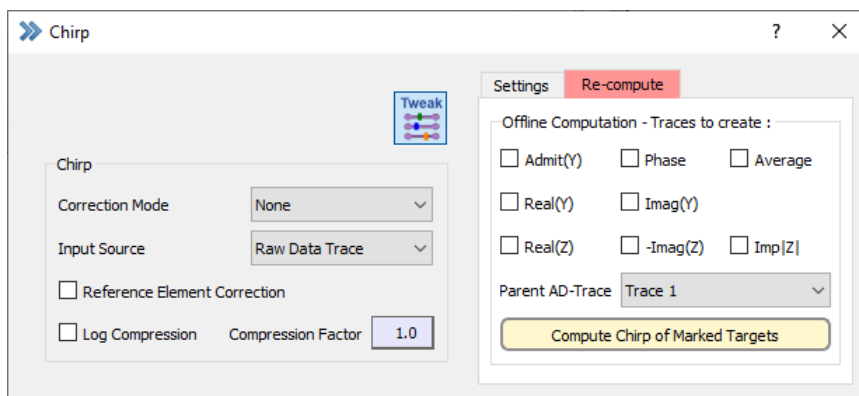


Figure 21.5: Chirp re-compute options of PATCHMASTER NEXT.

Offline Computation - Traces to create: You can mark by using the checkboxes which Traces (*Admit(Y)*, *Real(Y)*, *Real(Z)*, *Phase*, *Imag(Y)*, *-Imag(Z)*, *Average*, *Imp(Z)*) should be created during re-calculation of the spectra.

Parent AD-Trace: Select the corresponding Trace for computing chirp, either *All* or any other Trace (Trace 1...16).

Compute Chirp of Marked Targets: Recalculates and generates the selected Traces (select and mark the target (Sweeps or Series) first).

21.3 The Chirp Traces

Chirp_Avg

Average of the chirp response. In case multiple *Chirp* segments are used in one Sweep, the *Chirp_Avg* contains the average of all *Chirp* segments.

Chirp_Phase

The phase angle between stimulus and response versus frequency. The default unit is degree(°). This is a frequency based Trace (X-unit is Hertz).

Chirp_Admit(Y)

Contains the magnitude of the spectrum normalized to the stimulus amplitude. In case of a recorded current response this parameter can be interpreted as admittance. The default unit is Siemens (S). This is a frequency based Trace (X-unit is Hertz).

Chirp_Real(Y)

The real part of the admittance. This is a derived frequency based Trace (X-unit is Hertz), calculated as follows:

$$Real(Y) = \frac{Admittance}{\sqrt{1 + \tan(Phase)^2}} \quad (21.7)$$

The default unit is Siemens (S).

Chirp_Imag(Y)

The imaginary part of the admittance. This is a derived frequency based Trace (X-unit is Hertz), calculated as follows:

$$\text{Imag}(Y) = \tan(\text{Phase}) * \text{Real}(Y) \quad (21.8)$$

The default unit is Siemens (S).

Chirp_Real(Z)

The real part of the impedance. This is a derived frequency based Trace (X-unit is Hertz), calculated as follows:

$$\text{Real}(Z) = \frac{\text{Real}(Y)}{\text{Real}(Y)^2 + \text{Imag}(Y)^2} \quad (21.9)$$

The default unit is Ohm (Ω).

Chirp_Imag(Z)

The imaginary part of the impedance. This is a derived frequency based Trace (X-unit is Hertz), calculated as follows:

$$-\text{Imag}(Z) = \frac{\text{Imag}(Y)}{\text{Real}(Y)^2 + \text{Imag}(Y)^2} \quad (21.10)$$

The default unit is Ohm (Ω).

Chirp_Imp|Z|

The impedance. This is a derived frequency based Trace (X-unit is Hertz), calculated as follows:

$$\text{Imp}|Z| = \frac{1}{\sqrt{\text{Real}(Y)^2 + \text{Imag}(Y)^2}} \quad (21.11)$$

The default unit is Ohm (Ω).

21.4 Setting up a Chirp Acquisition

Create a new stimulation sequence with one *Chirp* segment as stimulus. When at least one segment contains a chirp wave form then a button *Chirp Wave* appears in the *Parameter* section of the *Stimulus Editor*.

A detailed description of the available chirp wave parameters can be found in chapter 21.1.1 on page 293.

21.4.1 Parametrization of the Chirp Stimuli

Possible Frequency Range: The maximal possible frequency in a chirp wave is half the sampling frequency ($\text{Min. Points} / \text{Cycle} = 2$).

- **Linear and Exponential Chirp:** The *Start* and *End Frequency* can be directly entered in the *Chirpwave Parameters* dialog. According to *Start* and *End Frequency* and given segment duration the rate of frequency increase (k) and the *Min. Points / Cycle* are calculated.

Parameter	Value
Amplitude	10.0 mV
Start Freq.	78.1 Hz
End Freq.	10.0 kHz
Min. Points	2.0
Segment Points	256

Figure 21.6: Linear Chirp settings of the Stimulus Editor. Exponential Chirp settings show the same parameters.

- **Spectroscopy Chirp:** *Start Frequency* and *End Frequency* can not be entered directly. Both parameters are defined by the *Sampling Frequency*, *Segment Duration*, and the *Min. Points / Cycle*. The *End Frequency* is given by *Sampling Frequency* divided by *Min. Points / Cycle*. The *Start Frequency* then depends on the duration of the segment. The longer the segment the smaller the starting frequency of the chirp.

Parameter	Value
Amplitude	10.0 mV
Start Freq.	78.1 Hz
End Freq.	10.0 kHz
Min. Points	2.0
Segment Points	256
Chirps to Skip	0

Figure 21.7: Spectroscopy Chirp settings of the Stimulus Editor.

We select the *Spectroscopy Chirp* option for our stimulation sequence here.

Useful Frequency Range: In the preceding section we have discussed the possible frequency range in a chirp stimulus. Correct analysis of the chirp response, however, has to obey some additional boundary conditions. The most important rule is to obey the *Sampling Theorem* or *Nyquist Theorem*! That means that the response has to be low pass filtered to prevent aliasing artifacts. The filtering can be either done by the system under study itself or by additional filters. When using filters with steep cut-off (e.g. 8-Pole filter) unbiased analysis can be extended to about 80 % of the possible frequency range.

21.4.2 Adding Chirp Analysis Traces

Once you have configured the chirp stimulus you can add multiple chirp analysis Traces to the AD selection in the Stimulus Editor:

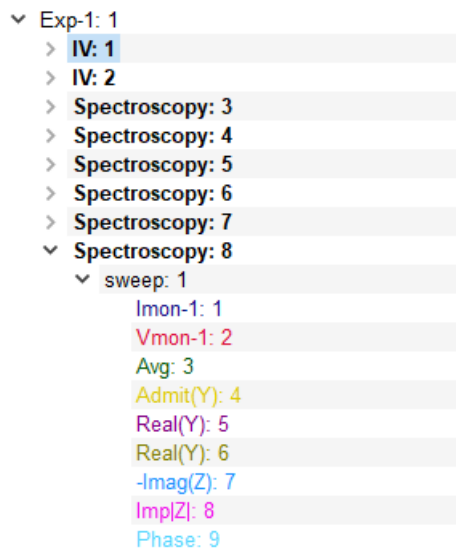


Figure 21.8: Available Chirp Traces of the Stimulus Editor when Spectroscopy Chirp was activated.

Please, have a look for chapter 21.3 on page 297 where we already provided an overview of all Chirp Traces available.

21.4.3 Minimizing Swing-In Effects

In order to minimize swing-in effects, two methods can be used:

1. Use at least two Chirp Segments: We duplicate the *Chirp* segment and set the *Start Segm* to "2". In case of very long chirp stimuli this method is very time consuming.

In the following screenshot you see a sample Stimulus Editor sequence for a chirp acquisition.

21.4. Setting up a Chirp Acquisition

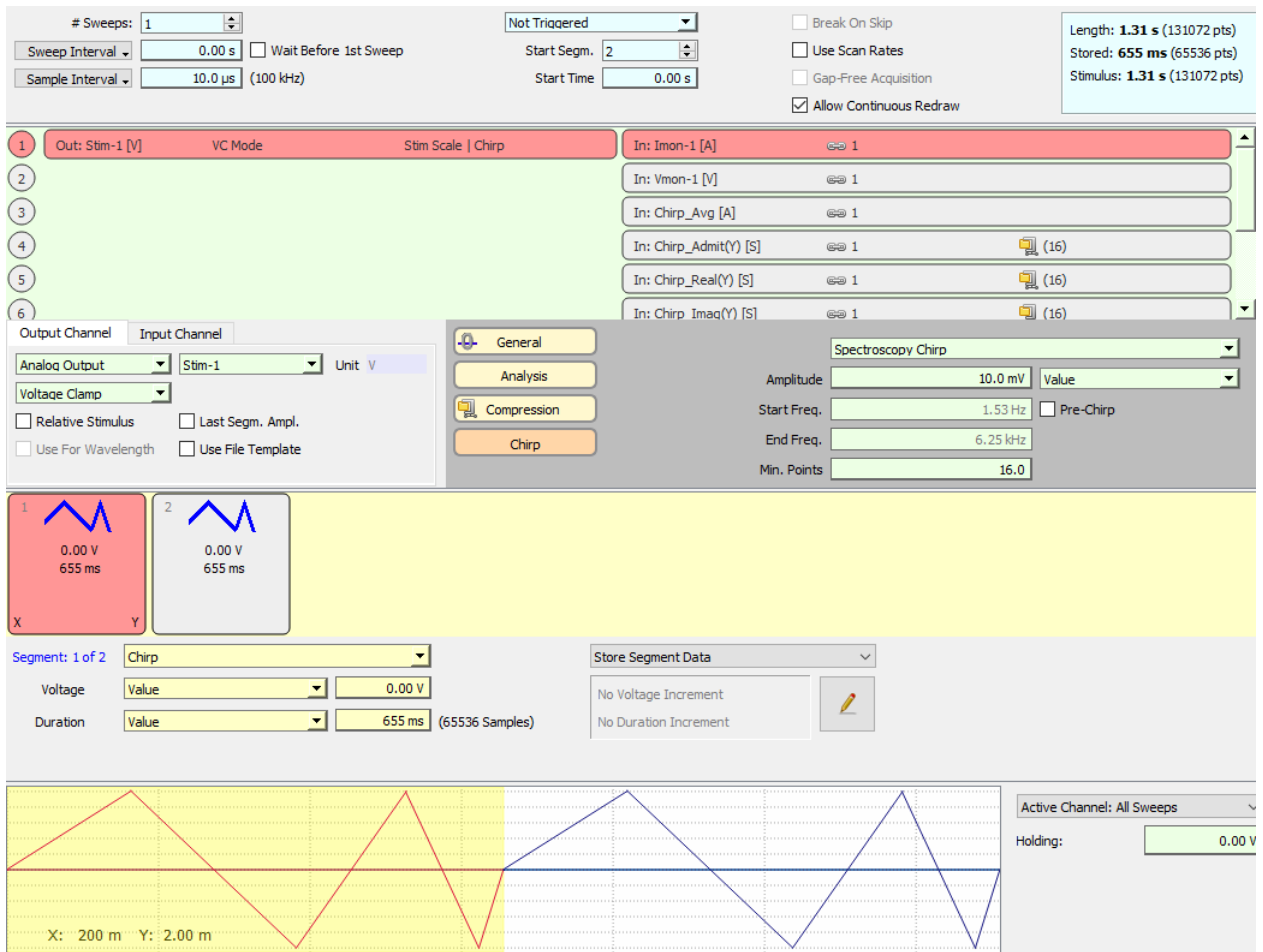


Figure 21.9: Example of a Chirp stimulation sequence. To avoid swing-in effects a second chirp segment was inserted.

2. Use of a Pre-Chirp Segment: In order to reduce the time for swing in to a minimum, a *Constant* segment of fixed length can be defined before the *Chirp* segment. When the option *Pre-Chirp* is activated in the Chirpwave Parameters dialog, then this segment is automatically filled with appropriate swing-in stimulus data. Please note that the *Pre-Chirp* segment should not be recorded (use *Start Segment "2"*) to ensure accurate chirp analysis.

21.4. Setting up a Chirp Acquisition

The screenshot displays the Heka software interface for configuring a Chirp acquisition. The top panel shows acquisition parameters: # Sweeps: 1, Sweep Interval: 0.00 s, Sample Interval: 10.0 µs (100 kHz), Start Segm.: 2, and Start Time: 0.00 s. A status box indicates: Length: 660 ms (66036 pts), Stored: 655 ms (65536 pts), Stimulus: 660 ms (66036 pts). The channel list includes 'Out: Stim-1 [V]' and 'In: Imon-1 [A]'. The 'Spectroscopy Chirp' configuration panel shows: Amplitude: 10.0 mV, Start Freq.: 1.53 Hz, End Freq.: 6.25 kHz, Min. Points: 16.0, and the 'Pre-Chirp' checkbox is checked. The waveform plot shows a chirp signal with a scale of X: 100 m, Y: 2.00 m. The segment configuration table is as follows:

Segment	1 of 2	Constant	Value	Duration
Voltage	Value	0.00 V		
Duration	Value	5.00 ms (500 Samples)		

Figure 21.10: Example of a Chirp stimulation sequence. To avoid swing-in effects a pre-chirp segment was used.

In the following screenshot the swing in signal of a *Pre-Chirp* segment is shown. The first 5 ms of the stimulus are the *Pre-Chirp* segment which provides an optimal swing-in of the chirp segment.

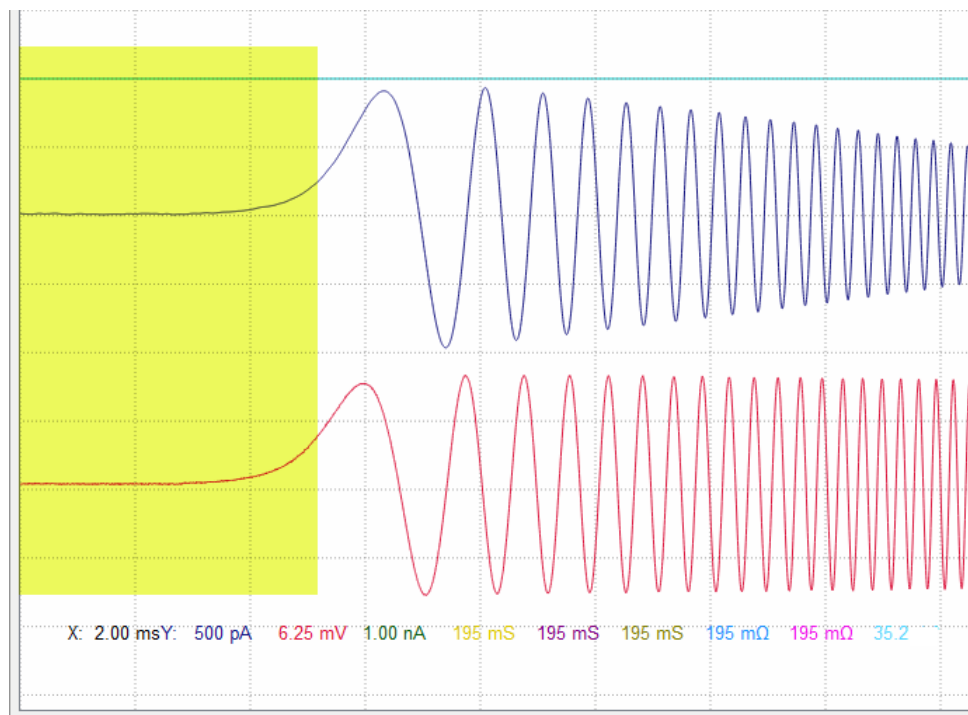


Figure 21.11: Spectroscopy Chirp measurement showing the current (blue) and voltage signal (red) of the chirp measurement. The model cell, “MC 10”, was used and set to “10 MOhm” for this recording. If one uses the pre-chirp stimulation sequence the first 5 ms of this recording (yellow area) would be discarded from the recording (swing-in phase).

21.5 Display of Spectra in the Oscilloscope Window

The chirp analysis Traces are stored in the Data Tree.

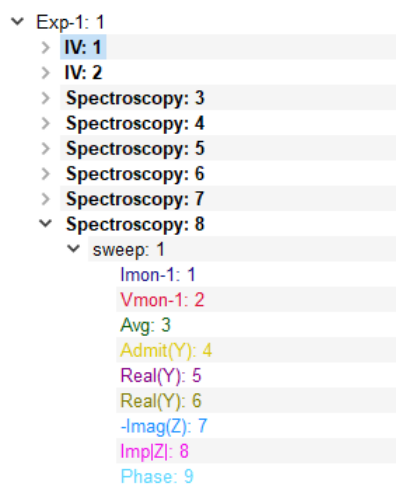


Figure 21.12: All traces from the chirp recording are stored in the Data Tree. There they are available for replay purposes.

Their X-unit is Hertz and the number of points differs from the raw data Trace. Since raw data Trace and spectra have different X-axis scaling it is difficult to show them at the same time on appropriate scaling in the Oscilloscope window, which supports only one X-scaling. By default you will see the raw data Trace in the Oscilloscope window, since the Oscilloscope window takes its X-scaling from the first Trace in the Sweep.

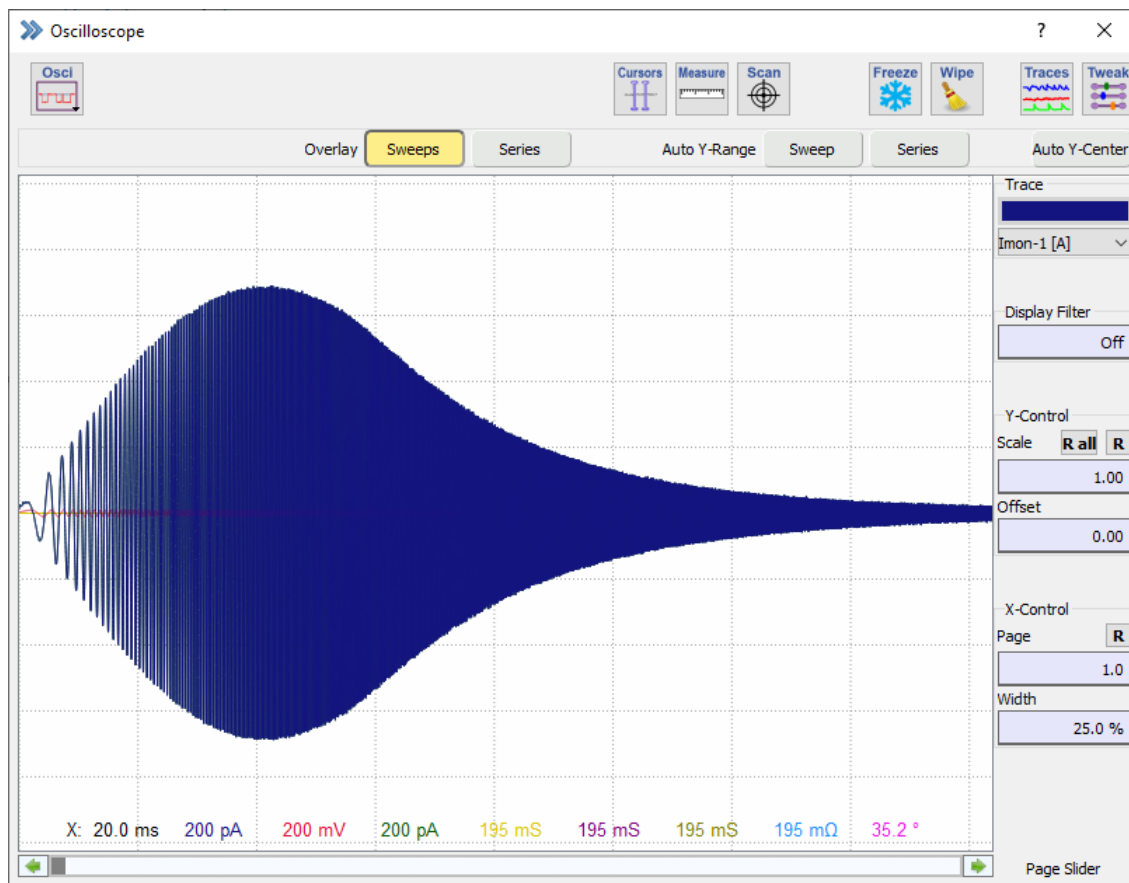


Figure 21.13: Current Trace of the Chirp stimulation sequence.

In order to show also the chirp analysis Traces in the Oscilloscope window, please select *Fixed X-width* from the Oscilloscope menu and enter as *X-max* (panel to the right in the Oscilloscope window) the maximum frequency in the spectrum (e.g. 6.25k, what stands for 6.25 kHz).

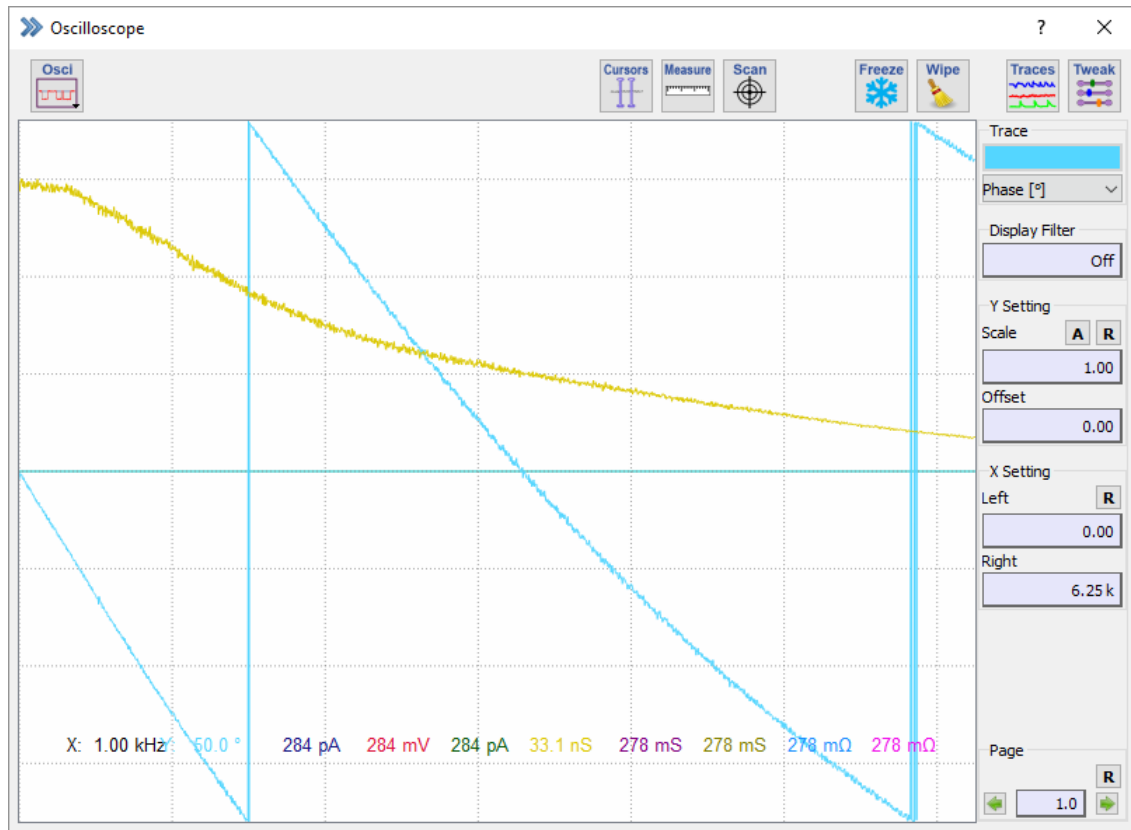


Figure 21.14: Chirp Traces, namely "Phase"(light blue) and "Admittance"(yellow) with proper X-axis scaling.

Trace 9 (light blue) and Trace 4 (yellow) show the phase and the admittance, respectively.

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