

PATCHMASTER

USER MANUAL

Multi Channel Systems MCS GmbH Phone +49 7121 90925 26
Aspenhaustrasse 21 Fax +49 7121 90925 11
D-72770 Reutlingen Website www.heka.com / www.elproscan.com
Germany Email eimeaorderscmt@harvardbioscience.com
 support@heka.com

Harvard Bioscience, Inc. Phone +1 800 597 0580
84 October Hill Road Fax +1 508 429 5732
01746 Holliston Website www.heka.com / www.elproscan.com
Massachusetts Email us-sales@smart-ephys.com
United States support@heka.com

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1 Introduction

1.1 Disclaimer

This *Product* relies on the tools of Microsoft Windows OS (Windows Vista, Windows 7, Windows 8, Windows 10) or Mac OS (10.6 - 10.14). HEKA is not responsible for: i) the contents of these third party products, ii) any links contained in these third party products, iii) changes or upgrades to these third party products and iv) 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

Extended analysis functions for PATCHMASTER data are provided by the FITMASTER software. FITMASTER features analysis and fitting routines designed to fully support the extended PATCHMASTER data structure. Analysis can be performed on the levels of *Traces*, *Sweeps* and *Series*. There are standard fit functions such as "Polynomial, Exponential, Gaussian, and Boltzmann" tailored functions to fit e.g. whole-cell current traces according to the Hodgkin & Huxley gating formalism, current-voltage relationships and dose-response curves.

1.3 Supported System Software

 PATCHMASTER is supported on all recent MS Windows Versions: Windows Vista, Windows 7, Windows 8, Windows 10.

 PATCHMASTER is supported on Mac OS (10.6 - 10.14). Older Mac OS X versions and Mac OS 9 are not supported.

1.4 Naming Conventions

1.4.1 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.

1.4.2 Syntax

- PATCHMASTER: Small Capitals denote a HEKA program / hardware or keys on the keyboard.
- Replay: Typewriter font denotes menu entries and chapters or filenames
- Protocol Editor: Sans serif font denotes window names or dialogs.
- Sinewave: Roman font denotes options or buttons
- *Italic*: Denotes general emphasis.
- Bullet list: Lists points.
- Numbered list: Lists actions to be performed sequentially.

 &  or (MS Windows) & (Macintosh) denotes system-specific keys and actions.

1.5 Windows and Mac Key Conventions

In PATCHMASTER, all key commands are saved in the file `Patchmaster.key` and will be read at the program start (see chapter 3 on page 13).

Note that all key commands specified in this manual refer to the default setting in `Patchmaster.key`. Since all commands can be customized by the user, the settings in your working version of PATCHMASTER might differ from these default settings.

Please also be aware that in case the file `Patchmaster.key` is not available at the program start, no key commands are available!

The basic conventions for the system keys on MS Windows and Mac are as follows:



- CTRL: Stands for the CONTROL key.
- ALT: Stands for the ALT key.



- CTRL: Stands for the CONTROL key.
- CMD: Stands for the COMMAND key (apple or cloverleaf symbol).
- ALT: Stands for the OPTION key.

1.6 Installing PATCHMASTER

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.

1.7 Starting PATCHMASTER

Upon clicking on PATCHMASTER the software starts and various controls and windows become available:

- The drop-down menus File, Edit, Windows, Replay, Display, Buffer, Notebook, Protocols, EPC10, Help.
- Frequently used PATCHMASTER windows such as Amplifier, Control Window, Analysis Window 1, Oscilloscope, Replay.
- A scrolling text window called Notebook.

Most of these windows can be minimized; they can be re-opened by clicking on the window bar or by selecting them in the drop-down menu Windows.

1.8 Get Online Help on Keys

The option Show Keys in the drop-down menu Help (compare chapter 4.10 on page 42) displays the shortcut keys that are assigned to various controls of the active windows.

By selecting List Keys in the drop-down menu Help you can list all keys in the Notebook window. This is useful e.g., if you want to print the complete list.

For further information on the assigned keys, see chapter 3 on page 13.

1.9 Get Online Help Controls

The option Show Tooltips in the drop-down menu Help (chapter 4.10 on page 42) displays a short description of the control under the mouse tip.

1.10 Closing PATCHMASTER

To exit from PATCHMASTER, choose Quit from the drop-down menu File or



press CTRL + Q.



press CMD + Q.

You have three possibilities:

- **Save + Exit:** Saves configuration and data files and quits the program.
- **Exit:** Saves data files and quits the program.
- **Cancel:** Exit process will be aborted, you return to the program.

Note: If you changed the protocols, the Pulse Generator pool, or the Analysis, then you will be asked independently if you want to save your changes.

Note: Data files are always saved.

1.11 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, POTMASTER or CHARTMASTER software
- The program version number: e.g. v2.65, v2.73.1
- Your operating system and its version: e.g. Mac OS 10.8, Windows 7 64-bit
- Your type of computer: e.g. Intel Core i3 3.2 GHz with 2 GB RAM
- Your acquisition hardware, if applicable: e.g. EPC 10 USB, EPC 800 + LIH 8+8 or PG 340 USB
- The serial number and version of your amplifier, if applicable: e.g. EPC 9 single, version "920552 D"
- The questions, problems, or suggestions you have
- Under which conditions and how often the problem occurs

We will address the problem as soon as possible.

Contact Address:

HEKA Elektronik GmbH
a division of Harvard Bioscience
Aspenhaustrasse 21
D-72770 Reutlingen
Germany
Phone: +49 7121 90925 26
Fax: +49 7121 90925 11
E-mail: support@heka.com
Web: www.heka.com
www.elproscan.com

2 User Interface

The following chapter describes the user interface of PATCHMASTER. It consists of four sections:

Dialog controls explains all types of control controls available in the PATCHMASTER user interface.

Modifying the Dialogs and Controls describes how size, color, and arrangement can be changed.

Saving modified Dialogs and Controls describes how to save the dialog settings.

Toggling between Windows describes how to switch very fast in between different PATCHMASTER dialog windows.

2.1 Dialog Controls

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

The general rules for dialog controls in this program are:

- **Whole Cell** : Box items with a drop shadow enclose changeable values, either as
 - list item or
 - pop-up menu list or
 - drag item
- **SETUP** : Rounded rectangles are items that perform some action.
- **496. MO** : Simple rectangles (without drop shadow) display a measured value.
- **Recording Mode** : Plain text is for titles only.

2.9 kHz Drag: A number in a box with a drop shadow. The parameter value in a drag item can be changed by clicking on it and dragging the mouse up and down. Alternatively, you can double-click on it, or SHIFT-click, or right-click (MS Windows), and then type in a new value. Terminate input with RETURN or ENTER. Using TAB will cycle through all *Drag* items of the active dialog window.

Whole Cell List: Similar in appearance to a *Drag* item. Clicking on it will pop up a menu list from which one can choose a setting.

Success! Edit Text: A text string in a box with a drop shadow. Clicking on it will allow editing the displayed string.

SETUP Button: Rounded corner rectangle. Clicking on it will cause the respective action to occur.

Store Switch: Rounded corner rectangle. Clicking on it toggles the parameter value. The switch is "On" or activated if the item is highlighted. A switch can optionally also execute some action.

Prediction Radio Button / Checkbox: Identical to the standard dialog items. Clicking on them will toggle the respective parameters.

-70 mV **Framed Text / Number / Boolean:** Simple box with optionally some text. The Boolean value is indicated by its color, inactive controls are gray .

Enter: Pressing ENTER on the extended keyboard always brings you back to edit the control that was edited last. The feature is very useful when one often edits the same control (e.g., a duration of a specific segment in the Pulse Generator or the *Display Gain* in the Oscilloscope window).

Background Color: The color that appears while the user is dragging or entering a value is set by the *Highlight Color* in the Mac OS control panel.



Be careful if changing – the user will not be able to read the edited number if the highlight color is set to a very dark color. The MS Windows version displays highlighted controls with white text on a black background.

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 (see table) are always displayed in scientific notation. The old value is erased as soon as the user starts to type. To preserve the old string, move the LEFT or RIGHT cursor first. To leave the previous value unchanged although a new one has been entered already, just clear the input by pressing ESC, then RETURN or ENTER.

Table 2.1: Numerical Input Options

Name	eng.	sci.
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 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.

String Buffer: Whenever an edit process is finished with RETURN the edited string is entered into a cyclic buffer of edit strings consisting of 10 entries. These strings can be accessed during editing using CURSOR UP and CURSOR DOWN. This feature is useful when identical or similar strings have to be typed into various string items.

2.2 Modifying Dialogs and Controls

Dialog items can be modified in many different ways, e.g. background or item color, text font, position of one item, position of all items in the window.

To modify dialogs and control items in the PATCHMASTER user interface, you have to select **Enable Icon Configuration** from the Windows menu.



Select **Enable Icon Configuration**. Now you can customize the windows:

- To drag and resize an item, right-click on the item and drag.
- To drag a group of items, press CTRL and right-click on the group and drag. A group of items is indicated by a grey background field.
- To bring up the Dialog Control window, press CTRL and left-click on the item. Here you can modify the item settings e.g. such as color, text font, dragging speed (see below).



Select **Enable Icon Configuration**. Now you can customize the windows:

- To drag and resize an item, press ALT and left-click on the item and drag. The new item position will be ignored if ALT is up when the mouse button is released.
- To drag a group of items, press CMD and ALT and left-click on the group and drag.
- To bring up the Dialog Control window, press CMD and click on the item. Here you can modify the item settings e.g. such as color, text font, dragging speed (see below).



Be careful when e.g. changing positions, sizes or colors of buttons. If you deselect the option visible in the Dialog Control window the button will disappear. Be reminded that it is not possible to get the button back after that action.

The following table summarizes all actions (Enable Icon Configuration has to be selected!):

Table 2.2: Icon Configuration Options

Action	Mac OS	MS Windows
Open a configuration dialog	CMD + click	CTRL + left-click
Move one item	ALT + left-click + drag	ALT + right-click + drag
Move group of items	CMD + ALT + left-click+drag	CTRL + right-click + drag

All windows except the Configuration window can be iconized i.e. reduced to a minimal size window. Such a window can easily be expanded to the original size (and shrunk again) by clicking in its zoom box.

2.2.1 Dialog Control Window

In the Dialog Control window, you can control the properties of an item.

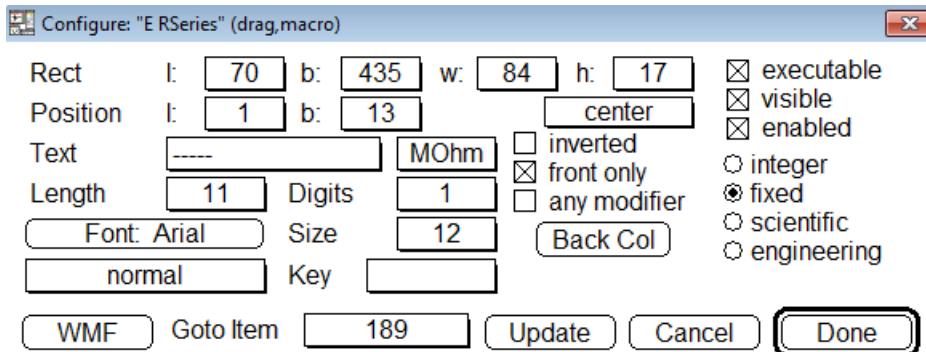


Figure 2.1: Icon Configuration Dialog

Here you can see the Dialog Control window for a *Drag* button, in this case the amplifier *R-series* button. For other buttons, e.g., with numbers or execution commands, there may be more or less controls available.

Here is the complete list of the controls:

Rectangle: Gives the position of the rectangle in the dialog window (left **l**, bottom **b**) and the size of the item (**width**, **height**) in pixel.

Position: Gives the position of the text in the item (left **l**, bottom **b**) in pixel. You can select a position from the list: left/center/right/last. "Last" is useful if you have text that may be longer than the specified button and where the end of the text is more important than the beginning, e.g. a complete file path and name. In this case, only the end of the text will be displayed, cutting off the beginning.

Text: Text label of the item.

Note: The label of a button can only be changed when there is already a name in the text field inserted. Buttons with the default entry "—" will not be effected by a change of the label.

Unit: Unit of numeric values.

inverted: Changes the text color from black to white or vice versa.

Length: Maximum number of characters for this field. If the number is too low no number/value is displayed.

Digits: Number of decimal places.

Font: Select a font type from the list of available fonts on the system.

Size: Change the font size of the text.

Style: Define the style of the text: normal, bold, italic, underline, outline, shadow, condense, extend.

Key: Define the character which is assigned to that item. This enables the item to be executed from the keyboard. When a key is applied to a **list** item, there are two keys assignments. E.g. one for increment and one for decrement.

front only: If marked, the key will only work if this window is in front, i.e. the active one. This prevents key command collision in case you want to use the same key settings in several windows.

any modifier: If marked, any of the modifier keys can be used.

Note: In newer MS Windows versions this function is limited.

executable: The button starts action by clicking. If not executable, the button will stay in its original color and is inactive.

visible: The button is visible.

Note: If not activated, the button will be invisible and cannot be edited anymore! To restore the original setting you have to delete the file Patchmaster.set.

enabled: The button can be activated or edited. If not enabled, the button will be gray and inactive.

Back Col: Color for a switch button that is *not* active (default: pink) or for any other button.

High Col: Color for a switch button that is active (default: red). For other buttons this feature is disabled.

Format of values:

- integer: Value has to be entered as integer, e.g., "5".
- fixed: Value has to be entered as floating point with maximum as many decimal places as given in **Digits**, e.g., "0.001".
- scientific: Value has to be entered in scientific format, e.g., "e-9" for "nano".
- engineering: Value has to be entered in engineering format, e.g., "n" for "nano".

WMF: Saves the window as Windows Meta File (WMF, MS Windows) or PICT (Mac).

Goto Item: Index number of the button. Please do not change this number, it is for internal handling only.

 Configure: "E Mode" (list,macro) In the caption of the dialog, you can see, if the item can be called from a *Macro Command* in a protocol ('macro').

To complete the input, you have the following possibilities:

- Click on Update to see the change in the item of the respective window.
- Click on Cancel to leave the Dialog Control window without changes.
- Click on Done to leave the Dialog Control window and save all changes.

2.2.2 Hiding Controls

If you want to hide controls that are usually not necessary for your tasks, the easiest way to hide them is to pull them to the side of the window.

Proceed as follows:

1. Select Enable Icon Configuration from the Windows menu.
2. Increase the size of a window. For that pull with the mouse on the lower end of the window.
3. Press CTRL and click with the right mouse button (MS Windows) or while holding the CMD key down click (Mac OS) on a control and drag it to the edge of the window. While moving, you will see a gray rectangle underneath it.
4. Re-size the window again to the original size. The controls are now hidden in the not visible area.
5. Now you can save it using the Save Front Dialog function in the Windows menu. The window setting will be saved as *.dia file.
6. To restore the old default settings, delete the *.dia files from the home path as set in the Configuration window.

2.2.3 Deleting Controls

If you want to customize PATCHMASTER for special purposes, e.g., for not allowing the user access to controls, the secure version is to delete the controls. For this, you have to edit the button properties in the Dialog Control window.

Proceed as follows:

1. Select `Enable Icon Configuration` from the `Windows` menu.
2. Press `CTRL` and click with the left mouse button (MS Windows) or while holding the `CMD` key down click (Mac OS) on a control. The Dialog Control window opens.
3. Deselect `enable` or `executable` to simply deactivate the control. It will still be visible then.
4. Alternatively deselect `visible` - the control disappears completely.
5. Click on `Update` to see the effect, or on `Done` to save the control setting.
6. Now you can save it using the `Save Front Dialog` function in the `Windows` menu. The window setting will be saved as `*.dia` file.

To prevent users from changing your settings, place the `.dia` files into access restricted folders and change the home path in the Configuration window accordingly.

To restore the old default settings, delete the `*.dia` files from the home path as set in the Configuration window.

2.3 Saving Dialogs and Controls

Position and size of all PATCHMASTER windows are saved in the configuration file (e.g., `Patchmaster.set`).

However, position, size, and state (iconized/not iconized) of each window can also be stored using the menu option `Save Front Dialog` in the `Windows` menu (e.g., `IO_Control.dia`). If a dialog file for a window exists, then the settings from that dialog file overrule the settings from the configuration file.

Note: When installing a new version of PATCHMASTER these customized dialogs are likely to become incompatible, because additional items may have been introduced in the new version. It is, therefore, best to discard these custom dialogs when upgrading.

Note: When you have saved a wrongly configured window by chance, delete the corresponding file from the PATCHMASTER directory, the naming convention is `[windowname].dia`, e.g., `Oscilloscope.dia`.

2.4 Toggling between Windows

Hitting the ESC key will close whatever window is in front. Systematically pressing the ESC key will close all windows one at a time (except the Notebook window).

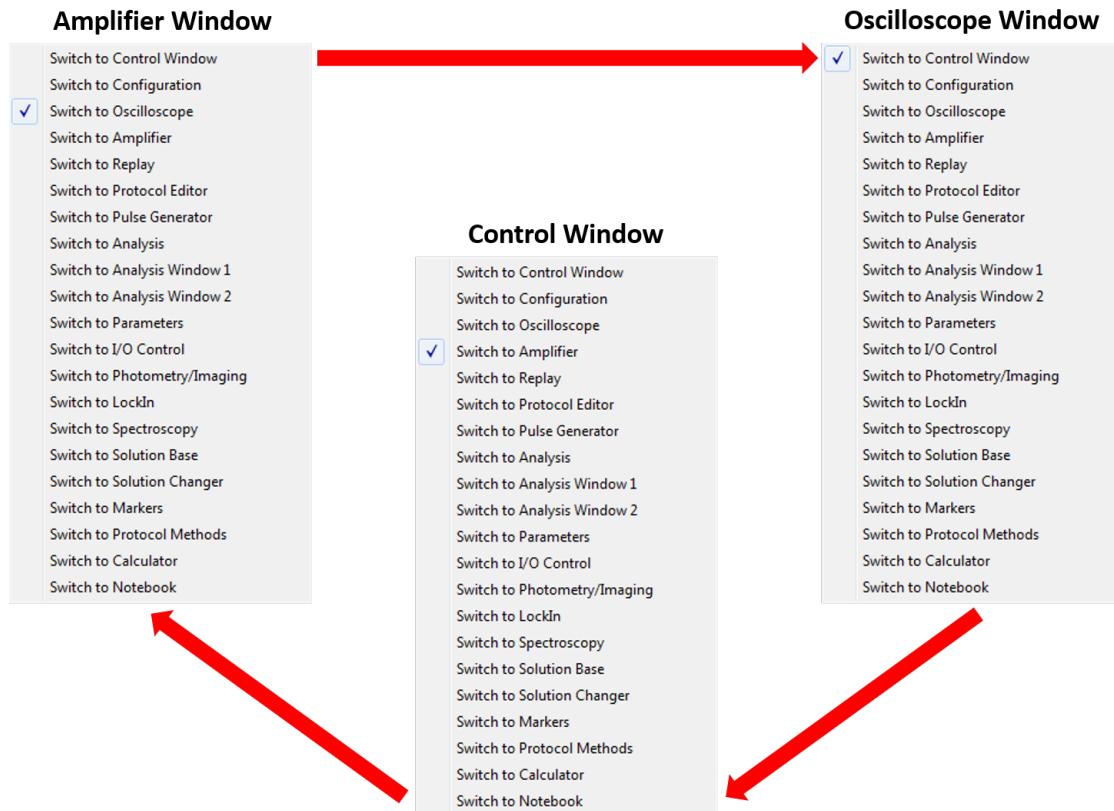


Figure 2.2: Windows Toggle Principle

The SPACE key can be used to quickly toggle between windows. The sequence of switching can be set in the *Switch to* control. This control is extremely useful in several respects, firstly, it enables you to switch from one window to another, depending upon what *Switch to* window option is selected.

Secondly, switching between windows can be performed very quickly and easily if you assign a keystroke to perform the function. Simply select the desired *Switch to* window option and then assign a key to this window in the previously described Icon Configuration window. To achieve this press the CTRL and select the *Switch to* button with your mouse. The SPACE key is the default key but this can be changed. Thirdly, having the *Switch to* control, enables window switching to be included within macro recordings in the Protocol Editor.

Note: Since this feature is not needed normally, it is available only if either *Enable Icon Configuration* is selected or you are in the Macro Recording mode. You can then find the control in the invisible window area.

Furthermore, this control is also available in the Protocol Editor, event *Switch Window* where it allows the inclusion of window switching in a protocol sequence.

2.5 Copy and Paste Functions

In this section we give an overview of the Cut and Paste functions within the different PATCHMASTER windows and dialogs.

While editing an item text in a dialog the Cut and Paste functions will operate on the edited text of the active, highlighted item.

The menu function `Edit → Copy` is enabled for the following windows:

- Notebook Window: Standard copy functionality
- Equation Editor Dialog: Complete equation is copied.
- Calculator Window: Result of the equation if the SHIFT is not pressed and equation plus result if the SHIFT is pressed.
- Amplifier Window: Amplifier state as defined in the Parameter window (Amplifier tab).
- Analysis Window 1 & 2: Respective *Analysis Graphs* are copied
- Analysis Window: The function list of the active *Analysis Method*, equivalent to the *List* function.
- Oscilloscope Window: Oscilloscope graph is copied.
- PGF Editor Window: Text description of selected stimulus is copied. The stimulus cartoon is copied instead of the text listing, if the *Cartoon View* tab is active.
- Protocol Editor Window: Parameters as defined in the active tab are copied.
- Replay Window: Text listing of the data tree is copied.
- Protocol Methods: The protocol method is copied.
- Markers Window: Copies *Series* and *Markers* to the clipboard, equivalent to the function "Write to Notebook".
- In most other functions or when the OPTION is down during the Copy function in the above functions the window content is copied to the clipboard as PICT and WMF for Mac OS and MS Windows, respectively. The graph is stored to disk, if the SHIFT is hold down while selecting the Copy function. Be aware that *Trace* representations will be a bit map (not vectors) when exported as part of the dialog window.

The menu function `Edit → Paste` is enabled for the following windows:

- Notebook Window: Standard paste functionality
- Equation Editor Dialog: Insert a complete equation
- Calculator Window: Insert an equation
- Protocol Editor Window: Insert a protocol. This includes a method copied in the **Protocol Methods** window. Events are inserted at the active position, if the pasted text contains events without a leading protocol definition. New protocols are appended to the present protocol pool.

Thus, Copy and Paste operations are target specific. E.g., pasting an equation while a sub-equation is being edited in FITMASTER, will paste the new text into the selected sub-equation string, while pasting when no item is selected, will replace the complete equation.

3 Keys

Controls within windows can be accessed from the keyboard. The key assignments are saved in the Patchmaster.key file and will be read upon starting the program. All commands can be customized by the user.

Please be aware that in case the Patchmaster.key file is not available when the program is started, no key commands are assigned!

The option Show Keys in the Help menu displays the key assignments in the various windows. For further information see chapter 1.8 on page 3.

To list the keys, choose List Keys in the Help menu. The complete key list is displayed in the Notebook window.

To save the keys, choose Save Keys in the Help menu. The keys are saved in the Patchmaster.key file. Old keyboard assignments will be automatically saved with an incrementing extension, e.g., *.k00, *.k01, *.k02....

You can freely customize the key commands by:

- changing and saving key assignments via the dialog control (chapter 2.2 on page 6) or by
- directly modifying the Patchmaster.key file, e.g., in a text editor.

3.1 The Key Listing

All keys can be listed in the Notebook by choosing List Keys in the Help menu. Alternatively open the Patchmaster.key file with a text editor.

In the following the notation of the key listing is described:

; denotes remark – will not be executed

@ denotes menu command.

B, P, N... denotes the window (B = Protocol Editor, P = Pulse Generator, N = Notebook...)

; B -> Protocol Editor
 B WindowSwitch Key SPACE any

char denotes a letter – simply press it.

R UnmarkIT Key Char U

any denotes that any modifier key may be pressed and the key will function.

R WindowSwitch Key SPACE any

3.2. The PATCHMASTER.key File

back denotes that this key will also work when the window is in the background (reverse to the "front only" function in the Dialog Configuration window).

E HoldDec2 Key CursorLeft option back

shift, option, caps, command, control requires that the respective modifier key must be pressed.

HOME, END denotes the corresponding keys on your keyboard.

R ScrollHome Key HOME any

NUM denotes that you have to use the numeric keypad for these numbers.

E Macro3 Key Numeric3

3.2 The PATCHMASTER.key File

```
MACRO-FILE                    900

; @ -> Menu Keys
@ File                        "New..."                    Key "N" [Press "Key" + CTRL]
@ File                        "Open Read Only..."      Key "O" [Press "Key" + CTRL]
@ File                        "Open Modify..."          Key "M" [Press "Key" + CTRL]
@ File                        "Update File"             Key "U" [Press "Key" + CTRL]
@ File                        "File Status"             Key "I" [Press "Key" + CTRL]
@ File                        "Quit"                      Key "Q" [Press "Key" + CTRL]

;        the standard keys should not be changed:
; @ Edit                      "Undo"                      Key "Z" [Press "Key" + CTRL]
; @ Edit                      "Cut"                        Key "X" [Press "Key" + CTRL]
; @ Edit                      "Copy"                        Key "C" [Press "Key" + CTRL]
; @ Edit                      "Paste"                      Key "V" [Press "Key" + CTRL]
; @ Edit                      "Select All"                Key "A" [Press "Key" + CTRL]
; @ Edit                      "Find..."                    Key "F" [Press "Key" + CTRL]
; @ Edit                      "Find Same"                Key "G" [Press "Key" + CTRL]
; @ Edit                      "Find Selection..."        Key "H" [Press "Key" + CTRL]
; @ Edit                      "Replace..."                Key "R" [Press "Key" + CTRL]
; @ Edit                      "Replace Same"             Key "T" [Press "Key" + CTRL]

@ Windows                    "Oscilloscope"             Key F12
@ Windows                    "Amplifier"                Key F11
@ Windows                    "Replay"                    Key F10
@ Windows                    "Protocol Editor"        Key F9
@ Windows                    "Pulse Generator"        Key F8
@ Windows                    "Analysis"                Key F7
@ Windows                    "Notebook"                Key F5
@ Windows                    "Close Front Window"     Key "W" [Press "Key" + CTRL]

@ Notebook                  "Save"                        Key "S" [Press "Key" + CTRL]
@ Notebook                  "Print..."                    Key "P" [Press "Key" + CTRL]
@ Notebook                  "Clear"                        Key "B" [Press "Key" + CTRL]
@ Notebook                  "Zoom In"                    Key "K" [Press "Key" + CTRL]
@ Notebook                  "Zoom Out"                    Key "L" [Press "Key" + CTRL]

; A -> Analysis
A WindowSwitch             Key SPACE any

; B -> Protocol Editor
B WindowSwitch             Key SPACE any
B End                        Key END any
B Home                      Key HOME any
```

3.2. The PATCHMASTER.key File

```
B DownPage           Key PageUp any
B UpPage             Key PageDown any
B Down               Key CursorUp any
B Up                Key CursorDown any

; C -> Solution Base
C Done              Key RETURN any

; D -> Parameters
D WindowSwitch      Key SPACE any

; E -> Amplifier
E WindowSwitch      Key SPACE any
E HoldDec2          Key CursorLeft option back [1 mV steps]
E HoldInc2          Key CursorRight option back [1 mV steps]
E HoldDec1          Key CursorLeft [10 mV steps]
E HoldInc1          Key CursorRight [10 mV steps]
E GainDec           Key CursorDown back
E GainInc            Key CursorUp back
E VOffsetDec        Key Numeric- shift option
E VOffsetInc         Key Numeric+ shift option
E VScaleDec          Key Numeric- option
E VScaleInc          Key Numeric+ option
E IOffsetDec         Key Numeric- shift
E IOffsetInc         Key Numeric+ shift
E IScaleDec          Key Numeric-
E IScaleInc          Key Numeric+
E TestOverlay        Key Char O
E AutoZero           Key Char Z
E SaveRpip           Key Char P
E RsOff              Key Numeric0 back
E ClampOff           Key Numeric0 back
E Setup              Key Numeric1
E Seal               Key Numeric2
E WholeCell          Key Numeric3

; G -> Probe Selectors
G WindowSwitch      Key SPACE any

; H -> Photometry
H WindowSwitch      Key SPACE any

; I -> I/O Control
I WindowSwitch      Key SPACE any

; J -> Calculator

; K -> Markers
K WindowSwitch      Key SPACE any

; L -> LockIn
L WindowSwitch      Key SPACE any
L LockInDone         Key RETURN any

; N -> Control Window
N WindowSwitch      Key SPACE any
N TimerSet           Key Char T any back
N Resume             Key Char R control back
N Wait               Key Char W control back
N Next               Key Char N control back
N Break              Key Char B control back
N Stop               Key Char S control back
N PG6                Key Char 6
N PG5                Key Char 5
N PG4                Key Char 4
N PG3                Key Char 3
```

3.2. The PATCHMASTER.key File

```
N PG2          Key Char 2
N PG1          Key Char 1

; O -> Oscilloscope
O WindowSwitch  Key SPACE any
O YOffsetDec   Key Numeric- shift back
O YOffsetInc   Key Numeric+ shift back
O YScaleDec    Key Numeric- back
O YScaleInc    Key Numeric+ back
O YCenter      Key Numeric. back
O ResetY       Key Numeric* back
O DispTrace    Key Numeric4 back
O DispTrace    Key2 Numeric5 back
O Wipe         Key DeleteLeft any back
O MoveRRight   Key Char > any [for "Scan" function, jumps 10 points]
O MoveRLeft    Key Char < any [for "Scan" function, jumps 10 points]
O MoveLRight   Key Char .  [for "Scan" function, point by point]
O MoveLLeft    Key Char ,  [for "Scan" function, point by point]

; P -> PGF-Editor
P WindowSwitch  Key SPACE any

; R -> Replay
R WindowSwitch  Key SPACE any
R ScrollEnd    Key END any
R ScrollPageDown Key PageDown any
R ScrollDown   Key CursorDown any
R ScrollUp     Key CursorUp any
R ScrollPageUp Key PageUp any
R ScrollHome   Key HOME any
R ScrollRight  Key CursorRight any
R ScrollLeft   Key CursorLeft any
R UnmarkIt    Key Char U
R MarkIt      Key Char M
R ShowIt      Key RETURN any

; S -> Configuration
S WindowSwitch  Key SPACE any

; T -> Trace Properties
T Done         Key RETURN any

; U -> Online_1
U WindowSwitch  Key SPACE any

; V -> Online_2
V WindowSwitch  Key SPACE any
```

4 Menus

The following section describes the various drop-down menus in PATCHMASTER.

4.1 File Menu

The **File** menu has all options to handle PATCHMASTER experiment files. A single PATCHMASTER "Experiment", that can hold a variable number of single electrophysiological experiments, can consist of several types of files:

- The `*.dat` file contains only the actual raw data without any timing or scaling information (=data).
- The `*.mac` file contains macros (=Macro). [Disposed since version 2x52]
- The `*.mrk` file contains the used marker information (=Marker).
- The `*.mth` file contains the used methods (=Method).
- The `*.onl` file contains Analysis information (=Online Analysis).
- The `*.pgf` file contains the used stimulus templates (=Pulse Generator File).
- The `*.pro` file contains Protocol Editor information (=Protocol Editor).
- The `*.pul` file contains the complete data tree (=Pulsed Tree).
- The `*.amp` file contains amplifier settings (=Amplifier Setting). This file is only required for EPC 10 Double, Triple, and Quadro amplifiers.
- If the solution database is used, then a file with the extension `*.sol` is stored with the data files (=Solution). It contains information on the solutions used in the stored experiments.
- In the `*.txt` file (=Text), the contents of the Notebook are stored (If *Auto Store* is activated in the Notebook menu).

If the option *Make Bundle File* is checked in the Configuration window (see chapter 5.5.2 on page 56), then the files `*.pul`, `*.pgf`, `*.onl`, `*.mrk`, `*.mth` and `*.dat` (and `*.amp`) are merged into one single `*.dat` file.

Raw data acquired by PATCHMASTER is only written to disk in the so called *Store* mode (i.e. if the *Store* button in the Control window is switched on).

The data are written to disk either:

- upon completion of a *Sweep* or *Series* or
- during the acquisition, in case of a continuous *Sweep*.

The structural information is kept in RAM; they are stored to disk only when:

4.1. File Menu

- a new file (File → New), a new group (File → New Group) or a new experiment (File → New Experiment) is created or
- the Update File function is executed (File → Update File) or
- the program is terminated (File → Quit)

New...: Creates a new, empty data file that is ready for data acquisition. The file has read and write permission.

Open 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.

Open 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.

Merge...: Appends the data tree from an existing data file to the data tree of the actually opened file.

Update File: Updates the whole experiment to disk. This includes all files involved (see above). If you encounter computer crashes leading to data loss, use this option frequently or enable one of the settings to be found via File → Write Tree Files.

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. A typical output could look as follows:

```
Storing to disk ENABLED,  
read-write file: "C:\HEKA\Data\Exp001.dat"  
length: 284 kb; free disk space: 44.33 GB.
```

New Group: Generates a new *Group* in the output data tree of the Replay 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.

Write Tree File: Defines when the data are flushed to disk. Choose one of these options:

- After Update File: Files are updated after an *Update File* command, and after creating a new experiment or *Group*.
- After Series: Writes raw data to disk after each acquired *Series*.
- After Sweeps: Writes raw data to disk after each *Sweep*.

The second option will make sure that the system file cache gets written to disk (i.e., "flushed") after acquiring a *Sweep*, and the third option performs a cache flush after acquiring a complete *Series*. Deselecting both options will suppress file cache flushing. In that case, the operating system will flush the file cache when it overflows.

New...	Ctrl+N
Open Read Only...	Ctrl+O
Open Modify...	Ctrl+M
Merge...	
Update File	Ctrl+U
Close	
File Status	Ctrl+I
New Group	
New Experiment	
Write Tree Files	▶
Convert To PPC Format	
Convert To Intel Format	
Page Setup...	
Page Properties Notebook...	
Page Properties Traces...	
Quit	Ctrl+Q

4.1. File Menu

The flushing of the file cache may take some seconds, depending on its size. Thus, if one lets the system decide when to flush, it may occur at an inappropriate moment, such as in the middle of a series. On the other hand, if PATCHMASTER would always force file cache flushing (as it does when the option *Write After Sweep* is active), one could not take advantage of the fact that writing to the file cache in RAM is faster than physically writing to disk.

It is safest to select the *Write After Sweep* option. This ensures that the data are immediately written to disk. The timing between *Sweeps* is also not interrupted by the system when a possibly large file cache is written to disk. If one must get the fastest disk performance possible, one can deselect the options. In that case, data are written to RAM, not directly to disk. However, this can only work as long as fewer data are acquired than there is space in the file cache.

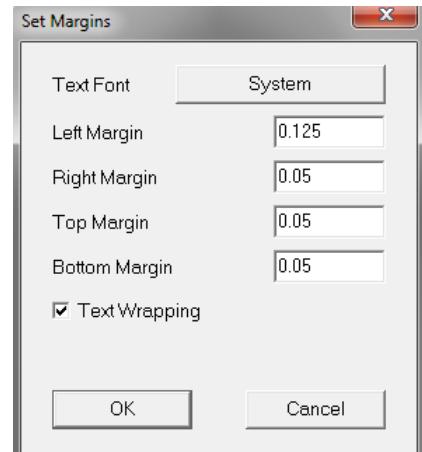
Note: PATCHMASTER writes only that part of the tree files which were added or moved, thereby drastically reducing the time required for updating the files to disk.

Convert to PPC: Converts the raw data to PowerPC format ("big endian"). This is required if a third party program is used that can not distinguish between Intel ("little endian") and PPC data format but requires data to be read in PPC format.

Convert to Intel Format: Converts the raw data to Intel ("little endian") format. This is required if a third party program is used that can not distinguish between Intel and PPC data format but requires data to be read in Intel format.

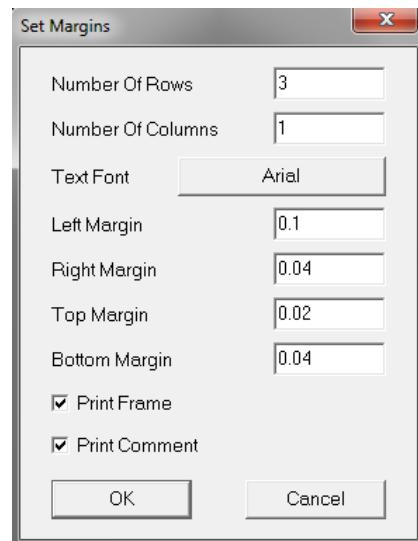
Page Setup...: Calls the Printer/Page Setup dialog of the operating system.

Page Properties Notebook...: Calls a dialog to set the page margins (left, right, top and bottom) and the font for printing. These settings apply for printing the Notebook.



4.1. File Menu

Page Properties Traces...: Calls a dialog to set the number of columns and rows, the page margins (left, right, top and bottom) and the font for printing. These settings apply for printing data graphs.



Quit: Exits PATCHMASTER.

The default quit options are:

- Save + Exit: Saves configuration and data files and then quits the program.
- Exit: Saves data files and quits program.
- Cancel: Exit process will be aborted, you return to the program.

These default behavior can be influenced by the options *Save Settings* and *Verify Quit* in the configuration dialog of PATCHMASTER.

Note: If you changed the Protocol Editor, the Pulse Generator pool or the Analysis you will be asked independently if you want to save your changes.



Data files are always saved.

4.2 Edit Menu

The Edit menu applies to text manipulation in the Notebook window. The **Copy** function can also be used to copy graphs from the Oscilloscope or from an Online window into the clipboard.

Please note that most entries of this menu are disabled unless the Notebook window is in front!

The menu entries conform to the typical functions of the actual operating system (Mac OS or MS Windows).

Undo: Cancels the last action performed in the Notebook window.

Cut: Cuts the text selection from the Notebook window.

Copy: Copies a text selection from the Notebook. The **Copy** function can also be used to copy graphs from the Oscilloscope or from an Online Window into the clipboard. The active window (Notebook, Oscilloscope or Analysis) determines what is copied.

Paste: Pastes text from or to the clipboard.

Clear: Removes any content of the Notebook window.

Select All: Selects the whole Notebook window content.

Find...: Finds the entered search string.

Note: The Find function is case-sensitive and only searches from the cursor position to the end of the string.

Find Same: Finds the next appearance of the entered search string.

Find Selection...: Finds the search string that was marked (highlighted) in the Notebook window.

Replace...: Replaces the entered search string by some new string.

Replace Same: Finds and replaces the next appearance of the entered search string.

Undo	Ctrl+Z
Cut	Ctrl+X
Copy	Ctrl+C
Paste	Ctrl+V
Clear	
Select All	Ctrl+A
Find...	Ctrl+F
Find Same	Ctrl+G
Find Selection...	Ctrl+H
Replace...	Ctrl+R
Replace Same	Ctrl+T

4.3 Windows Menu

The Windows menu applies to the windows in PATCHMASTER. Clicking on a menu entry either opens the respective window or brings the already open window to the front. For most windows there are key commands assigned which will be displayed in the menu (see image beneath and also chapter 3 on page 13).

Control Window: Opens the Control Window or brings it to the front (see chapter 8 on page 99).

Configuration: Opens the Configuration window or brings it to the front (see chapter 5 on page 43).

Oscilloscope: Opens the Oscilloscope window or brings it to the front (see chapter 6 on page 71).

Amplifier: Opens the Amplifier window or brings it to the front (see chapter 7 on page 75).

Replay: Opens the Replay window or brings it to the front (see chapter 9 on page 103).

Protocol Editor: Opens the Protocol Editor window or brings it to the front (see chapter 11 on page 139).

Pulse Generator: Opens the Pulse Generator window or brings it to the front (see chapter 10 on page 107).

Analysis: Opens the Analysis window or brings it to the front (see chapter 13 on page 187).

Analysis Window 1, 2: Opens the specified Analysis Window window or brings it to the front (see chapter 13.4 on page 202).

Parameters: Opens the Parameters window or brings it to the front (see chapter 14 on page 211).

I/O Control: Opens the I/O Control window or brings it to the front (see chapter 16 on page 225).

Photometry/Imaging: Opens the Photometry Configuration or Imaging Configuration window or brings it to the front if the Photometry or Imaging Extension is activated (see chapter 5.4.8 on page 51 or chapter 5.4.6 on page 51).

LockIn: Opens the LockIn Control window or brings it to the front if the LockIn Extension is activated (see chapter 5.4.4 on page 50).

Spectroscopy: Opens the Spectroscopy Control window or brings it to the front if the Spectroscopy Extension is activated (see chapter 5.4.5 on page 51).

Solution Base: Opens the Solution Base window (see chapter 17 on page 229). If no solution base file is loaded you will be prompted to create a *.sol file.

Solution Changer: Opens the Solution Changer dialog (see chapter 18 on page 233).

Markers: The Markers window allows to set sweep markers and/or trace markers during a continuous recording (see chapter 19 on page 235).

Protocol Methods: Opens a dialog which provides information about the used *Protocol Method*.

Calculator: The Calculator window allows to perform numeric calculations in PATCHMASTER (see chapter 20 on page 239).

Notebook: Opens the Notebook window or brings it to the front (see chapter 15 on page 223).

Control Window	
Configuration	
Oscilloscope	F12
Amplifier	F11
Replay	F10
Protocol Editor	F9
Pulse Generator	F8
Analysis	F7
Analysis Window 1	
Analysis Window 2	
Parameters	
I/O Control	
Photometry/Imaging	
LockIn	
Spectroscopy	
Solution Base	
Solution Changer	
Markers	
Protocol Methods	
Calculator	
Notebook	F5
Close Front Window	Ctrl+W
Save Front Dialog	
Reset Front Window Position	
Enable Icon Configuration	

Probe Selector: Opens the Probe Selector window or brings it to the front.

Note: This menu item is only visible, if the Probe Selector has been activated in the configuration dialog (see chapter 5.4.11 on page 54).

Close Front Window: Closes the window that is in front display.

Save Front Dialog: Dialogs modified by the user can be saved via this menu entry (see chapter 2 on page 5).

Reset Front Window Position: The position of the active (front) window is reset to a predefined "home" position. This function is essential when accidentally the window position has a huge offset and cannot be brought back into the field of view.

Enable Icon Configuration: If selected, the dialogs and controls in the PATCHMASTER user interface can be modified (see chapter 2.2 on page 6).

4.4 Replay Menu

The Replay menu functions are active when the Replay window is selected.

Show: Displays the content of the selected target. Traces are displayed according to the settings specified in the Display menu and the Oscilloscope window. If the *Root* 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 Oscilloscope window.

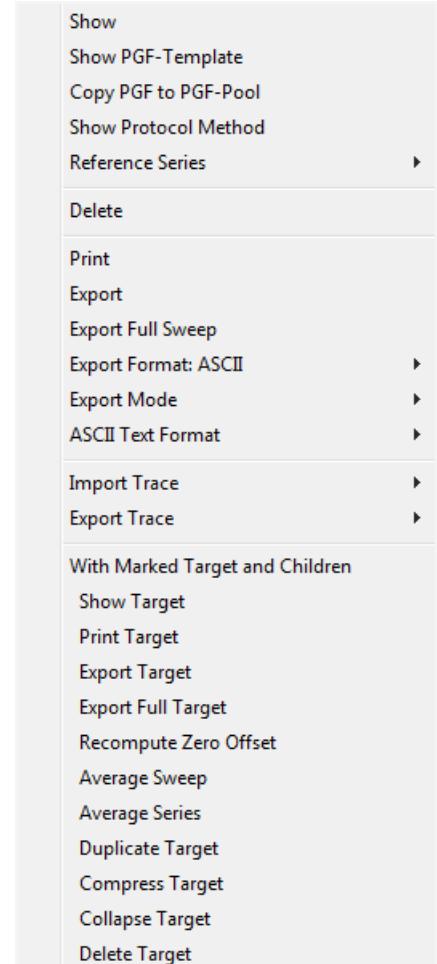
Show PGF Template: This option opens the stimulation file (PGF-Template) of the corresponding selected *Series* in the Replay tree (or the first *Series*, if a *Group* is the target). Leave the window open and scroll through your acquired data in the Replay tree to get an overview of the stimulation settings of your recordings (read-only).

Copy PGF to PGF-Pool: Copies the stimulus protocol of a selected *Trace*, *Sweep* or *Series* into the current Pulse Generator file.

Show Method: Displays the name and the used settings of the *Protocol Method* of the selected target (read-only). There are two additional options:

- **Copy To Analysis:** Copies the used *Analysis Method* of the *Protocol Method* to the *Analysis Methods* pool of the *Analysis*.
- **Copy To Protocol:** Copies the protocol event lines of the used *Protocol Method* into the active protocol at the selected position of the *Protocol Editor*.

Note: By using the *Copy* function from the *Edit* menu one can copy the the method text to the clipboard. Then one can paste the method at any position in the *Protocol Editor* by the *Paste* option.



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 *Display* menu is active. The subtraction of the Reference Series not only affects the display of the data in the Oscilloscope window, but also data analysis (see chapter 4.5.2 on page 31) and export. A previously selected Reference Series can be turned off with the *Off* function.

Delete: Deletes the selected target.

Print: Prints the selected target and the *Analysis Graphs* if an *Analysis Method* was active. The *Analysis Graph* gets only printed when the *Series* level is the selected target.

Export: Exports the selected target. Display gain, leak subtraction or zero line subtraction will be applied (special case for *Igor Binary* export see below).

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 or make sure that the display is set to full time range, i.e. Start Time = 0%, End Time = 100% .

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.

Export Format: This determines the output device and the type of output to be created. Output is generated in the way the data are displayed in the Oscilloscope window, e.g. when the digital filter is on, filtered data are output.

The following export formats are implemented:

- ASCII: 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 Replay menu.

Please note, that this may create huge ASCII files when the output target is a *Group*, for example.

- Igor Pro: Exports Igor Pro format. For further format options, see the lower part of the menu.
- MatLab: Sweeps are exported as MatLab file.
-  PICT: Sweeps are exported as Mac OS PICT file. Each file contains a single Sweep. When a Series is output, the Sweep files are generated automatically with the same name convention as waves for Igor Pro files: indices of "Group_Series_Sweep" are appended to the name.
-  WMF: Sweeps are exported as Windows Meta Files. Each file only contains a single Sweep. When a Series is output, the Sweep files are generated automatically with the same name convention as waves for Igor Pro files: indices of "Group_Series_Sweep" are appended to the name.

Note: Instead of exporting graphs in PICT or WMF format, you might want to copy (CTRL+C) the traces and to paste (CTRL+V) them into another application.

- Printer: Defines a printer for the export of the selected data. In contrast to the direct Print option where you always print the data and the Analysis Graphs, if available, the Printer allows to set either Traces and/or Analysis - Graphs in the Export Mode. If one wants to have individual Sweeps rather than a complete Series plotted in the page sections, one has to turn off Overl. Ser. in the Oscilloscope window or use the export option Export Full Sweep.

Further export parameters can be set here:

- Trace Time relative to Sweep: Individual Sweeps will be exported in an overlaid fashion. Time of the first data point of each Sweep starts with 0.
- Trace Time 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.

- Trace Time relative to Timer: Individual *Sweeps* will be exported in a concatenated fashion. The *Trace* time is offset by the *TimerTime* at *Sweep* start.
- Relative Trace Time: 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 *Sweep* starts with 0.

The following format options refer to Igor Pro format only:

- Igor - Allow Raw Data Access: Whenever possible an Igor Info file is generated allowing access to raw PATCHMASTER data which should be stored in the same folder. Using this output option assures fastest output and the smallest output files.
- Igor - 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 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.

- Igor - 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".
- Igor - Make Graphs: The *Sweeps* are exported as Igor graphs as they appear in PATCHMASTER 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.
- Igor - 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.
- Igor - 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.

Export Mode: Shows a sub-menu that allows determining what will be exported:

- 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.

4.4. Replay Menu

- Analysis - Graphs: The graphs of the Analysis get exported.
- Analysis - Notebook: The Analysis function results which are displayed in the Notebook get exported. Please note that the option *Notebook* in the Analysis window has to be selected.

Note: This option is not available when using the Igor Pro Export Format.

ASCII-Text Format: Shows a sub-menu that allows to specify the type of separator used when generating ASCII tables, and the format of the exported text:

- Space/Comma/Tab Separator: Specifies how values are separated.
- Include Headers: If checked, a header that specifies various parameters of the exported data will precede the actual values.

Import Trace: Imports data into a selected *Trace*.

When importing data you first have to generate a corresponding data structure (*Tree*) in PATCHMASTER. Make sure that the *Gain* and *Sampling Interval* of the data structure match the data you intend to import:

- *Gain* mismatch might result in loss of resolution or saturation of the data.
- The length of the imported *Trace* must be equal or greater than the *Trace* selected in the Replay window. Points exceeding the length of the *Trace* in the Replay window are discarded.

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.

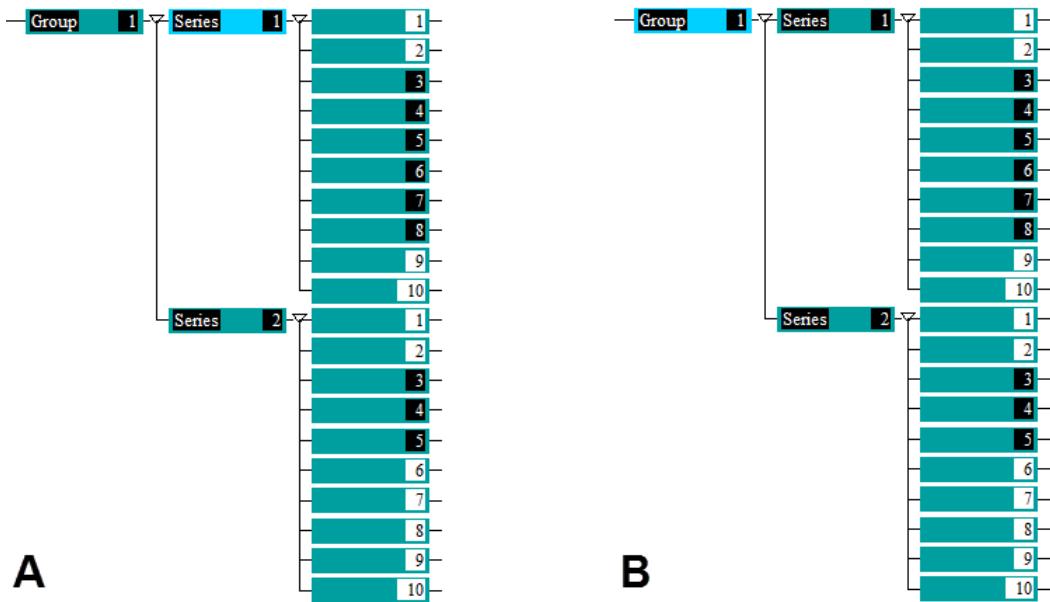
Note: Since the Traces will be imported into the open file, the import function does not work when your file is read-only.

Export Trace: Exports data. You have four possibilities:

- as ASCII file: Exports data in ASCII file format.
- as Binary file: Exports data in Binary file format.
- as Igor Wave: Exports data in Igor wave format.
- as Stimulus Template file: Exports data as a stimulus template file.

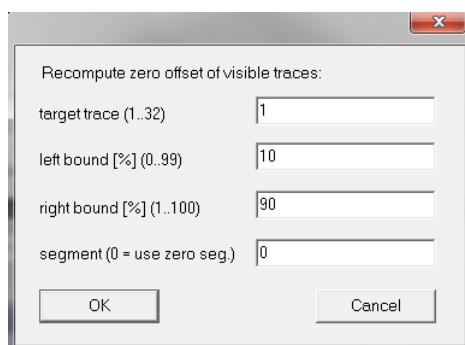
With marked Target and Children...

Actions executed from this section of the Replay menu are performed on all marked children of the selected Target in the data tree:



In example "A" the With Marked Target and Children → Export command would export only Sweep 3 to 8 of the first IV, while in example "B" all marked Sweeps would be exported.

- Show: Displays marked Targets in the selected tree branch.
- Print: Prints the marked Targets and children of the selected tree branch.
- Export Target: Exports marked targets and children of the selected tree branch. The display options in the Oscilloscope window define the export options.
- Export Full Target: 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 4.5 on page 29).



- target trace (1...32): Selects the target Trace for the zero offset recomputing.
- left bound [%](0...99) & right bound [%](0...100): Defines the range in between PATCHMASTER should recompute the zero offset.
- segment (0=use zero seg.): Defines the Segment (of the PGF) 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.

4.4. Replay Menu

Note: The zero offset of leak Traces are only recomputed if the "Show Leak Traces" is selected from the Display 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: Creates a new Group containing all marked targets. This is e.g. convenient when one needs to compress a Trace for export.
- Compress: 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: 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: Deletes the marked targets and children of the selected tree branch.

Note: Delete and Delete Marked Target Children are disabled when a data file is opened with File → Open Read Only.

4.5 Display Menu

This menu sets some parameters for the display of data in the Oscilloscope window.

Auto Show: Automatically displays the data selected in the Replay window. When this option is disabled, a double-click on the targets in the tree is required for displaying the data.

Trace Properties: In the Trace Properties dialog the user can set *Show*, *Vector* (connect individual data points) and *Center* flags, select the *Marker* type and the *Reference Series* properties. All settings are explained in detail in chapter 4.5.2 on page 31.

Also, the *Trace Properties* can be set via macro commands to allow automatic display adjustment depending on type of acquisition. Have a look for the macro commands starting with T (Help menu → List All Macro Items).

The color of *Traces* can be set in the Line Colors dialog of the Configuration window (see chapter 5.6.1 on page 58).

Show Leak Traces: Shows the P/n leak pulse signals in the Oscilloscope like a *Trace*. As a prerequisite you have to activate the *Leak Subtraction* in the Pulse Generator (see chapter 10.14 on page 135).

Show Zero Line: Draws a reference zero line in the Oscilloscope window.

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 PGF (see chapter 10.9.2 on page 128). 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 *Replay* menu (see chapter 4.4 on page 27).

Note: 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 *Replay 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 item is checked, then the Reference Series is subtracted from all replayed data (see chapter 4.5.2 on page 31).

Subtract Trace Buffers: If this item is checked, then the *Trace Buffer* is subtracted from all replayed *Traces* (see chapter 4.5.2 on page 31).

Dimmed Overlay: Turns the *Dimmed Overlay* mode on or off. In *Dimmed Overlay* mode, the last displayed *Trace* is drawn in another color than the previously plotted *Traces*.

Overlay Pages: During continuous acquisition the data are displayed in pages. The pages can be overlaid.

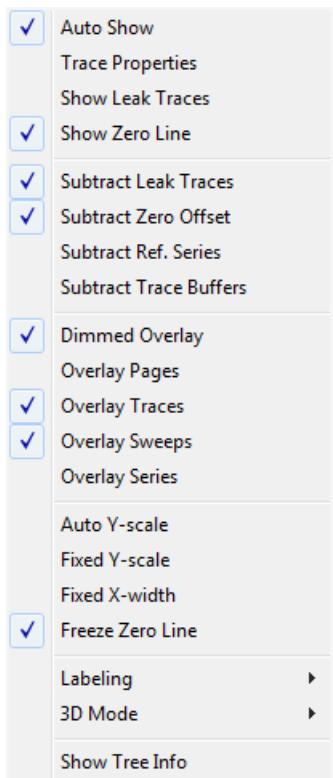
Overlay Traces: Overlay of all *Traces* of a selected *Sweep*.

Overlay Sweeps: Overlay of all *Sweeps* of a *Series*.

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

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.

Fixed Y-scale: The Y-scale is fixed. 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 Fixed Y-scale.

Fixed X-width: The X-scale is fixed. In the Oscilloscope window the controls *Start Time* and *End* turn into *X-min* and *X-max*.

Freeze Zero Line: This checkbox determines the behavior of the display scaling in the Oscilloscope window. When *Freeze Zero Line* is selected, the Oscilloscope 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.

Labeling: Determines the labels in the main display of the Oscilloscope window and the *PGF-Editor Grid* independently:

- Labels Only: Draws calibration bars.
- Grids + Labels: Draws a grid and units/division.
- Grids + Values: Draws a labeled grid.
- No Labels: No labels, no grids.

Show Tree Info: When this option is selected, information about the replayed target is shown in the Notebook window.

4.5.1 3D-Mode

Shows a sub-menu for selection of the pseudo three-dimensional graphing.

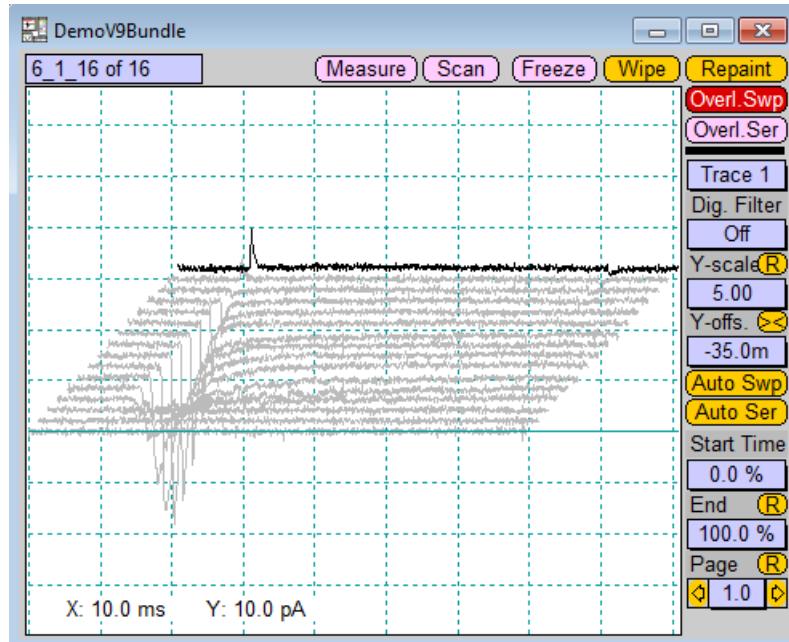


Figure 4.1: 3D Mode of the Oscilloscope Window

The following settings can be made:

- 3D-Graph - On: The results are displayed in black and white in pseudo three-dimensional mode by displaying subsequent *Sweeps* with a horizontal and vertical offset. The *dX* and *dY* values for the 3D feature can be entered in the Configuration window. The default value for both is 0.010.

4.5. Display Menu

- 3D-Graph - Color: The results are displayed in pseudo colors leading to a two-dimensional height profile. You can set the color selection and the contrast by changing the values in Y-scale and Y-offs.
- 3D-Graph - On and 3D-Graph - Color: The results are displayed in pseudo colors and 3D-mode to give the impression of contour lines, thus leading to a 3D height profile.

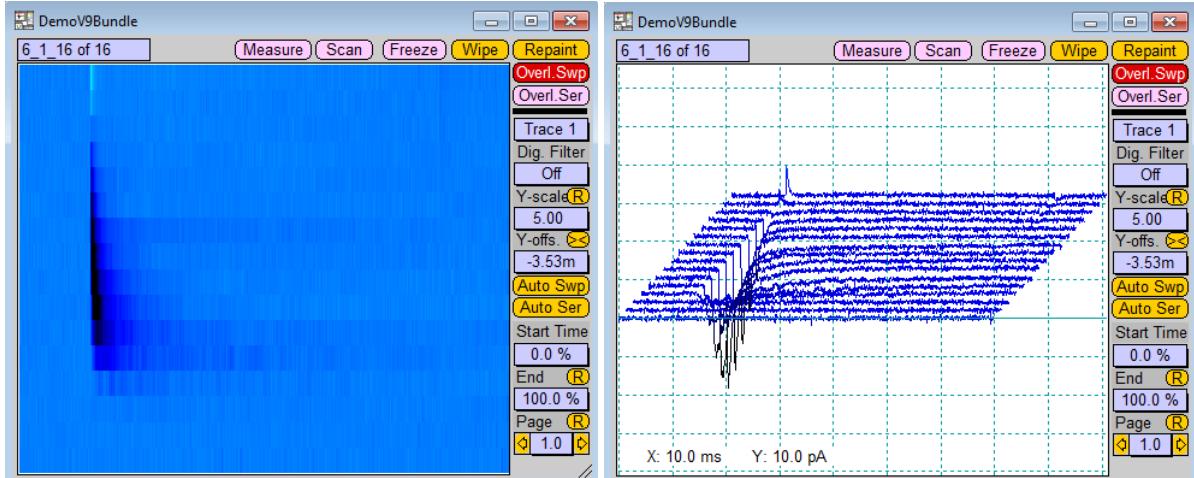


Figure 4.2: Pseudo Color Data Representation

4.5.2 Trace Properties Dialog

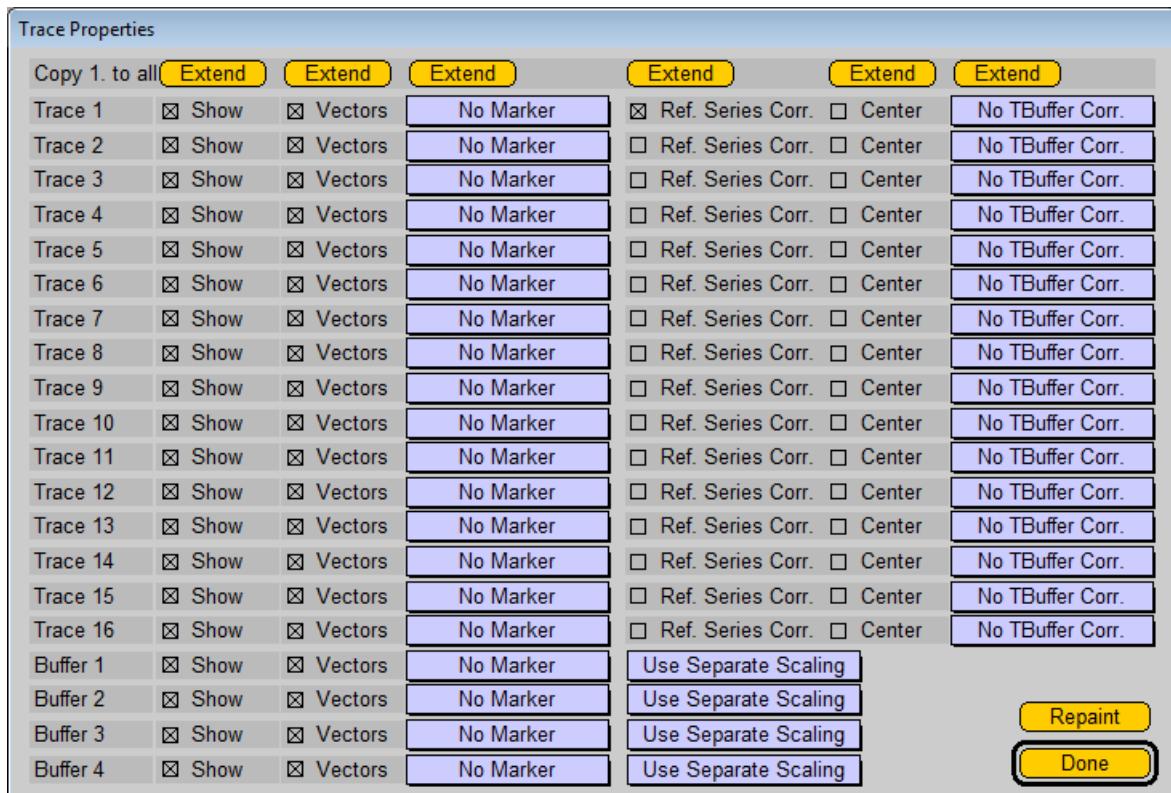


Figure 4.3: Trace Properties Dialog

The Trace Properties dialog provides access to certain display settings of individual *Traces* and *Buffers*.

Extend: Extends the setting of the first *Trace* to all other *Traces*.

Trace 1...16, Buffer 1...4: Up to 16 *Traces* and 4 buffers can be individually modified.

Note: The amount of traces (default: 16) is defined in the Configuration (General pane).

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

Vectors: When option Vectors is checked then the markers are connected by lines.

Marker: Markers, such as

- Point
- Plus
- Star
- Diamond
- Cross
- Square

can be used for plotting data points. This is particularly useful, if LockIn or FURA data are acquired.

Ref. Series Corr.: If Ref. Series Corr. 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 item from the Replay menu. The Ref. Series Corr. can be turned 'On' or 'Off' globally in the Display menu.

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.

Trace Buffer Corr.: If Trace Buffer Corr. 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 Trace Buffer subtraction can be turned 'On' or 'Off' globally in the Display menu.

Use Trace Scaling: With this option, the display scaling of a *Trace Buffer* can be coupled to the display scaling of a certain *Trace*.

Repaint: Repaints the last displayed Sweep in the Oscilloscope with the new *Trace Properties*.

Done: Closes the *Trace Properties* dialog.

Note: Like other menu settings, the *Trace Properties* are stored in the PATCHMASTER configuration file.

4.6 Buffer Menu

PATCHMASTER offers four independent *Trace Buffers* for storing and processing of intermediate *Trace* data.

Buffer 1... Buffer 4: Select (make active) the buffer to work on.

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* (*Buffer 1...4*).

Clear: Clears the selected *Buffer*.

Show: Shows the selected *Buffer* in the Oscilloscope.

Scale: Scales the selected *Buffer* with *Scale* and *Offset*.

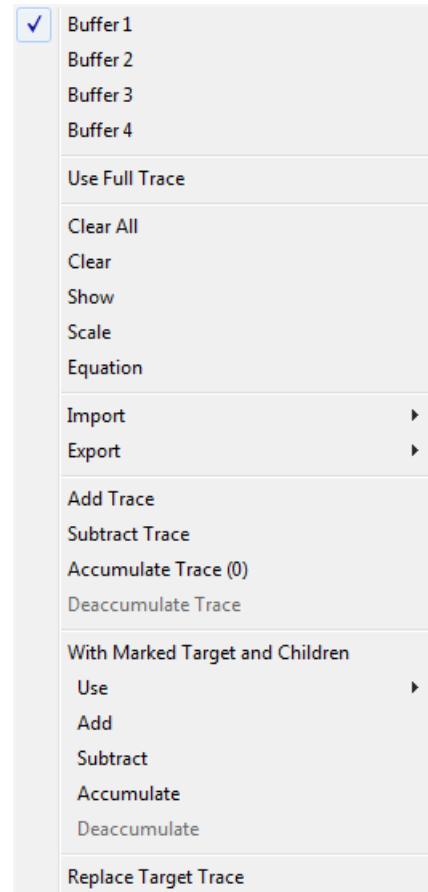
Equation: Allows to perform a *Buffer* calculation based on an equation. For details see chapter 20 on page 239 and chapter 20.2.2 on page 243.

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 *Replay* 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*. You have four possibilities:

- as ASCII file: Exports data in ASCII file format.
- as Binary file: Exports data in Binary file format.
- as Igor Wave: Exports data in Igor wave format.
- as Stimulus Template file: Exports data as a stimulus template file (see Using a Recorded Waveform as Stimulus in the PATCHMASTER Tutorial).

Add Trace: Adds the *Trace* selected in the *Replay* window to the active *Buffer*.

Subtract Trace: Subtracts the *Trace* selected in the *Replay* window from the active *Buffer* (*Trace*).

Accumulate Trace: Accumulates the *Trace* selected in the *Replay* window 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 *Replay* 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 *Replay* window and processed simultaneously.

- Use: Specify which *Trace* of the marked *Sweeps* should be processed.
- Add, Subtract, Accumulate, Deaccumulate: Work on all marked targets. For further explanations of the functions refer to individual functions explained above (see chapter 4.4 on page 26).

Replace Target Trace: The *Trace* selected in the *Replay* window (*target Trace*) will be replaced with the active *Buffer*.

4.6.1 Handling of Parameters by the Buffer

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.

4.7 Notebook Menu

The Notebook is used to display experiment information. The ASCII-table separator setting of the *Replay* menu is used for the Notebook as well. This enables e.g. to transfer data directly to spread sheets requiring a TAB separator, such as MS Excel, via "Cut and Paste". The options in the Notebook menu are:

Save: Saves the Notebook 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.

Clear: Clears the Notebook.

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.

Zoom In: Shrinks the Notebook window to default (i.e. small) size.

Save	Ctrl+S
Save As...	
Merge...	
Print...	Ctrl+P
<input checked="" type="checkbox"/> Clear when Saved	
Clear	Ctrl+B
Set Length... (20000)	
Line Numbers	
Font Size...	
Zoom In	Ctrl+K
Zoom Out	Ctrl+L
Scientific Notation	
Auto Store	

Zoom Out: Expands the Notebook window to full program screen size.

Scientific Notation: 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 Replay menu.

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.

4.8 Protocols Menu

The Macros menu of older PATCHMASTER versions has been replaced by the new Protocols menu (since version 2x52). This drop-down menu can be used for the conversion of macro files from older PATCHMASTER versions to protocols.

Append Macro File: Converts an existing macro file (*.mac) to a protocol and appends this new protocol to the actually opened protocol file (*.pro).

Execute while Recording: If selected, every button you press during macro/protocol recording will be executed. If not selected, this option allows recording a protocol without executing the buttons. Thus, during protocol recording, commands and values are recorded, listed in the Notebook, and then the parameters are immediately set back to their previous value.

Note: Macro/protocol recording can be started from the Protocol Editor dialog (see chapter 11.1 on page 139).

Execute: Executes the selected, user defined protocol. Alternatively, protocols can be executed from:

- the Control window, if the option *Hide Protocol-Bar* in the Control window is not activated on the Display tab (see chapter 5.6.3 on page 59).
- the Amplifier window, if the option *Show Amplifier Protocols* is activated on the Display tab (see chapter 5.6.3 on page 59).

<input checked="" type="checkbox"/>	Append Macros File
<input type="checkbox"/>	Execute while Recording
<input type="checkbox"/>	Execute Example1
<input type="checkbox"/>	Execute Example2
<input type="checkbox"/>	Execute Link
<input type="checkbox"/>	Execute Buffer
<input type="checkbox"/>	Execute SETUP
<input type="checkbox"/>	Execute SEAL
<input type="checkbox"/>	Execute WHOLE-CELL
<input type="checkbox"/>	Execute 8
<input type="checkbox"/>	Execute 9
<input type="checkbox"/>	Execute 10
<input type="checkbox"/>	Execute 11
<input type="checkbox"/>	Execute 12
<input type="checkbox"/>	Execute 13
<input type="checkbox"/>	Execute 14
<input type="checkbox"/>	Execute 15
<input type="checkbox"/>	Execute 16
<input type="checkbox"/>	Execute 17
<input type="checkbox"/>	Execute 18
<input type="checkbox"/>	Execute 19
<input type="checkbox"/>	Execute 20
<input type="checkbox"/>	Execute 21
<input type="checkbox"/>	Execute 22
<input type="checkbox"/>	Execute 23
<input type="checkbox"/>	Execute 24
<input type="checkbox"/>	Execute 25
<input type="checkbox"/>	Execute 26
<input type="checkbox"/>	Execute 27
<input type="checkbox"/>	Execute 28

4.9 EPC 10 USB Menu

Enable Background: This allows to keep the test pulse running and thus *I-mon*, *V-monitor*, and *R-memb* being updated even when PATCHMASTER is in the background.

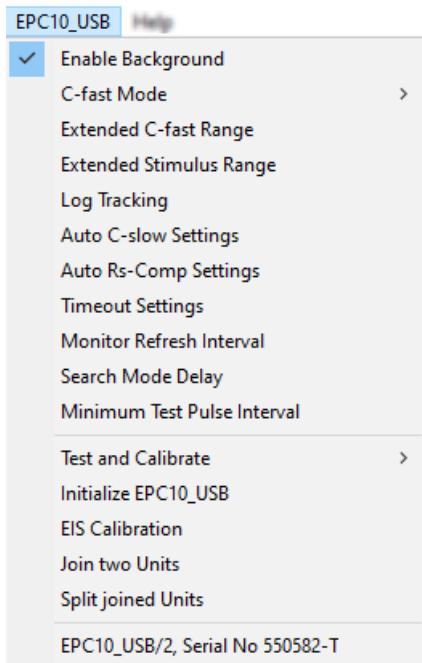
C-fast Mode: The following options are available:

- Use Lookup Table
- Computed

Use Lookup Table results in faster compensation procedure than the *Computed* mode. The latter mode is preferable when compensating large capacitances.

Extended C-fast Range: Activates the extended *C-fast* compensation range of newer EPC 10 amplifiers. The *C-fast* range can be extended by a factor of about 10x. With this extended compensation range, the noise in lipid bilayer experiments can be significantly reduced, by fully compensating for the huge and fast capacitances in these experiments.

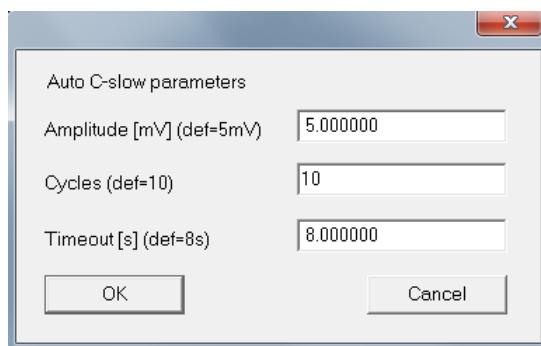
Extended Stimulus Range: Activate this option to increase the stimulus range of the amplifier to ± 2 V.



Note: Activating this option does also turn on the option Extendend C-fast Range.

Log Tracking: Writes the updated values of *C-slow* and *G-leak* to the Notebook when capacitance tracking is on.

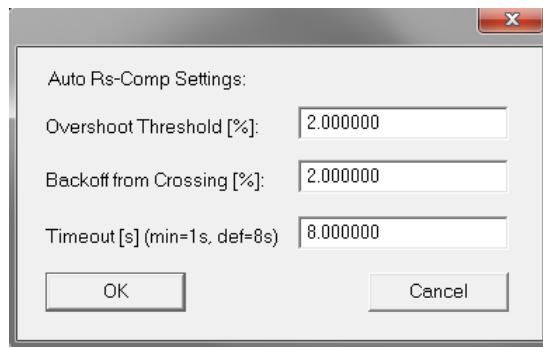
Auto C-slow settings



Three parameters of the *Auto C-slow* setting can be modified:

- Amplitude[mV](def=5mV): The amplitude of the voltage pulses used to compute *C-slow* and *G-series*. The default value is 5 mV.
- Cycles(def=10): The number of voltage pulses used to compute *C-slow* and *G-series*. The minimum and maximum cycle numbers are '4' and '38', respectively. The default entry is '10'.
- Timeout[s](def=8s): The time after which PATCHMASTER will stop trying to perform an *Auto C-slow* compensation. If the compensation routine does not find appropriate parameters within the timeout period, then *C-slow* and *R-series* in the amplifier dialog will remain unchanged. The minimum value is 1 s, the default value is 8 s.

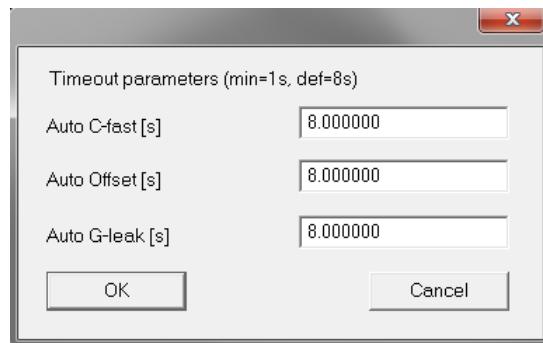
Auto Rs-Comp Settings



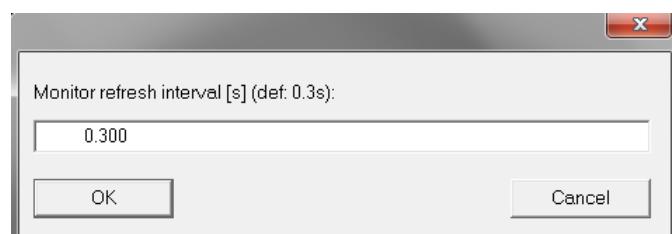
Three parameters of the *Auto Rs-Comp* setting can be modified:

- **Overshoot Threshold[%]:** This parameter defines the extend of overshoot the procedure is searching for. The default value is 2 %.
- **Backoff from Crossing:** This parameter defines how much the *Rs-comp* value is reduced from the settings at which the % overshoot is obtained. The default value is 2 %.
- **Timeout[s](min=1s,def=8s):** The time after which PATCHMASTER will stop trying to perform an *Auto C-slow* compensation. If the compensation routine does not find appropriate parameters within the timeout period, then *C-slow* and *R-series* in the amplifier dialog will remain unchanged. The minimum value is 1 s, the default value is 8 s.

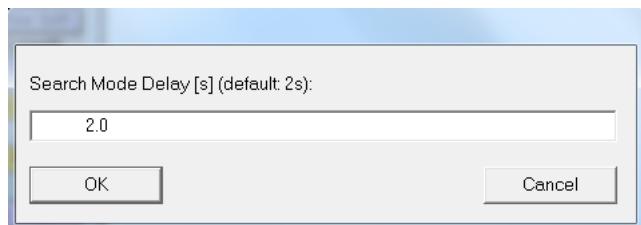
Timeout Settings: The time after which PATCHMASTER will stop trying to perform an *Auto C-fast*, *Auto Offset* or *Auto G-leak* compensation. If the compensation routine does not find appropriate parameters within the timeout period, the settings in the amplifier dialog will remain unchanged. The minimum value is 1 s, the default value is 8 s.



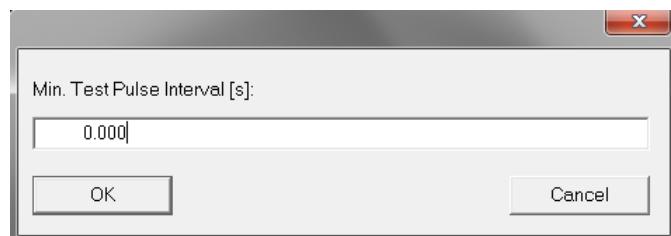
Monitor Refresh Interval: This option defines the refresh interval time of *I-mon* and *R-memb* depicted in the *Monitor* tab of the *Amplifier* window (see chapter 7.1 on page 76). *I-mon* and *R-memb* computed from an acquired *Sweep* are not affected by the *Monitor Refresh Interval* value. The default value is 0.3 s.



Search Mode Delay: The *Search Mode* (see *Operating Modes* in the EPC 10 manual) is essentially a repetitive *Auto-V₀* procedure at a holding potential of 0 mV. The delay specifies at what rate *Auto-V₀* is performed. The default value is 2 s.



Minimum Test Pulse Interval: By default, the *Test Pulse* is repeated "as fast as possible" (*Min. Test Pulse Interval* = 0). This item allows to reduce the test pulse frequency by setting a desired *Test Pulse* interval.



4.9.1 Test and Calibrate

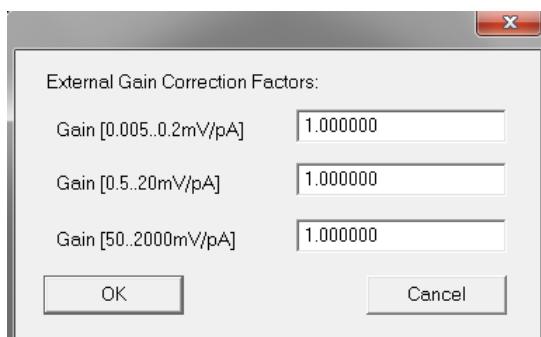
Calibrate: Calibrate the EPC 10. The calibration procedure creates a new scale file and a new *C-fast* table.

Full Test: Make a full test of the EPC 10. If the *Full Test* should detect hardware problems, it will generate a test report, which can be send to HEKA for further diagnosis.

Note: The Calibration and the Full Test procedure may take several minutes. We also recommend to switch on the amplifier at least one hour before executing these procedures.

Calibrate
Full Test
External Gain Calibration
External Zero Calibration
<input checked="" type="checkbox"/> Scale from/to EEPROM
Scale from/to Disk
Save Protocol
Print Protocol File

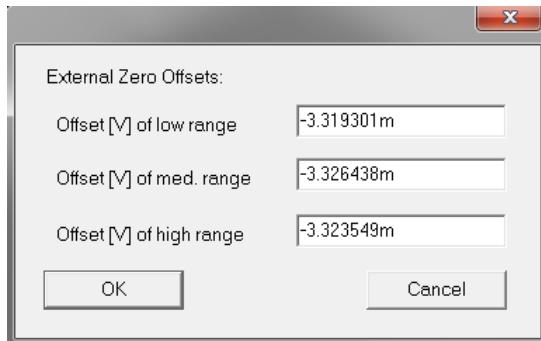
External Gain Calibration: Enter here *Gain* correction factors for the low (top), medium (middle) and high (bottom) *Gain* range.





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

External Zero Calibration: Enter here the *Offset* 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.

Scale from/to EEPROM: If this option is selected, then the calibration file (scale file) is loaded from the EEPROM of the amplifier. The calibration procedure also writes the scale file to the EEPROM. This option only exists for newer EPC 10 amplifiers (since revision 'R' for USB amplifiers and revision 'S' for PCI amplifiers) and iTEV 90 amplifiers.

Scale from/to Disk: If this option is selected, then the calibration file (scale file) is saved/loaded to/from a file stored on the hard-disk of the host computer. The calibration procedure also writes the scale file to a file on disk.

Save Protocol: Save the protocol of the *Full Test* as an ASCII file.

Print Protocol File: Select and print a protocol file from disk.

4.9.2 Initialize EPC 10

This is used to restart the AD/DA interface; e.g., in case PATCHMASTER was started with the interface being turned off.

4.9.3 EIS Calibration

Selecting this option start the calibration routine for EIS (Electrochemical Impedance Spectroscopy) measurements. The Protocol Editor opens and you are prompted to attach the model cell MC 61 EIS (Order number: 895336) to the headstage of the amplifier. Follow the instructions of the software to finalize the calibration process.

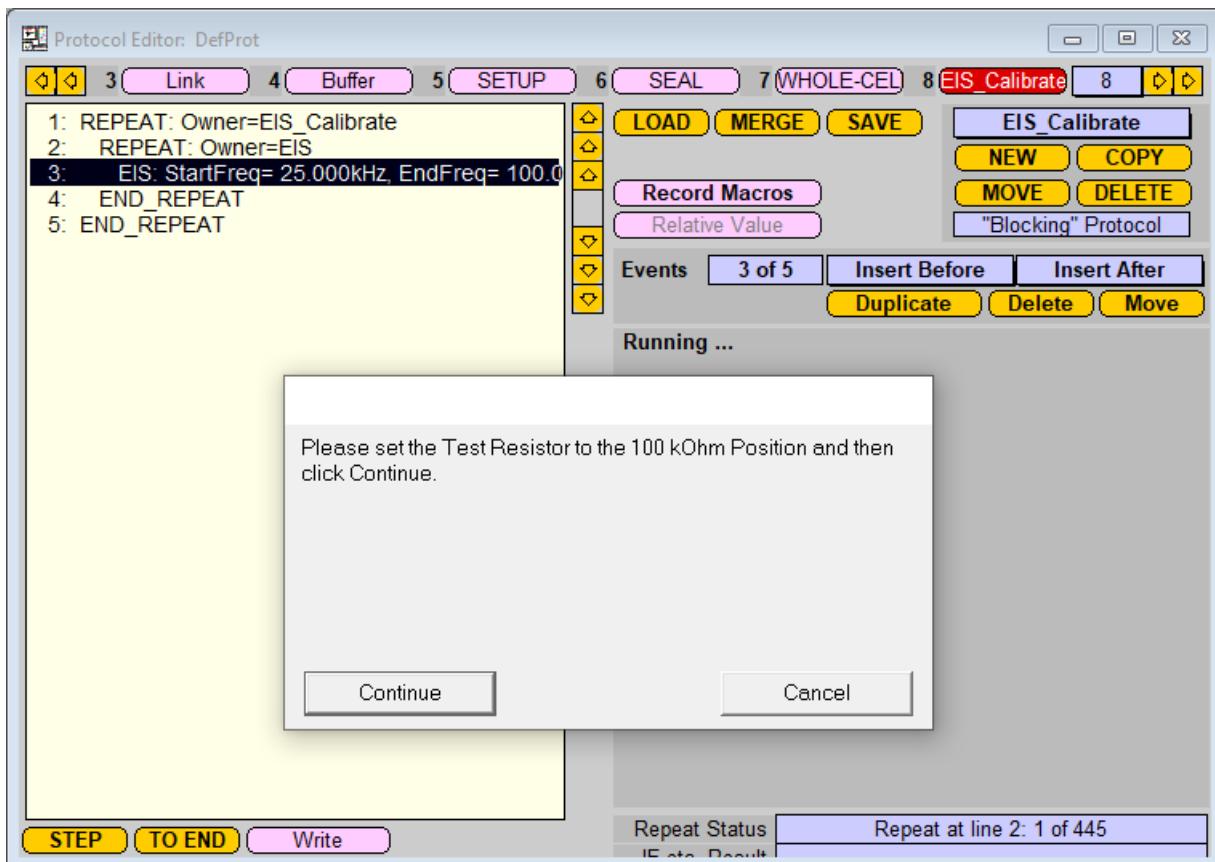


Figure 4.4: EIS Calibration opens the Protocol Editor Window.

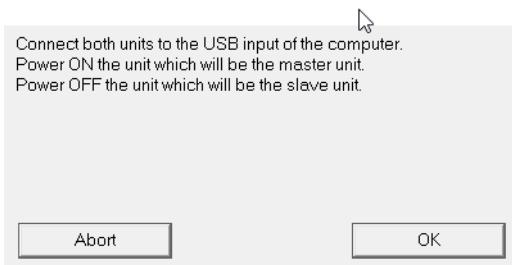
Once the calibration process has finished a lookup table is saved and one can now use the *EIS Method* in the Protocol Editor (see 12.2 on page 176).



The EIS calibration is absolutely necessary to do before starting the EIS experiments.

4.9.4 Joining / Splitting Units

Join two Units: Use this option if you want to control two EPC 10 USB patch clamp amplifiers (either Single, Double, Triple, Quadro), one EPC 10 USB patch clamp amplifier and one LIH 8+8, or two LIH 8+8 data acquisition interfaces.

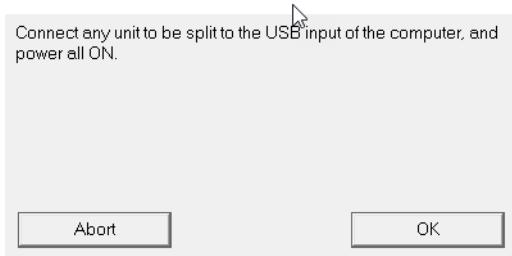


Note: Restarting PATCHMASTER is necessary to make the changes effective.



Follow the instructions provided by the software carefully!

Split joined Units: Use this option if you want to split again two units (EPC 10 USB patch clamp amplifiers or LIH 8+8 data acquisition interfaces) which have been joined before (see 4.9.4 on the facing page).



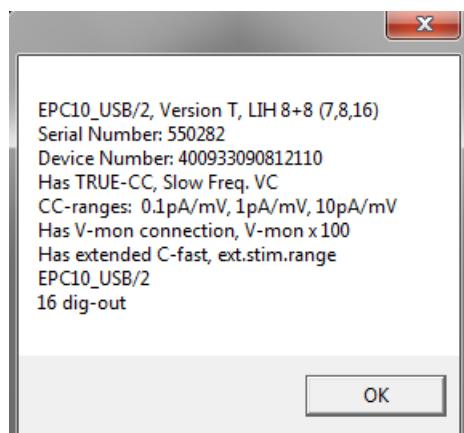
Note: Restarting PATCHMASTER is necessary to make the changes effective.



Follow the instructions provided by the software carefully!

4.9.5 Amplifier Version / Serial Number

EPC10_USB/2, Serial No 550282-T: Displays the number of amplifiers ('USB/2'), if an EPC 10 Double, Triple or Quadro is used, the serial number ('550282') and the board version ('T'). A click on this item opens a dialog with more detailed information on the capabilities of the amplifier.

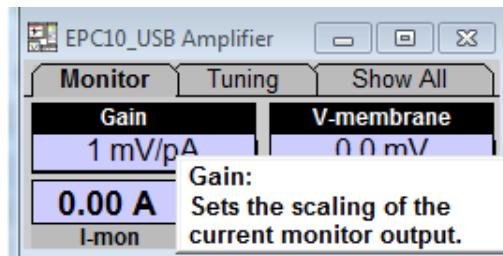


4.10 Help Menu

With the additional tools listed in the Help menu you can get more familiar with the available functions of PATCHMASTER.

Show Tooltips
Show Keys
Hide Keys
List Keys
Save Keys
List All Items
List All Macro Items
About PatchMaster

Show Tooltips: When activated information about the button that is located under the mouse tip is displayed.



Show Keys: Displays the key commands with the corresponding buttons.



Hide Keys: Hides the key commands.

List Keys: Lists all keys to the Notebook window (see chapter 3 on page 13).

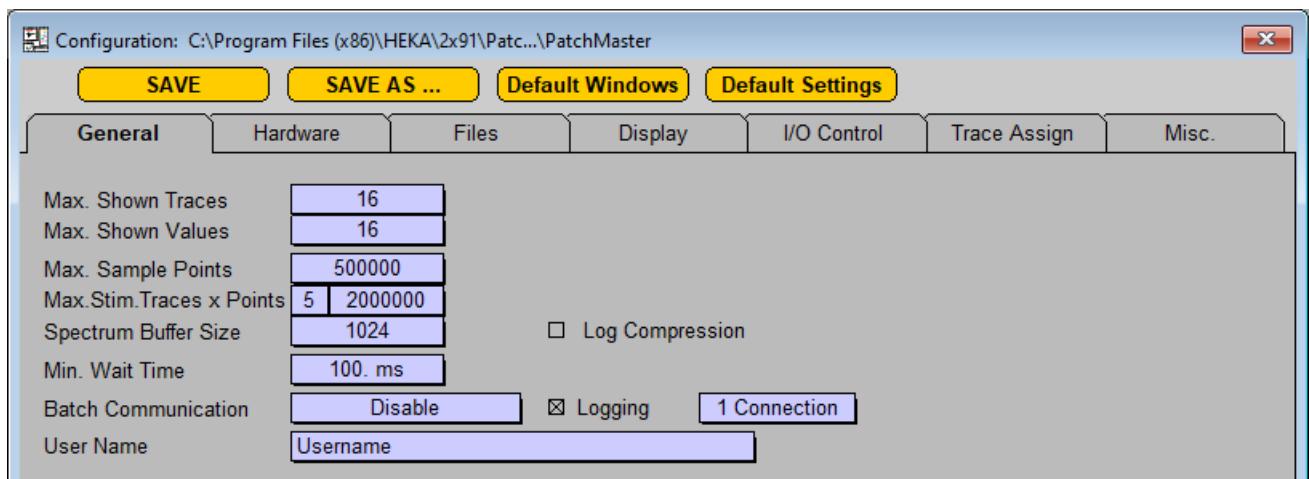
Save Keys: Saves the actual key command settings to the default key file. The old Patchmaster.key file will be saved with the extension *.kXX (X = consecutive index number).

List all Items: Lists all items and their values in the Notebook window.

List all Macro Items: Lists all macro items and their values in the Notebook window.

About PATCHMASTER: Displays the PATCHMASTER software version and contact information.

5 Configuration Window



Settings like sources for external parameters, default values, display settings, colors, fonts, default files, ... can be edited in the Configuration window. To access the Configuration window select the drop-down menu Windows → Configuration. These and other settings can be stored in *.set files; by default this file is Patchmaster.set. By means of different *.set files every user can define their individual program layout to meet specific requirements.

5.1 Individualize PATCHMASTER

 The name of a PATCHMASTER setting file can be used as a command line parameter upon starting the program. That way, one can start PATCHMASTER with various configurations which is very useful when several users work on one setup and have a need for different PATCHMASTER settings.

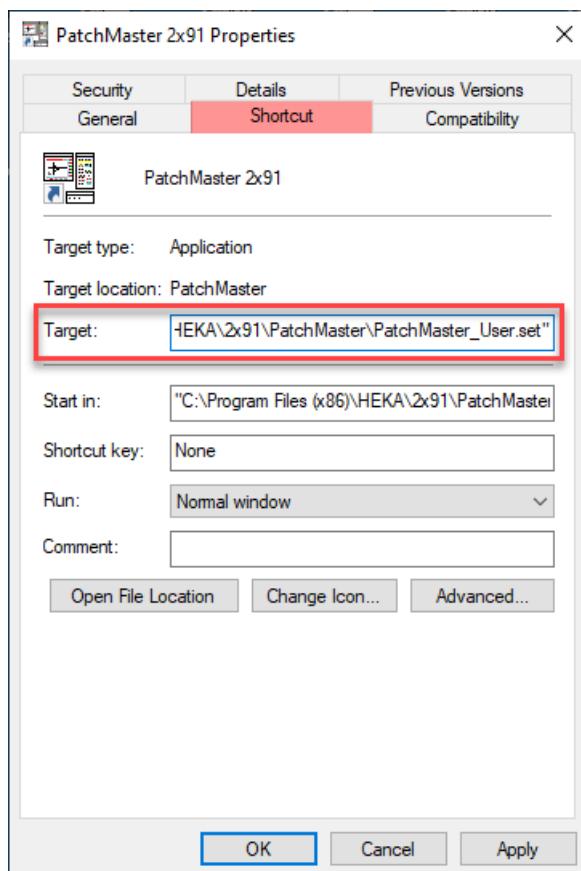
The procedure for an individualized PATCHMASTER starting procedure is as follows:

- Rename the *.set file in your HEKA folder (e.g. "Patchmaster_User.set").

Note: The term "User" is just a place holder for individual names.

- Create a shortcut of Patchmaster.exe on the desktop.
- Rename the shortcut (e.g. "Patchmaster_User").
- Right-click on the shortcut: Properties - Shortcut - Target.
- The following command line should be written in the "Target" field, including the inverted commas and empty spaces:

```
"C:\Program Files (x86)\HEKA\Patchmaster\Patchmaster.exe" "C:\Program Files (x86)\HEKA\Patchmaster\Patchmaster_User.set".
```



Note: Please be aware that your installation path of your PATCHMASTER may vary on your PC. The "Patchmaster" definition on the screenshot corresponds to POTMASTER or CHARTMASTER, respectively.

5.2 Save



SAVE: Saves the configuration file with its current name.

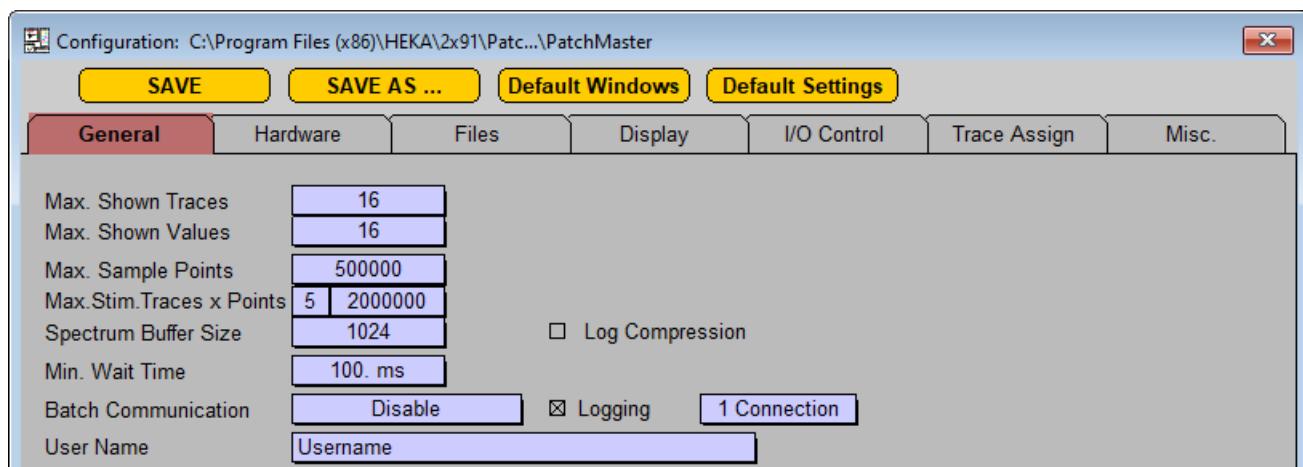
SAVE AS...: Saves the configuration file under a new name.

Default Windows: Resets the position of most windows. This helps to restore the windows position once one gets dragged off screen (for example after reducing the screen resolution).

Default Settings: Sets all settings back to the original settings. Note that this needs a restart of the program!

Note: There is no Load option because PATCHMASTER has to be restarted upon substantial changes of the settings. Thus, in order to use another setting, quit PATCHMASTER and restart. Before you do so, make certain that there is no Patchmaster.set file available. In this case, upon start-up, PATCHMASTER will prompt you to select a new setup file (this can have any name).

5.3 General



5.3.1 Max Shown Traces and Values

Max. Shown Traces: This number defines how many Traces are used by PATCHMASTER. The user interface is adapted to show only the defined number of Traces. A small number makes the dialogs and lists within PATCHMASTER shorter. The new setting only takes effect after saving the configuration file and upon restarting the program. When using the *Probe Selector* it may be necessary to increase *Max. Shown Traces*.

Max. Shown Traces	16
Max. Shown Values	16

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

5.3.2 Memory Allocation

Max. Sample Points: Sets the maximum number of points that can be acquired in one *Trace* (see chapter 10.7 on page 112). This parameter is limited by the amount of available memory.

Max. Sample Points	500000
Max.Stim.Traces x Points	5 2000000

Max. Stim. 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. Stim. Traces to make more memory available for allocating sample points.

Spectrum Buffer Size: Maximum number of samples used for spectrum analysis calculation (Fast Fourier Transformation). 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 Spectrum Buffer Size 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.

5.3.3 Minimum Wait Time

Min. Wait Time: Sets the time PATCHMASTER reserves to wait for the correct time to start when executing individual repeat loops such as acquisition of a Series of Sweeps or an Repeat loop in the Protocol Editor.

Min. Wait Time	100. ms
----------------	---------

For example, to ensure an *Acquire Each Sweep* repeat loop keeping the same repeat time as set by the *Duration* in the *Repeat* event, the program will wait *Min. Wait Time* before executing the next acquisition to hit the scheduled starting time of the next *Sweep* as exactly as possible.

Note: In case you are observing incorrect and unstable repeat times, please try to increase the Min. Wait Time.

This parameter can also be set via the *Acquire Properties* event in the Protocol Editor.

5.3.4 Batch Communication

Batch Communication: The *Batch Communication* with other applications can be disabled or enabled (see Controlling PATCHMASTER in the PATCHMASTER Tutorial).

Batch Communication	Disable	<input checked="" type="checkbox"/> Logging	1 Connection
---------------------	---------	---	--------------

- Disable: If this option is checked, the *Batch Communication* is turned off.

5.4. Hardware

- Enable as Receiver: PATCHMASTER is configured to serve as *Receiver* and will wait for command inputs of other applications.
- Enable as Sender: PATCHMASTER is configured to serve as *Sender*, thereby controlling another program, e.g. FITMASTER.
- 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 sent, when the data file is closed. The *Receiver* will then open, update, or close the same data file as the *Sender* application.
- Polling: In the *Polling* mode, the master application polls the slave application for commands. The slave then answers to the query and appends all pending commands, i.e., it no longer sends commands independently. In doing so, commands do no longer get lost, if heavy bi-directional communication is ongoing.

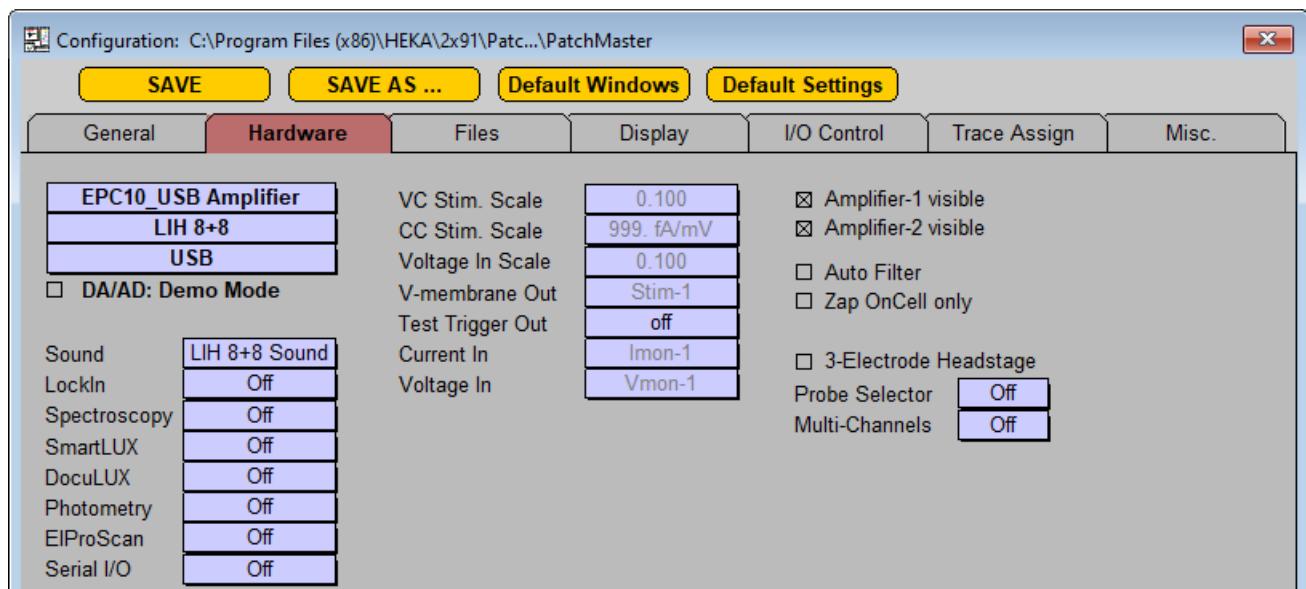
Logging: If the checkbox is activated the communication via the *Batch Communication* interface is written to the Notebook.

Connections: Specify the number of connections to other software instances which can communicate via batch communication.

5.3.5 User Name

User Name: A user name that is entered here will be stored with the raw data.

5.4 Hardware



5.4.1 Amplifier and Digitizer Selection

Amplifier: Select your amplifier from this list.

PATCHMASTER supports the following amplifiers:

- iTEV 90 Amplifier: An iTEV 90 computer controlled two-electrode voltage/current clamp amplifier.
- EPC 10 PLUS Amplifier: An EPC 10 PLUS Single or Double amplifier.
- EPC 10 USB Amplifier: An EPC 10 USB Single, Double, Triple or Quadro amplifier.
- EPC 10 Amplifier: An EPC 10 Single, Double, Triple or Quadro amplifier.
- EPC 9 Amplifier: An EPC 9 Single, Double, or Triple amplifier.
- EPC 800 Remote: An EPC 800 USB amplifier, controlled by the software.
- EPC 800 Local: An EPC 800 USB amplifier, controlled by the knobs on the front panel of the amplifier. PATCHMASTER monitors *Gain*, amplifier modes, and filter settings.
- EPC 8 Remote: An EPC 8 amplifier, controlled by the software.
- EPC 8 Local: An EPC 8 amplifier, controlled by the knobs on the front panel of the amplifier. PATCHMASTER monitors *Gain*, amplifier modes, and filter settings.
- EVA 8 Remote: An EVA 8 amplifier, controlled by the software.
- EVA 8 Local: An EVA 8 amplifier, controlled by the knobs on the front panel of the amplifier. PATCHMASTER monitors *Gain*, amplifier modes, and filter settings.
- EPC 7 Amplifier: For working with any amplifier with **no** telegraphing options.

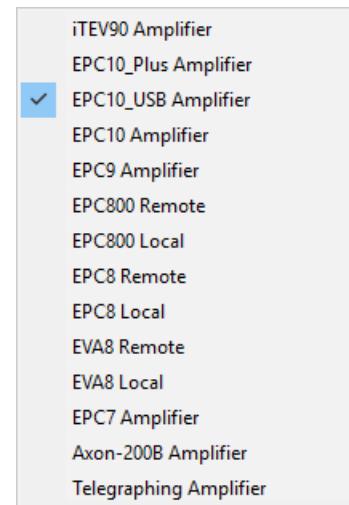
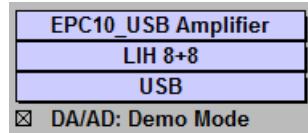
- Axon-200B Amplifier: *Lookup Tables* for interpreting the *Gain* and *Bandwidth* telegraphing channels of the Axopatch-200B amplifier are loaded automatically. Please double check, if the current and voltage monitor as well as the telegraphing channels are connected to the specified AD-input channels.
- Telegraphing Amplifier: For connection of an amplifier **with** telegraphing capabilities.

Digitizer Selection: Select the appropriate AD/DA-converter, if it is not automatically assigned via the amplifier selection.

PATCHMASTER supports the following AD/DA-converters:

- ITC-16
- ITC-18
- LIH 1600 / ITC-1600
- LIH 8+8

Board Type: Select the used *Board Type*. There are two options:



PCI
PCI
PCI
<input checked="" type="checkbox"/> USB
USB
USB

- 1...3 PCI: Additional set-up information, if there is more than one possible card slot to connect to the digitizer. The type of the PCI board depends on the EPC version (e.g., PCI-1600 for the EPC 10, PCI-18 for the EPC 10 Plus).
- USB: InstruTech's USB-16 or USB-18 adapters allow for connecting an ITC-16, EPC 9, ITC-18 to the computer via an USB 2.0 port. In case, such an adapter is used, it should be enabled here.

Demo Mode Activate the checkbox to use the program in *Demo mode*. The functionality of PATCHMASTER is restricted in the *Demo Mode*. You can only read your own data files or read and analyze the demo data files (`DemoFitData.dat`, `DemoV9Bundle.dat`).

Stand-Alone mode: If PATCHMASTER is only used for data review or export functions, the AD/DA hardware is not necessarily required. In this case PATCHMASTER will come up with the message "*AD/DA initialization failed: check power!*" or "*EPC 10 initialization failed! Please, check power and connections*". One can now enter *Continue* and select one of the *Demo modes* (e.g., EPC 10 or EPC 7 *Demo mode*). This ensures that the program does not try to access the hardware. This mode is also called *Stand-Alone mode*. Any created output is taken as input; i.e., if a *Stim. Scaling* of 0.1 is selected, the system now behaves as if an amplifier is connected with a pipette having a resistance of 10 MΩ.

If a *Telegraphing Amplifier* is used, the *Gain* and/or *Bandwidth* settings for the amplifier are read from an AD-channel and encoded by means of a *Lookup Table*. Upon selection of this amplifier type, the user must specify the AD-channel for the *Current Gain* and *Bandwidth* and load the appropriate *Gain* and *Bandwidth* tables. Both settings are made in the *I/O Parameter* section (see chapter 5.7 on page 59).

Some *Lookup Tables* for common amplifiers are already supplied (see folder `Patchmaster → Lookup Tables`). If your amplifier is not in the list, select the EPC 7 table or create your own table.

Lookup Tables can be created easily as they are ASCII files with a simple structure with a series of text lines each containing:

Voltage | Gain (mV/pA)

or

Voltage | Bandwidth (Hz)

The voltage is the threshold above which the following *Gain* or *Bandwidth* setting applies. The voltage values are scanned beginning with the first value. Thus, voltages must be in *descending* order! The voltage thresholds for discrimination between adjacent settings should be halfway between the nominal telegraph voltages.

Note: The `DacLookup` file in the `LookupTables` folder (inside the PATCHMASTER folder) depicts exemplary how to create your own lookup table.

5.4.2 Output and Input Channels

5.4. Hardware

If an EPC 9, EPC 800 or EPC 10 is used, *VC Stim. Scale* and *CC Stim. Scale* are set internally and can not be changed by the user in the Configuration.

If amplifiers other than EPC 9 or EPC 10 are used then the *VC Stim. Scale* and *CC Stim. Scale* settings have to be adjusted to match the *Ext. Stim. Input Scaling* factors of the amplifier.

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

VC Stim. Scale	0.100
CC Stim. Scale	100. fA/mV
Voltage In Scale	0.100
V-membrane Out	Stim-1
Test Trigger Out	off
Current In	Imon-1
Voltage In	Vmon-1

Note: The EPC 9, EPC 800 and EPC 10 amplifiers have an internal *VC Stim. Scale* fixed at 0.1.

CC Stim. 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 *Ext. Stim 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 9 and EPC 10 amplifiers feature a so-called CC-Gain (see chapter 7.1.21 on page 88) 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 Stim. 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.

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

Test Trigger Out: Assigns the output channel of the trigger that is set at the beginning of each *Test Pulse*. The *Test Trigger Out* should be turned off if the digital oscilloscope of PATCHMASTER (Oscilloscope window) is used. Turn it on if you would like to trigger an external oscilloscope.

The test trigger pulses might interfere with your stimulus output of the PGF sequence if identical Test Trigger Out and stimulus DA channels are chosen.

Current In: Assigns the input AD channel for the current *Trace*. This input channel is scaled by the *Current Gain* (see chapter 5.7.2 on page 63).

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

5.4.3 Sound

The output device of the sound monitor can be specified.

- MIDI Sound: Uses the sound card of the computer.
- PSA-12: HEKA USB sound generator.
- LIH 8+8 Sound: Uses the built-in sound generator of LIH 8+8 interfaces or EPC 10 USB amplifiers.

5.4.4 LockIn

Here, the LockIn amplifier of PATCHMASTER can be activated. For a detailed description see chapter Software LockIn Extension, 23 on page 257, and the PATCHMASTER Tutorial "Capacitance Measurements using the LockIn Extension".

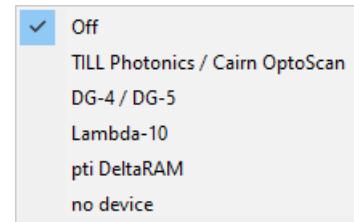
5.4.5 Spectroscopy

Here, the Spectroscopy Extension of PATCHMASTER can be activated. For a detailed description see chapter Spectroscopy, 24 on page 275, and the PATCHMASTER Tutorial "Using the Spectroscopy Extension".

5.4.6 SmartLUX

Here, the Imaging Extension (SMARTLUX) of PATCHMASTER can be activated. Depending on the fluorescence excitation light source or wavelength switcher you have installed in your setup, you can choose between different kinds of Imaging Extensions:

- TILL Photonics/Cairn OptoScan: "TILL Photonics" or "Cairn OptoScan", can be controlled via an analog output of the amplifier or any AD/DA converter supported by PATCHMASTER. The exposure of the camera should then be triggered via a digital output channel.
- DG-4/DG-5: The "DG-4/DG-5" can be controlled via the digital output of the EPC 10, ITC-16 or ITC-18. The exposure of the camera should then be triggered via an analog output channel.
- Lambda-10: The "Sutter filter wheel Lambda-10" can be controlled via the digital output of the EPC 10, ITC-16 or ITC-18. The exposure of the camera should then be triggered via an analog output channel.
- pti DeltaRAM: The "High-Speed Random-Wavelength Illuminator" can be controlled via the digital output of the EPC 10, ITC-16 or ITC-18. The exposure of the camera should then be triggered via a digital output channel.
- no device: No light source control.



For details on the Imaging Extension aka "SMARTLUX" please refer to the SMARTLUX manual.

5.4.7 DocuLUX

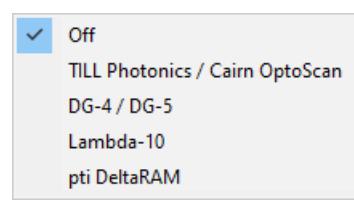
Enable DocuLUX features for PATCHMASTER here. Please be aware, that you need to buy the DocuLUX bundle (order number: 895286 or 895287) first.

5.4.8 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 Photonics/Cairn OptoScan: "TILL Photonics" or "Cairn OptoScan", can be controlled via an analog output of the amplifier or any AD/DA converter supported by PATCHMASTER.
- DG-4/DG-5: The "DG-4/DG-5" can be controlled via the digital output of the EPC 10, ITC-16 or ITC-18.
- Lambda-10: The "Sutter filter wheel Lambda-10" can be controlled via the digital output of the EPC 10, ITC-16 or ITC-18.



5.4. Hardware

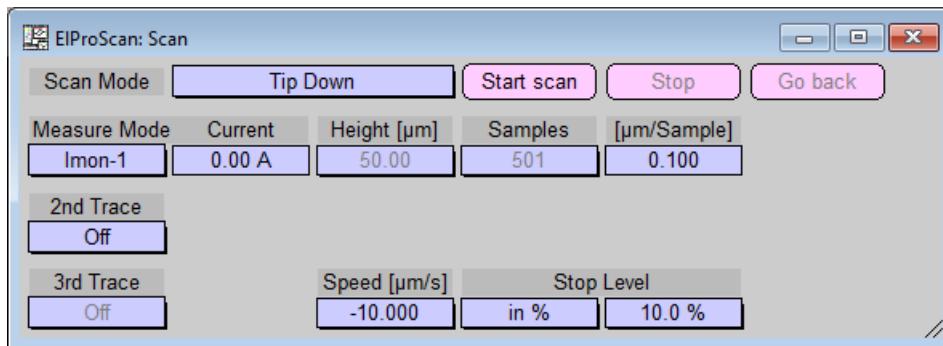
- pti DeltaRAM: The “High-Speed Random-Wavelength Illuminator” can be controlled via the digital output of the EPC 10, ITC-16 or ITC-18.

For details on the Photometry Extension please refer to the chapter Photometry, 21 on page 245.

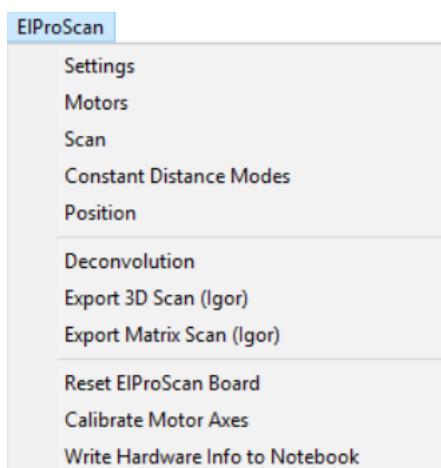
5.4.9 ElProScan

The ElProScan Extension can only be used with additional hardware. Further, our ELPROSCAN customers will get a special hardware configuration file. If you activate the extension without having the configuration file you will get an error message indicating that the *ElProScan Hardware file* is missing.

After the activation of the ElProScan Extension two additional windows will pop up:



Further, there is an additional menu entry in PATCHMASTER named ElProScan:



The functions and options of the new windows and the new menu are explained in detail in our ELPROSCAN manual.

5.4.10 Serial Out

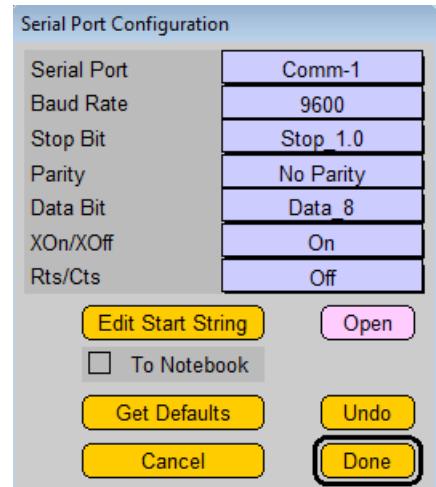
This control is used to open and set up the serial port communication mode. If a so-called *Serial Communication* has been established between the computer and another device, PATCHMASTER can send strings over a serial port, but it will not receive instructions.

The options for the serial communication are:

- Off: No connected device.
- Comm 1...4: Any device that can receive strings through the serial port. Up to 4 ports can be defined and triggered.

When opening a serial communication, PATCHMASTER will allow you to configure the serial device. Make sure that the settings match on both communicating machines.

- Serial Port: No Port, Comm 1... Comm 8.
- Baud Rate: 300, 600, 1200, 1800, 2400, 3600, 4800, 7200, 9600, 19200, 57600, 115200, 128000 or 256000 bps.
- Stop Bit: 1.0, 1.5 or 2.0.
- Parity: No Parity, Even Parity or Odd Parity.
- Data Bit: 5, 6, 7 or 8 data bits.
- XOn/XOff: On or Off.
- Rts/Cts: On or Off.



To change all settings or leave the dialog, do one of the following:

- Edit Start String: Allows to enter a specific start string.
The *Start String* (Initialization String) is send once after opening the COM port.
- Open: Opens the configured port (icon text changes to "Close")
- To Notebook: Commands sent and received are written to the Notebook.
- Get Defaults: Sets the default settings given in PATCHMASTER.
- Undo: Cancels all changes without closing the dialog.
- Cancel: Cancels all changes and closes the dialog.
- Done: Saves all changes and closes the dialog.

If a serial out is active then command strings can be entered and sent via the I/O Control window (see chapter 16 on page 225). There is also a *Serial Out* event available in the Protocol Editor, 11.4.3.2 on page 152.

5.4.11 Further Options

Amplifier Visible: In case of the use of Double, Triple or Quadro amplifiers, you can check which amplifier main controls should be visible in the Amplifier window.

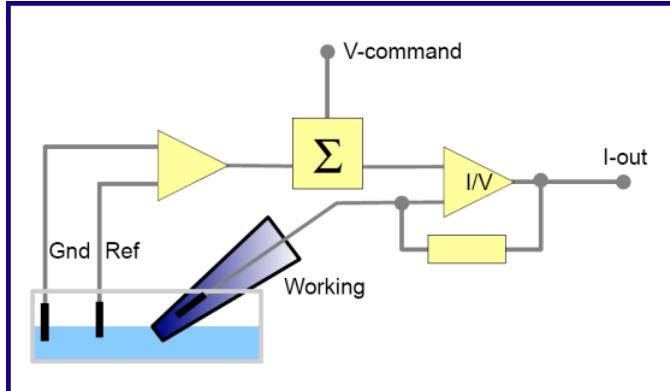
Auto Filter: The Pulse Generator window provides the option to automatically set a hardware filter *Filter 1*, *Filter 2* according to the chosen sample interval (see chapter 10 on page 107). *Auto Filter* enables and disables this automatic filter setting, which is only supported for the EPC 10 and EPC 9 with its built-in hardware filters.

Zap OnCell only: If this checkbox is selected, the *Zap* feature is restricted to *On Cell* mode. This ensures that one does not accidentally zap a patch or a whole cell. (For more information on the *Zap* function, please refer to *Various Controls*, 7.1.17 on page 87.)

3-Electrode Headstage: If you want to use a *3-Electrode Headstage* you have to activate this checkbox. Further you have to switch from *2 Electrode Mode* into *3 Electrode Mode* (see chapter 7.1.16 on page 87). Typical applications using the *3 Electrode Mode* are:

- Accounting changes of the *Liquid Junction Potential*
- Precise electrochemical measurements
- Multi-electrode pair configurations

In the *3 Electrode Mode* you can measure the potential difference between a third electrode (reference) and the ground (counter) electrode without having a current flow through the reference electrode. The reference electrode stays in equilibrium. The potential difference is added to the command potential for the working electrode.



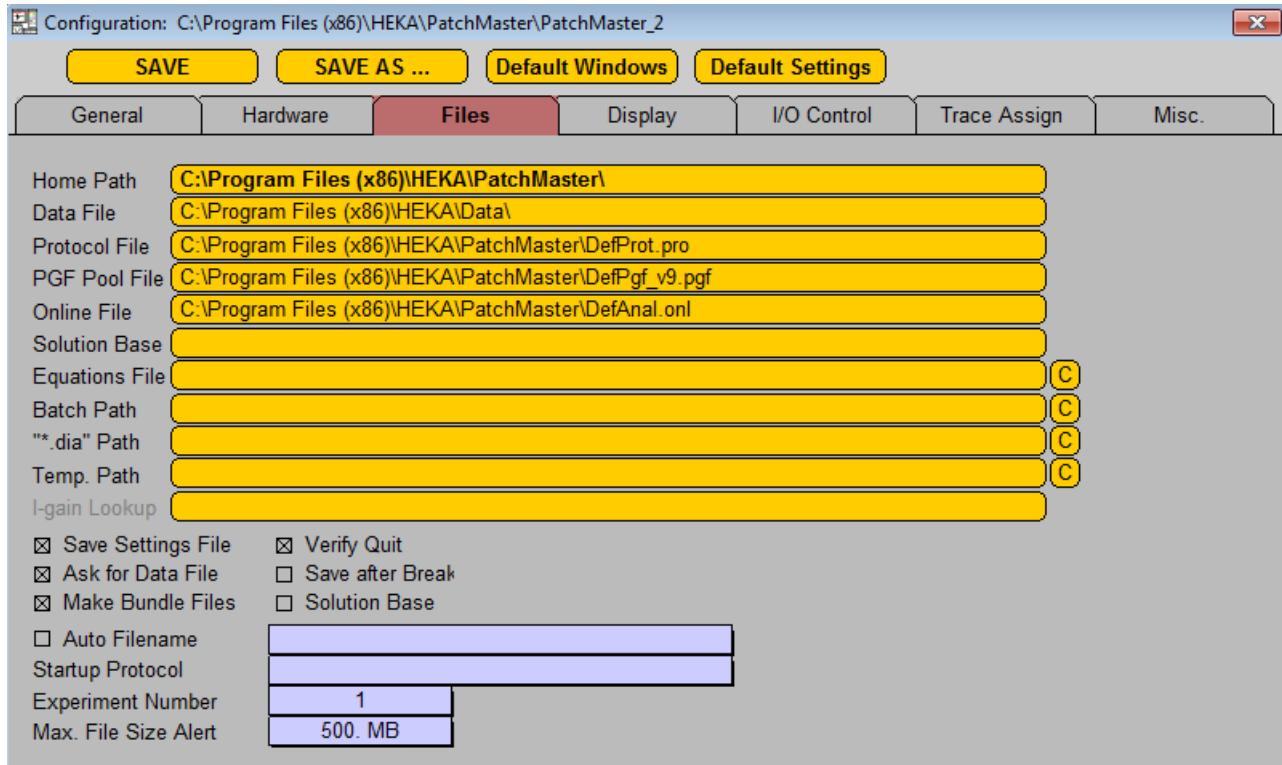
Probe Selector: Enables the *Probe Selector* (optional accessory). For further information we refer to the manual of the *Probe Selector*.

Multi-Channels: Enabling *Multi-Channels* has major implications on the *Oscilloscope* window (see chapter 6.7 on page 74) and the *Analysis* (see chapter 13.6 on page 208).

<input checked="" type="checkbox"/> Amplifier-1 visible	<input checked="" type="checkbox"/> Amplifier-2 visible
<input type="checkbox"/> Auto Filter	<input type="checkbox"/> Zap OnCell only
<input type="checkbox"/> 3-Electrode Headstage	
Probe Selector	Off
Multi-Channels	Off

5.5 Files

In this section, the user has to specify the paths for several files. PATCHMASTER will use these paths when saving or retrieving files.



To rename a path, click on the button and set a new path.

Home Path: Sets the path to the folder `LookupTables` and to the key assignment file (`Patchmaster.key`). It is also the path where the default files `DefPgf_v9.pgf`, `DefAnal.onl` and `DefProt.pro` are searched automatically as long as no configuration file was saved.

The path to the Configuration file is shown in the title of the Configuration window. This path can be changed by saving the Configuration under another path using the *SAVE... AS* function.

Data File: Sets the path to the data file (file extension: `*.dat`).

Protocol File: Sets the path to the protocol files (file extension: `*.pro`).

PGF Pool File: Sets the path to the Pulse Generator file (file extension: `*.pgf`).

Analysis File: Sets the path to the Analysis file (file extension: `*.onl`).

Solution Base: Sets the path to the solution data base files (file extension: `*.sol`).

Equations File: Sets the path to the equation file, which can contain equation formulas. This path can be cleared by use of the button.

Batch Path: Path for batch file control protocols, e.g., `E9BatchIn` and `E9BatchOut`. This path can be cleared by use of the button. For further information we refer to the PATCHMASTER Tutorial: "Controlling PATCHMASTER".

Dialog Files: Sets the path to the dialog files, in which button placements of the different windows can be stored in case they should deviate from the default. This path can be cleared by use of the button.

Temp. Path: Optionally a path different from the home path can be specified for storing temporary data. This path can be cleared by use of the button.

I-gain Lookup: Path to the *Current Gain Lookup Table* if selected in the configuration (see chapter 5.7.2 on page 63). Only necessary for telegraphing amplifiers.

5.5.1 General Advice on Naming Folders and Files

Here is some advice to keep in mind when naming folders and files:

- The use of invisible characters and spaces is not recommended (a blank in a file name is very often overlooked).
- Names should not begin with a number, because some other applications, e.g., Igor, do not allow names with a number as the first character (exported Igor waves inherit characters of the data file).
- PATCHMASTER only shows the first 14 letters of the file name in the title of the Oscilloscope window.
- The first few letters of a name are the more important ones, because they ease file selection with the file selector. The file selector continuously selects the files while the user types.
- Experiment data should not be stored within the HEKA software folder. This will ease upgrading the software without moving the data.

5.5.2 Miscellaneous Settings

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

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

Make Bundle Files: When this option is selected, all files generated for a data set (e.g. the raw data *.dat, *.pgf, *.pul,...) are combined into one *.dat file.

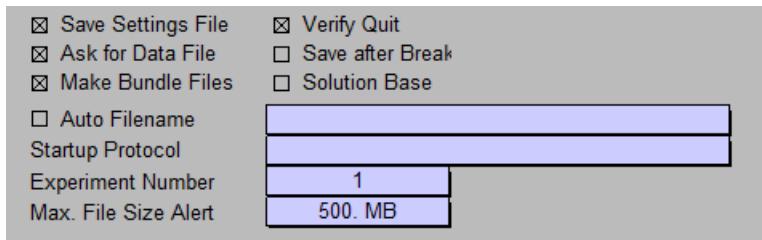
Verify Quit: When this option is enabled, PATCHMASTER will ask to save modified setting files (*.pgf, *.pro, *.onl) when you quit the program. When it is not enabled, the Configuration settings will not be saved on quit!

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

Solution Base: The Solution Base is activated by default, therefore the default solution base (*DefSolutionBase.sol*) is loaded (see chapter 17 on page 229).

When *Show Solutions* or *Show Amplifier Solutions* is selected in the *I/O parameters* section of the Configuration window (see chapter 5.7.2 on page 64). The I/O Control window is extended to include, not only the solution index, but also the solution names.

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)

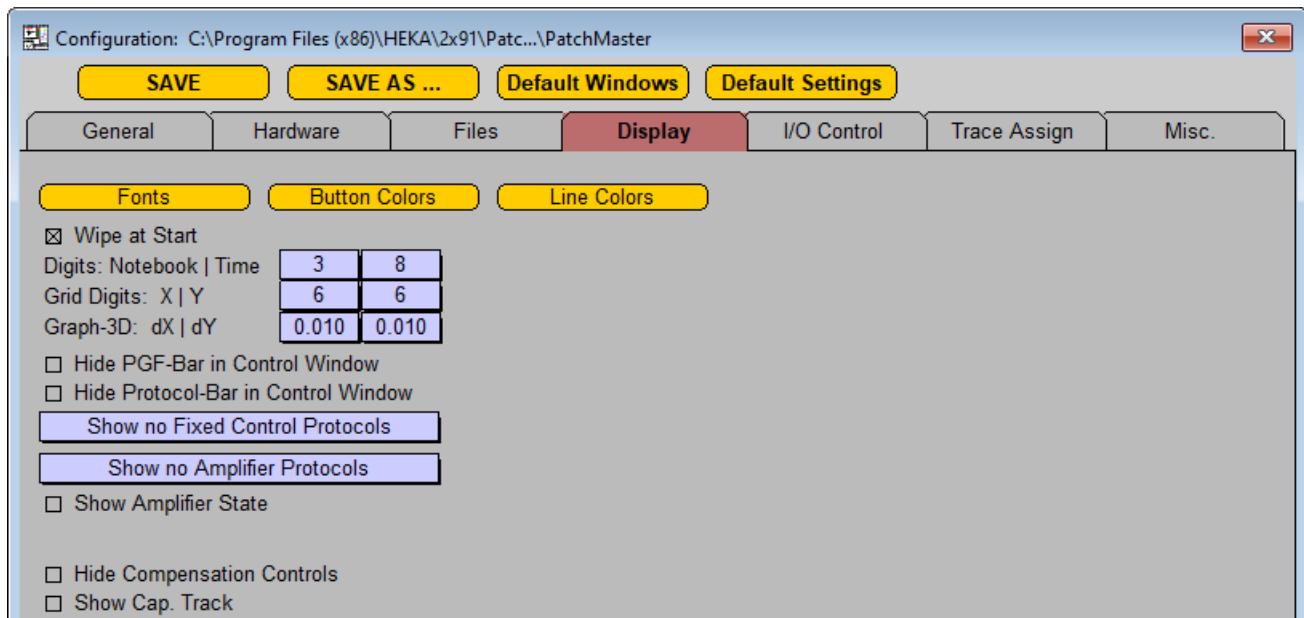
Startup Protocol: Here, you can enter the name of a protocol that is executed after the end of program initialization (i.e. after the configuration file is loaded, the amplifier initialized and the other settings activated). E.g., this allows for additional settings like the immediate activation of the second amplifier of an EPC 10 Double at start-up.

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.

5.6 Display



5.6.1 Fonts and Colors

Fonts / Button Colors / Line Colors:

Colors and text fonts for the program layout can be selected here. These are global settings for all window dialogs and they are installed upon restart of PATCHMASTER. The colors and fonts are stored in the configuration file (*.set), i.e., they are independent of the dialog files (see chapter 2 on page 5).

Note: If dialog files are present, they will overwrite the Configuration settings.

Note: You can make the background colors of the windows dark (useful when doing light-sensitive experiments) by selecting the option Button Colors. In this case, you may also have to change the color of lines, like the Trace Colors, for example, using the option Line Colors.

5.6.2 Notebook, Display, and Analysis

Wipe at Start: If this option is activated the Analysis and Oscilloscope windows will be wiped before data acquisition.

Digits:

<input checked="" type="checkbox"/> Wipe at Start
Digits: Notebook Time
Grid Digits: X Y
Graph-3D: dX dY

- Notebook: Numbers of digits for data display in the Notebook window (range 3 – 24).
- Time: Numbers of digits for the *Time* and *Timer* function in the Oscilloscope window (range 8 – 11). The maximal number is 11, this results in "hh:mm:ss:ms".

Grid Digits X|Y: Number of digits to be shown in grid annotation of the Oscilloscope and the stimulation template preview of the Pulse Generator (range 6 – 10).

Graph 3D dX|dY: Allows to specify the horizontal and vertical offset of subsequent *Sweeps* to be displayed in the 3D graphing mode (range 0.001 – 1.000). You can also enter 0 for one or both. Note that the *3D-Graph* feature has to be on for this option to take effect (see chapter 4.5 on page 29).

5.6.3 Show Options

The appearance of some windows can be configured using the following options:

Hide PGF-Bar in Control Window: The complete PGF pool in the Control window can be hidden.

Hide Protocol-Bar in Control Window: The complete protocol pool in the Control window can be hidden.

Show Fixed Control Protocols: In case the user does not want to scroll through the protocol pool, a specified number of protocols (0, 7, 15, 23, 31 or 39) can be shown as buttons in the Control window.

Show Amplifier Protocols: Protocols can be started via a button click from the Amplifier window. Here, the user can specify how many protocol buttons (0, 4, 8, 12, 16, 20 or 24) should be created in the Amplifier window. To see the new protocol buttons you have to enlarge the Amplifier window by activating the *Show All* tab.



Note: When using Amplifier Protocols please be aware of some restrictions (see chapter 11.3 on page 141).

Show Amplifier State: After changing the selected amplifier or the *Recording Mode* of the selected amplifier in the Amplifier window the amplifier state is constantly displayed beneath the amplifier selection field (see chapter 7.1.3 on page 77).

Note: This function is only available when multiple amplifiers are used (e.g. Double, Triple...).

Hide Compensation Controls: If you activate this option the compensation controls (*C-fast*, *C-slow*, *Rs Comp*) are hidden in the Amplifier window and not available any more.

Show Cap. Track: If you activate this option the *Cap. Track* options (*Cap. Track*, *Delay*) are shown in the Amplifier window.

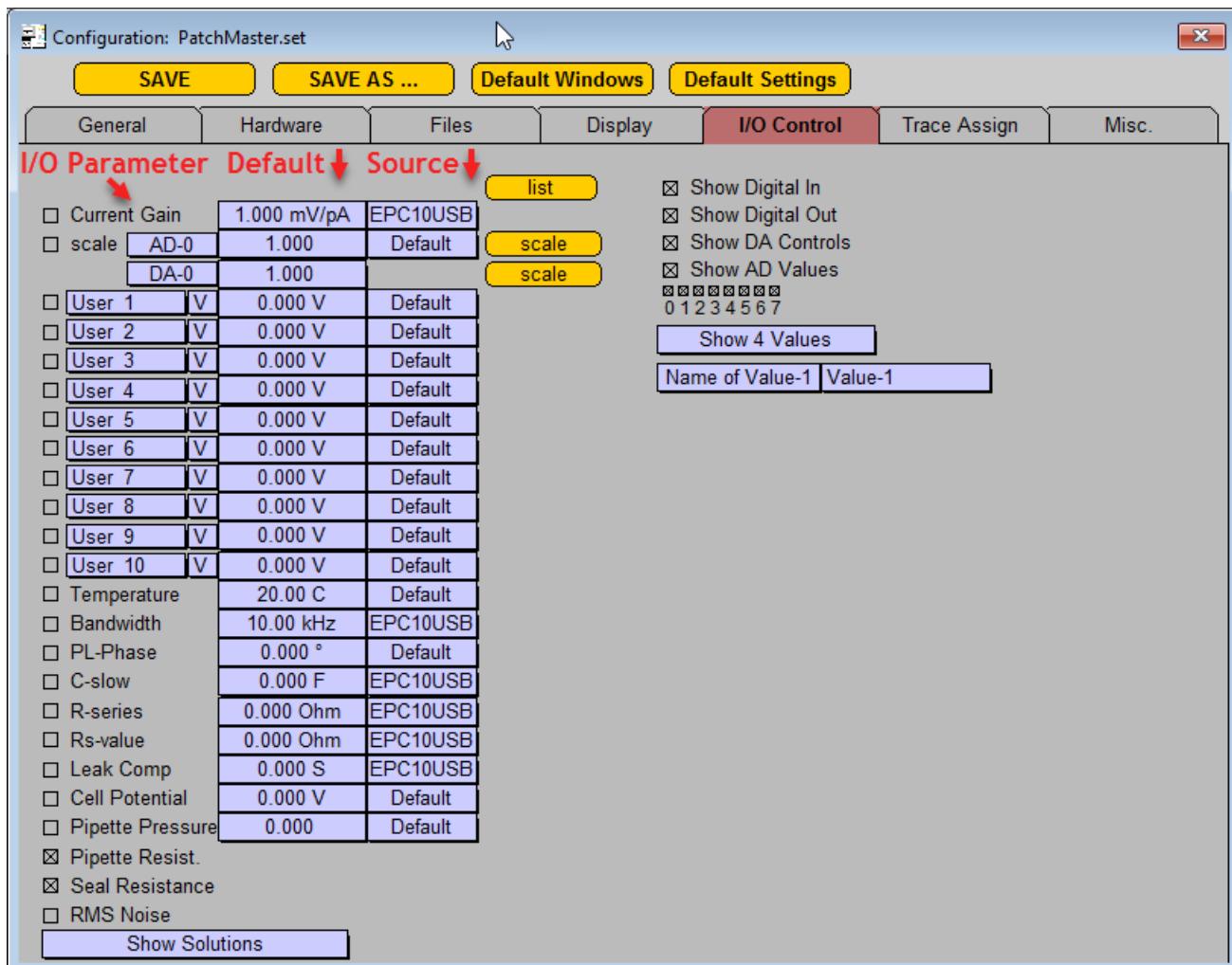
5.7 I/O Control

Selected options in this section of the Configuration window will appear in the I/O Control window (see chapter 16 on page 225) and can be edited there.

Parameters such as e.g. *Temperature*, *Gain*, *R-series*, are used to customize the recording environment of a given experimental setup.

Several settings are automatically preset when using an EPC 10 or EPC 9 amplifier. Other amplifiers will require specific settings in order for PATCHMASTER to know how to perform scaling of input and output.

The checkbox on the left determines whether the parameter is displayed (and updated) in the I/O Control window. For each I/O parameter you have to enter a default value and a source.



Default: The default values can be edited in this column.

Source: In this row one can determine how the value is obtained. For most of the parameters there are three alternatives:

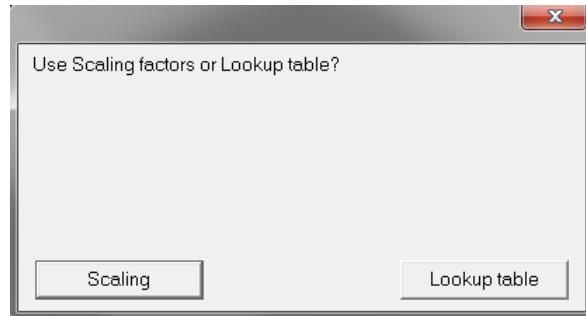
- Default: The specified default value is taken.
- EPC 10: Parameters are obtained directly from the given EPC 10 amplifier.
- AD-Channels: Parameters are sampled via a specified AD-channel.

[list]: Pressing this button writes all scaling and offset values for the I/O parameter in the Notebook window. Use this feature for getting a quick overview on the scaling of all your AD- and DA-channels.

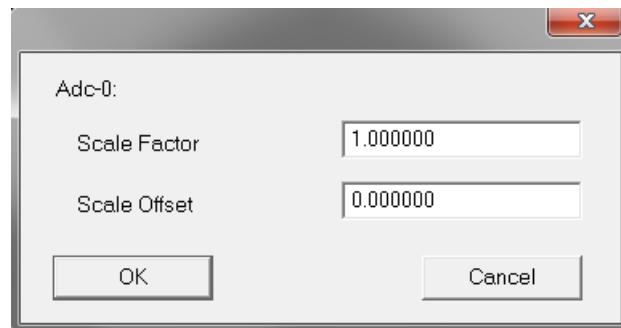
5.7.1 AD/DA Input/Output Scaling

In the I/O Control pane of the Configuration window you can define the scaling methods for the DA- and AD-channels. Please note that the I/O Control window always displays the raw (unscaled) voltage values of the channels. The scaling of DA- and AD-channels can be used for stimulus output and for data acquisition via the Pulse Generator.

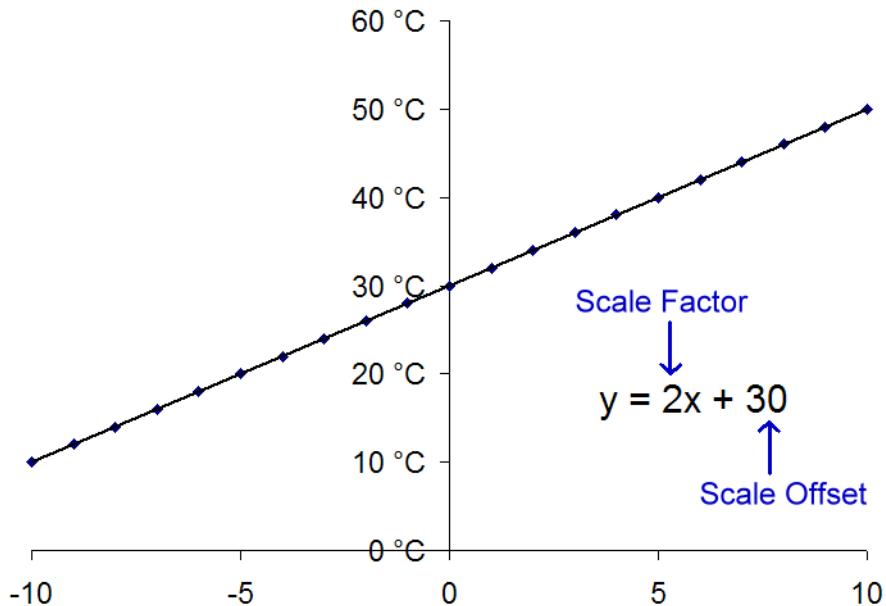
In case of an AD-channel you can choose between a *Scaling Factor* and a *Lookup table*:



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

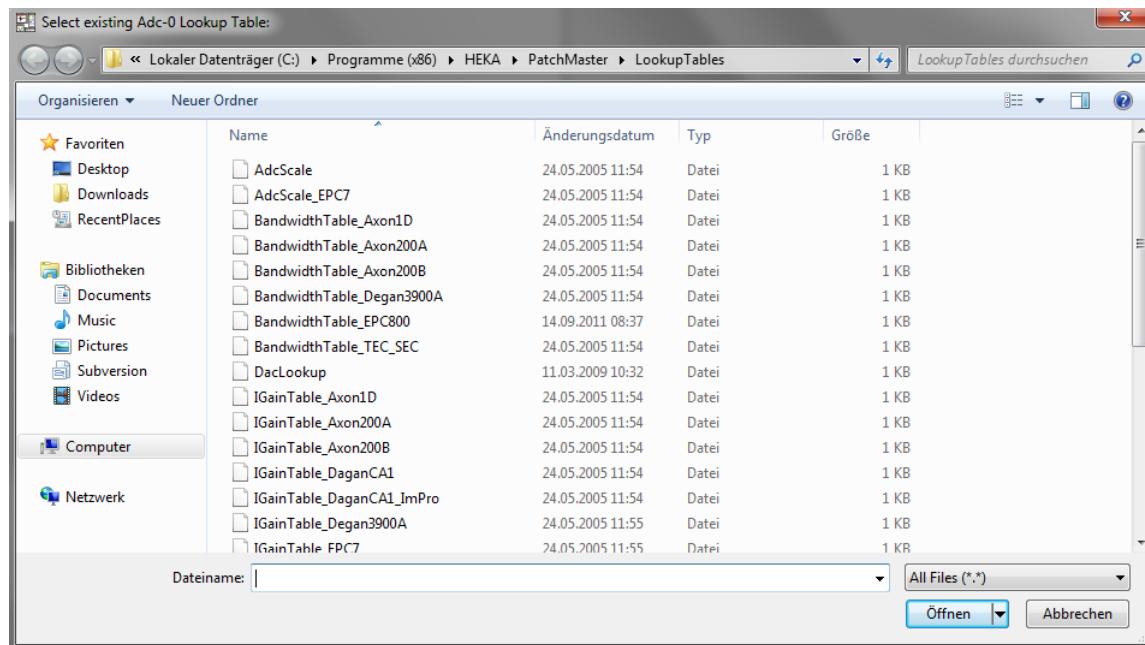


Example: A temperature controller has a measure range from 10 °C to 50 °C and a corresponding voltage output range of +/- 10 V. The correlation between measured temperature and voltage output is linear (see figure below).

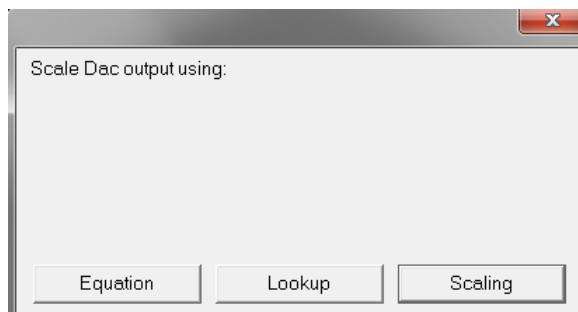


In this example, *Scaling Factor* should be set to "2" and *Scale Offset* should be set to "30". If the correlation between measured signal and voltage output is not linear then a *Lookup table* should be used. For input via a *Lookup table*, you have to choose the file from which the program will read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

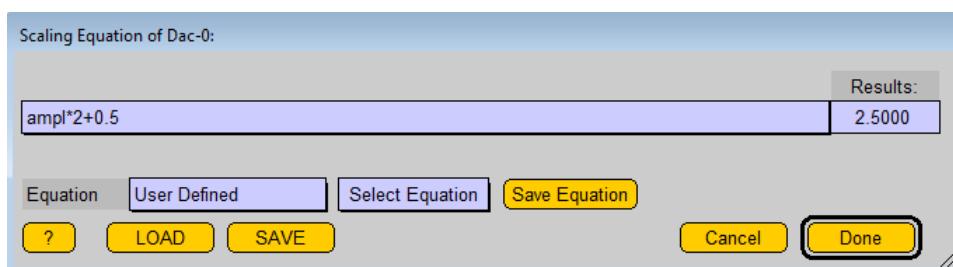
5.7. I/O Control



In case of a DA-channel you have in addition the possibility to apply a scaling based on an equation. The equation has to be entered here in the Control window, whereas the scaling has to be activated in the Pulse Generator (see chapter 10.9.1 on page 123).



The options *Lookup* and *Scaling* work similar to the methods described for the AD-channel scaling. In case you choose *Equation* the following dialog appears:



In the command field an equation can be entered, the calculated result is displayed in the *Results* field beneath.

Equation : A label for the equation can be entered in this field.

Select Equation : Already saved equations can be selected in this pop-up menu.

Save Equation : After defining a name for the entered equation it can be saved. After doing this the formula is available in the pop-up menu of *Select Equation*.

Note: The new equations is saved in a default *Equations.txt* file. If you want to change the file or the file name for your equations you have to this via the Save button.

 : Prints a help text on the equation syntax to the Notebook.

 : Brings up a file selector to open an equation file.

 : Defines the place and the name of the file in which your equations are stored.

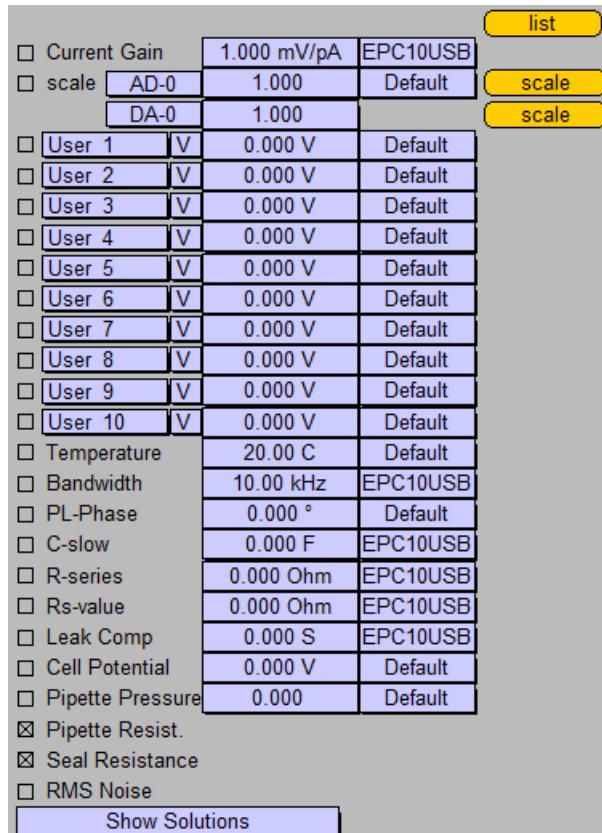
For a detailed description on the equation syntax please refer to the chapter Calculator and Equations, 20 on page 239.

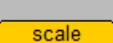
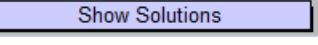
As was mentioned earlier, *Lookup tables* for commonly used amplifiers are supplied. If your amplifier is not one of those provided, a *Lookup Table* can be created as an ASCII file.

Note: Have a look for our video tutorial "Using Telegraphing Inputs" to learn how to create your own *Lookup table*.

Note: It takes CPU time to read AD-channels and to convert the data to the desired values. In order to minimize handling overhead, only activate those parameter fields that are actually used.

5.7.2 List of I/O Parameters



<input type="checkbox"/> Current Gain	1.000 mV/pA	EPC10USB	
<input type="checkbox"/> scale	AD-0	1.000	Default
	DA-0	1.000	
<input type="checkbox"/> User 1	V	0.000 V	Default
<input type="checkbox"/> User 2	V	0.000 V	Default
<input type="checkbox"/> User 3	V	0.000 V	Default
<input type="checkbox"/> User 4	V	0.000 V	Default
<input type="checkbox"/> User 5	V	0.000 V	Default
<input type="checkbox"/> User 6	V	0.000 V	Default
<input type="checkbox"/> User 7	V	0.000 V	Default
<input type="checkbox"/> User 8	V	0.000 V	Default
<input type="checkbox"/> User 9	V	0.000 V	Default
<input type="checkbox"/> User 10	V	0.000 V	Default
<input type="checkbox"/> Temperature	20.00 C	Default	
<input type="checkbox"/> Bandwidth	10.00 kHz	EPC10USB	
<input type="checkbox"/> PL-Phase	0.000 °	Default	
<input type="checkbox"/> C-slow	0.000 F	EPC10USB	
<input type="checkbox"/> R-series	0.000 Ohm	EPC10USB	
<input type="checkbox"/> Rs-value	0.000 Ohm	EPC10USB	
<input type="checkbox"/> Leak Comp	0.000 S	EPC10USB	
<input type="checkbox"/> Cell Potential	0.000 V	Default	
<input type="checkbox"/> Pipette Pressure	0.000	Default	
<input checked="" type="checkbox"/> Pipette Resist.			
<input checked="" type="checkbox"/> Seal Resistance			
<input type="checkbox"/> RMS Noise			
			

Current Gain: Gain of the *Current Monitor* input. This parameter scales the current signal sampled from the *Current Monitor* channel specified in the Output and Input Channel Settings. When using a telegraphing amplifier the AD-channel that samples the gain telegraph voltage has to be specified and the *Gain Lookup Table* has to be chosen.

scale:

- **AD-Channel:** If data Traces from AD-channels are acquired in a PGF sequence, the voltages can be scaled to convert them to appropriate units. For scaling you can use a fixed value or *Lookup tables* (see chapter 5.7.1 on page 60). In case you have an EPC 8 or EVA 8 amplifier connected as second amplifier then use EPC 8 *Gain* to scale the AD-channel that samples the *Current Monitor* signal of the EPC 8. The corresponding EPC 8 has to be connected via the parallel digital I/O interface and the amplifier set to local mode. If you want to use external noise filters you should pick up the *Filter 1* or *Filter 2* signal of the EPC 10. After filtering your signal you have to select the corresponding AD-input channel (AD-0...). Furthermore you have to select the *Epc 10 Gain* option in the *Source* pop-up menu. Changes in the *Gain* adjustment of the active amplifier are now displayed in the *Default* box.
- **DA-Channel:** Free DA output channels that can be used for stimulation during a PGF sequence can be scaled with a factor entered in the *Value* field.

User 1, 2: There are two 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.

Bandwidth: Recording bandwidth.

PL-Phase: Phase angle of a LockIn amplifier.

Temperature: Temperature (from a recording device).

C-slow: Membrane capacitance.

R-series: Series resistance.

Rs-value: Series resistance compensation.

Leak Comp: Leak conductance for leak compensation.

Cell Potential: Cell potential (from an external device).

Pipette Pressure: Pressure (from an external device).

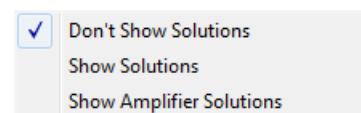
Pipette Resist.: Value of *R-membrane* after pressing W (this will write the value of *R-membrane* into *Pipette Resistance*).

Seal Resistance: Value of *R-membrane*.

RMS Noise: Value of the RMS (=Root Mean Square) noise.

Show Solutions: From a drop list you can choose if external and internal solution numbers will be displayed and can be edited in the I/O Control window.

In case you run an EPC 10 with multiple headstages the option *Show Amplifier Solutions* allows to specify different internal solutions for each amplifier.



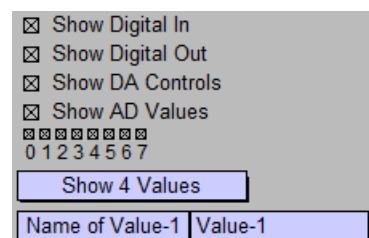
5.7.3 Show Digital In / Out

Show Digital In: If the checkbox is deselected the Digital Inputs in the I/O Control window will be hidden.

Show Digital Out: If the checkbox is deselected the Digital Outputs in the I/O Control window will be hidden.

Show DA Controls: If the checkbox is deselected the drag-able DA-channel fields in the I/O Control window will be hidden.

Show AD Values: If the checkbox is deselected the AD-channel display fields in the I/O Control window will be hidden.

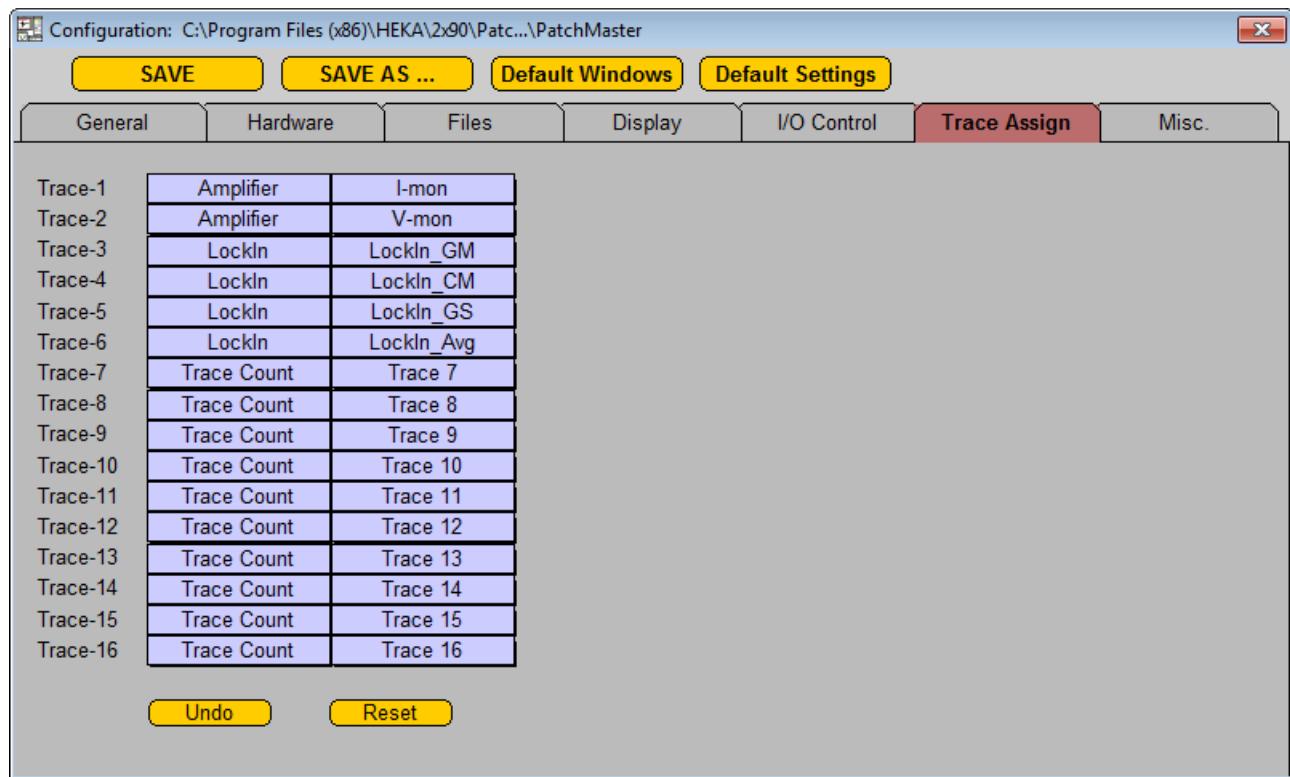


Show AD Channels: Via these checkboxes the user can deactivate any *AD Value* field in the I/O Control window.

Show Values: You can specify how many values (4, 8, 12, 16, 20, 24, 28 or 32) should be listed in the I/O Control window.

Name of Value: A user defined name can be specified for each value (right text field) in order to make the parameter input and output more meaningful. A list of all value names pops up if you select the left box.

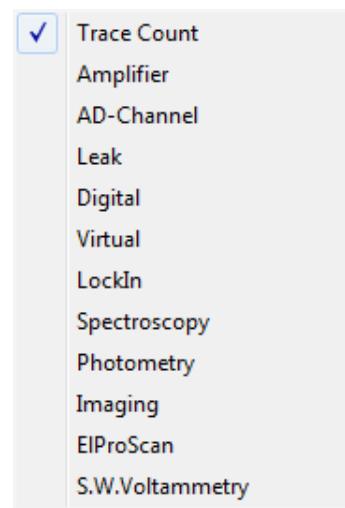
5.8 Trace Assign



The *Trace Assignment* function is very useful if your number of input and output channels of a PGF is increasing. This function allows you a defined labeling of each acquired *Trace*. E.g. if you define acquisition channels for current, voltage, ADC input, LockIn and Leak, they get labeled according to their origin, i.e. *I_{mon}-1*, *V_{mon}-1*, *Adc-0*, *Adc-1*, *LockIn_CM*, *LockIn_GM*, *LockIn_GS* and *Leak-8*, instead of simple *Trace* counts (e.g. *Trace 1...8*). It is also possible to assign several *Traces* with the same input signal, so you can define e.g. three times *I_{mon}-1* but the compression rate for each channel can be set differently. The benefit comes with the consistent labeling of these *Traces* within PATCHMASTER (e.g. Analysis, Trace Properties, Oscilloscope etc.) which helps the user to keep the overview of the data.

In the *Trace Assign* dialog it is possible by default to assign labels for up to 16 *Traces* (*Trace 1...16*). If you increase the number of *Max. Shown Traces* in the *General* tab of the Configuration window this number will also increase.

For each *Trace Assignment* you have to select the type of channel:



Trace Count: Define a *Trace* number for the selected *Trace*:

- *Trace 1...32*

Amplifier: Define a current or voltage monitor for the selected *Trace*:

- *Imon*
- *Emon*

AD-Channel: Define an AD-channel for the selected *Trace*:

- *Adc-0...2*
- *Mux*
- *Adc-8...15*

Leak: Define a leak channel for the selected *Trace*:

- *Leak-1...32*

Digital: Define a digital channel for the selected *Trace*:

- *Dig-in(word)*
- *Dig-in 0...15*

Virtual: Define a virtual channel for the selected *Trace*:

- *Virtual-1...32*

LockIn: Define a *LockIn* channel for the selected *Trace*. The following channels are available:

- *LockIn_CM*
- *LockIn_GM*
- *LockIn_GS*
- *LockIn_Real(Y)*
- *LockIn_Img(Y)*
- *LockIn_DC*
- *LockIn_Avg*
- *LockIn_CV*
- *LockIn_GP*
- *LockIn_Admit(Y)*
- *LockIn_Phase*
- *LockIn_Real(Z)*

5.8. Trace Assign

- $\text{LockIn_Imag}(Z)$
- $\text{LockIn_IMP}|Z|$

Spectroscopy: Define a spectroscopy channel for the selected *Trace*. The following channels are available:

- Chirp_Avg
- Chirp_Phase
- $\text{Chirp_Admit}(Y)$
- $\text{Chirp_Real}(Y)$
- $\text{Chirp_Imag}(Y)$
- $\text{Chirp_Real}(Z)$
- $\text{Chirp_Imag}(Z)$
- $\text{Chirp_Imp}|Z|$

Photometry: Define a photometry channel for the selected *Trace*. The following channels are available:

- Photo_W1
- Photo_W2
- Photo_W3
- Photo_R
- Photo_Ca

Imaging(SmartLUX): Define an imaging channel for the selected *Trace*. The following set of channels is available for six times:

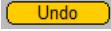
- Image_W1
- Image_W2
- Image_W3
- Image_R
- Image_Ca

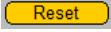
ElProScan: Define an ELPROSCAN channel for the selected *Trace*. The following channels are available:

- SF-ampl
- SF-phase
- z_pos
- z_slope

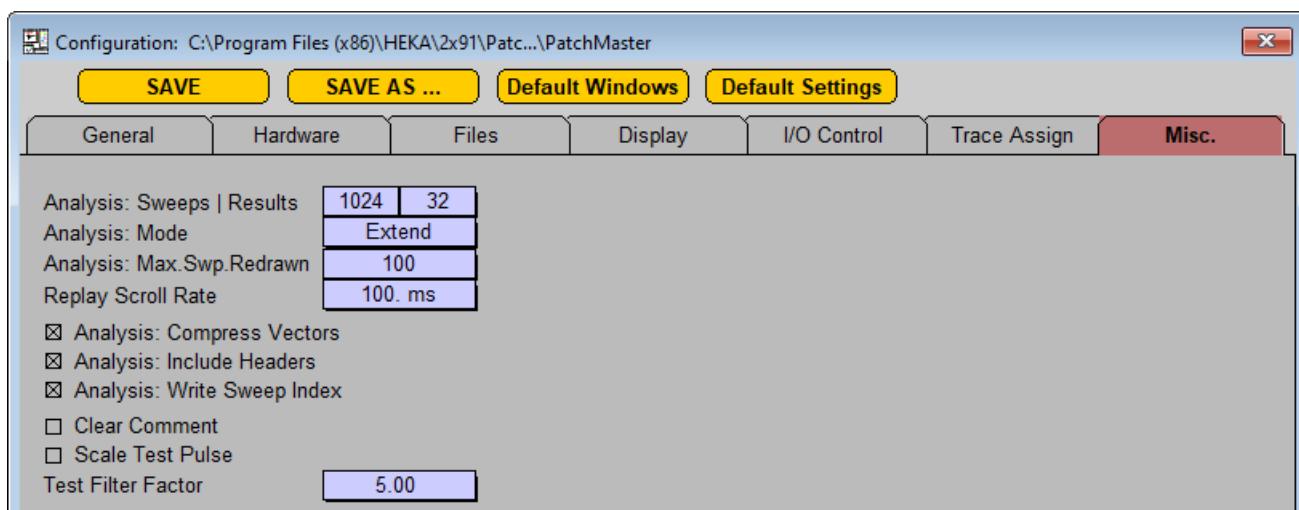
S.W.Voltammetry: Define a square wave voltammetry channel for the selected *Trace*. The following channels are available:

- *I-forward*
- *I-reverse*
- *I-diff*
- *E-mean*

 : The last change will be reset.

 : Resets all *Trace assignments* to the default value.

5.9 Miscellaneous



Analysis: Sweeps | Results: The Analysis needs to allocate memory for storage of its results. The *Analysis Result* buffer is a 2-dimensional buffer (table).

- Analysis: Sweeps: Maximum Analysis results per Analysis function (default = 1024)
- Analysis: Results: Maximum Analysis functions (default = 36)

For example if you use the Analysis for chart like recording during your experiment. The experiment lasts for about 10 minutes and you analyze one set of *Analysis: Results* per second then the online buffer has space for 600 *Analysis: Sweeps*.

Note: The Analysis buffer is cleared after each Wipe.

In case you select more Analysis functions in your Analysis Method than specified here, then all results exceeding this number will not be computed, hence not displayed!

Analysis Mode: Handling of *Analysis: Sweeps | Results*:

- Fixed: The number of *Analysis: Sweeps | Results* given above is fixed, i.e. limited to that number. For long chart like recordings and many Analysis results the number of Sweeps and results might have to be set to such high values that available memory become a problem.

- Extend: The number of *Analysis: Sweeps | Results* given above will be extended, if more data are acquired. With this mode selected the system might run into trouble when no further free memory is available.

Analysis | Max.Swp.Redraw: Defines the maximum number of *Sweeps* which can be displayed in an *Analysis* window.

Replay Scroll Rate: Defines the time interval when scrolling through the *Replay* tree.

Analysis: Compress Vectors: If this checkbox is selected, the data of the *Analysis* results get compressed to increase the drawing speed of the graphs in the *Analysis Windows*.

Analysis: Include Headers: If this checkbox is selected, the column headers of the *Analysis* results written into the *Notebook* is displayed. If you deactivate the checkbox the headers will not be shown.

Analysis: Write Sweep Index: If this checkbox is active, the sweep index for each *Sweep* is printed into the *Notebook* and exported.

Clear Comment: Clears the *Comment* field in the *Control* window after the *Comment* is stored with the data.

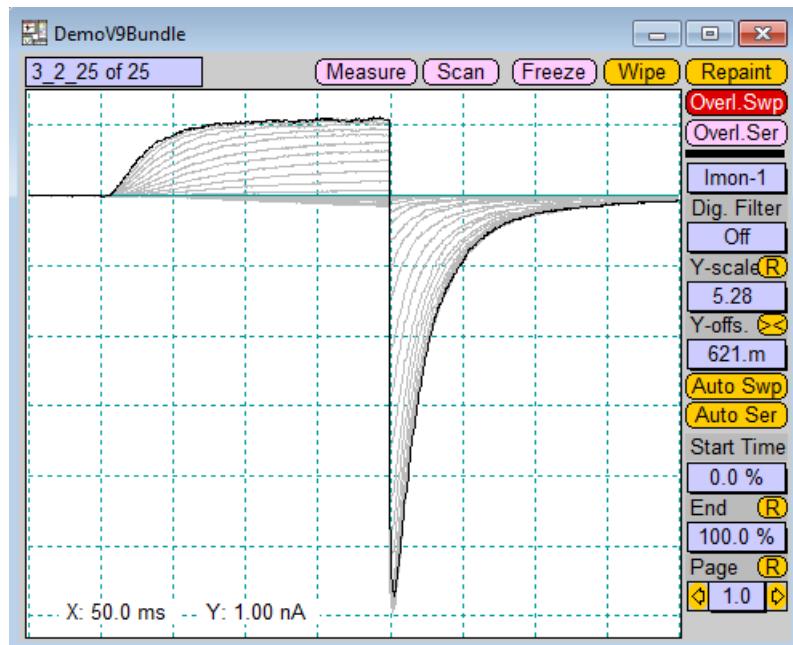
Scale Test Pulse: If this checkbox is selected, the display scaling factors in the *Amplifier* window (*I-scale*) will apply to the *Test Pulses*. Note that you can choose a *Test Pulse* series in the *Amplifier* window. For more information on the *Test Pulse* function, please refer to *Test Pulse*, 7.1.8 on page 80.

Test Filter Factor: The EPC 10 filter is set according to the *Sample Interval* of the built-in *Test Pulse* using this *Test Filter Factor*. Of course, this only is active when *Auto Filter* is on. The *Test Filter Factor* can be turned off by setting the value to zero.

5.10 Special Features of EPC 10 Double, Triple and Quadro

PATCHMASTER will automatically detect a connected EPC 10 Double, Triple or Quadro and will pre-configure most settings. The fields *V-membrane Out*, *Current In*, and *Voltage In* cannot be specified. These AD- and DA-channels are internally connected and, therefore, cannot be changed. For more information about these amplifiers please refer to the respective hardware manuals.

6 Oscilloscope Window



The Oscilloscope window is mainly used for monitoring the data. The *Traces of a Sweep* are displayed versus the time. Controls for display scaling and data handling are provided. The title of the window contains the currently active data file name.

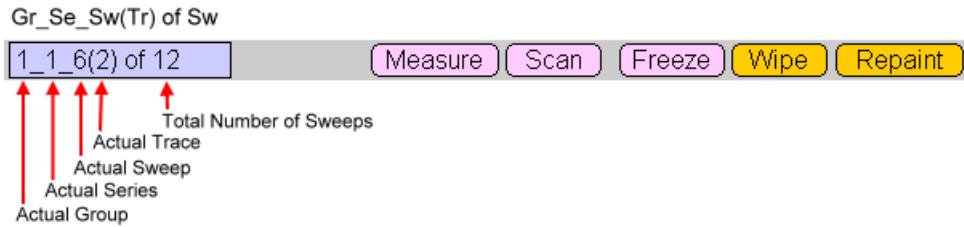
6.1 Display

Zooming – Lassoing: It is possible to set the display scaling in the Oscilloscope window by "lassoing" a screen region while pressing the left mouse button. When you release the mouse button, the marked area will be set to fill the Oscilloscope 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 *Y-scale* or *Start/End Time* or drag the mouse *outside* the active screen area. The display gains gets reduced by 20% as long as the mouse is outside, and stops when it moves back on the active display area. When the mouse is below or above the screen, the *Y-scale* is changed, and when the mouse is to the left or right of the screen, the *X-scale* is changed.

Cursor shapes: To accommodate different user preferences there are multiple cursor shapes available. Press the CTRL key while the "cross" cursor is displayed and you can cycle through all available cursor shapes.

6.2 Navigation



Group_Series_Sweep of Sweep: Currently active *Group*, *Series* and *Sweep* number within the data tree. The total number of *Sweeps* per *Series* is given after "of". The numbers are updated with every start of a new *Sweep* acquisition. During data replay these information are replaced by the information of the replayed data. Note that this can interfere with the above mentioned updating process if data are replayed in the wait time between data acquisition cycles.

6.3 Measurements on Traces

[Measure] : Allows to measure amplitudes and duration. With this option the user can measure the displayed data by pointing and dragging the mouse or by clicking on $\leftarrow\rightarrow$ and $\downarrow\uparrow$. Two mouse-driven horizontal lines are provided to measure signal differences. By clicking on the button **To Notebook**, the measurement data is written to the Notebook window.

[Scan] : Allows to measure signal amplitudes by scanning the individual data points. When selected, a marker is displayed on the *Trace*. This marker can be moved forward and backward by dragging with the mouse or by clicking on $\leftarrow\rightarrow$ and $\ll\gg$. By clicking on the button **To Notebook**, the measurement data are written to the Notebook window.

Note: The functions "Measure" and "Scan" work on the selected *Trace* in the *Replay* window. If anything other than a *Trace* is selected in the *Replay* window, then the functions work on the *Trace* that is selected in the *Oscilloscope* window (right panel).

6.4 Display Refreshing

[Freeze] : Freezes the present content of the Oscilloscope. Any change in display parameters, such as scaling etc. will unfreeze the display again, as does a *Wipe* or switching to the *Amplifier* window.

[Wipe] : Clears the Oscilloscope completely.

[Repaint] : Clears the Oscilloscope first, then redraws the last displayed *Trace*. This is useful e.g., if you had the *Overlay Sweep* option activated, but then decided that you only want to see the last *Sweep* of the *Series*.

6.5 Overlay Options

[Over.Swp] : Displays all *Sweeps* of a *Series* without erasing the screen in between *Sweeps*. The next *Series*, however, will erase the screen.

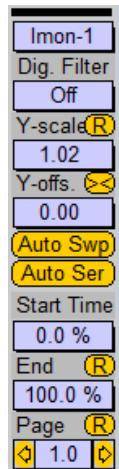
[Over.Ser] : Displays all incoming *Sweeps* without erasing the screen. This allows *Sweeps* of different *Series* to be computed. The screen can be wiped by pressing BACKSPACE.

6.6 Display Scaling

Trace Color: Displays the color of the selected *Trace*.

Trace 1...16 (default): Selects the *Trace* to which the scaling setting applies. The color of the selected *Trace* is displayed above the *Trace* control.

Note: When using the Trace Assignment function (see chapter 5.8 on page 65) the names of the Traces may be different.



Dig. 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-scale: For each *Trace* you can control the display scaling. The value of 1 corresponds to full scale of ± 10.24 V. This scaling does not affect the display of *Test Pulses*. Pressing + from the numeric keypad (take care not to have NUMLOCK activated!) increases the display scaling by a factor of 2 and - decreases it by a factor of 2. It is also possible to auto-adjust (*Auto Y-scale*) or fix the Y-scale (*Fixed Y-scale*) of the Oscilloscope. The reset button (R) sets the Y-scale to "1". For further information we refer to chapter 4.5 on page 29.

Note: This display scaling does not affect the display of the *Test Pulse Current Trace*. If you want to be able to scale the *Test Pulse* as well, activate the option *Scale Test Pulse* in the *Configuration* window and use *I-scale* in the *Amplifier* dialog. We advise against activating that option because one can easily overlook that the amplifier Gain is not correctly set, when the current *Trace* is scaled by the display scaling.

R: Resets the Y-scale to the default values.

Y-offs.: 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 + and SHIFT - (from the numeric keypad) increase and decrease the display offset by 0.1.

Center: Clicking on the *Center* button automatically centers the active *Trace* on the screen. When pressing the SHIFT key together with this button all *Traces* will be centered.

Auto Swp: Pressing the *Auto Swp* button calculates the maximum Y-scaling factors (Y-scale, Y-offs.) to display the selected *Trace* of a *Sweep* as large as possible in the Oscilloscope. The basis for the calculation are the Y-min and Y-max values of the selected *Trace* of the *Sweep* (see chapter 14.1.5 on page 215).

Auto Ser: Pressing the *Auto Ser* button calculates the maximum Y-scaling factors (Y-scale, Y-offs.) to display the selected *Traces* of a *Series* as large as possible in the Oscilloscope. The basis for the calculation are the Y-min and Y-max values of the *Sweep Traces* of the *Series* (see chapter 14.1.5 on page 215).

Start Time / End: Section of the *Sweep* to be shown on the screen in % (*Start* - *End*).

R: The Reset button sets the full *Sweep* length (0 to 100 %).

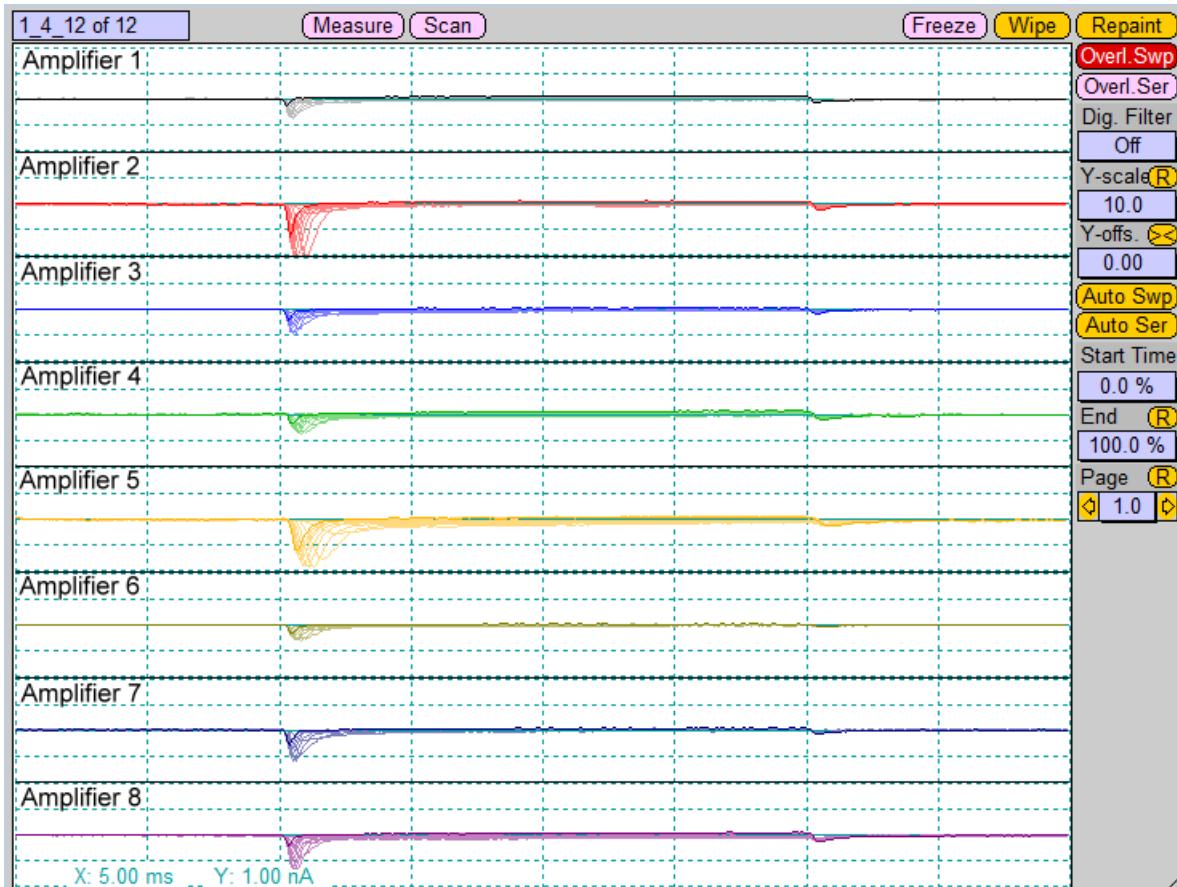
Note: The full time scale provided for *Sweep* display is based on the longest *Sweep* within a *Series*. Alternatively, one can use the *Fixed Scale* option (see below).

Page: Page of display during replay of continuous data *Sweeps* or when time axis is chosen to be less than 100%. Clicking on the right/left arrow control will display the next/previous page of the current *Sweep*. Dragging the page number scrolls the data forward or backward; entering a page number will display that particular page. Page is highlighted whenever there is more than one page available.

R: The Reset button sets the page number to "1.0".

6.7 Multi-Channels

If the *Multi-Channel* option is activated in the Configuration the *Traces* are displayed in separate, clipped viewports, one beneath the other. All viewports share the same scaling in the Oscilloscope. The figure below shows an example for data recorded with two joined EPC 10 Quadro and *Multi-Channels* set to "8".

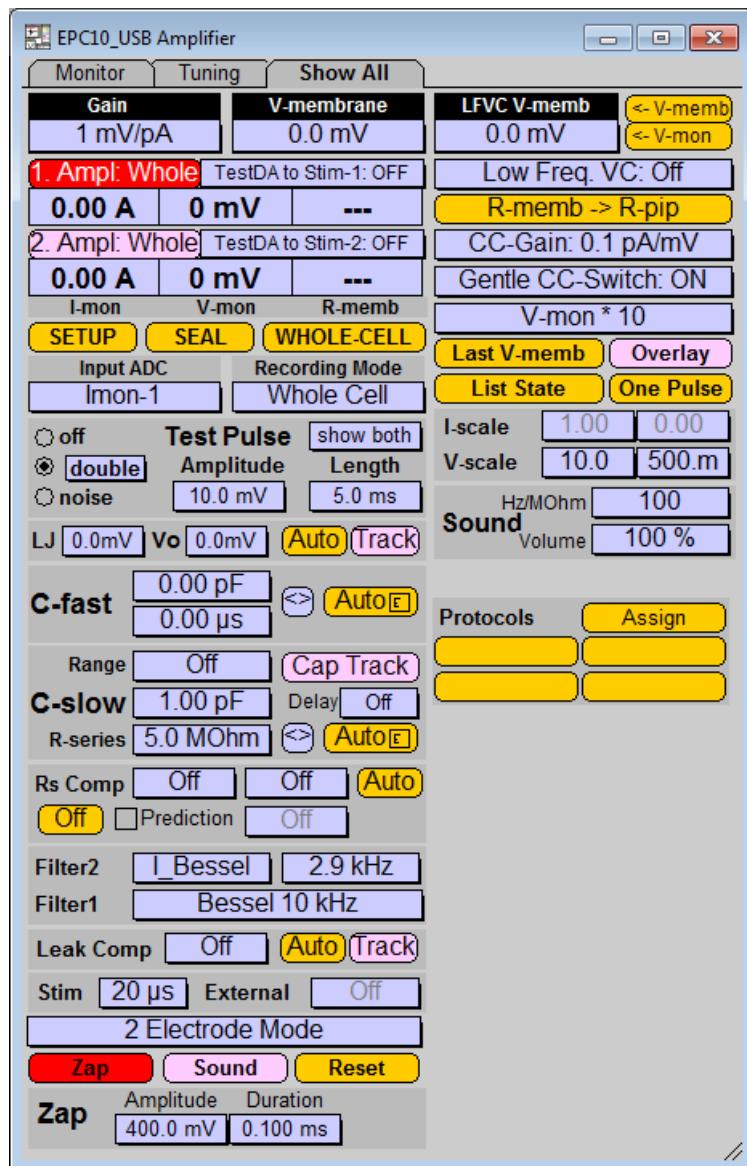


Note: When using Multi-Channels only 1 AD channel (Trace) per amplifier is allowed.

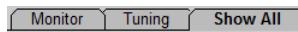
7 Amplifier Window

The Amplifier window is used for controlling, adjusting and displaying the amplifier settings. The window might differ depending on the type of amplifier you are controlling with PATCHMASTER. We will describe the window of the EPC 10 in most detail and focus to special details only when describing the windows of other amplifiers.

This is the screen shot of the complete amplifier window for an EPC 10 Double amplifier:



7.1 EPC 10 Amplifier

 The Amplifier window has three different tabs (*Monitor*, *Tuning*, and *Show All*) to allow switching between the display of different sets of controls.

7.1.1 Amplifier Gain

Gain: Sets the scaling of the current monitor output. For EPC 10 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 *Gain* setting automatically selects one of the three available current measuring feedback resistors in the probe (5 MΩ, 500 MΩ, and 5 GΩ), corresponding to low, medium and high *Gain* ranges. The table below summarizes the main features and limitations of the *Gain* ranges:



Table 7.1: Gain Range Specifications of the EPC 10.

	Low	Medium	High
Feedback Resistor	5 MΩ	500 MΩ	50 GΩ
Gain	0.005-0.2 mV/pA	0.5-20 mV/pA	50-2000 mV/pA
I_{max}	$\pm 2 \mu A$	$\pm 20 nA$	$\pm 200 pA$
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 *Gain* range, the background noise is larger than in the high *Gain*, but up to about 20 nA can be recorded. This range is used mainly for whole-cell recordings.

The high *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 *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 *Gain*, then the *Gain* is set to the next highest valid *Gain* before changing to current clamp.

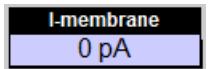
Clipping: A blinking box labeled "Clip" in the *Amplifier* title indicates saturation of the amplifier in the current monitor circuitry. Like the *Clipping* LED on the EPC 10 main unit, this is a warning that excess artifacts or noise may occur due to the saturation of the amplifier.

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

Note: In the case of an EPC 10 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.

7.1.2 Holding Potential and Holding Current

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 $\pm 1000/2000$ 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.

7.1.3 Amplifier Selection

For an EPC 10 Double, Triple or Quadro, there will be all amplifiers displayed (example for EPC 10 Double below).

The amplifier marked in red is the *active* amplifier. When switching to another amplifier, the Amplifier window updates all parameters 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.

1. Amplifier	TestDA to Stim-1: OFF		
117. fA	0 mV	35.2 GO	
2. Amplifier	TestDA to Stim-2: OFF		
1.19 pA	0 mV	---	
I-mon	V-mon	R-memb	

On the right to the amplifier button there is a drop-list for setting the behavior of the stimulation channel of the amplifier.

TestDA to Stim-1: OFF: Usually, the amplifier uses the *V-membrane out* channel as defined in the Configuration window. These so-called stimulation channels are assigned per default as follows:

- *Amplifier 1* uses DA-3 as Stim-1 channel
- *Amplifier 2* uses DA-2 as Stim-2 channel
- *Amplifier 3* uses DA-1 as Stim-3 channel
- *Amplifier 4* uses DA-0 as Stim-4 channel

With this setting selected the *default* behavior is set. Each amplifier uses its own stimulation channel. The special setting *TestDA to Stim-1* is set to *OFF*.

TestDA to Stim-1: ON In case multiple amplifiers should be stimulated simultaneously, then the following has to be done:

First use the *TestDA* channel as DA channel in the stimulation template of the Pulse Generator sequence. Second, select this option in the Amplifier window, to apply the *TestDA* output to the *Stim* channel of the amplifier. 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: When designing a TestSeries in the way described above, a user defined Test Pulse can be send to multiple amplifiers simultaneously.

Note: When using the default Stim channel for each amplifier, then - depending on the type of hardware and the number of channels - a slight time delay (in the μs range) between the different stimulation channels might occur. This delay is displayed in the Pulse Generator window, section *Stimulus Template Preview*, chapter 10.12 on page 134.

Ext. Stim. Input: ON Select this option to switch the external stimulus input on. The external input will be added to the internal stimulus (holding potential and stimulus generated by the Pulse Generator.)

AutoCSlow → TestDA When *Auto CSlow* wants to be used e.g. in Gap Junction measurements it is required that no current is flowing through the junction during compensation. This current would falsify the capacitance estimate. Hence, both cells have to be stimulated absolutely simultaneously to prevent currents from flowing through the junction. To do so, set this option and the option *TestDA to Stim-1: ON* (see above).

7.1.4 Monitoring

I-mon: Displays the DC current monitor signal.

1.29 pA	0 mV	---
I-mon	V-mon	R-memb

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* feature.

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

Membrane resistance is computed using the difference between the pipette current before and during the first *Test Pulse* amplitude.

$$R_{memb} = \frac{V_{test}}{I_{test} - I_{leak}}$$

This algorithm is quite insensitive to problems such as pipette offsets, reversal potential, and liquid junction potential.

- Computing *R-memb* when no *Test Pulse* is applied:

Membrane resistance is computed by using the pipette current only.

$$R_{memb} = \frac{V_{mon}}{I_{mon}}$$

This second algorithm is quite sensitive to many problems such as pipette offsets, reversal potential, and liquid junction potential. In most cases the membrane resistance computed by this second algorithm will differ from that computed by the first algorithm (which is more trustworthy!).

- Computing *R-memb* when a *TestSeries* is used as *Test Pulse*:

Membrane resistance is computed using the voltage and current difference between two consecutive segments of the *TestSeries*. The segments, which should be used for the calculation are defined by the *Zero* segment setting in the *Pulse Generator* dialog.

$$R_{memb} = \frac{V_{zero+2} - V_{zero+1}}{I_{zero+2} - I_{zero+1}}$$

Note: A calculation of *R-memb* is only possible when at least three constant segments are available.

I-mon, *V-mon* and *R-memb* are continuously updated, even when the Amplifier window is not in front, during the wait period between the acquisition of *Sweeps* and *Series*, and in current clamp mode. *I-pipette* and *R-membrane* are also computed and updated after every acquired *Sweep*.

7.1.5 Main Protocols

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

SETUP **SEAL** **WHOLE-CELL**

SETUP: Resets all parameters (with the exception of LJ and V_0), sets the *Recording Mode*, the *Gain*, the initial *C-slow* and *R-series* estimates and defines *Test Pulse* parameters. The protocol should be applied after the pipette has entered the bath.

SEAL: Sets the *Recording Mode*, the *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 *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.

These protocols can be edited in the Protocol Editor dialog.

7.1.6 Input ADC

Input ADC: The *Input ADC* selection determines which AD channel is displayed in the Oscilloscope in the *Test Pulse* mode.

AD-0
AD-1
AD-2
AD-3
AD-4
Vmon
Imon2
Imon1
AD-8
AD-9
AD-10
AD-11
AD-12
AD-13
AD-14
AD-15

AD-0...15: Any of the free and available AD channels

Vmon: Voltage monitor output

Imon2: Current monitor after *Filter 2*

Imon1: Current monitor after *Filter 1*

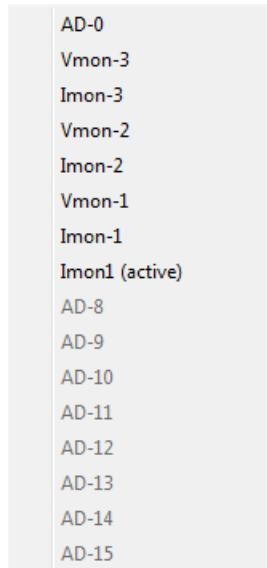
The Input ADC channel selection described here is for an EPC 10 Single amplifier only. The naming of the Input ADC channel selection for EPC 10 Double, Triple and Quadro is slightly different and described thereafter.

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The following naming of the *Input ADC* selection applies for an EPC 10 Double, Triple and Quadro. An example for an EPC 10 Triple is shown below:

Vmon-X, Imon-X: The pop-up menu for the AD selection contain the channel names. When changing the *active* amplifier the *Input ADC* will be adjusted automatically. The default is that *Vmon-1* and *Imon-1* belong to the first amplifier, *Vmon-2* and *Imon-2* to the second amplifier and so on. However, you can set the *Input ADC* to any other channel manually. Then this channel is always displayed when this amplifier is the *active* one until the user sets the *Input ADC* back to the default channel.

Imon1 (active): The current monitor after *Filter 1*. This channel is only available in EPC 10 Double and Triple amplifiers.



7.1.7 Recording Mode

Recording Mode: PATCHMASTER 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 Pulse Generator). As for the liquid junction potential, the polarity is always set correctly, regardless of the recording mode (see EPC 10 Manual: Compensation Procedures).

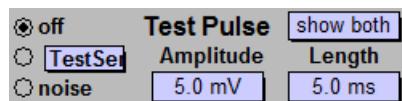
Out Out: Outside-Out configuration (voltage clamp).

Whole Cell: Whole-Cell configuration (voltage clamp).

C-Clamp: Current Clamp mode.

7.1.8 Test Pulse

Test Pulses are added to the holding potential and applied to the pipette; the current responses are sampled and displayed. *Test Pulses* are applied at maximal rates depending on the pulse length specified.



There are two *Test Pulse* modes:

1. Built-in *Test Pulses* (double or single)
2. *TestSeries* (which uses a stimulation template from the Pulse Generator as *Test Pulse*).

There are various options for the *Test Pulse* generation:

Off: Turns the *Test Pulse* "Off".

Test Pulse Mode:

- double pulse: A bipolar pulse pattern is generated and the current signal is sampled and displayed.
- single pulse: A mono pulse pattern is generated and the current signal is sampled and displayed.
- ramp: Loads a *Test Pulse* with a ramp pulse pattern. This kind of *Test Pulse* is especially for customers working with bi-layers (capacitance determination) or for testing purposes of the interface.

Note: The display scaling methods of the *Oscilloscope* window do normally not apply to *Test Pulses*. For further information see chapter 7.1.8.1.

- TestSeries: Loads the predefined PGF sequence *TestSeries* from the PGF pool.
- Enter Name: Loads customized PGF sequences from the PGF pool. You have to insert the exact name of the PGF you want to use for the *Test Pulse*. The big advantage of these *Test Pulses* is that they provide the full flexibility in pattern generation of the Pulse Generator. This includes that the sampled responses are handled like normal *Series* and that they can be stored to disk. For this type of *Test Pulse* the Analysis is available.

Note: If you use a predefined PGF sequence for the *Test Pulse* please be aware that you need at least three segments for the R-memb calculation (see chapter 7.1.4 on page 78). The first segment starts with the segment segment defined for the Zero Offset calculation in your PGF sequence. Thereafter, the next two segments are important for the calculation of R-memb. By shifting the Zero Offset calculation segment you will also shift the segment numbers for the R-memb calculation.

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). It is sampled in subsections of 10 times 256 points with a *Sample Interval* of 100 µs, i.e., a total length of 256 ms.

Note: Make sure to turn off the Auto Filter option (see chapter 5.4.11 on page 53) 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.

Test Pulse Display Option: Displays either the current *Trace* selected by the *Input ADC*, the voltage or both.

Amplitude/Length: Amplitude (mV) and duration (ms) of the single pulse and the double pulse can be specified in the dialog. The minimum pulse duration is 0.1 ms. No analysis of these pulses is provided, which makes the repetition interval quite short.

Note: Short *Test Pulses* will require a high sampling rate, i.e., a high filter bandwidth. If the Auto Filter option is activated, PATCHMASTER automatically selects the appropriate current input channel (Imon1 or Imon2) and sets the filter bandwidth (= Sampling Rate / Test Filter Factor). For bandwidths greater than approximately 9 kHz, PATCHMASTER automatically selects Imon1 as input, if an EPC 10 or EPC 9 amplifier is used; otherwise PATCHMASTER selects Imon2. Therefore, it can happen that the channel selection automatically toggles between Imon1 and Imon2 if *Test Pulses* are followed by a sequence from the *Pulse Generator* or vice versa.

7.1.8.1 Test Pulse Display Scaling

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For the *Test Pulses* the display scaling and *Trace offset* of the Oscilloscope window is not effective, because these *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.

I-scale	1.00	0.00
V-scale	10.0	500.m

If, however, amplification is needed you should enable the setting *Scale Test Pulse* in the Configuration window, (see chapter 5.9 on page 69) and use the *I-scale* and *V-scale* controls to set the display scaling of the *Test Pulse*.

I-scale, V-scale: Here, you can change the display scaling of current and voltage for the *Test Pulse*. You will find these controls on the right side of the Amplifier window.

7.1.9 Voltage Offsets

LJ: *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 V_0 operation. An online correction requires an *Auto V_0* operation to be performed before seal formation and LJ to be set to an appropriate value. No correction is performed if LJ = 0.

LJ [0.0mV] V_0 [0.0mV] **Auto** **Track**

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 or the bath solution is changed during an experiment. 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_0* 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.

V_0 : 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 V_0* operation or by manually dragging the mouse after clicking into the item. Furthermore, V_0 is changed automatically by the controlling program whenever the user changes the variable LJ. This is necessary for LJ and the *Auto V_0* operation to interact properly. It is not recommended to change V_0 manually, because this interferes with the software features for automatic offset correction.

Auto V_0 : The *Auto V_0* button calls a procedure for automatic zeroing of the pipette current. Thereby, an offset voltage (V_0) to the pipette potential is systematically varied until pipette current is zero. Range of V_0 is ± 200 mV. *Auto V_0* is typically performed before seal formation. It only works when a pipette is inserted into the bath.

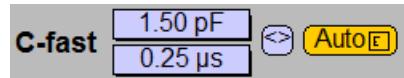
The *Auto- V_0* procedure interacts with the variable LJ to provide for online correction of *Liquid Junction* potentials and other offsets. This requires that *V-membrane* is set to the value of LJ (for *On Cell* and *In Out* recording modes) or to the opposite polarity of LJ (for *Whole Cell* and *Out Out* recording modes), before the actual zeroing operation is performed. *Auto V_0* does this automatically and leaves *V-membrane* at that value.

Note: V_0 is not changed by the *Reset* function.

Track: This option implements a *Search Mode*, which is essentially a *Auto V_0* procedure at a holding potential of 0 mV. The rate at which *Auto V_0* is performed is determined by the entry *Search Mode Delay* in the EPC 10 menu.

7.1.10 C-fast

The *C-fast* compensation circuit is used to cancel fast capacitive currents that charge the pipette and other stray capacitances (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.



Note: The range of the fast capacitance can be extended to 0-80 pF if an EPC 10 amplifier with revision letter 'T' is used.

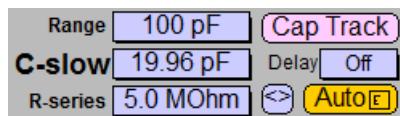
C-fast: This is the total *C-fast* value.

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

: This will select both, amplitude (vertical) and time constant (horizontal) of *C-fast*. Values for each may be changed manually depending on whether the mouse is moved horizontally or vertically.

Auto C-fast: Selection of this button performs an automatic compensation of *C-fast* and τ -*fast*. 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: R_s Comp is turned off, the stimulation is interrupted and the Gain is internally set to an appropriate value (0.2, 2 or 50 mV/pA, depending on the effective 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 small box in 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.

7.1.11 C-slow



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 Gain range only).

The program will prevent oscillations by limiting compensation according to the selected range and the effective capacitance values (R -series * *C-slow* > 5 μ s). Selecting the 1000 pF range will limit the Gain adjustment to a maximum of 20 mV/pA.

C-slow: 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 R -series * *C-slow* greater than 5 μ s 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.

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R-series: Adjusts the resistance in series with the slow capacitance (range: 0.1 MΩ - 10 GΩ) to determine the time constant of the C-slow transient and for R_s Comp. 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 on Auto.

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

 This will select both C-slow and R-series. Values for each are changed depending on whether the mouse is moved horizontally or vertically.

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 EPC 10 menu (see chapter 4.9 on page 36) by C-slow Num. Cycles and C-slow Peak 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 Gain is internally set to an appropriate value (0.2, 2 or 50 mV/pA, depending on the effective 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 Gain ranges, and the 100 pF range in the high 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  in 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.

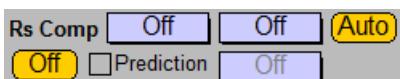
Cap Track: Selecting this button will perform repetitive Auto C-slow compensation. Clicking on Cap Track again will turn it off.

Note: If you are using the Cap. Track feature to measure small capacitance changes, it is advantageous to use a higher C-slow range when possible. Counter-intuitive as it may seem, the digital control of the C-slow circuitry is more precise in higher ranges, resulting in higher resolution, consistent with the Gain range and capacitance.

Delay: This determines how many seconds to wait before the next Auto-compensation is started. With short delays (e.g., 1 ms), membrane capacitance will be tracked at rates of >10 Hz (depending on the computer hardware).

7.1.12 Rs Comp

Rs Comp: 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%). The compensation is based on the value of R-series and will be effective only when R_s Comp is not off, i.e., set to a speed value. The following settings determine the speed of feedback compensation:



- Off: Turns compensation off
- 100 μs: Slow compensation
- 10 μs: Medium speed compensation

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- $5 \mu\text{s}$: Fast compensation
- $2 \mu\text{s}$: Very fast compensation

The choice of speed depends on the recording time constant and the degree of compensation desired. Fast R_s 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.

Auto R_s Comp: A click on the *Auto* button automatically adjust the R_s *Comp* 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 via the *Epc 10* menu (see chapter 4.9 on page 36).

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

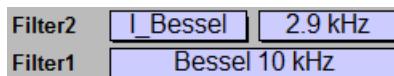
Prediction: Switches "On" or "Off" the R_s *Prediction* by selecting/deselecting the checkbox. The *Prediction* accelerates the charging of the membrane capacitance by imposing large, transient voltages on the pipette when step changes are commanded. The compensation of the *Prediction* is set independent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

In current clamp mode, R_s *Comp* will act as a bridge compensation. Optimal

Bridge Off 10 μs Auto

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%.

7.1.13 Filter



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 R_s *Comp* is active PATCHMASTER sets an appropriate *Filter 1* according to the speed value of R_s *Comp*.

Table 7.2: RS Compensation and Filter 1 Settings

R_s Comp	Filter 1
$2 \mu\text{s}$	HQ 30 kHz
$5 \mu\text{s}$	Bessel 30 kHz
$10 \mu\text{s}$	Bessel 10 kHz
$100 \mu\text{s}$	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*, i.e., the bandwidth of the signal actually recorded at *Imon2*.

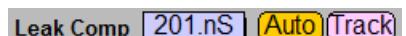
If you want to use an external filter rather than *Filter 2*, you can proceed as follows: Connect current monitor 1 (*Imon1*) to the external filter input, feed the filter output back into one of the AD channels (AD-0...AD-4), and select this channel as input channel.

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*.

7.1.14 Leak Compensation

Leak Comp: 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 *Auto*. The maximal values attainable depend on the *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 does not consider a possible hardware "Leak" correction of the EPC 10. The "Leak" value is stored and displayed together with the series parameters if Leak was activated in the PGF (i.e., $\text{Leak} \geq 0.1 \text{ pS}$). 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: Automatically determines and corrects the leak conductance.

Track: Continuously updates the *Leak Compensation*.

7.1.15 Stimulus

Stim: The stimulus 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:

Stim 20 µs External Off

- 2 µs
- 20 µs

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

External: The *External Stim. Input* is scaled by an editable factor (range: ± 0.1 – 1x), to allow for different external stimulators. It is strongly recommended to set *External* to *Off* (i.e., equal to zero) if no external stimulator is connected to the *Stim. Input*. This will prevent pick-up of external noise. When using an EPC 10 Single amplifier this option is always disposable. For users of multiple amplifiers we refer to *Amplifier Selection*, chapter 7.1.3 on page 77.

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.

7.1.16 3 Electrode Mode

Electrode Mode: The default of the EPC 10 amplifier is the *2 Electrode Mode* which is sufficient for most applications. But for some applications a third electrode (*3 Electrode Mode*) might be reasonable (for activation and further reference we refer to the chapter 5.4.11 on page 53).

2 Electrode Mode

7.1.17 Various Controls

Zap: A high voltage pulse is applied to the pipette in order to rupture the patch membrane. The parameters of the *Zap* pulse (duration and amplitude) can be specified in the lower part of the *Amplifier* window (see chapter 7.1.18).

Zap Sound Reset

In the Configuration window (see chapter 5.4.11 on page 53) it can be further specified whether *Zap* is always enabled or restricted to the *On Cell* recording mode.

Sound: If this control is 'On', a sound is played proportional to *R-membrane*. In addition you have to select the proper output device in the Configuration (see chapter 5.4.3 on page 50).

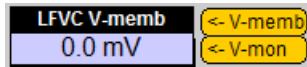
Reset: Selecting this button will reset the EPC 10 to its initial default configuration. It will reset the DA channels to zero, which is useful e.g. to cancel the output of *C-slow* and *R-series* set by *Cap Track*. In addition, *Reset* is very useful to define the initial state of the EPC 10 when recording a macro.

7.1.18 Zap Settings

Zap: Sets the parameters for the *Zap* pulse, namely *Amplitude* and *Duration*. A *Zap* pulse is a simple, square voltage pulse of a given amplitude and duration at the *Stim-DA* of the active amplifier. These 2 values are stored in the Configuration file. The *Zap* amplitude can also be a negative value.

Zap Amplitude Duration
400.0 mV 0.100 ms

7.1.19 Low Frequency Voltage-Clamp



LFVC V-memb: Sets the membrane potential used for *Low Frequency Voltage-Clamp* (=LFVC).

V-memb / V-mon: Copies the values of *V-memb* respectively *V-mon* into *LFVC V-memb*.

Low Freq. VC: 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 (*LFVC V-memb*). 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 *LFVC V-memb* 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 *Gain* range. In the medium *Gain* range, 1-100 is approximately the time in seconds, whereas in the high *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.

7.1.20 R-membrane

R-memb → R-pip: Copies the value of *R-memb* into *R-pip*. This is used to store the pipette resistance in the data file before forming a seal.

R-memb → R-pip

7.1.21 Current Clamp Settings

Four current-clamp scaling ranges can be selected:

CC-Gain: 0.1 pA/mV

- CC-Gain: 0.1 pA/mV
- CC-Gain: 1 pA/mV
- CC-Gain: 10 pA/mV
- CC-Gain: 100 pA/mV

CC-Gain: When the *CC-Gain* 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 *Gain* setting. The maximal output current in current clamp mode is 1 / 10 / 100 / 1000 nA, respectively.

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 *CC-Gain* options for the EPC 9 depend on the hardware version:

Table 7.3: CC-Gain Options of the EPC 9 version.

EPC 9 version	CC-Gain (pA/mV)
A	0.1 / 1
B	0.1 / 1
C	0.1 / -
D	0.1 / 1

Note: The Gain range cannot be switched in current clamp mode. Please go back to voltage clamp and use the *CC-Gain* setting to switch to another Gain range. Then go back to current clamp mode.

Gentle CC-Switch: This control selects between the two alternate modes of switching between voltage and current clamp and back.

Gentle CC-Switch: ON

- Gentle CC-Switch: OFF
I-hold will be set to 0 pA when switching to current clamp mode.
- Gentle CC-Switch: ON
I-hold 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-memb* commanded under voltage clamp. In analogy, upon returning to voltage clamp, *V-memb* will be set according to the membrane potential under current clamp and rounded to the next 2 mV.

7.1.22 Further Options

V-mon Gain: The measured voltage can be amplified by a factor of 10 or 100 before digitization. In the *Vmon*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.

V-mon * 10

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

Last V-memb : If changing from voltage clamp to current clamp mode, the software remembers the previously set holding potential. *Last V-memb* will set this holding potential after changing modes.

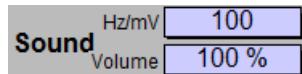
Overlay : The *Test Pulse* traces will be overlaid in the *Oscilloscope* window.

List State : Sends the actual amplifier state information to the *Notebook*.

One Pulse : As the name suggests, this button executes one *Test Pulse*. This is useful when *Test Pulses* are off and simply one *Test Pulse* is to be output.

7.1.23 Sound Settings

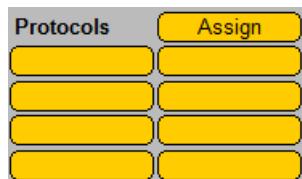
Hz/MΩ - volume: Here the settings for the sound feature can be entered. The sound is played proportional to R -membrane. The sensitivity (Hz/MΩ) and volume (in %) of the sound encoding of R -membrane can be specified there.



Note: The sound will only be produced if the sound control on the left side of the EPC 10 Amplifier window is active and when the proper sound device is selected in the Configuration (see chapter 5.4.3 on page 50).

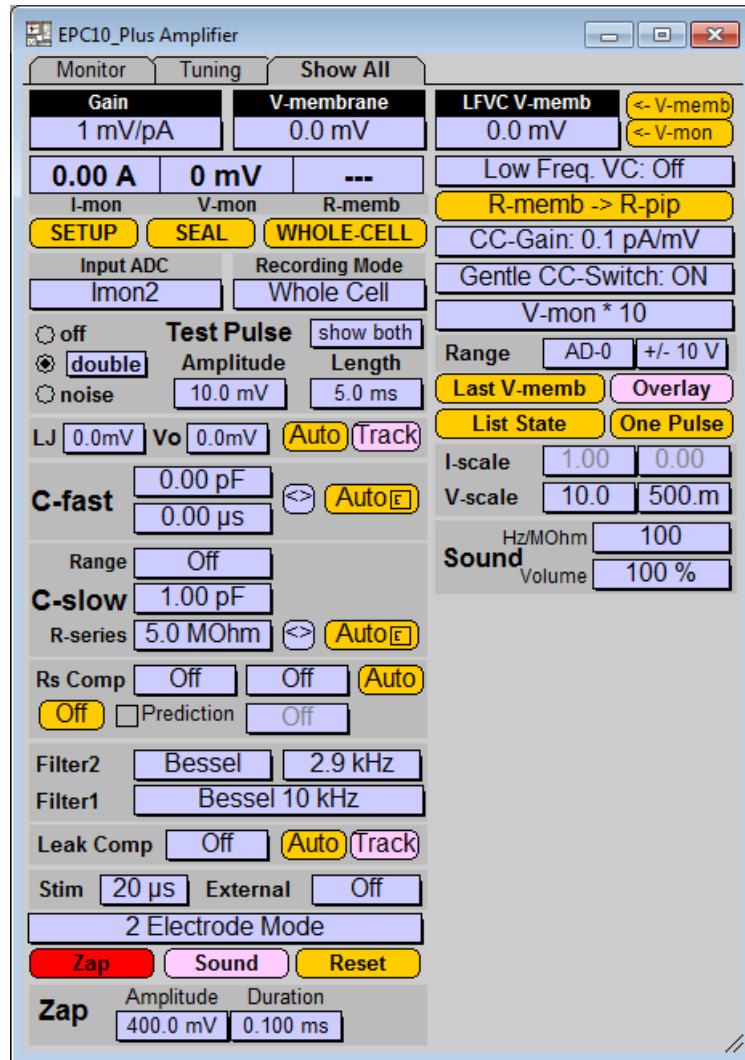
7.1.24 Amplifier Protocols

On the *Display* tab of the Configuration you can activate to show a specific number of *Protocol* buttons in the Amplifier window (see chapter 5.6.3 on page 59). In this example we show 8 so-called *Amplifier Protocols*. Use the *Assign* button to assign a specific protocol to the button.



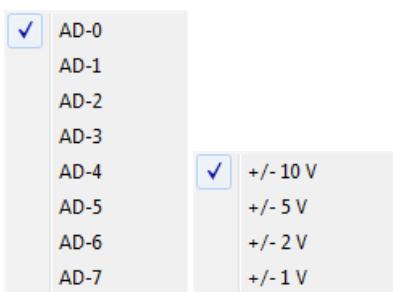
7.2 EPC 10 PLUS

The EPC 10 Plus patch clamp amplifier has an ITC-18 interface built-in. All amplifier functions are identical to the EPC 10 except some features that relate to the interface board.



AD-channel input range: In the Amplifier window there is an additional option for the input range of all AD channels. For each of the eight AD channels (AD-0...7) an input range (+/- 10, 5, 2, 1 V) can be selected.

Range AD-0 +/- 10 V



7.3 EPC 800 Remote/Local

The following screenshots show the Amplifier windows for an EPC 800 in *Remote* and *Local* mode.

7.4. EPC 8 Remote/Local



The EPC 800 window provides a subset of functions of the EPC 10. For details on the individual functions please refer to the section on EPC 10 amplifier controls (see chapter 7.1 on page 76) and to the hardware manual of the EPC 800.

The following controls are not available in the EPC 800 Local Amplifier window:

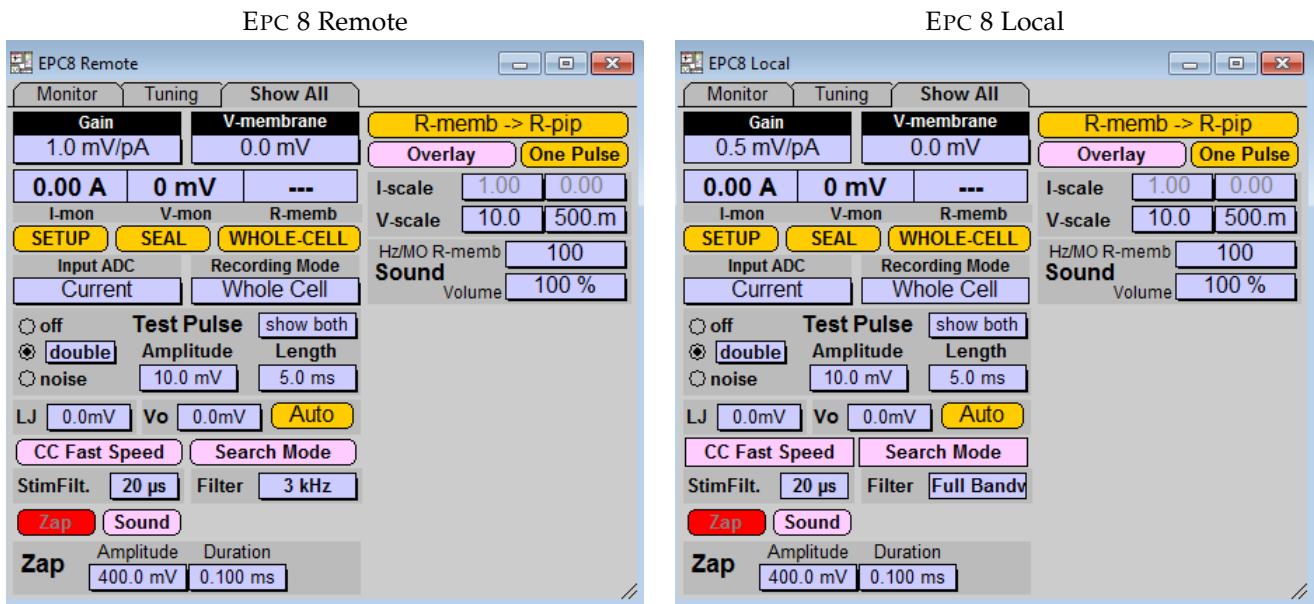
- Auto C-fast
- Auto C-slow
- Reset
- V-memb to LFVC Holding
- V-mon to LFVC Holding

The function of the *Zero Tracking* button is identical to the *Track* button explained in chapter 7.1.9 on page 82.

7.4 EPC 8 Remote/Local

The EPC 8 window provides a subset of functions of the EPC 10. For details on the individual functions please refer to the section on EPC 10 amplifier controls (see chapter 7.1 on page 76) and the hardware manual of the EPC 8.

The following screenshots show the Amplifier windows for an EPC 8 in *Remote* and *Local* mode.



7.4.1 EPC 8 Specific Controls

- CC Fast Speed: Compared to an EPC 7 the current clamp circuitry has been improved in EPC 8. The fast current clamp mode (*CC Fast Speed*) allows to better follow rapid changes in membrane potential, such as in neuronal action potentials. For further details we refer to the manual of the EPC 8.
- Search Mode: This option implements a *Search Mode*, which is essentially a *Auto V₀* procedure at a holding potential of 0 mV. The rate at which *Auto V₀* is performed is determined by the entry *Search Mode Delay* in the EPC 8 menu.

7.4.2 Telegraphing Parameters

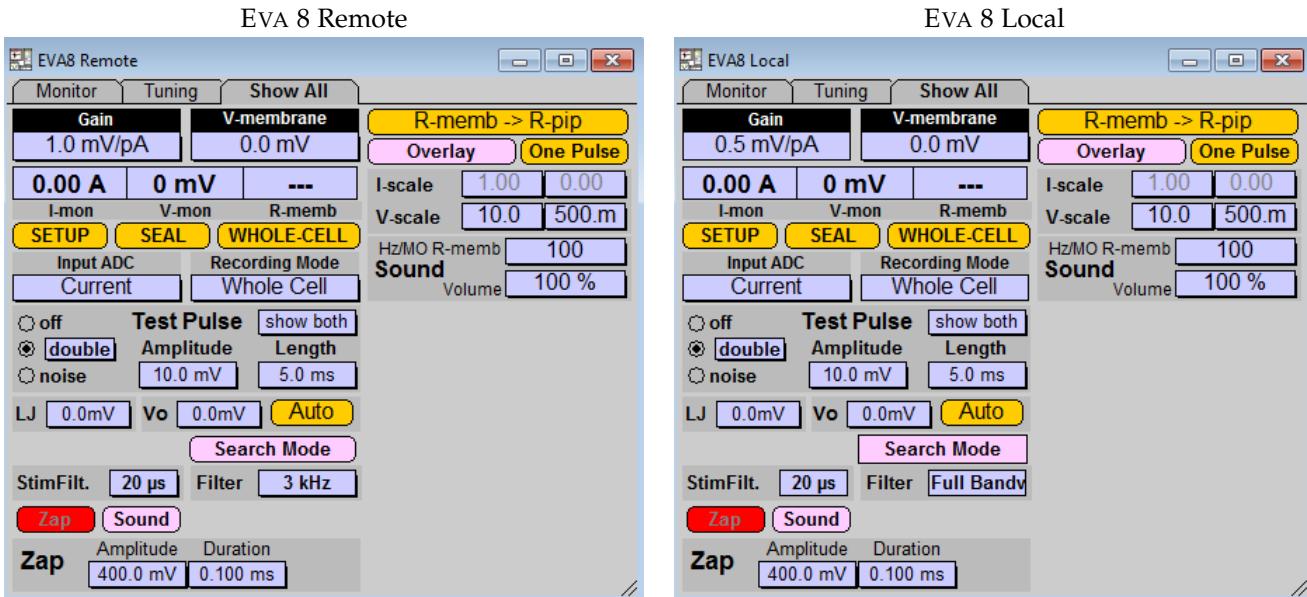
The following functions can be set in *Remote* mode and read in *Local* mode:

- Gain
- Recording Mode
- StimFilt.
- Filter
- CC Fast Speed
- Search Mode

7.5 EVA 8 Remote/Local

The EVA 8 window provides a subset of functions of the EPC 10. For details on the individual functions please refer to the section on EPC 10 amplifier controls (see chapter 7.1 on page 76) and to the hardware manual of the EVA 8.

The following screenshot shows the Amplifier window for an EVA 8 used in *Remote* mode.



7.5.1 EVA 8 Specific Controls

- **Search Mode:** This option implements a *Search Mode*, which is essentially a *Auto V₀* procedure at a holding potential of 0 mV. The rate at which *Auto V₀* is performed is determined by the entry *Search Mode Delay* in the EVA 8 menu.

Note: The EVA 8 does not have a current clamp recording mode.

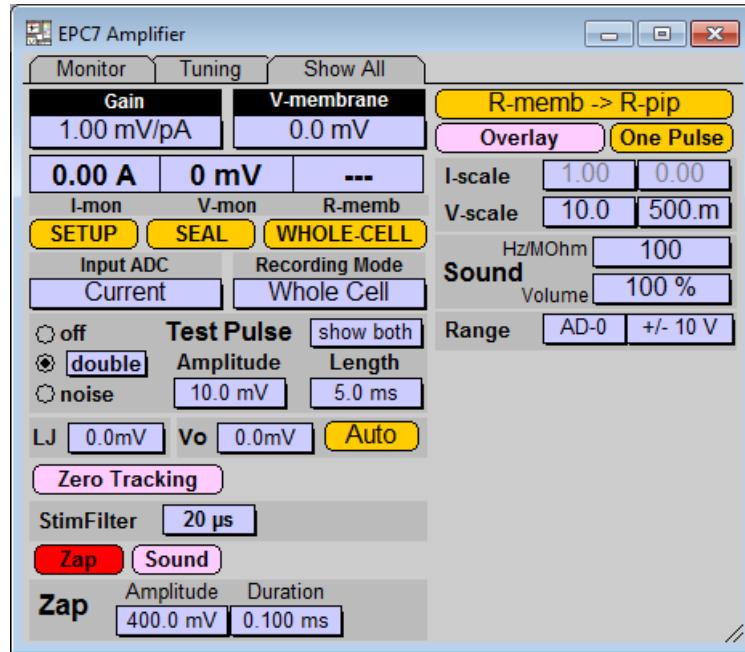
7.5.2 Telegraphing Parameters

The following functions can be set in *Remote* mode and read in *Local* mode:

- Gain
- Recording Mode
- StimFilter
- Filter
- Search Mode

7.6 EPC 7 and Telegraphing Amplifiers

All non-telegraphing controls have to be set manually in the Amplifier window.

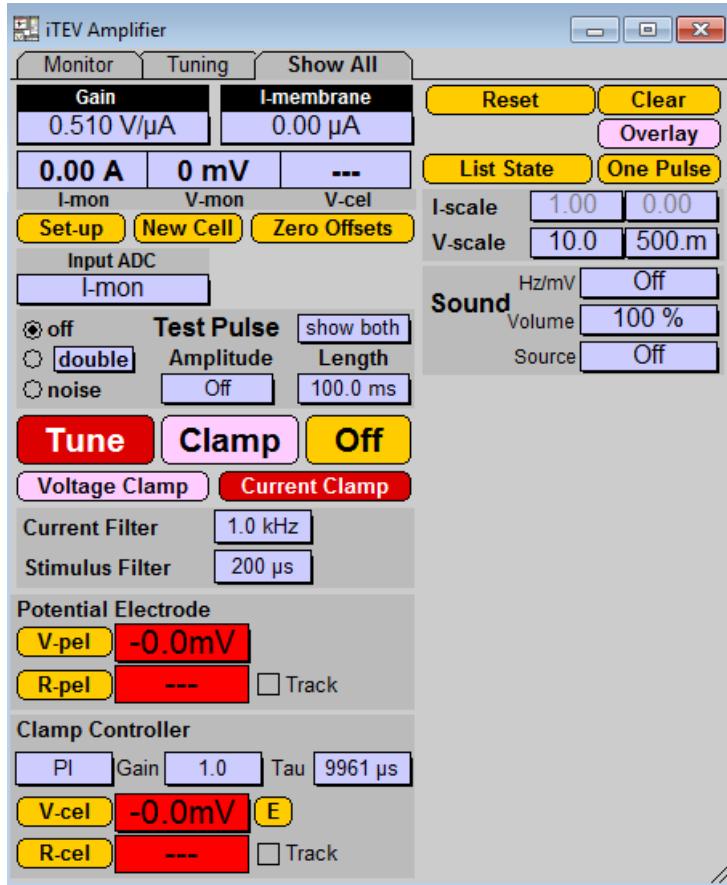


Note: The option Range is only available when using an ITC-18. It allows to set the voltage range of the AD input channels.

The amplifier settings will be saved together with the data. The user has to make sure that the settings in the Amplifier window are identical to the settings on the connected amplifier.

7.7 iTEV 90 Multi-Electrode Clamp

The iTEV 90 window provides a subset of functions of the EPC 10 as well as some iTEV 90 specific functions. The special iTEV 90 related functions are shortly explained below and in length in the hardware manual of the iTEV 90. All other functions are explained in the section on EPC 10 amplifier controls (see chapter 7.1 on page 76).



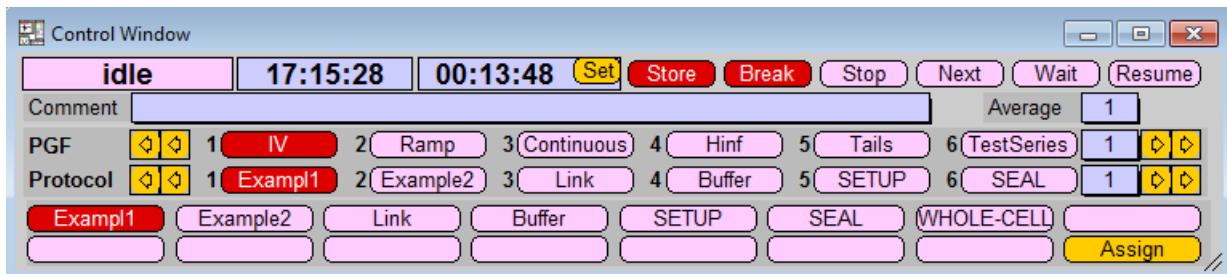
7.7.1 iTEV 90 Specific Controls

- V-cel: Voltage of the Current Electrode.
- Main Protocols: The buttons *Set-up*, *New Cell* and *Zero Offsets* are used to call protocols of equivalent names provided in the Protocol Editor.
- Tune: When active the amplifier is in the current clamp mode with a very small *Gain* - it is effectively inactive. This mode is used to zero potential offsets and to measure electrode resistances.
- Clamp: The amplifier is put into an active mode and all settings of this window apply.
- Off: The amplifier is turned off, i.e., the high voltage source is inactivated and the feedback loop is opened.
- Current Filter: Cut-off frequency of an 4-pole *Bessel* filter used to process the *I-mon* signal prior to sampling.
- Potential Electrode:
 - V-pel: For measurement and compensation of the electrode offset potential. The corresponding offset potential value will be displayed in the field on the right. In addition, the field will turn from red to blue to indicate that this electrode is ready for the experiment.

- R-pel: For measurement of the electrode resistance. If within an acceptance range, *R-pel* will be displayed in the field on the right and this field will turn from red to blue to indicate that *R-pel* was successfully checked before a new experiment.
- Track: Checkbox to activate continuous measurement of *R-pel*.
- Clamp Controller:
 - Controller type: Select between "P" (proportional) or "PI" (proportional / integral) mode of the clamp controller.
 - Gain: Proportional gain of the controller.
 - Tau: Time constant (μs) of the integrator used in the PI-mode.
 - V-cel: For measurement and compensation of the electrode offset potential. The corresponding offset potential value will be displayed in the field on the right. In addition, the field will turn from red to blue to indicate that this electrode is ready for the experiment.
 - E: The Current Electrode can be enforced if it was disabled due to high compliance voltage.
 - R-cel: For measurement of the electrode resistance. If within an acceptance range, *R-cel* will be displayed in the field on the right and this field will turn from red to blue to indicate that *R-cel* was successfully checked before a new experiment.
 - Track: Checkbox to activate continuous measurement of *R-cel*.
- Clear: Sets *V-pel*, *R-pel*, *V-cel* and *R-cel* to default values.

7.7. iTEV 90 Multi-Electrode Clamp

8 Control Window



From this window, protocols or Pulse Generator sequences are started or stopped. It allows comments to be entered and it provides timing information. The number of buttons for executing protocols can be set in the Display section of the Configuration dialog (see chapter 5.6.3 on page 59).

8.1 Information about the Experiment

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

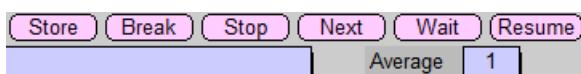
- "aq: waiting": PATCHMASTER is waiting for the user to click on the *Resume* button during an acquisition (because the *Wait* button was activated by the user).
- "acquiring": Acquiring data in the *Interactive* mode.
- "ex: waiting": PATCHMASTER is waiting for the user to click on the *Resume* button during a protocol (because of a *Wait* event with the wait type "Resume Icon" or "wait for key").
- "gap-free": Acquiring data in the *Gap-free* mode.
- "idle": All else.
- "locked": User is locked out (PATCHMASTER PRO only).
- "noise": Measures noise.
- "recording": Recording a macro.
- "run macro": Runs a macro (may be part of a protocol).
- "run protocol": Runs a protocol.
- "test": *Test Pulse* is running.
- "test (demo)": *Test Pulse* in *Demo* mode.

Time: Actual system time measured since midnight.

Timer: This item functions as a stopwatch or timer called *TimerTime*. It can be reset at any time by clicking on the *Set* item or by pressing T. It may be useful to keep track of the experiment (e.g., to monitor the time spent in a specific experiment mode). The *TimerTime* is updated also during the *Series* execution. The timer value is stored at the beginning of the *Sweep* acquisition. The internal *Timer* tick corresponds to 1 ms. When you click on *Set*, the *Timer* will be reset to zero and started again.

Comment: Comment to the currently active *Series*. This field can be edited. It will result in a modification of the text of the present *Series* if a file was opened with write permission. This means that one has to enter a text to a *Series* after it has been acquired. The new comment will be copied into all incoming new *Series* until a new text is entered.

8.2 Controlling Data Acquisition



Store: Store is used to save the data that will be acquired from now on. A dialog window opens and you have to enter the name of the new data file.

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.

Break: Break is used to stop *Series* acquisition or replay. The *Break* flag is reset, when the next target is displayed or executed. If *Break* 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 *Pulse Generator*. The *Break* button can also be used in *Replay* mode when performing a lengthy operation.

Stop: This button is used similarly to *Break*. However, during acquisition of a *Sweep*, this particular *Sweep* will be completed. The *Stop* button can also be used in *Replay* mode when performing a lengthy operation.

Next: At the end of the actually acquired *Sweep* the *Series* is stopped and the next protocol event will be executed.

Wait: This button is used to pause *Series* execution. Its command key is W. *Series* execution is resumed by a click on *Resume* or by R. Wait will become effective after completion of the currently acquired *Sweep*. The *Wait* button can also be used in *Replay* mode when performing a lengthy operation; e.g., it allows inspecting a particular *Sweep*, when replaying a collection of *Sweeps*.

Resume: Opposite of *Wait*. Press *Resume* to continue data acquisition or replay.

Average: By use of this function, a PGF sequence can be repeated several times. Only the average is stored to disk. Please enter the number of averages acquired for one *Sweep*.

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 10.14 on page 136).

Note: 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.

8.3 Starting Pulse Generator Sequence



If you have not activated the option *Hide PGF-Bar in Control Window* on the Display tab of the Configuration (see chapter 5.6.3 on page 59) then you will see a row with buttons listing the various sequences of the loaded Pulse Generator file (*.pgf). Six buttons are shown at a time. But the row of buttons can be scrolled using the arrow buttons on the left and right side.

- A single click on one of those buttons will start the respective Pulse Generator sequence.
- By entering a number into the field to the right of the button row, the sequence with this index is started.

8.4 Starting a Protocol



If you have not activated the option *Hide Protocol-Bar in Control Window* on the Display tab of the Configuration (see chapter 5.6.3 on page 59) then you will see a scrollable row with buttons listing the various protocols of the loaded protocol file (*.pro). Six buttons are shown at a time. But the row of buttons can be scrolled using the arrow buttons on the left and right side. If you have activated the option *Show fixed Protocols in Control Window* on the Display tab of the Configuration (see chapter 5.6.3 on page 59) then you will see a buttons listing the various protocols of the loaded protocol file (*.pro). A single click on one of those buttons will start the respective Protocol.

- A single click on one button in the scroll bar will start the respective protocol.
- By entering a number into the field to the right of the button row, the protocol with this index is started.
- A single click on one button in the row of the fixed protocols will start the respective protocol.

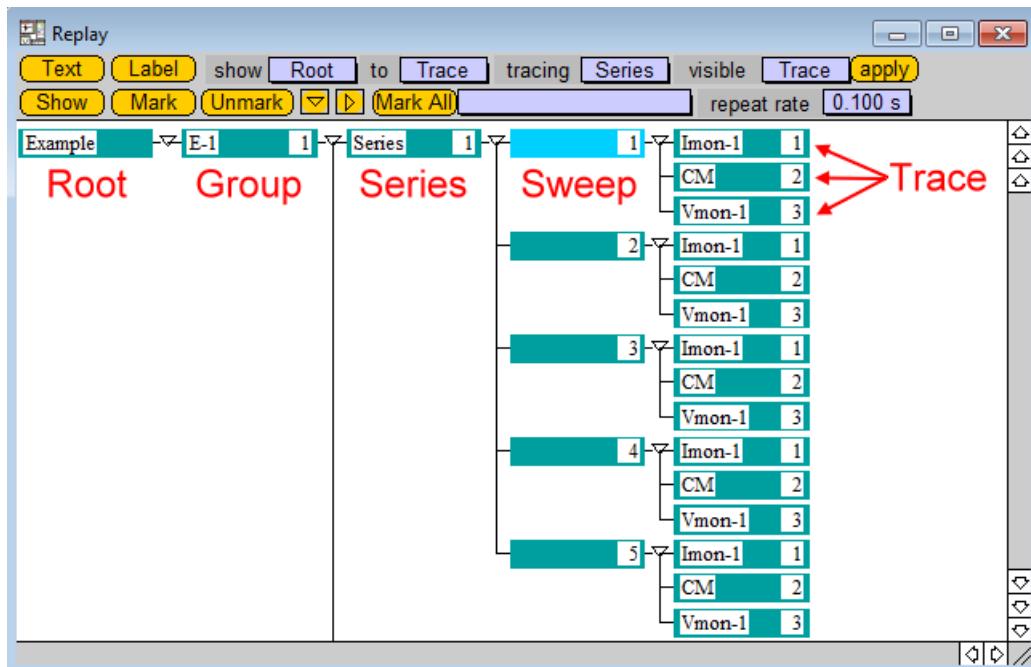
8.5 Fixed Control Protocols

If you have activated the option *Show X Fixed Control Protocols* in the Configuration window (see chapter 5.6.3 on page 59) there will be a certain number of buttons available below the protocol bar.



The position of the protocols is fixed. The order can only be changed when using the *Assign* button. In the upcoming dialog the user is asked to enter the name of an existing protocol and the position to which button it should be moved within the *Fixed Control Protocols*.

9 Replay Window



If data has been acquired in the *Store* mode or if an old data file has been opened, they can be reviewed and edited in the **Replay** window. To open it select **Windows** → **Replay**. Up to five levels of the data tree are displayed.

9.1 Main window functions

Text **Label** **show** **Root** **to** **Trace** **tracing** **Series** **visible** **Trace** **apply**

Text: Displays the comment to the target (either *Root*, *Group* or *Series*). Use this function to edit the comment. The entered text can be seen in the field *Text* of the tab for *Root* and *Group* or in the field *Comment* of the tab for *Series* in the Parameter window.

Note: A comment for a *Series* can be written directly in the field *Comment* of the *Oscilloscope* window.

Label: Displays the target label (*Groups/Series/Sweep/Trace*). All objects have default labels assigned:

- **Group:** E-1
- **Series:** Name of Pulse Generator sequence

- Sweep: No label
- Trace: Name of acquired AD channel or assigned *Trace name* of the Pulse Generator

You may use the *Label* function to edit the label.

When creating a new *Experiment* or a new *Group* with the *File Operation* event or acquiring a *Series* with the *Acquire* event in the *Protocol Editor*, then you can specify a custom label.

show / to: Defines which part of the data tree will be displayed. The highlighted part of the data tree is referred to as target throughout this manual. Available parts of the data tree are *Root*, *Group*, *Series*, *Sweep*, and *Trace*.

tracing: Specify the part of the data tree that will be traced during replay. This feature might be of importance if the *Replay* window is very narrow (allowing to display three levels of the tree only) and many tree entries exist. Setting the *Tracing* item to *Series* then the active items during replay are the series objects and still *Root*, *Group* and *Series* are shown in the *Replay* window. If you would set the *Tracing* item to *Trace* keeping the same narrow *Replay* window, then the whole tree would be scrolled to the right and only *Series*, *Sweep* and *Trace* are shown in the *Replay* window.

visible/Apply: This feature can be used to expand or minimize the whole tree. If we specify *Series* and click *Apply* then the tree is reduced to the level of the *Series*, all *Sweep* and *Trace* objects are hidden.



Show: Replay the selected target. Alternatively, you can perform a double-click on the target.

Mark: Mark the selected target. You can mark more than one entry. Several functions in the *Replay* menu apply for the marked entries (see chapter 4.4 on page 23). Note that set marks will be written into the *.pul file of the data when you save the data. That means that marks will also be reloaded when you open such a marked data file.

Unmark: Unmark the selected marked target.

Note: You can also quickly mark or unmark your targets by selecting them and pressing 'M' or 'U', respectively.

Show/Hide Arrows: These buttons can be used to expand and close the child tree.

Mark All: Mark all targets in the selected data range with the named label.

repeat rate: Enter a time interval for controlling the scroll speed when keeping a scroll button (arrows) pressed. Every time value between 0.01 and 1 s is possible.

(\leftarrow | \rightarrow , \uparrow , \downarrow) **Window Scroll Arrows:** The arrow buttons on the window frame can be used to scroll/jump through the data tree.

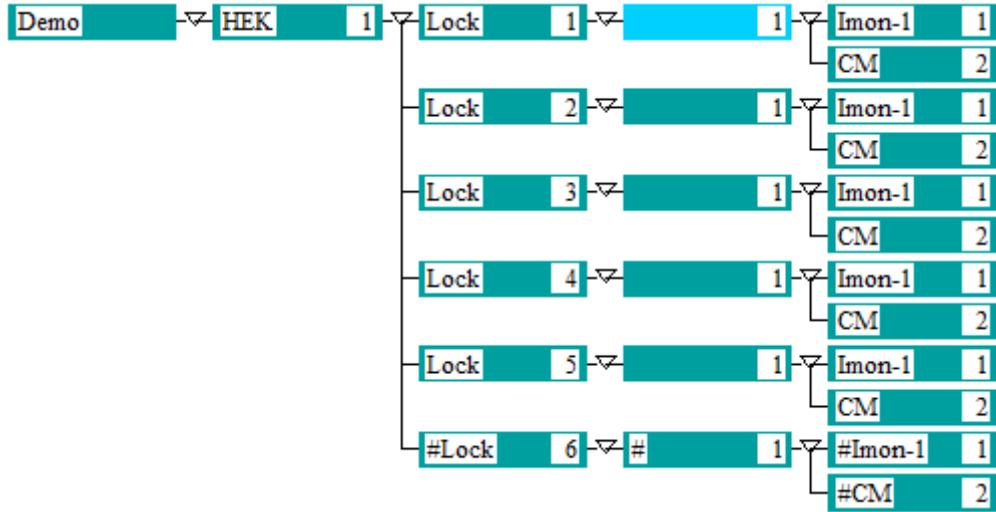
9.1.1 Tree Handling

Interpreting the Tree objects: Besides a running index, text is displayed in the icons of the tree entries. The displayed text is the *Label* of the object. Usually the *Root* icon contains the file name, the *Group* icon contains the experiment number, and the *Series* icon shows the name of the *Stimulation Sequence*. The *Sweep* icon holds the *Sweep* index and the *Trace* icon the *Trace* name.

Example: In the screenshot of the *Replay* window below you can see the following entries in the first line:

- File name: Demo
- Group name: HEK; The group "HEK" is the first group of the file Demo.

- Series name: Lock 1; The *Series Lock* is the first *Series* of the *Group*.
- Sweep name: 1; the *Sweep* carries no name or label. It is the first *Sweep* of the *Series*.
- Trace names: *Imon-1, CM 2*



Labels starting with a "#" (Series "6") denote a currently acquired object that will not be stored after acquisition.

Maneuvering through the Tree: The cursor keys (LEFT, RIGHT, UP and DOWN) of the keyboard allow you to scroll through the data structure of the tree. PAGE UP and PAGE DOWN can be used to scroll one window up or down. HOME and END will move to the start or end of the tree.

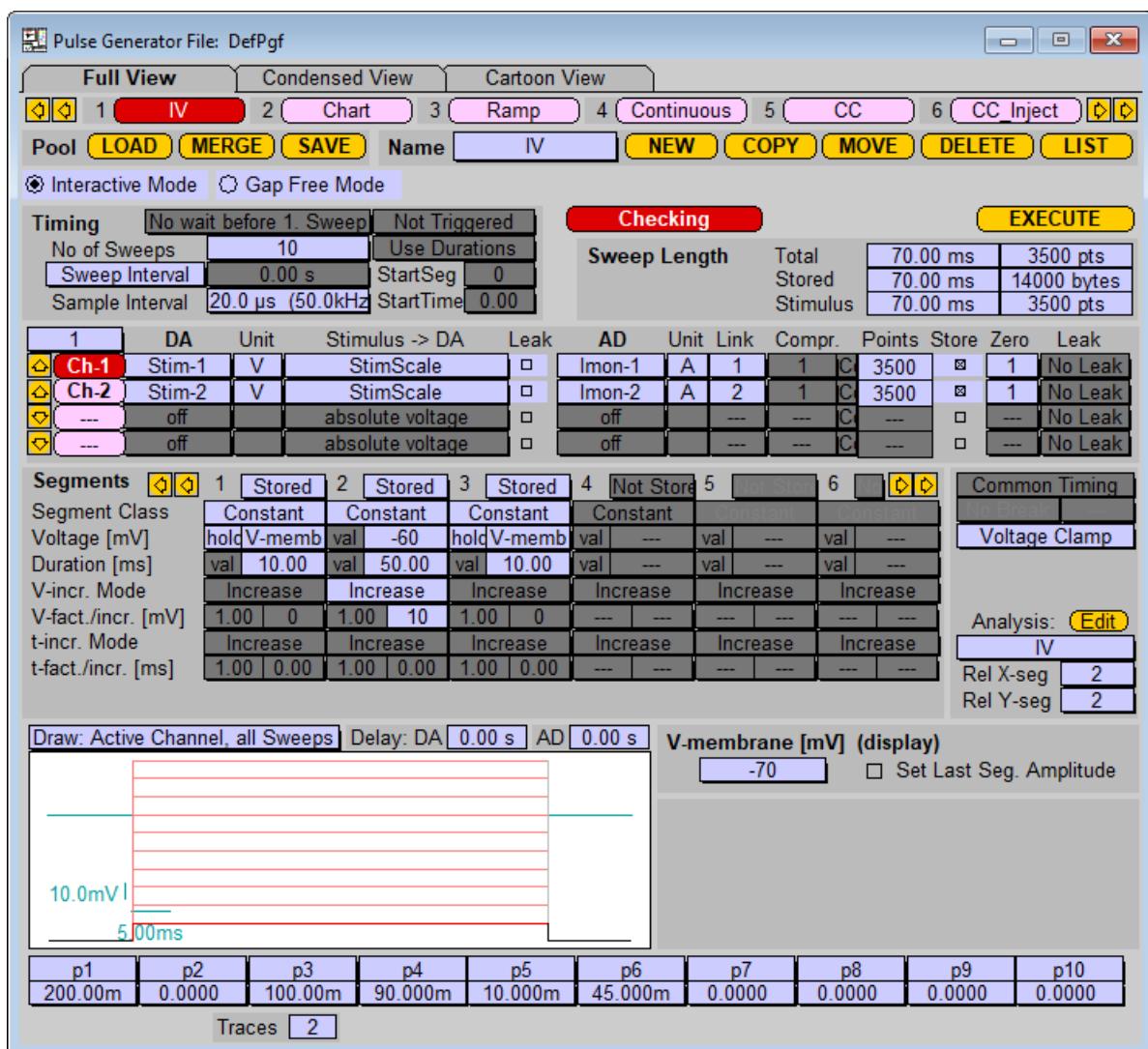
Selecting/Marking multiple objects: An example: For analysis purposes you **Mark All** **Lock** would like to export all data obtained with Lock protocols for a given cell. Thus, enter Lock in the name field, select the Group for the cell under consideration, and click on *Mark All*. Then all Series named Lock of this group can be exported with *Replay* → *Export Marked Target Children*.

Mark a Target and its Children:

SHIFT+CLICK = Marks Target and Children between two selections.

STRG+CLICK = Unmark a specific Target Children

10 Pulse Generator Window



The Pulse Generator 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.

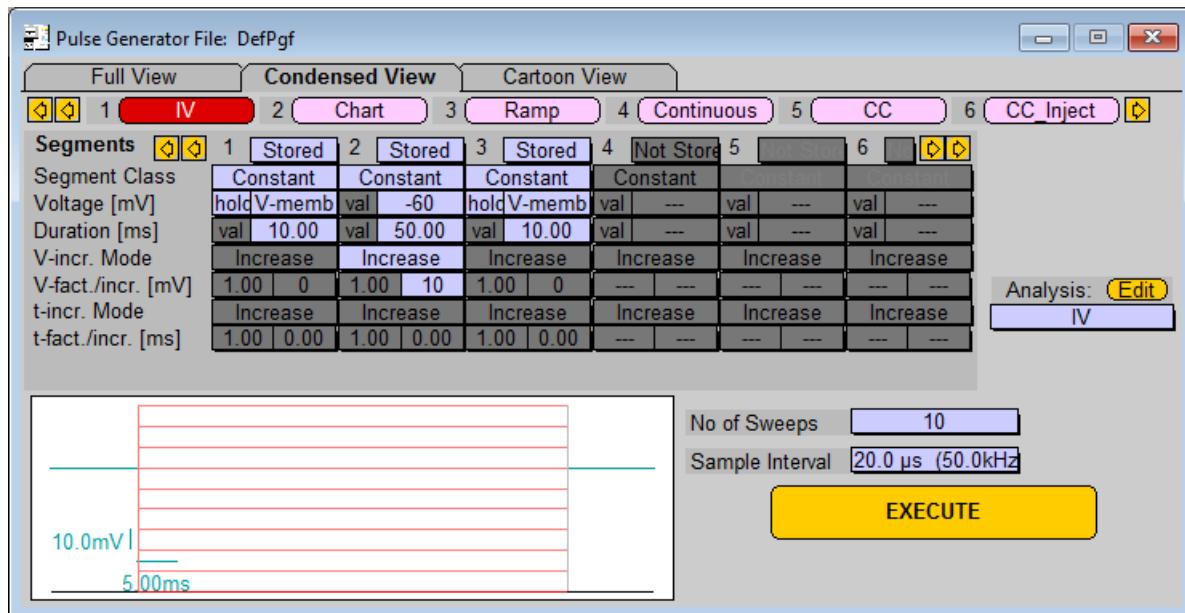
10.1 Different Views of the Pulse Generator

There are three different views available for the Pulse Generator window.

Full View: Shows the complete Pulse Generator window (see figure above).

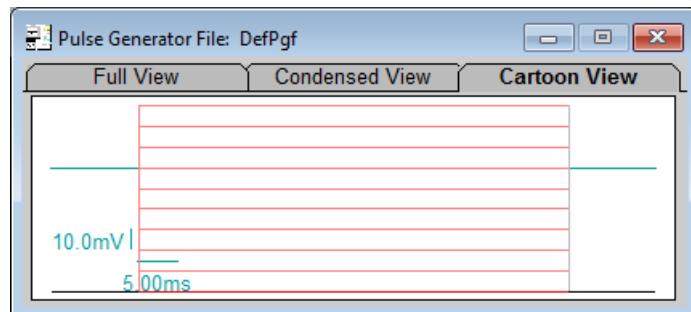
Note: You can use the "Copy to Clipboard" function to copy the text listing of the PGF setting somewhere else.

Condensed View: The condensed view provides access to the most important functions of a simple experiment. In case segment parameters have to be changed frequently, then the Pulse Generator window may stay opened during the entire experiment with minimal space requirement.



Cartoon View: Shows only the graphical representation of the selected Pulse Generator sequence.

Note: If you use the "Copy to Clipboard" function when the Cartoon View pane is active, the stimulus cartoon is copied instead of the text listing of the PGF settings.



10.2 Sequence Selector



Sequence Buttons: The first row displays a section of the pool of available sequences. It is a paging bar in units of six sequences. Two arrows at each side allow scrolling through the available pulse protocols (the innermost arrows move in increments of one page, i.e., six sequences; the outermost arrows move to the start/end of the sequence list). A sequence is selected by clicking on it.

The pool of sequences is read from a file, usually the default file `DefPgf_v9.pgf`.

Note: If no Pulse Generator file is available, PATCHMASTER creates the default file. This file only contains one stimulation sequence, called "Test". This sequence can also be edited.

More sequences are added to the pool by copying an already existing sequence (COPY button, see below) or by creating a new sequence (NEW button, see below).

10.3 Sequence Pool Handling



LOAD: Loads the pool of available stimulation sequences (*.pgf file). The present file name is indicated in the title bar of the Pulse Generator window, e.g., "Pulse Generator File: DefPgf_v9".

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

SAVE: Saves the pool of available stimulation sequences. After modification of the existing pool of sequences, the entire Pulse Generator File (PGF) should be saved to disk (Save button). It can be saved under any name. PATCHMASTER automatically appends the extension *.pgf to the file name. If this new PGF file should be loaded into the Pulse Generator as a default, the new name of the PGF file has to be specified in the Configuration window and the configuration file has to be saved.

Name: Editable name of the present sequence.

NEW: Creates a new stimulation sequences in the loaded PGF pool.

COPY: Duplicates the actual sequence into the first free position. A new name has to be entered.

MOVE: Moves the sequence to a new position. A number for the new position has to be entered. Use this option to move the most commonly used sequences to one of the handy first six positions, or to rearrange your pool.

DELETE: Removes the present sequence from the pool.

LIST: Writes the settings of the actual Series into the Notebook window. Use this option, if you want to create a listing of your sequence to be able to recreate it on another machine. A PGF listing could look as follows:

```
PGF-stimulus: pulse, Sequence: 4 - polarogr
TIMING:
NumberSweeps: 1, Averages: 0, SweepInterval: 0.000 s,
SampleInterval: 10.00 us
Wait before 1. sweep: FALSE
```

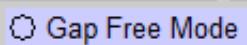
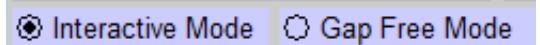
```

CHANNEL: 1, channel 1 time.
AD-Channel [A]: AD-6, Comp.Factor: 1, Comp.Mode: 00000000,
CompSkip: 0
Rel.DacChannel: 1, Rel.Segments - X: 2, Y: 2, Write: TRUE,
Holding: 0.000 V
LEAK SUBTRACTION:
Rel.DacChannel: 1, Rel.Segments - X: 2, Y: 2, Write: TRUE,
Holding: 0.000 V no leak pulses
DA-Channel [V]: DA-3, use StimScalere relative to Vmemb
Amplifier mode: VoltageClamp, Set last Seg. Ampl.: FALSE
SEGMENTS: Voltage Duration VFact VIincr TFact Tincr

```

10.4 Acquisition Modes

Interactive Mode: In the *Interactive Mode* there is a minimum waiting period between the recording of single *Sweeps*, the so-called "Sweep Gap". The size of the gap depends on the speed of your computer system and the complexity and duration of the stimulus to be calculated and output. If you need to do any modification (e.g. *C-slow*, recording modes...) during the acquisition which effects the *Sweep* recording you have to select the *Interactive Mode*. During the "Sweep Gap" the set *Holding Potential* will be applied.



Gap Free Mode: The *Gap Free Mode* 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 the *Gap Free Mode*.

10.5 Timing

Timing:

- Wait before 1. Sweep: Forces PATCHMASTER to wait the time indicated by *Sweep Interval* before executing the sequence after activating it.
- No Wait before 1. Sweep: If you want the sequence to start immediately without a delay.
- 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.

Timing	No wait before 1. Sweep	Not Triggered
No of Sweeps	10	Use Durations
Sweep Interval	0.00 s	StartSeg 0
Sample Interval	20.0 μ s (50.0kHz)	StartTime 0.00

No. of Sweeps: Determines the number of *Sweeps* within one sequence, e.g., how many times the specified pulse pattern is run.

Sweep Interval/Sweep Delay: One can select either a *Sweep Interval* time or a *Sweep Delay* time. The *Sweep Interval* defines a waiting time from the start of one *Sweep* to the start of the subsequent *Sweep*. The *Sweep Delay* defines a waiting time from the end of the last *Sweep* to the beginning of the subsequent *Sweep*.

Note: Only one selection, the active one, is taken into consideration for the recording.

Sample Interval: The timing of data acquisition is given as *Sample Interval* (in seconds) and as sampling frequency (in Hz). The shortest *Sample Interval* (in case only one channel is used) is 5 μ s (200 kHz); the longest interval is 1 s (1 Hz). Only the *Sample Interval* has to be entered, the sampling frequency is just displayed as a reference.

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

Trigger Mode: Determines if and how 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. Otherwise you have to activate the sequence and then one or more external triggers have to be applied.

- Not Triggered: No external triggering.
- Trigger Series: One trigger at the start of a sequence.
- Trigger Sweeps, Main + Leaks: A trigger is required for the main pulse and for each single leak pulse.
- Trigger Sweep, Main only: One trigger is required to start the complete Sweep, main as well as leak pulses.

Use Durations / Use Scan Rates:

Segments	◀▶	Store 1	Store 2	Store 3
Segment Class		Constant	Ramp	Constant
Voltage [mV]	hold	V-memb	val 50	hold
Duration [ms]	val	50.00	val 800.00	val 50.00
V-incr. Mode	Increase		Increase	Increase
V-fact./incr. [mV]	1.00 0	1.00 0	1.00 0	
t-incr. Mode	Increase		Increase	Increase
Scan-fact./incr.	1.00 0.00	1.00 0.000	1.00 0.00	
Scan Rate [V/s]	---	62.50m	---	

Ramp segments can be specified either by their duration or by their *Scan Rate*. If the *Scan Rate* is given by the user, the duration will be calculated, and vice versa. The desired mode can be selected from the list:

- Use Durations: The time between the ending and beginning of a *Ramp* is set in seconds in the *Duration* field of the sequence.
- Use Scan Rates: The *Scan Rate* is set in 'V/s' in the *Scan Rate* field of the sequence and is calculated as *Voltage/Duration*.

Start Seg: Sets the start segment.

Start Time: Sets the start time within the start segment.



Start Seg and Start Time determine the start from where acquired data are stored. The full stimulation template is always output.

10.6 Check and Execute

Not Checking / Checking: This option determines whether a check is performed for any inconsistencies that might occur when entering values.

NOT Checking : When *Not Checking* is enabled, 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.

Checking : When *Checking* is enabled, the active sequence is checked after any modification of segments and when storing, switching, or leaving the PGF 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.

EXECUTE: Allows to output the presently active stimulation sequence. Upon termination of data acquisition, the program returns to the Pulse Generator window. In this way, pulse patterns can be adjusted interactively without changing windows until they yield the desired responses.

10.7 Sweep and Channel Length

Sweep Length: Is the duration of the *Sweep*. It is given by the longest *Trace* (stored or not stored) in a *Sweep*.

Sweep Length	Total	30.00 ms	1500 pts
	Stored	25.00 ms	5000 bytes
Channel Length	Stimulus	30.00 ms	1500 pts

Shows the length of the executed *Sweep* and stored data as determined by the timing, compression and segments settings.

- Total: Denotes the total duration of the longest *Trace* of the *Sweep* in 'ms' and 'points'.

Note: If the total Sweep length exceeds Max. Sample Points (see chapter Memory Allocation, 5.3.2 on page 45) 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 window.

- Stored: Denotes the total duration stored of one *Sweep* of the given sequence in 'ms'. The *Sweep* size is given in 'bytes'.

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

Channel Length: Length of the actual DA stimulation. This can be shorter than the *Sweep* length, e.g., a short trigger impulse.

- Stimulus: Denotes the duration of the selected stimulus signal in 'ms' and 'points'.

10.8 Wave Parameters

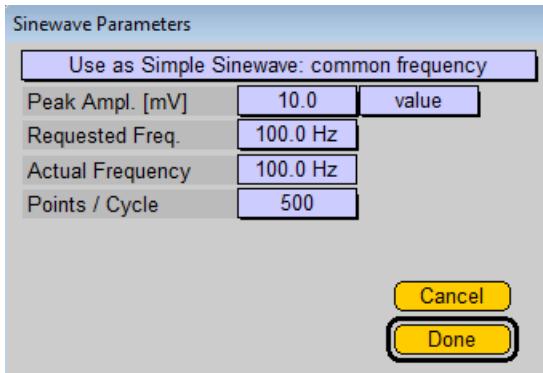
Depending on the *Segment* type (see chapter Segment Classes, 10.10.1 on page 129) and the *Stim* → *DA* setting (see chapter 10.9.1 on page 123) different wave parameter buttons will appear.

10.8.1 Sine Wave Parameters

Sine Wave For segments of the *Sine* type. This button will appear between the buttons *Checking* and *Execute* and allows the specification of the wave characteristics.

10.8.1.1 Use as Simple Sinewave: Common Frequency

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



Peak Ampl. [mV]: Defines the size of the *Peak Amplitude*. The amplitude is half of the peak-to-peak amplitude.

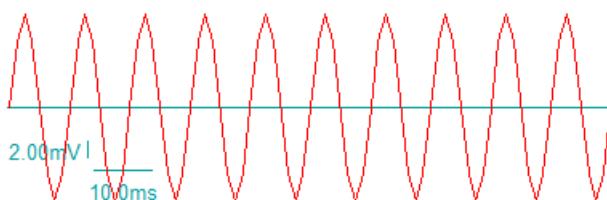
Value or parameter: If *Value* is selected the amplitude of the sine wave can be entered in the field *Peak Ampl.*. Alternatively, the *Peak Ampl.* can be determined by a *PGF parameter*. This allows to automatically change the *Peak Ampl.* of the sine wave between different series acquisitions.

Requested Freq.: Desired frequency of the sine wave in Hertz (Hz). Once the *Wave Parameters* window is closed, the *Requested Freq.* is set to the *Actual Frequency*.

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 Freq.*. The *Actual Frequency* is a function of the *Requested Freq.*.

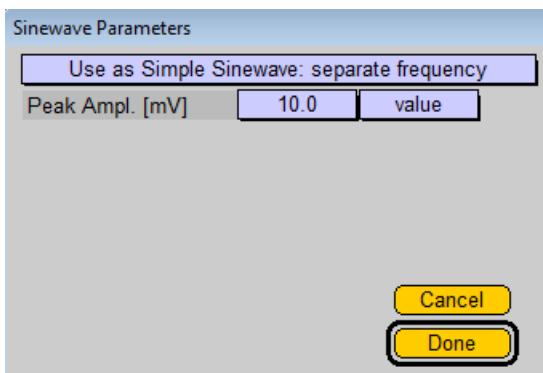
Points / Cycle: Number of sample points per full wave length. This number is calculated and can not be edited. It is a display value only and gives the number of points per sinus wave which is the cycle length divided by the sample interval.

Below a sine wave created with a *Peak Ampl.* of 10 mV and a *Actual Frequency* of 100 Hz is shown.



10.8.1.2 Use as Simple Sinewave: Separate Frequency

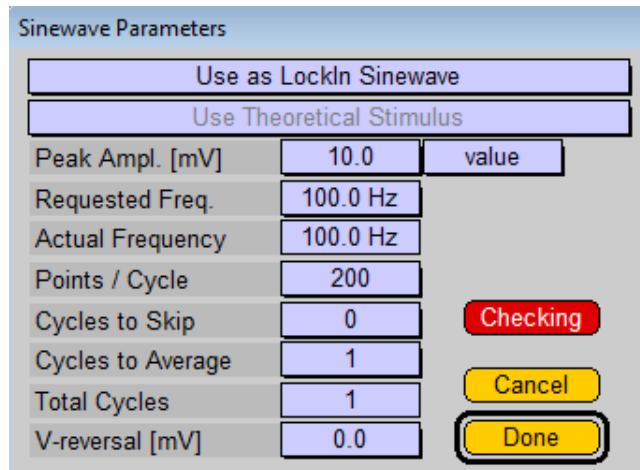
Use this setting if the frequency of the wave is determined by the segment duration. Each segment will contain one wave cycle only. This option allows to create a *Sweep* containing wave cycles of different frequencies.



All available settings have been explained before (see chapter 10.8.1.1 on the preceding page).

10.8.1.3 Use as LockIn Sinewave

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



Note: For the LockIn Sinewave to be an active option, the LockIn Extension must first be turned on in the Configuration window (see chapter 5.4.4 on page 50).

Use Theoretical Stimulus: Specify whether to use the theoretical stimulus or a measured stimulus Trace for computing real and imaginary traces.

Note: This option is only available if the LockIn Mode is set to "Basic Spectroscopy" and the Calibration Mode to "Manual". Further, it is needed when using the Protocol Method "Impedance Spectroscopy", also known as "EIS".

Peak Ampl. [mV]: See chapter 10.8.1.1 on page 112.

Requested Freq.: See chapter 10.8.1.1 on page 112.

Actual Frequency: See chapter 10.8.1.1 on page 112.

Points / Cycle: See chapter 10.8.1.1 on page 112.

Cycles to Skip: When a Sinewave 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 Sinewave 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 Cycle to Skip is recommended.

Cycles to Average: 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: Number of all cycles.

V-reversal [mV]: 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, V-reversal is the zero current potential extrapolated from the slope conductance about V_{membr} , 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 V-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.

Checking: Allows to deactivate the internal checking procedures during the editing process.

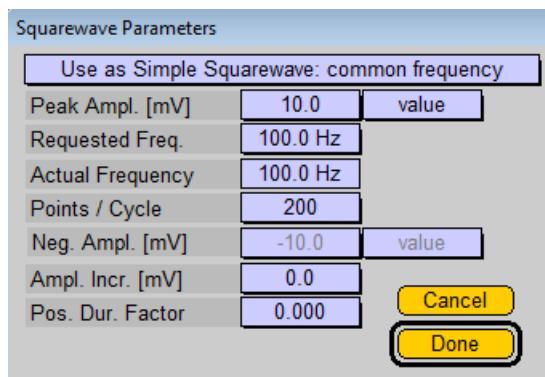
For more information concerning the LockIn extension, we refer to the chapter Software LockIn Extension, 23 on page 257, and the PATCHMASTER Tutorial Capacitance Measurements using the LockIn Extension.

10.8.2 Square Wave Parameters

Square Wave For segments of the *Square* type. This button will appear above the *Execute* button and allows the specification of the wave characteristics.

10.8.2.1 Use as Simple Squarewave: Common Frequency

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



Peak Ampl. [mV]: Defines the size of the *Peak Amplitude*. The amplitude is half of the peak-to-peak amplitude.

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

Requested Freq.: Desired frequency of the square wave in Hertz (Hz). Once the *Wave Parameters* window is closed, the *Requested Freq.* is set to the *Actual Frequency*.

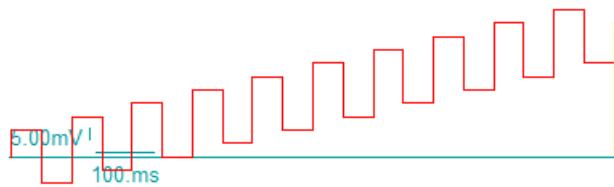
Actual Frequency: Only certain frequencies are possible because they are generated by dividing a fundamental clock frequency by an integer. Nevertheless, the *Actual Frequency* is usually within a couple of percent of the *Requested Freq.*. The *Actual Frequency* is a function of the *Requested Freq.*.

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.

Neg. Ampl. [mV]: If a *Pos. Dur. Factor* unequal zero is used, the negative amplitude of the square wave can be entered.

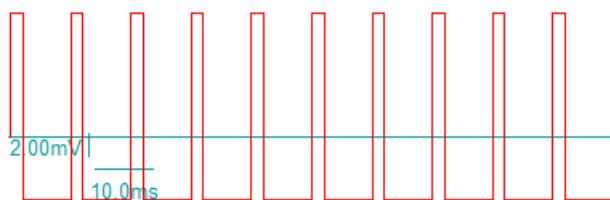
Ampl. Incr. [mV]: Defines an increment amplitude which causes a baseline shift of the square waves ("Staircase" effect).

Below a square wave created with a *Peak Amplitude* of 10 mV and an *Ampl. Incr.* of 5 mV) is shown.



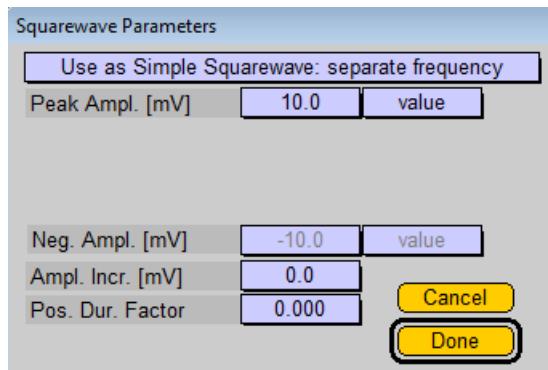
Pos. Dur. Factor: Factors between 0 and 1 are allowed. A factor of '0.5' means that both half cycles have the same duration. A factor smaller than '0.5' shortens the positive half cycle of the square wave.

Below a square wave created with a *Pos. Dur. Factor* of '0.2' and a *Peak Ampl.* of 10 mV and a *Neg. Ampl.* of -5 mV is shown.



10.8.2.2 Use as Simple Squarewave: Separate Frequency

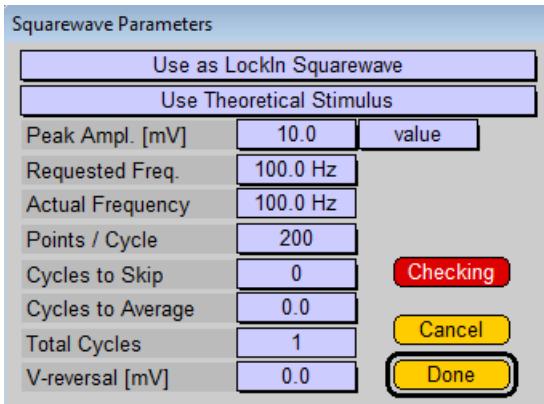
Use this setting if the frequency of the wave is determined by the segment duration. Each segment will contain one wave cycle only. This option allows to create a *Sweep* containing wave cycles of different frequencies.



All available settings have been explained before (see chapter 10.8.2.1 on the preceding page).

10.8.2.3 Use as LockIn Squarewave

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



Note: For the LockIn Squarewave to be an active option, the LockIn Extension must first be turned on in the Configuration window (see chapter 5.4.4 on page 50).

Use Theoretical Stimulus: Specify whether to use the theoretical stimulus or a measured stimulus Trace for computing real and imaginary traces.

Note: This option is only available if the LockIn Mode is set to "Basic Spectroscopy" and the Calibration Mode to "Manual". Further, it is needed when using the Protocol Method "Impedance Spectroscopy", also known as "EIS".

Peak Ampl. [mV]: See chapter 10.8.2.1 on page 115.

Requested Freq.: See chapter 10.8.2.1 on page 115.

Actual Frequency: See chapter 10.8.2.1 on page 115.

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.

Cycles to Skip: When a Squarewave 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 Squarewave 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' Cycle to Skip is recommended.

Cycles to Average: 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: Number of all cycles.

V-reversal [mV]: Applicable in the Square + 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, V-reversal is the zero current potential extrapolated from the slope conductance about V_{membr} , 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 V-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.

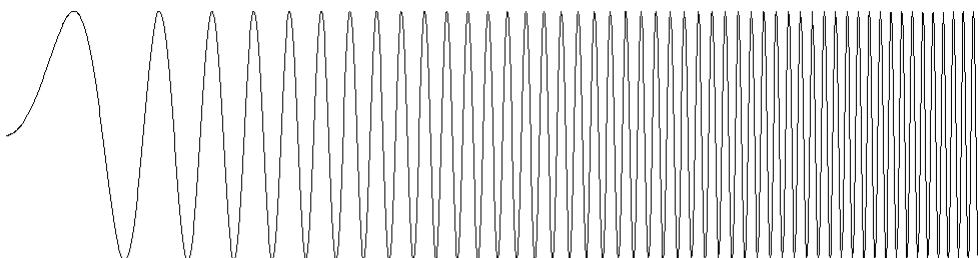
Checking: Allows to deactivate the internal checking procedures during the editing process.

For more information concerning the LockIn extension, we refer to the chapter Software LockIn Extension, 23 on page 257, and the PATCHMASTER Tutorial Capacitance Measurements using the LockIn Extension.

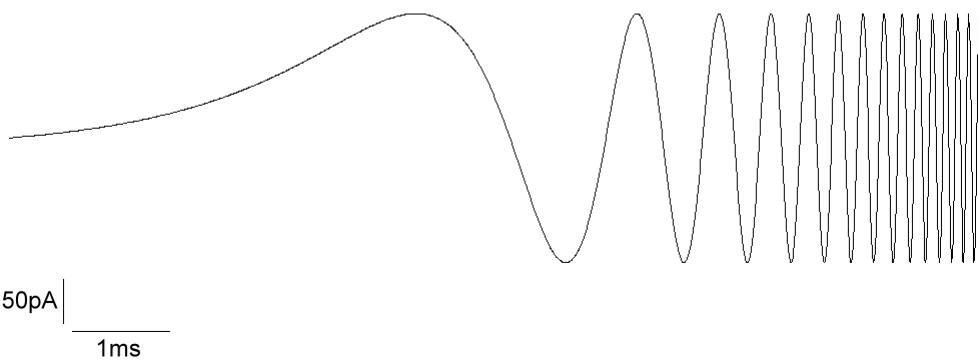
10.8.3 Chirp Wave Parameters

Chirp Wave For segments of the *Chirp* type. This button will appear between the buttons *Checking* and *Execute*, allowing specification of the wave parameters. 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:

Linear Chirp



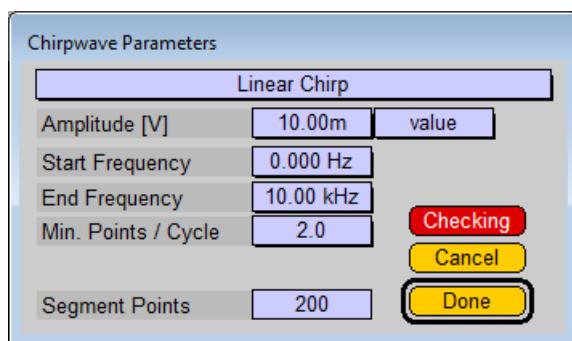
Exponential Chirp



50pA
1ms

10.8.3.1 Linear Chirp

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



Amplitude [mV]: Defines the size of the *Peak Amplitude*. The amplitude is half of the peak-to-peak amplitude.

Value or parameter: If *Value* is selected the amplitude of the chirp wave can be entered in the field *Peak Ampl.*. Alternatively, the *Peak Ampl.* can be determined by a *PGF parameter*. This allows to automatically change the

Peak Ampl. of the chirp wave between different Series acquisitions.

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

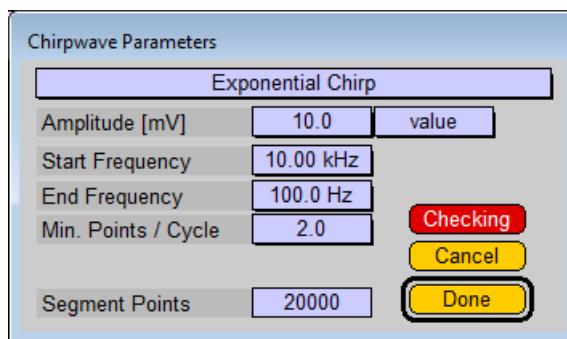
End Frequency: Defines the frequency where the chirp wave ends.

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

Segment Points: Total amount of points for the *Chirp* segment. It is a display value only.

10.8.3.2 Exponential Chirp

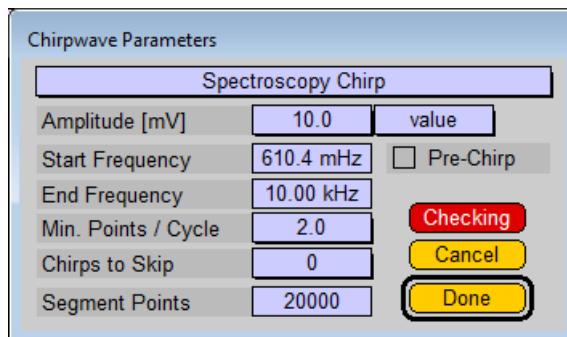
Use this setting if the chirp frequency should change in an exponential manner.



All available settings have been explained before (see chapter 10.8.3.1 on the facing page).

10.8.3.3 Spectroscopy Chirp

Use this setting if the *Chirp* wave should be analyzed with the Spectroscopy Extension.



Note: For the Spectroscopy Chirp to be an active option, the Spectroscopy Extension must first be turned on in the Configuration window of Patchmaster (see chapter 5.4.5 on page 51).

Amplitude [mV]: See chapter 10.8.3.1 on the facing page.

Value or parameter: See chapter 10.8.3.1 on the preceding page.

Start Frequency: See chapter 10.8.3.1 on the facing page.

End Frequency: See chapter 10.8.3.1 on the preceding page.

Min. Points / Cycle: See chapter 10.8.3.1 on the facing page.

Chirps to Skip: Defines the number of skipped chirp segments.

Segment Points: See chapter 10.8.3.1 on page 118.

Note: End Freq. = Min. Points / Cycle * Sampling Freq.

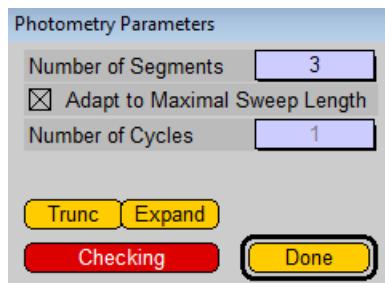
Pre-Chirp: Attaches a small chirp fragment to the beginning of the chirp wave to avoid

Checking: Allows to deactivate the internal checking procedures during the editing process.

For details on the parametrization of *Chirp* wave form we refer to the chapter Spectroscopy Extension, 24 on page 275 and the PATCHMASTER tutorial Using the Spectroscopy Extension.

10.8.4 Photometry Wave Parameters

Photometry Wave If the option *Use for Wavelength* is active for a DA output channel, then the *Photometry Wave* button will appear above the *Checking* button. It assists you in creating repetitive stimulation pattern for controlling the excitation wavelengths.



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.

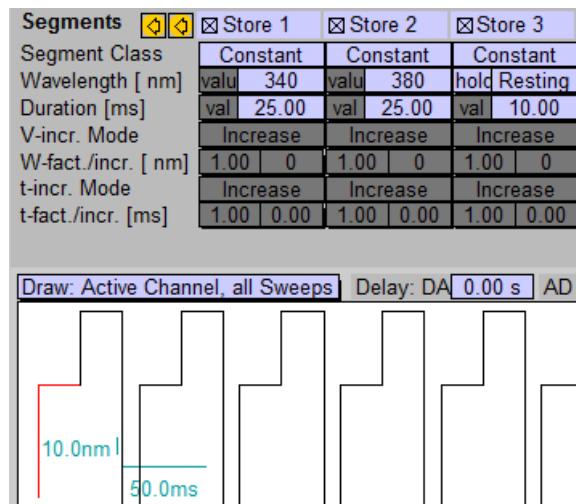
Adapt to Maximal Sweep Length: If checked, then the number of cycles is calculated by the software in respect to the duration of the longest output channel.

Number of 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.

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.

Trunc: 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.

Checking: Allows to turn off the internal consistency checks. Turning this transiently off might be necessary when multiple changes of parameter would lead to intermediate invalid configurations.

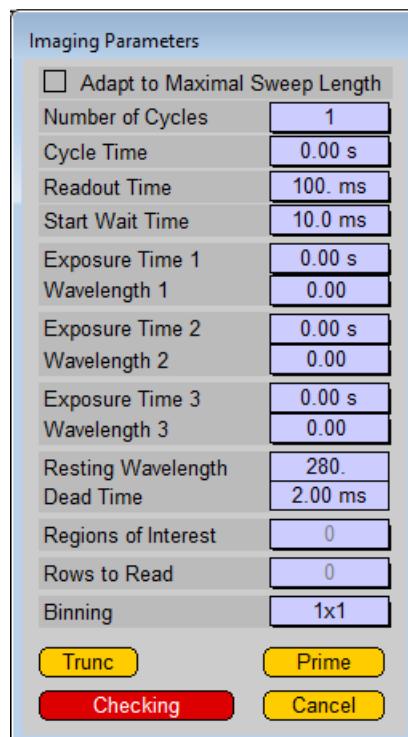


In this example a single cycle consists of 3 segments. You have just to edit the first three segments. A click on *Expand* adds 15 additional segments to the PGF sequences. The resulting PGF sequence then consists of $6 * 3 = 18$ segments. *Trunc.* removes all segments except the initial 3 segments.

10.8.5 Imaging Wave Parameters

In case you want to perform image acquisition during the execution of a *Pulse Generator* sequence, please we recommend reading first the chapter *Imaging Extension*, 22 on page 255.

The *Imaging Wave* parameters are usually set in the *PGF Primer* of SmartLUX and automatically integrated into the Pulse Generator sequence by the *Prime* function of SmartLUX. Hence, usually **it is not necessary to modify the parameters in this dialog**.



For a detailed description of the imaging parameters we refer to the SMARTLUX manual.

10.9 Channel Settings for DA Output and AD Input

1	DA	Unit	Stimulus -> DA	Leak	AD	Unit	Link	Compr.	Points	Store	Zero	Leak	
Ch-1	Stim-DA	V	StimScale	<input checked="" type="checkbox"/>	Imon2	A	1	1	C	1250	<input checked="" type="checkbox"/>	1	Store Avg
Ch-2	off	V	absolute voltage	<input type="checkbox"/>	Vmon	V	1	1	C	1250	<input checked="" type="checkbox"/>	0	No Leak
---	off		absolute voltage	<input type="checkbox"/>	off		---	---	C	---	<input type="checkbox"/>	---	No Leak
---	off		absolute voltage	<input type="checkbox"/>	off		---	---	C	---	<input type="checkbox"/>	---	No Leak

Ch-1...16: Settings for each channel. The default is "1", the other channels (Channels = 2...) may be used to simultaneously record the potential or an amperometric signal, for example.

The DA section allows you to set the properties of the DA output, e.g., the stimulus signal.

The AD 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 *Link* in the AD settings! This allows you to associate several AD inputs to the same DA stimulation.

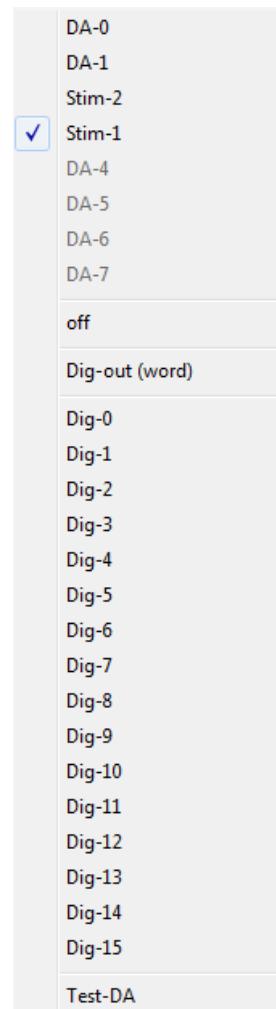
10.9.1 DA output channel settings

DA: The DA channel for stimulation has to be specified. The figure below shows the options for the EPC 10 Single amplifier.

- DA-0...2: Analog output.
- Stim-DA: Standard output channel.
- off: No output of stimulus, disables this channel.
- Dig-out (word): Output as digital word.
- Dig-0...15: Digital output channel.
- Test-DA: Test-DA can be used to simultaneously stimulate multiple amplifiers (compare chapter [Amplifier Selection](#), 7.1.3 on page 77).

Note: Test-DA is only available with multiple amplifiers such as EPC 10 Double, Triple or Quadro

Note: With the EPC 10/2, -10/3 and -10/4 the pop-up list for DA selection contains the amplifier index: Stim-1 is the explicit stimulus output of the first amplifier, Stim-2 is the output of the second one and so on.



Unit: Units for output channels. Defaults are "V" for voltage, and "A" for current.

Stimulus → DA: Conversion between the stimulus and the actual analog output.

- apply StimScale: Apply standard conversion.
- relative to V-memb.: Calculates the signal relative to V-memb.
- load from file template: Load a stimulus template from file. A detailed description how to use a recorded waveform as a stimulus template can be found in the chapter Using a Recorded Waveform as Stimulus in the PATCHMASTER Tutorial.
- use for LockIn: Has to be activated if the stimulus should be used for the software LockIn. For details please refer to the chapter Software LockIn Extension, 23 on page 257.
- use for Wavelength: Has to be activated if the stimulus should be used to control a wavelength. Amplitudes of segments can then be entered in "nm". This option is available if the Photometry or Imaging Extension is activated.
- use DA-Scaling: Has to be activated if the stimulus should be scaled depending on the settings made in the I/O Control tab of the Configuration. For further information we refer to the chapter AD/DA Input/Output Scaling, 5.7.1 on page 60.
- use for Spectroscopy: Has to be activated if a chirp wave form should be used for impedance or admittance analysis with the Spectroscopy Extension. For details please refer to the chapter Spectroscopy Extension, 24 on page 275.
- use for Camera Trigger: Has to be activated if the stimulus should be used to control the exposure of the camera. This option is available if the Imaging Extension is activated. For further information we refer to the chapter Imaging Extension, 22 on page 255.

<input checked="" type="checkbox"/>	Stim to DA
	apply StimScale
	relative to V-memb
	load from file template
	use for LockIn
	use for Wavelength
	use DA-Scaling
	use for Spectroscopy
	use for Imaging

If nothing is selected, "absolute voltage" will be applied.

Use of digital Outputs: Digital outputs are mainly used for controlling (triggering) other devices, such as perfusion systems, filter wheels, flash lamps, or others. The EPC 10 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 "Dig-0", "Dig-1", and "Dig-2" in the Pulse Generator dialog of PATCHMASTER.

There are two ways to control the digital lines:

If only one digital line is intended to be used, then one of the "Dig-Out" lines ("Out-0" to "Out-15") can be selected as output channel. In the *Segments* section of the Pulse Generator 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).

The *Dig-Out 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: $1 * 10^2 + 1 * 10^1 + 1 * 10^0 = 4 + 2 + 1 = 7$.

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

Leak: If selected, leak templates are output (see chapter P / n `Leak Correction`, 10.14 on page 135). This option allows a rapid deselecting of leaks without changing the leak settings. By activating this option the `Leak Traces` storing option is set by default to "Store Average".

Note: *The leak template options are hidden in the `Pulse Generator` as long as this flag is not activated.*

10.9.2 AD input channel settings

AD: The AD-channel for input.

- AD-0...2: AD channel.
- Vmon: Voltage monitor of the amplifier.
- Imon2: Current monitor of the amplifier.
- Imon1: Current monitor 1 (filter 1) signal of the *active* amplifier.
- off: No input.
- Virtual: Virtual Trace is used to generate a Trace where the data come from a source different from an ADC, e.g., external data loaded via the option *Import Trace*, or data which generate virtual Traces, such as the LockIn.
- Dig-in (word): Digital input.
- Dig-0...15: Digital input channels.
- Chirp Traces: Storage of particular Spectroscopy values, such as e.g. *Admittance*, *Phase*

AD-0
AD-1
AD-2
AD-3
AD-4
Vmon
<input checked="" type="checkbox"/> Imon2
Imon1
AD-8
AD-9
AD-10
AD-11
AD-12
AD-13
AD-14
AD-15
off
Virtual
Dig-in (word)
Dig-0
Dig-1
Dig-2
Dig-3
Dig-4
Dig-5
Dig-6
Dig-7
Dig-8
Dig-9
Dig-10
Dig-11
Dig-12
Dig-13
Dig-14
Dig-15
Chirp_Avg
Chirp_Phase
Chirp_Admit(Y)
Chirp_Real(Y)
Chirp_Img(Y)
Chirp_Real(Z)
Chirp_Img(Z)
Chirp_Imp Z

- LockIn Traces: Storage of particular *LockIn* values, such as e.g. C_m , G_m , G_s ...
- Imaging/Photometry Traces: Storage of particular *Imaging* or *Photometry* values (e.g. mean fluorescence, calcium concentration, fluorescence ratio.)

LockIn_Avg
LockIn_Phase
LockIn_Real(Y)
LockIn_Img(Y)
LockIn_Admit(Y)
LockIn_Real(Z)
LockIn_Img(Z)
LockIn_Imp[Z]
LockIn_CM
LockIn_GM
LockIn_GS
LockIn_DC
LockIn_CV
LockIn_GP
Photo_W1
Photo_W2
Photo_W3
Photo_R
Photo_Ca

Unit: Unit for input channels. Default is "A" for current (in voltage clamp mode). Use "V" if a plain AD channel is sampled or any other applicable unit if virtual *Traces* are used or the channel is scaled.

Link: 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*.

Compr.: Enter the number of points for the compression (compression factor) and choose the compression method from the list. The compression factor 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.

Note: Normal Traces are acquired and can be compressed. Virtual Traces are generated.

- Compression Mode:
 - single sample: Takes one point per compression interval.
 - mean: Averages all points of a compression interval.
- 2-byte integer / 4-byte real: Data type for storage.
- Set Skip: With the *Set Skip* function, 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.
- Set Offset: With this function, the start of the compression interval can be influenced. 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.

<input checked="" type="checkbox"/>	Compression Mode: single sample mean
<input checked="" type="checkbox"/>	2-byte integer 4-byte real
	Set Skip (Skip = 0)
	Set Offset (Offset = 0)
	Digital Filter
	Set Defaults
	Build Instructions: empty

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.

- 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.
- Set Defaults: Sets all settings back to default.

Build Instructions: Allows computing a virtual *Trace* from other *Traces* and inputs. The instruction string starts with the math 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
 - Bn: *Trace* buffer with index n
 - Cn: Cathodic integral of *Trace* with index n
 - Dn: Differential of the *Trace* with index n
 - In: Integral of the *Trace* with index n
 - 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)"
 - Tn: *Trace* with index n
 - Vn: Value with index n
 - Zn: Zero data of *Trace* with index n
 - Operators: +, -, *, /
 - Brackets: ()
- Other Assignments:
 - CC-Switch: Switches the recording mode to Current Clamp mode at the beginning of the specified segment. CC-Gain needs to be in 0.1 pA/mV or 1 pA/mV range. Example: ";CC-Switch=2" = Switch to Current Clamp Mode at the beginning of segment 2.
 - Name="String" (separated from the math operation by a semicolon ';'). "String" is just a placeholder for any name.
 - No LockIn: Suppressing LockIn computation
 - Tcount: *Trace* count with a value between 1 and 16
 - 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=[number]: Copying the *Trace* data from the "slave" PATCHMASTER instance via the batch communication protocol. The equal sign must immediately follow "slave", e.g. "Slave=2". The given number is the *Trace* count of the *Trace* to get. In the given example data from 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, e.g.: "StimSwp(1,3-5,7-9)".
- VC-Switch: Switches the recording mode to Voltage Clamp mode at the beginning of the specified segment. Current Gain needs to be in medium gain range. Example: ";VC-Switch=2" = Switch to Voltage Clamp Mode at the beginning of segment 2.
- Yrange=real (separated by a colon ','). 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.
- 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.

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

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 μ C). The *Trace* # is set to "5".



No other operator priority is considered besides the brackets.

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 *Replay* window.

It is also possible to specify an equation as a build instruction similar to the equation handling in the *Analysis* (see chapter 13.3.2.8 on page 200), the *Buffer* menu (see chapter 4.6 on page 33) or the *Calculator* dialog (see chapter 4.3 on page 22). 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)".

Points: Number of resulting points for this channel.

Store: If checked, 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.

Zero: Number of the segment on which base 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 *StartSeg* and a *StartTime* >0. Then the Zero Offset will be calculated between *StartTime* and end of the segment.

Note: When the segment number is set to "0", no Zero Offset subtraction will be calculated.

Note: 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 Replay Tree and then back to the just acquired Trace. A simple "Wipe" or "Redraw" of the displayed data is not sufficient.

Leak: Defines how the leak responses are stored.

- No Leak: No leak correction applied.
- Store Avg.: Stores the leak responses as average.
- Store Each: Stores every leak response separately.
- Store None: Leak correction is applied but the leak response is not stored.

Note: Leak correction will only be performed and stored when leak correction is activated (see chapter DA output channel settings, 10.9.1 on page 122)

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".

10.10 Segments

10.10.1 Segment Classes

Segments	1	2	3	4	5	6	7
Segment Class	Stored	Stored	Stored	Not Stored	Not Stored	Not Stored	Not Stored
Voltage [mV]	Constant	Constant	Constant	Constant	Constant	Constant	Constant
Duration [ms]	hold	V-memb	val	-60	hold	V-memb	val
val	10.00	val	10.00	val	10.00	val	---
V-incr. Mode	Increase	Increase	Increase	Increase	Increase	Increase	Increase
V-fact./incr. [mV]	1.00	0	1.00	10	1.00	0	---
t-incr. Mode	Increase	Increase	Increase	Increase	Increase	Increase	Increase
Scan-fact./incr.	1.00	0.00	1.00	0.00	1.00	0.00	---
Scan Rate [V/s]	---	---	---	---	---	---	---

A pulse pattern consists of an arbitrary number of segments. Segments are shown as a horizontally scrolling matrix; clicking on the arrows does scrolling.

Store: On the top of the segment column there is a *Store* field with a selection list determining whether or not the data of that segment is to be stored to disk.

The following options are available:

- Not Stored: The data for that segment are sampled but removed from the *Trace* before storage. A non-stored segment must *not* be a *StartSegment* or a *Relevant Y-segment*. For a description on the usage of non-stored segments please read Non-Stored segments in the Pulse Generator in the PATCHMASTER Tutorial.
- Stored: The data are sampled and stored.
- First Sweep: These segments are output only with the first *Sweep* of the *Series* but are not stored.
- Last Sweep: These segments are output only with the last *Sweep* of the *Series* but are not stored.

Note: There are some important consequences when using P/n leak correction (see chapter *P/n Leak Correction*, 10.14 on page 135). 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.

Segment Class:

Segments can be the following:

Segment Class	Constant	Ramp	Sine	Square	Chirp	Continuous
---------------	----------	------	------	--------	-------	------------

- Constant: Segment of constant amplitude.
- Ramp: Segment with ramp from the amplitude of the previous segment to the amplitude of this segment.
- Continuous: Identifier for continuous data acquisition.

Note: Only the last segment can be of that class.

- Sine: Segment with sine characteristics. Amplitude and cycle duration are defined in *Sine Wave* parameters (see chapter 10.8.1 on page 112).
- Square: Wave segment with rectangular characteristics. Amplitude and cycle duration are defined in *Square Wave* parameters (see chapter 10.8.2 on page 115).
- Chirp: Wave segment with increasing sine wave frequency. Wave characteristics are defined in *Chirp Wave* parameters (see chapter 10.8.3 on page 118).

<input checked="" type="checkbox"/>	Constant
<input type="checkbox"/>	Ramp
<input type="checkbox"/>	Continuous
<input type="checkbox"/>	Sine
<input type="checkbox"/>	Square
<input type="checkbox"/>	Chirp
<hr/>	
<input type="checkbox"/>	Insert
<input type="checkbox"/>	Duplicate
<input type="checkbox"/>	Delete
<input type="checkbox"/>	Duplicate...
<input type="checkbox"/>	Delete...

The list entries *Insert*, *Duplicate*, and *Delete* are used to create or remove segments.

- Insert: Inserts a constant segment (default duration = 0) at the actual location (moves the selected segment to the right).
- Duplicate: Creates a copy of the selected segment and inserts it at the actual location.
- Delete: Deletes the selected segment at once.
- Duplicate...: Creates multiple copies of a number of segments. You have to enter how many segments you want to copy. Segments are counted starting with the selected segment. How often specifies the number of copies from these segments. They will be inserted at the actual location.
- Delete...: Deletes a specified number of segments at once.

Voltage: The *Voltage* of a segment is either a numeric value (*val*, in mV), a holding value at time of sequence execution (*hold*), or a value from the *PGF parameters* list (*p1-p10*). The *Voltage* field may turn into "Current (pA)" in *Current Clamp* mode, into "Wavelength (nm)" when the segments are used for photometry stimulation or into "Amplitude" when the *Use for DA scaling* 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 milliseconds (ms). The value may be adjusted by dragging the mouse or typing the number. For segment duration, *PGF parameters* can also be used. In this case, all data are taken in seconds. Make sure that the duration of segments are even multiples of the *Sample Interval*. A warning is given if they are not, in case *Checking* is activated.

val / hold / p1...10: Allows setting the value.

- val: Standard value.
- hold: Keeps the potential at $V_{membrane}$ during voltage clamp measurements. If the channel is used for photometry, the resting wavelength as specified in the Photometry window is set.
- p1...10: Sets the value to the value given in the corresponding PGF parameter. For a description of usage of the PGF parameters please read PGF Parameters, chapter 10.15 on page 138.

10.10.2 Scan Rates

The Scan Rate options are only available if *Use Scan Rates* is activated (see chapter 10.5 on page 111).

- Scan-fact./incr.: If a value for the increment is entered the field *t-incr. Mode* gets active.
- Scan Rates [V/s]: 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 Voltage/Duration.

10.10.3 Increment Modes

The various increment modes are flexible tools to create sequences with varying durations or amplitudes. Please note that first the amplitudes or durations in logical order are calculated as specified by the parameters *Voltage/Duration*, *V-fact./t-fact* and *V-incr./t-incr.*. Then the logical order is converted to the physical output by applying the selected *Increment Mode*.

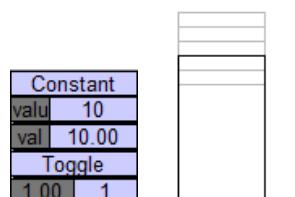
Increment Mode: This determines the order of incrementing the segment voltage and/or duration. The options are as follows (the numbers give an example for a Series with 6 Sweeps):

<input checked="" type="checkbox"/>	Increase
<input type="checkbox"/>	Decrease
<input type="checkbox"/>	Interleave +
<input type="checkbox"/>	Interleave -
<input type="checkbox"/>	Alternate
<input type="checkbox"/>	Toggle
<input type="checkbox"/>	<i>t * Factor</i>
<input checked="" type="checkbox"/>	<i>V * Factor</i>
<input type="checkbox"/>	<i>dV * Factor</i>

- Increase: first Sweep comes first (e.g. 1, 2, 3, 4, 5, 6)
- Decrease: last Sweep comes first (e.g. 6, 5, 4, 3, 2, 1)
- Interleave +: ascending interleaved (e.g. 1, 3, 2, 5, 4, 6)
- Interleave -: descending interleaved (e.g. 6, 4, 5, 2, 3, 1)

Example: If you apply a Series of 6 Sweeps at an increment of 10 mV starting at -40 mV the logical sequence will be: -40, -30, -20, -10, 0 and +10 mV. With the mode *Interleave -* activated the pulses will be output in the following (physical) order: +10, -10, 0, -30, -20 and -40 mV.

- Alternate: first, last, second, penultimate... (e.g. 1, 6, 2, 5, 3, 4)
- Toggle: Starts with the first Sweep, second the voltage increment is added and third the voltage segment is subtracted. Then twice the voltage segment is added.... E.g. using 6 Sweeps with a *V-incr.* of 1 mV and the first Sweep steps to 10 mV. The output voltages are as follows: 10, 11, 9, 12, 8, 13 mV.



10.10.3.1 Logarithmic increment modes

V * Factor: In mode *V * Factor* the logarithmic increment is based on the voltage of the first Sweep. Therefore the voltage cannot be zero. The segment's voltage of the *i*th Sweep is then calculated as:

$$V_i = \text{Voltage} * V\text{Factor}^{i-1} + (i - 1) * dV\text{incr}$$

In mode *V * Factor* the increment may be zero. Let *Voltage* be 10 mV, *ΔVIncr.* = 0 mV, and *VFactor* = 2 then the Series 10, 20, 40, 80 mV, ... is obtained.

dV * Factor: In mode *dV * Factor* the logarithmic increment is based on the linear increment step. Therefore the voltage increment cannot be zero. The segment's voltage of the *i*th Sweep is then calculated as:

for *V-factor* = 1:

$$V_i = \text{Voltage} + (i - 1) * dV\text{-incr}$$

for *V-factor* ≠ 1:

$$V_i = \text{Voltage} V_{i,i>1} = \text{Voltage} + dV\text{-incr} * V\text{-Factor}^{i-2}$$

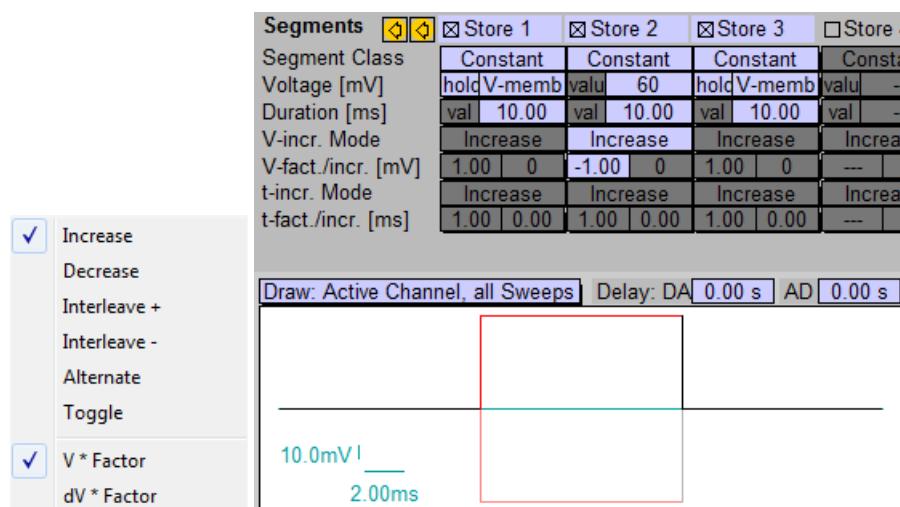
In mode *dV * Factor* the first segment may be zero and is then logarithmically incremented. Let *Voltage* = 0 mV, *dV-Incr* = 1 mV, and *V-Factor* = 2 then the Series 0, 1, 2, 4 mV, ... is obtained.

In some kind of experiments it might be useful to toggle between two fixed potentials. To achieve this, you have to:

- Define your starting "Voltage".
- Select "Increase" and "V*Factor".
- Select "V-fact." to "-1".

There are three cases for setting the voltage increment:

1. "0": E.g. +100 mV, -100 mV, +100 mV, -100 mV,...
2. "positive" (e.g. +20 mV): E.g. +100 mV, +80 mV, +100 mV, +80 mV,...
3. "negative" (e.g. -20 mV): E.g. +100 mV, +120 mV, +100 mV, +120 mV,...



In analogy to the logarithmic amplitude increments the duration increments are calculated as follows:

t * Factor: In mode *t * Factor* the logarithmic increment is based on the duration of the first Sweep. The segment's duration of the *i*th Sweep is then calculated as:

$$t_i = \text{Duration} * t\text{Factor}^{i-1} + (i - 1) * dt\text{Incr}$$

In mode *t * Factor* the increment may be zero. Let *Duration* be 10 ms, *dt-Incr.* = 0 ms, and *t-Factor* = 2 then the Series 10, 20, 40, 80 ms, ... is obtained.

dt * Factor: In mode *dt * Factor* the logarithmic increment is based on the linear increment step. Therefore the duration increment cannot be zero. The segment's duration of the *i*th Sweep is then calculated as:

for t-Factor = 1:

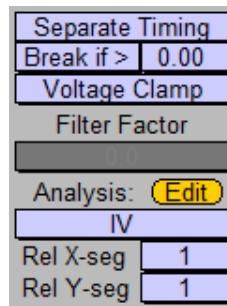
$$t_i = \text{Duration} + (i - 1) * dt\text{-incr}$$

for t-Factor \neq 1: $t_1 = \text{Duration}$

$$t_{i,i>1} = \text{Duration} + dt\text{-incr} * t\text{-Factor}^{i-2}$$

In mode *dt * Factor* the duration of the first segment may be zero and is then logarithmically incremented. Let *Duration* = 0 ms, *dt-Incr* = 1 ms, and *t-Factor* = 2 then the Series 0, 1, 2, 4 ms, ... is obtained.

10.11 Miscellaneous



Common Timing / Separate Timing: If *Common Timing* is selected, the segments of all channels are timed like the first one. If *Separate Timing* is selected, all channels are timed separately, i.e. segment boundaries may be different in different channels.

Break Condition: For each acquired *Trace* of the AD input that is longer than 1 second a *Break* condition can be specified. The first condition that is *TRUE* terminates the acquisition of the *Sweep*.

- No Break: No break condition for that *Trace*.
- Break if >: Terminates the acquisition if the acquired values become larger than the value entered in the field to the right.
- Break if <: Terminates the acquisition if the acquired values become smaller than the value entered in the field to the right.
- Break if abs >: Terminates the acquisition when an absolute sample value of that trace exceeds the given threshold.
- Break on "next": Terminates the acquisition of the *Sweep* if the user presses the *Next* button in the Control window. The acquisition then continues with the next *Sweep*.

Note: The delay between the break condition = *TRUE* and leaving the acquisition routine can be up to 50 ms.

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

- Any Mode: Allows the stimulus to be executed in both modes, e.g. for photometry.
- Voltage Clamp: Restricts the protocol to *Voltage Clamp* mode. Stimulus amplitudes are entered in (mV). *Holding* corresponds to *V-membr*. If the stimulus is output at a DA channel, the stimulus amplitude is scaled with the *VC: Stim Scale* factor specified in the Configuration.

- **Current Clamp:** Restricts the protocol to Current Clamp mode. Stimulus amplitudes are entered in (pA). *Holding* corresponds to *I-membr*. The range of the stimulus amplitude depends on the *CC-Gain* setting (see chapter 7.1.21 on page 88). If the stimulus is output at a DA channel, the stimulus amplitude is scaled with the *CC: Stim Scale* factor specified in the Configuration.

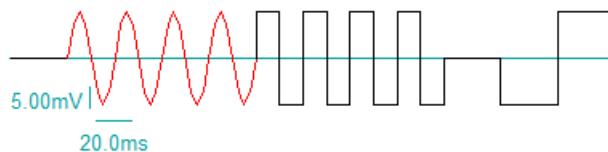
Filter Factor: The *Filter Factor* is implemented for the EPC 9 and EPC 10 amplifiers. It is used to define the automatic filter setting relative to the sample rate (activated by *Auto Filter* in the Configuration window).

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. The suggested filter frequency is shown in parentheses.

Analysis: Enter an *Analysis Method* that should be executed automatically after the data acquisition. Note that *Automatic Stimulus Control* has to be activated in the Analysis window for this feature to become effective.

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

10.12 Stimulus Template Preview



After each editing operation, the stimulus template is refreshed to reflect the changes made. Sweep other than the first one are shown as dashed lines. If part of the pulse pattern exceeds the DA limits, the forbidden voltage region is indicated in the picture by shading. The corresponding warning box "Segment with too large voltage encountered" only appears once. Segments drawn in red color refer to the relevant Y-segment. The type of graph scaling is chosen in Display → Labeling → PGF-Editor Grid.

Preview Mode: Select from the list what shall be displayed in the preview:

- Draw: Active Channel, all Sweeps: Displays the settings of the active channel for all Sweeps. Recommended if you use increment modes in your settings.
- Draw: Active Channel, 1. Sweep: Displays the settings of the active channel for the first Sweep.
- Draw: Active Channel, Last Sweep: Displays the settings of the active channel for the last Sweep.
- Draw: All Channels, all Sweeps: Displays the settings of all channels for all Sweeps. Recommended if you use Increment modes in your settings.
- Draw: All Channels, 1. Sweep: Displays the settings of all channels for the first Sweep.
- Draw: All Channels, Last Sweep: Displays the settings of all channels for the last Sweep.
- Draw no cartoon: Disables the display of the stimulus template.

Delay: Displays the hardware delay time for the DA output and the AD input with respect to the first DA output channel.

Delay: DA 0.00 s AD 0.00 s

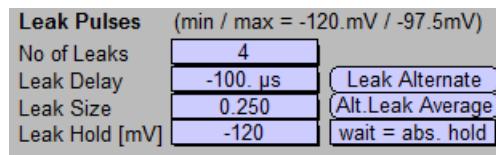
10.13 V-membrane



V-membrane (mV): This control displays the presently selected membrane potential. It is **only** used for the sequence cartoon as reference and can be changed without actually affecting the membrane potential in the Amplifier window (*V-membrane*). When the PGF template of a stored file is reviewed, V-membrane is the holding voltage of that particular experiment.

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

10.14 P/n Leak Correction



PGF Leak Pulse settings: *No of Leaks*: 4, *Leak Delay*: 100 ms, *Leak Size*: 0.250, *Leak Hold*: -120 mV. The *V-Holding* is set to -70 mV.

Leak min/max: 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:

$$L(i) = (V(i) - Vh) * size + Lh \quad (10.1)$$

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

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

$$L(2) = (-50mV + 70mV) * 0.25 - 120mV = \underline{-115mV} \quad (10.2)$$

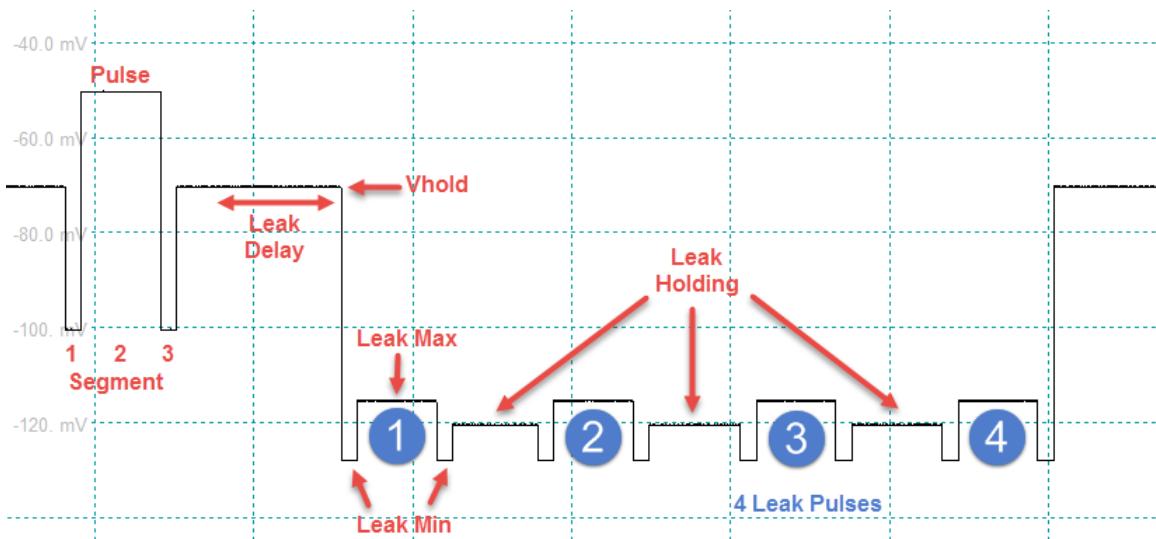
No of Leaks: Arbitrary numbers of so-called "P/n pulses" are supported.

Leak Delay: This is 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 .

Leak Size: Determines the amplitude of the leak pulses relative to the main pulse.

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

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



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 software: the leak pulses are summed and scaled with a factor of $1 / (\text{No of Leaks} * \text{Leak Size})$.

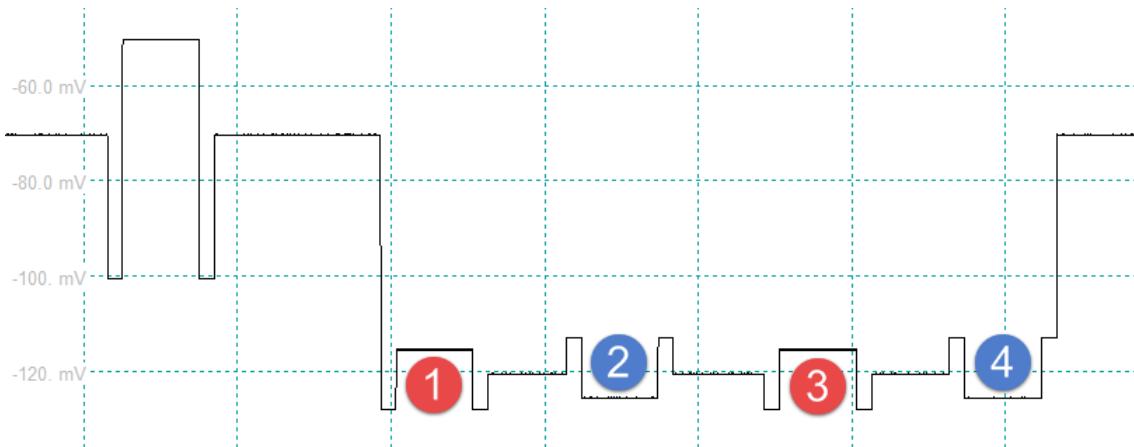
Note: You can improve the signal-to-noise ratio if you increase *Leak Size* and *No of Leaks*, but be sure that the leak pulses do not reach the activation level of the channels you want to study.

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 when you want to scale the leak Traces.

Software/Hardware Leak: In case an iTEV 90 amplifier is used the user can select between Software P/n and Hardware P/n function.

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

The following figure illustrates a pulse template.

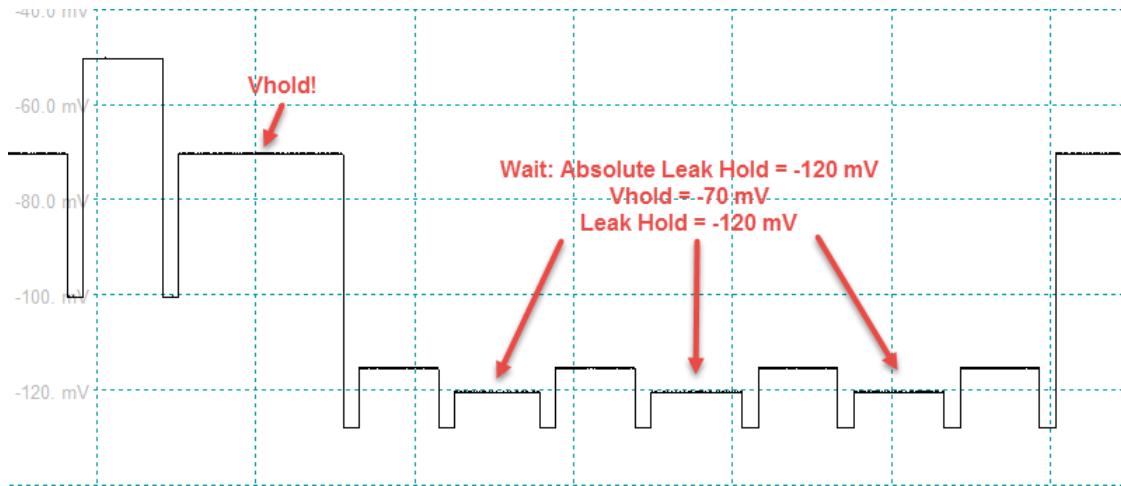


Alt. Leak Average: This procedure was intended to be used with the *Average* function provided in the Control Window (see chapter 8.2 on page 100). The *Average* function acquires a given number of averages acquired for one Sweep. Only the average is stored to disk. *Alt. Leak Average* provides the option 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*.

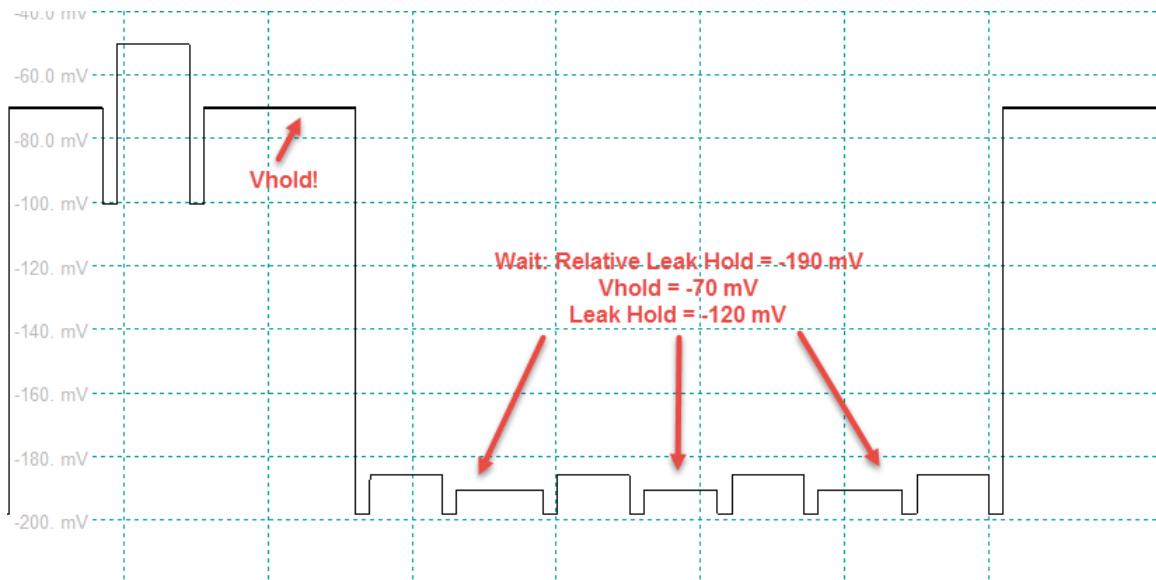
Note: An even number of Sweeps to average is required to give correct results. The entire leak template of every other Sweep is then inverted.

Wait:

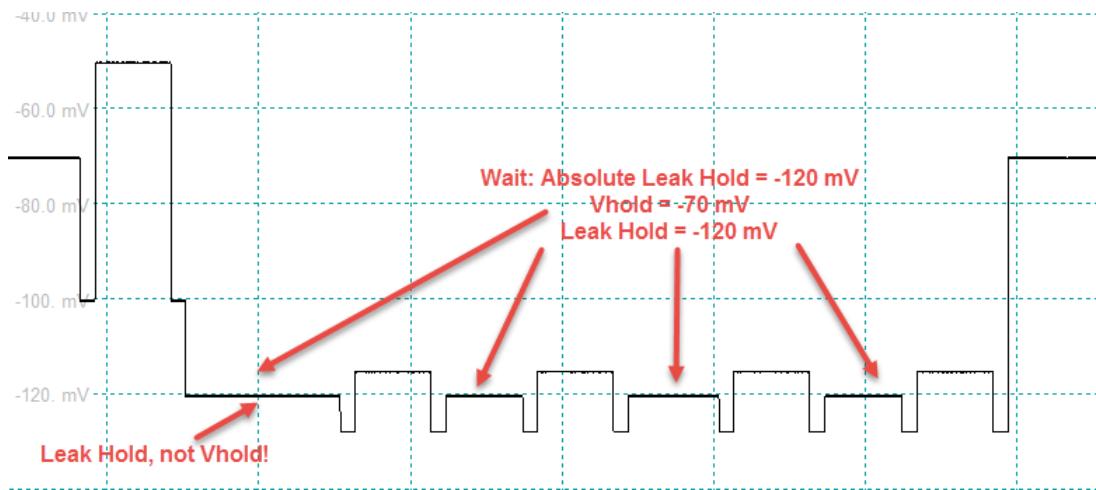
- wait = abs. hold: Keeps the cell potential at the chosen absolute holding potential during wait periods.



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



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



10.15 PGF Parameters

p1	p2	p3	p4	p5	p6	p7	p8	p9	p10
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Use the *PGF parameters* as variables in the segment settings for *Voltage* or *Duration*. This allows you to change multiple settings in one or more sequences with changing only one parameter. PATCHMASTER provides 10 *PGF parameters* which will be stored with the *Pulse Generator* file. Instead of *p1* to *p10* you can assign specific names to make the parameter input more intuitive.

Example: You want to increase the duration of three segments during a loop in a protocol. Enter the start duration in field "p1", set the duration of all segments to "p1" and then increase "p1" during loop execution.

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' (= $0.1 * 1 \text{ nA}$).

For a description of usage of the PGF parameters please refer to the chapter Global Variables in PATCHMASTER in the PATCHMASTER Tutorial.

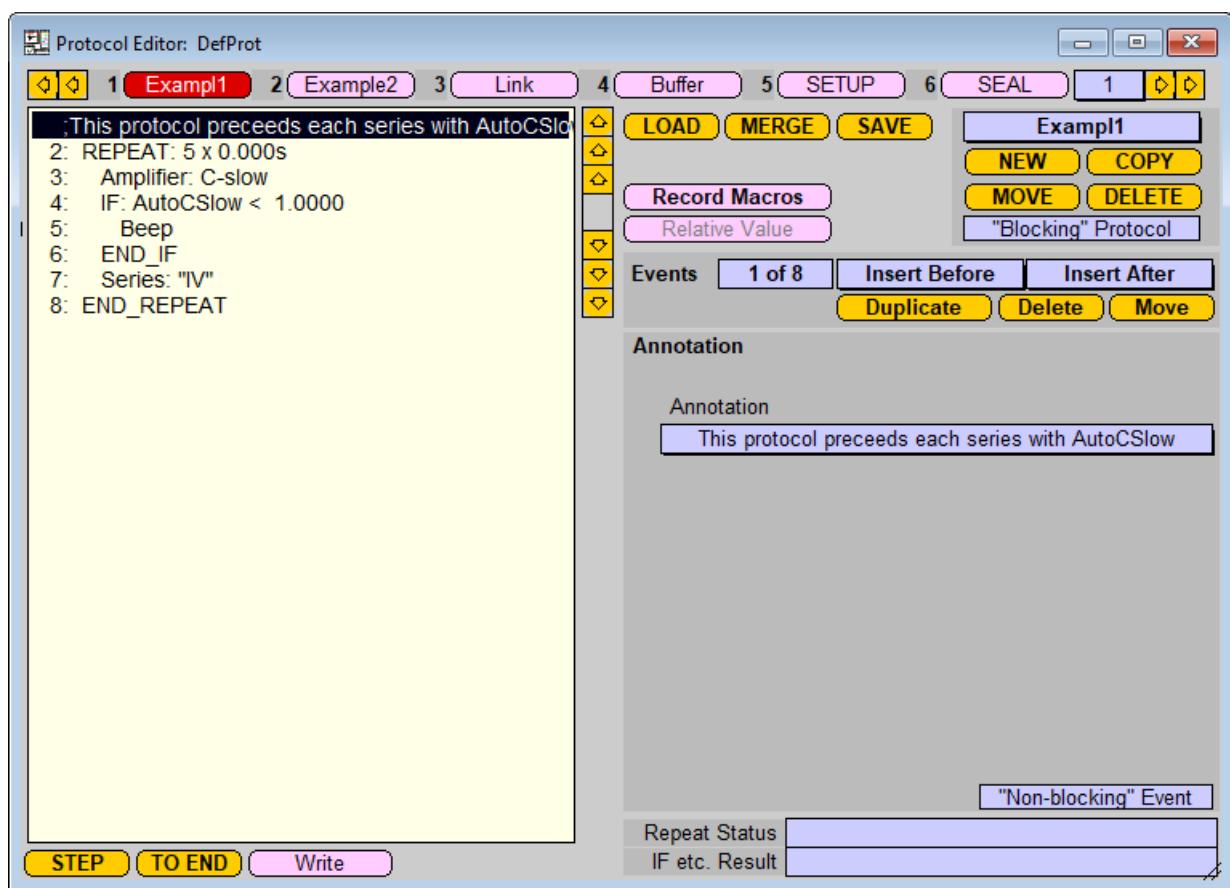
10.16 Error Handling

If PATCHMASTER encounters an unreasonable value in the Pulse Generator window, the user is requested to change the corresponding parameter before proceeding. In case of multiple errors, one cannot exit the dialog until all parameters are set correctly. If it seems impossible to solve the error situation and you consistently get error messages, you can load a valid PGF file from disk.

Values in the Pulse Generator controls are rounded to the exact values as displayed. This prevents unexpected results caused by rounding problems, such as the *Sample Interval* being an odd number.

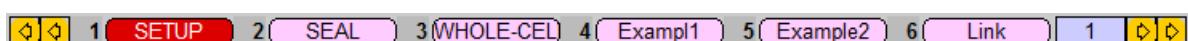
11 Protocol Editor Window

In the Protocol Editor window you can assemble complex experimental arrangements by combining PGF-templates with other operations (e.g. breaks, IF-THEN loops, setting changes). This window is the heart of the PATCHMASTER software concerning the automation of experiments.



With protocols, very complex sequences can be edited. 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.

11.1 Protocol Handling



Protocol Pool: The first row displays a section of the pool of available protocols. It is a paging bar in units of six protocols. Two arrows at each side allow scrolling through the available protocols (the innermost arrows move in increments of one page, i.e., six protocols; the outermost arrows move to the start/end of the sequence list). A protocol is selected by clicking on it or by entering the protocol number in the blue field on the right side next to the arrows.

After modification of the existing pool of protocols, the entire Protocol Editor file (*.pro) should be saved to disk (see below). It can be saved under any name. The default name is DefProt.pro. If a custom *.pro file should be loaded into the Protocol Editor as a default, the new name of the *.pro file has to be specified in the Configuration window as *Protocol File* (see chapter 5.5 on page 55) and the *Configuration File* has to be saved.

LOAD : Loads the pool of available protocols (*.pro file).

MERGE : Loads protocols from another (.pro file) 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 pool of available protocols (*.pro file).

Example : The name of the present protocol is displayed in the blue field. To edit this name, click into the field. End editing either by pressing RETURN or by clicking with the mouse into another area of the window.

NEW : Creates a new protocol in the pool.

COPY : Creates a copy of the active protocol and inserts it at the end of the protocol pool.

MOVE : Moves the present protocol to another position in the protocol pool.

DELETE : Removes the present protocol from the pool.

non-blocking / **blocking** : Status indicator if the whole protocol is handled as a "non-blocking" or a "blocking" protocol. If one of the used events contains the label "blocking" (see chapter 11.3 on the next page) the whole protocol will be handled as a "blocking" protocol. This gets important when using the option *Show Amplifier Protocols* (see chapter 5.6.3 on page 59) and the three predefined main protocols "SETUP", "SEAL" and "WHOLE-CELL". There, it is not allowed to execute protocols out of the Amplifier window containing events with the label "blocking".

Note: It is necessary to block the execution of "blocking" protocols out of the Amplifier window because this may cause infinite protocol loops.

Record Macros : Activates the macro recording. In the following the command lines for all activated events are recorded and inserted into the active protocol. Press again on *Record Macros* to finish the macro recording.

Relative Value : Interprets the subsequent *Vhold* potential as a relative value ("True"). It is a macro command and has to be activated via the *Record Macro* button. It has to be repeated prior to each *Vhold* macro command.

STEP : Steps to the next line in the event list and executes the event.

TO END : Runs the protocol through to its end.

Write : Writes the events to the Notebook window during execution of a protocol. The *Write* button should also be activated if you want to log the execution of single PGFs in the Notebook.

11.2 Event Handling

In the event area, you can select events for the actual event list that should be performed during the experiment.



Events: Here the position of the present event in relation to the total number of events is displayed.

Insert Before / Insert After: Here you can choose a new event from the drop-down list. It will be positioned either above or below the present entry.

Duplicate: Duplicates the present event. The duplicate will automatically be inserted above the duplicated event.

Delete: Deletes the present event from the list without further notice.

Move: Allows you to move the present event in the event list to another position by entering a new position number.

Note: Multi-line events such as Repeat loops or conditional statements can not be moved.

Note: Since protocol files are stored in text format, editing of protocols with standard text editors is also possible.

11.3 Recurring Functions

These functions and information fields exist in every event area.

Skip : Omits this step in the protocol execution. This option is included to allow the user a quick disabling of events without losing all settings.

Delay : Sets a delay time in seconds. The total time of the event is subtracted from the entered delay time value. E.g. an *Acquire Series* event needs in total 8 s for the execution and a *Delay* of 10 s is specified for this event. This would result in a delay (waiting time of the event) of 2 s (Delay time - Event execution time). The delay is executed after the event.

Repeat Status	Repeat at line 2: 2 of 5
IF etc. Result	Auto C-slow = 1.0000 < 1.0000 => FALSE

Repeat Status: Displays the status of loops. The following example shows that the repeat step happens at line 2 of the protocol and that there have been 2 repeats in a 5 cycle loop.

IF etc. Result: Displays the last IF condition that has been triggered.

non-blocking / **blocking** : This field indicates if the selected event is considered as "non-blocking" or "blocking" (see chapter 11.1 on the preceding page).

11.4 Events - Overview

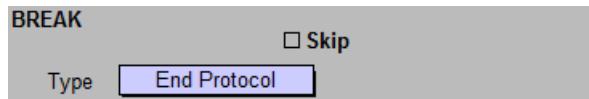
The available events are categorized into eight groups. Not all of the events are explained in detail here. Please follow the given links to other chapters.

	Data/Display
Protocol Sequence	
Break	Analysis
Chain Protocol	Digital Filters
Clear Key	Display Properties
Goto	Export
Goto_Mark	File Operation
IF...Then	Replay
ElseIf...Then	Trace Buffer
Else	Value/Parameter
Launch	PGF Parameters
Macro Command	Set Solutions
Repeat	Set Value
Switch Window	Messages
Wait	Annotation
Acquisition	Beep
Acquire Each Sweep	Write Icon Value
Acquire Properties	Extensions
Acquire Series	LockIn
Set Sweep Label	EIPROScan
Test Pulse	Photometry/Imaging
Hardware	DocuLUX
Amplifier	E-Chem Methods
Serial Output	Cyclic Voltammetry
Set DAC	Impedance Spectroscopy
Set Digital Bit	Open Circuit Potential
Set Digital Word	Square Wave Voltammetry
Set Solution Changer	Optical Spectroscopy

11.4.1 Protocol Sequence

11.4.1.1 BREAK

Use to terminate a repeat loop or the protocol.



Type:

- End Protocol: This kind of *BREAK* stops the protocol execution completely at this point.
- End Repeat: This kind of *BREAK* ends the execution of a repeat loop (e.g. *Acquire Each Sweep...*) and jumps out of it to the following events. If this *BREAK* is used outside of a loop, the execution jumps to the end of the protocol.
- End If: This kind of *BREAK* ends the execution of an IF-THEN statement and jumps out of it to the following events. If this *BREAK* is used outside of a loop, the execution jumps to the end of the protocol.

11.4.1.2 Chain Protocol

Use this event to link one protocol to another protocol.



Protocol Name: The name of the protocol is displayed in this field after one protocol is selected in the *Select Protocol* selection list.

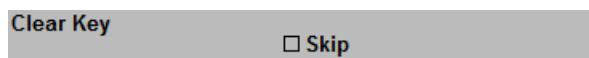
Return: If this option is not checked the protocol sequence will stop after executing the selected protocol. If the *Return* option is enabled the protocol sequence will come back to the main protocol and will be continued.

Edit Protocol: Opens the protocol named in the field *Protocol Name*.

Select Protocol: Select a protocol to define the link. After a selection is made the name of the protocol is displayed in the field *Protocol Name*.

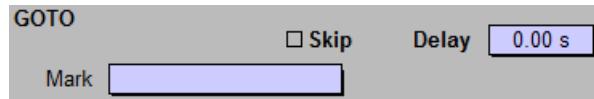
11.4.1.3 Clear Key

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.



11.4.1.4 GOTO

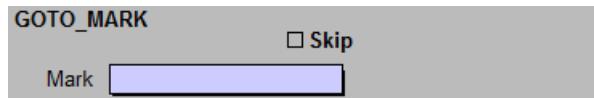
Use to jump to a *GOTO_Mark*.



Mark: Enter the name of the mark that denotes the destination of the GOTO jump (see below).

11.4.1.5 GOTO_MARK

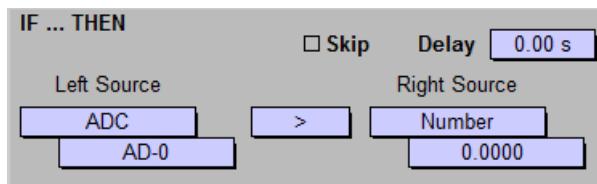
Creates a *GOTO_Mark*.



Mark: Enter the name of the mark for a possible destination of a GOTO jump.

11.4.1.6 IF...THEN

Allows the definition of conditional events. Note that the list of available channel variables depends upon the chosen source.



Left Source:

- ADC: AD channel to be read
- Digital In: Digital Input
- Parameters: 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 *Break* command or condition in the *Pulse Generator* sequence.
- Selection Failed: If the defined condition for a selection fails the loop can be interrupted.
- 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.

<input checked="" type="checkbox"/>	ADC
<input type="checkbox"/>	Digital In
<input type="checkbox"/>	Parameter
<input type="checkbox"/>	Analysis
<input type="checkbox"/>	Value
<input type="checkbox"/>	Key
<input type="checkbox"/>	Icon Value
<input type="checkbox"/>	Repeat Count
<input type="checkbox"/>	Sweep Count
<input type="checkbox"/>	Last Sweep
<input type="checkbox"/>	Sweep Aborted
<input type="checkbox"/>	Selection Failed
<input type="checkbox"/>	Break Alert
<input type="checkbox"/>	Batch Comm.
<input type="checkbox"/>	Serial Comm.
<input type="checkbox"/>	Series Name
<input type="checkbox"/>	Overflow
<input type="checkbox"/>	TRUE
<input type="checkbox"/>	FALSE

Note: The Title strings must not include double quotes ("), since these double quotes are used as separators for splitting the event text.

- Batch Comm.: Checks if the last received batch control string contains a user-defined phrase (see *Controlling PATCHMASTER* in the *PATCHMASTER Tutorial*).
- Serial Comm.: Checks if the last received string of the serial port communication contains a user-defined phrase (see chapter 5.4.10 on page 53).
- 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 / False: Choose if the IF loop shall be used (True) or not (False).

Right Source:

- ADC: AD channel to be read
- Digital In: Dig-0...15. The operators for the Digital Input set the voltage.
- Parameters: 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 / False: Choose if the IF loop shall be used (True) or not (False).

<input checked="" type="checkbox"/>	ADC
<input type="checkbox"/>	Digital In
<input type="checkbox"/>	Parameter
<input type="checkbox"/>	Analysis
<input type="checkbox"/>	Value
<input type="checkbox"/>	Number
<input type="checkbox"/>	TRUE
<input type="checkbox"/>	FALSE

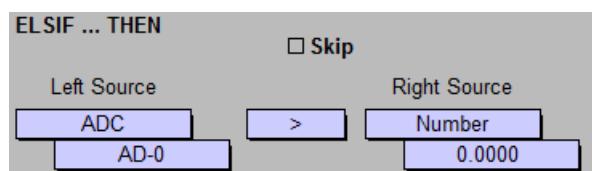
If Operators: Select the appropriate logical operator for the *IF... THEN* operation.

- >: 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*.

<input checked="" type="checkbox"/>	>
<input type="checkbox"/>	<
<input type="checkbox"/>	ABS >
<input type="checkbox"/>	ABS <
<input type="checkbox"/>	=
<input type="checkbox"/>	<>
<input type="checkbox"/>	MOD
<input type="checkbox"/>	invalid

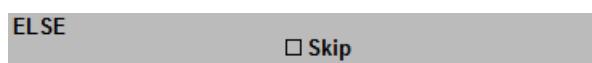
11.4.1.7 ELSEIF... THEN

Allows a secondary conditional event, e.g. a check after an IF-THEN step. Works identically to IF-THEN, see above.



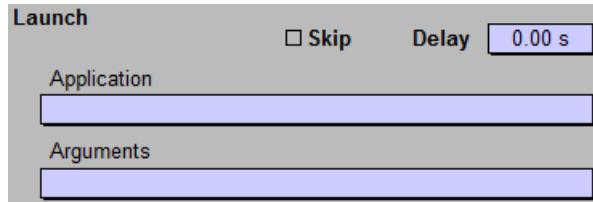
11.4.1.8 ELSE

Final step of the IF condition.



11.4.1.9 Launch

The *Launch* event allows to start another application. The path to the application is defined relative to PATCHMASTER's home path.



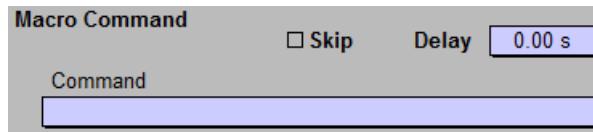
- Application: Enter path where the application is located.
- Arguments: Enter command line arguments if necessary.

11.4.1.10 Macro Command

Use to execute an individual *Macro Command*. Many controls that can not be accessed via special events can be controlled using *Macro Command*.

A listing of all *Macro Command* can be plotted to the Notebook using the function *List All Macro Items* from the Help menu (see chapter 4.10 on page 42.)

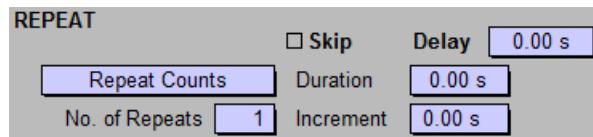
Alternatively, you can view the *Macro Command* for individual icons via opening the Dialog Control window (see chapter 2.2 on page 6.)



Command: Enter the name of the *Macro Command* you wish to include, e.g., "E Zap".

11.4.1.11 REPEAT

Inserts a repeat loop. There are two types of repeat loops.



- Repeat Counts: Repeats for a given number of repeats.
- Repeat Indefinite: Repeats in an indefinite loop.

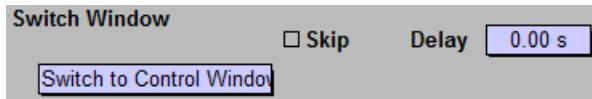
Duration: Time between repeats.

Increment: Increments duration.

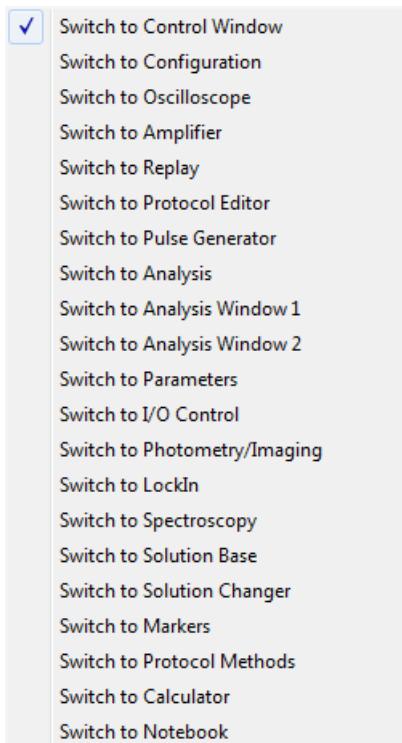
Note: REPEAT events cannot be moved in the event list!

11.4.1.12 Switch Window

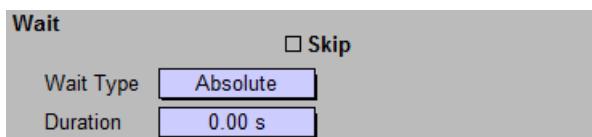
Brings the selected window in front.



In the drop-down menu you can select the a PATCHMASTER window you want to switch to:



11.4.1.13 WAIT



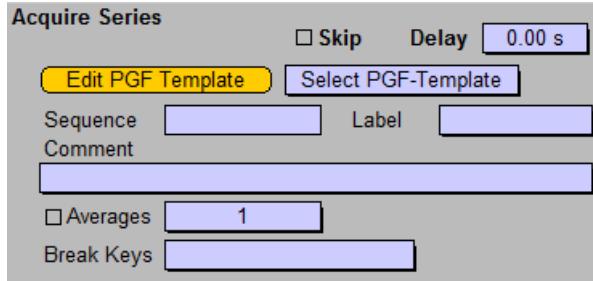
Wait type:

- Absolute: Waits exactly for the given delay time after the previous event.
- Relative: Waits for the given delay time after the actual end of the previous event (e.g., plus additional delay times).
- Resume Icon: Waits until the *Resume* button is clicked (i.e. sets the *WAIT* 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.

11.4.2 Acquisition

11.4.2.1 Acquire Series

Used to execute an entire PGF sequence.



Edit PGF Template: Opens the PGF template of the selected sequence in the Pulse Generator window.

Sequence: Indicates the name of the selected PGF.

Label: Enter a Series label, e.g. "Drug A".

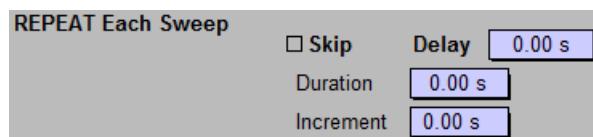
Comment: Enter a comment to the *Series*. The entry is stored with the *Series* and therefore available in the *Comment* field for the *Series* in the Parameter window.

Averages: A PGF sequence can be repeated several times. Only the average is stored to disk. Please enter the number of averages acquired for one *Sweep*. If the box in front of the "Averages" label is not checked, then this setting is ignored.

Break Keys: Define a key to break the acquisition of the running *Series*.

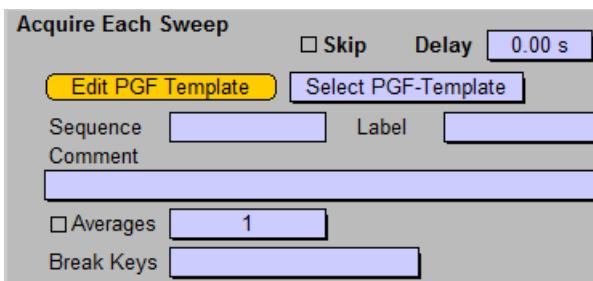
11.4.2.2 Acquire Each Sweep

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.



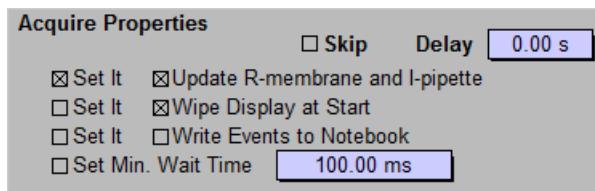
Duration: Defines the total cycle time for one *Sweep*.

Increment: Sets a defined time increment for the *Sweeps*.



For a description of the *Acquire Each Sweep* event please refer to the *Acquire Series* event above.

11.4.2.3 Acquire Properties



Update R-membrane and I-pipette: Compute and update the calculated *I-pipette* and *R-membrane* after every acquired Sweep. Enable the checkbox *Set It* in front of this option if you really want to execute it.

Wipe Display at Start: Determines whether the display is cleared at the start of a protocol or not. Enable the checkbox *Set It* in front of this option if you really want to execute it.

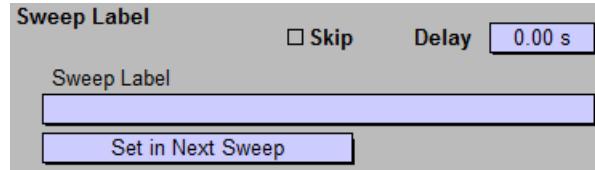
Write Events to Notebook: Writes the events to the Notebook window. This function is identical to the *Write* button. Enable the checkbox *Set It* in front of this option if you really want to execute it.

Set Min. Wait Time: Sets the time PATCHMASTER reserves to wait for the correct time to start when executing individual repeat loops such as acquisition of a *Series of Sweeps* or an *Repeat* loop in the Protocol Editor. Enable the checkbox *Set It* in front of this option if you really want to execute it. The *Min. Wait Time* can also be set in the Configuration window (see chapter 5.3.3 on page 46).

Note: A long Min. Wait Time will make the program to react very slowly. A very short Min. Wait Time might reduce the accurate timing of the starting of events. The timing within an acquisition; e.g sampling rate, is not affected by this parameter. Sampling within a Sweep is always accurate.

11.4.2.4 Set Sweep Label

Allows to put a label on the Sweep to be acquired next.



Sweep Label: Enter the name of the label into this entry field.

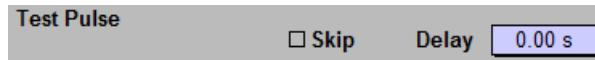
Sweep Label Target:

- **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 acquisition in the Replay window by pressing the *Label* button.

11.4.2.5 Test Pulse

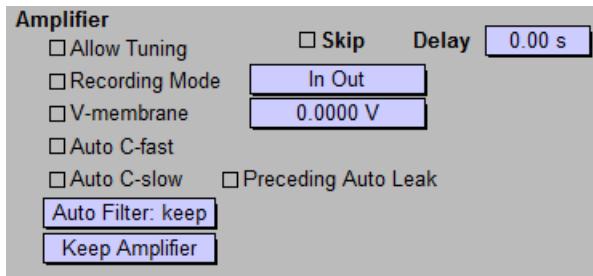
This event has no parameters. It executes a single *Test Pulse*.



11.4.3 Hardware

11.4.3.1 Amplifier

This event is for controlling the main amplifier settings.



Allow Tuning: The user can manually switch to the Amplifier window and adjust settings manually or just turn the *Test Pulse* on. The duration of the total event can be set by the *Delay*. It should have a minimal duration of 5 seconds to activate the tuning.

Recording Mode: Change the *Recording Mode* of the EPC 10.

- 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

For a more detailed description we refer to chapter 7.1.7 on page 80.

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. For a more detailed description we refer to chapter 7.1.10 on page 83.

Auto C-slow: Perform an *Auto C-slow* compensation. For a more detailed description we refer to chapter 7.1.11 on page 83.

Preceding Auto Leak: The *Preceding Auto Leak* option assures that a *Hardware Leak Subtraction* is performed prior to the *Auto C-slow* compensation. Such an operating sequence may be necessary if very leaky cells are measured.

Auto Filter: This option automatically sets a hardware filter according to the chosen sample interval.

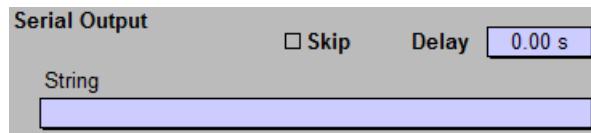
- keep: The status of the *Auto Filter* option is unaffected.
- ON: The *Auto Filter* option is enabled.
- OFF: The *Auto Filter* option is disabled.

Amplifier Selection: Choose which amplifier should be selected.

Note: This option is only available when using an EPC 10 Double, Triple or Quadro amplifier.

11.4.3.2 Serial Output

Use to send a string to the opened serial interface. For configuration of the *Serial Output* see Configuration Window, chapter 5.4.10 on page 53.

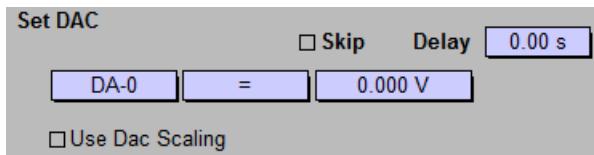


String: Enter a string that will be sent to a device via the *Serial Output*.

Examples:

- GO The command "GO" is send.
- GO\r The command "GO" + <Carriage Return> is send.
- GO\l The command "GO" + <Line Feed> is send.
- GO\032 The command "GO" + ASCII Code 32 (<Space>) is send.

11.4.3.3 Set DAC



Channel: Select a channel for analog output.

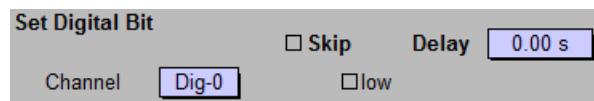
Operation: See 11.4.5.3 on page 159.

Voltage: Enter a voltage that will be output via the given channel.

Use Dac Scaling: If this option is active then the given value is multiplied by the DAC scaling factor specified in the Configuration dialog.

Note: The Set DAC event does not consider any scaling applied by equation or lookup table. Only entered scale factors are considered if the Use Dac Scaling is active.

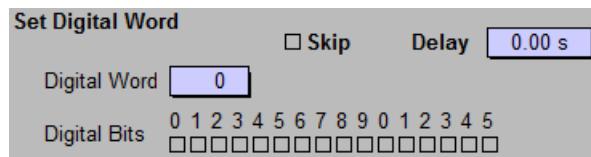
11.4.3.4 Set Digital Bit



Channel: Select a channel for digital output.

low: If selected, 0 Volt will be sent via the channel. If not selected, 5 Volt (standard TTL setting) will be sent.

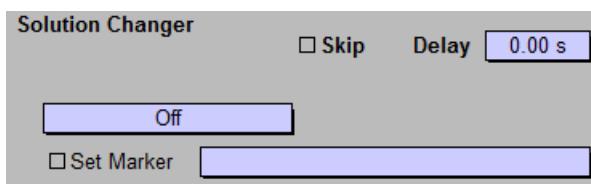
11.4.3.5 Set Digital Word



Digital Word: The entered number will be automatically translated into the digital bits.

Digital Bits: Allows selecting the digital bits individually.

11.4.3.6 Set Solution Changer



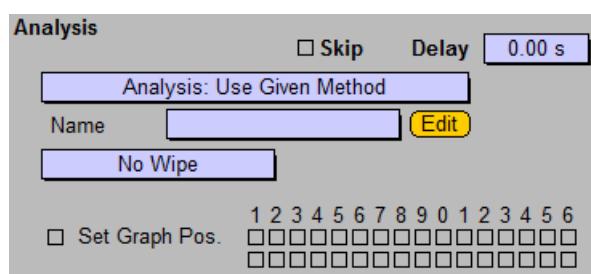
Solution: Select a *Solution* (1-16) at the connected *Perfusion System*.

Note: The output channels and their aligned solutions have to be defined first in the *Solution Changer* dialog (chapter 18 on page 233).

Set Marker: If selected, a *Marker* will be set when the *Solution* is changed by the *Solution Changer* event. The label of the *Marker* can be entered in the entry field behind.

11.4.4 Data/Display

11.4.4.1 Analysis



Analysis Mode:

- Analysis: Keep: Preserves the current settings of the Analysis.
- Analysis: Use Given Method: Sets the Analysis mode to *Use Selected Method* and selects the *Analysis Method* specified in the field *Name*.
- Analysis: Use Selected Method: Sets the Analysis mode to *Use Selected Method* and uses the active *Analysis Method*.

11.4. Events - Overview

- Analysis: Auto Stim Control – Pool: Sets the Analysis mode to *Automatic Stimulus Control: Pool* and uses the *Analysis Method* specified in the PGF sequence.
- Analysis: Auto Stim Control – Assigned: Sets the Analysis mode to *Automatic Stimulus Control: Assigned* and uses the *Analysis Method* which was assigned to the PGF sequence.
- Analysis: No Analysis: The Analysis is deactivated.

Name: Enter the *Analysis Method* you wish to start.

Edit: Opens the Analysis window with the given method for direct editing.

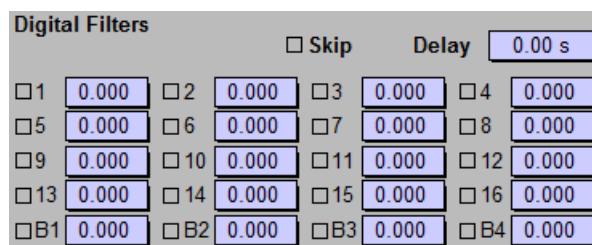
Wipe: Can be used to wipe individual graphs or all graphs at once.

- No Wipe
- Wipe All Graphs
- Wipe Graph 1...16

Set Graph Pos.: Activate the checkbox in front to apply the *Graph Positions* to the selected *Analysis Method*.

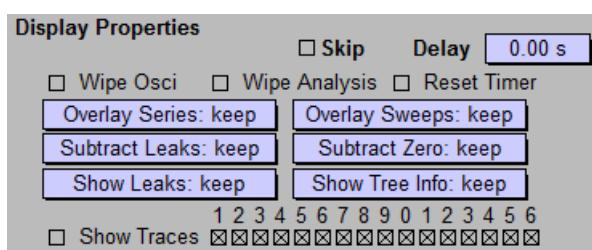
11.4.4.2 Digital Filters

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.



11.4.4.3 Display Properties

Use to control settings of the Display menu and the Oscilloscope window.



Wipe Osci: Deletes all displayed data in the Oscilloscope window.

Wipe Analysis: Deletes all displayed data in the Online windows.

Reset Timer: Resets the timer in the Oscilloscope window. These options are also described in section 4.5 on page 29.

Overlay Series and Overlay Sweeps:

- keep: Keep the state that was set before this event.
- ON: Overlay on.
- OFF: Overlay off.

Subtract Leaks:

- keep: Keep the state that was set before this event.
- ON: Subtract leak signals on.
- OFF: Subtract leak signals off.

Subtract Zero:

- keep: Keep the state that was set before this event.
- ON: Subtract zero offset on.
- OFF: Subtract zero offset off.

Show Leaks:

- keep: Keep the state that was set before this event.
- ON: Show leak signals on.
- OFF: Show leak signals off.

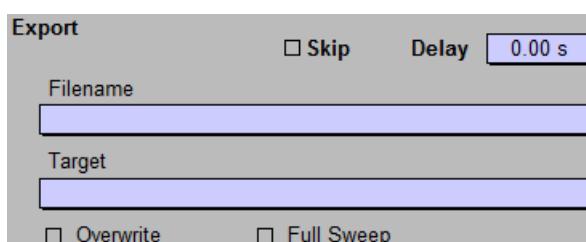
Show Tree Info:

- keep: Keep the state that was set before this event.
- ON: Show Tree Info in the Notebook - on.
- OFF: Show Tree Info in the Notebook - off.

Show Traces 1...9, 0...6: Changes the show flag of all 16 *Traces*. This function can be used to show only a subset of acquired *Traces* in the Oscilloscope window (see chapter 4.5 on page 29). Note that you have to mark *Show Traces* for the changes to take effect.

11.4.4.4 Export

Exports the acquired data according to the export settings made in the Replay menu. Unless you activate the checkbox *Full Sweep* only the displayed data is exported (see chapter 4.4 on page 23).



Filename: Define a name for exported file according to the auto filename components described in the Configuration (see chapter 5.5.2 on page 56).

Target: Declares the *Target Specifier* in the tree. 4 comma separated integers are allowed, specifying the index of *Group*, *Series*, *Sweep* and *Trace* (Gr_Se_Sw_Tr). "+" and "-" define relative index from the present selection. An index of "0" defines the present selection.

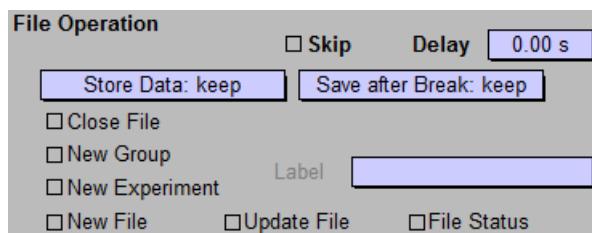
Examples:

- "0": Exports the present *Group*.
- "0,0": Exports the present *Series*.
- "0,0,0": Exports the present *Trace*.
- "0,-1": Exports the previous *Series* of the present *Group*.
- "1,4": Exports the 4th *Series* of the 1st *Group*.
- "1,1,4,1": Exports the 1st *Trace* of the 4th *Sweep* of the 1st *Series* of the 1st *Group*.

Overwrite: Enables to overwrite already existing output files.

Full Sweep: The full *Sweep* is exported independent of what is shown in the Oscilloscope.

11.4.4.5 File Operation



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 Break:

- **keep**: Keeps the status that was set before this event (either ON or OFF of the *Save after Break* parameter in the Configuration window).
- **ON**: Acquired *Sweep* data will be stored if the *Sweep* was terminated with the *Break* command.
- **OFF**: *Sweep* data will not be saved to disk if the *Sweep* was terminated with the *Break* command.

Close File: The data file will be closed.

New Group, New Experiment, New File: A new group, experiment or file can be created. The ability to be able to create a new data file is very convenient when the auto file name generation is used.

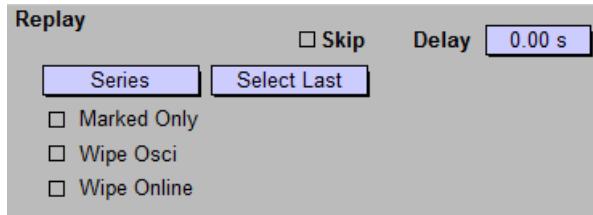
Label: It is active when a new group or a new experiment is created and it specifies the group label.

Update File: Updates and saves the file.

File Status: Prints the file status to the Notebook (see chapter 4.1 on page 17).

11.4.4.6 Replay

Data of the opened file can be replayed.



Select the target level of data which should be replayed:

- Nothing: Nothing is selected.
- Root: Select the *Root*.
- Group: Select the *Group*.
- Series: Select the *Series*.
- Sweep: Select the *Sweep*.
- Trace: Select the *Trace*.

The options refer to the target level selection (see above).

- Select Next: Selects the next target (no replay).
- Select Present: Selects the present target (no replay).
- Select Last: Selects the last target (no replay).
- Replay Next: Selects the next target and replays the data.
- Replay Present: Selects the present target and replays the data.
- Replay Last: Selects the last target and replays the data.

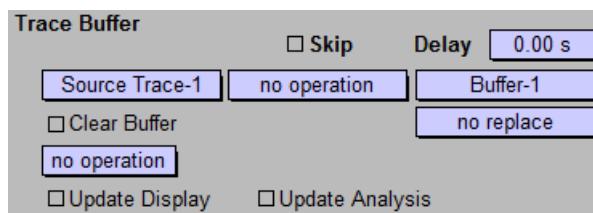
More options can be selected via the checkboxes:

Marked Only: Only marked data in the Replay tree will be affected.

Wipe Osci: Deletes all displayed data in the Oscilloscope window.

Wipe Online: Deletes all displayed data in the Online window.

11.4.4.7 Trace Buffer



Trace Buffer Source: Define the data source you want to operate with.

Operation: Define the operation which should be applied to data before storing it in a *Buffer*.

- no operation
- add to
- subtract from
- accumulate in
- deaccumulate from

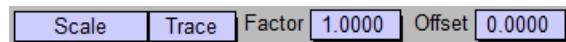
Target: Select the target *Buffer* (*Buffer 1...4*).

Replace: Replaces the selected *Trace* with the *Buffer*

Clear Buffer: Clears the target *Buffer* before storing the data.

Scaling:

- no operation
- Scale: Scales the *Trace Buffer Source* (*Trace* is selected) or *Buffer* (*Buffer* is selected) with a specified *Factor* and *Offset*.



- Equation: Calculates the scaling of the *Buffer* via an equation. For details on the equation syntax please refer to [Equation Syntax](#), chapter 20.1 on page 240.



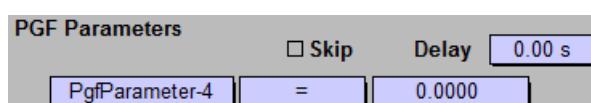
Update Display: Updates the display of the *Buffer* in the Oscilloscope window.

Update Analysis: Updates the display of the *Buffer* in the *Analysis* graphs.

11.4.5 Value/Parameter

11.4.5.1 PGF Parameters

PGF parameters can be set or modified during the protocol to automatically adapt the acquisition sequences.



PGF Parameters 1...10: Specify a PGF parameter to be set.

Operation: To modify a PGF parameter the following functions can be used.

- Functions working on manually entered *Values*:
 - =
 - increase by
 - decrease by
 - multiply by
 - divide by

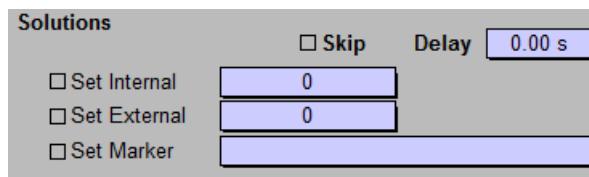
- Functions working on global Values:

- = value
- inc by value
- dec by value
- mul by value
- div by value

Value: Manually entered value or selection of a global Value (see PGF Parameters, chapter 10.15 on page 138).

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" (= $0.1 * 1 \text{ nA}$).

11.4.5.2 Set Solutions



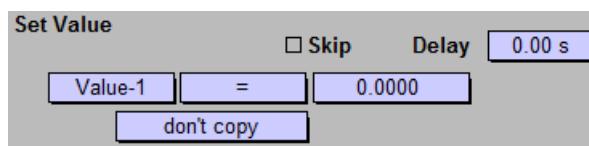
Set Internal: User-defined index number for the internal solution. Activate the checkbox to apply the setting.

Set External: User-defined index number for the external solution. Activate the checkbox to apply the setting.

Set Marker: Set a solution marker when the solution is changed. In the entry field a label for the marker can be defined. Activate the checkbox to apply the setting. See also Solutions, chapter 17 on page 229.

11.4.5.3 Set Value

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. The values can also be set via "batch control", thus allow triggering an IF decision by an external input, see Controlling Patchmaster in the PATCHMASTER Tutorial. In addition, results from the Analysis can be stored in these values (see Analysis Functions, chapter 13.3 on page 189). The values, therefore, constitute an important interface between the Protocol Editor and the Analysis.



Value 1...16, User 1-2: Refers to the global Values and the User Parameters. User Parameters can be defined in the Configuration window, see I/O Parameters, chapter 5.7.2 on page 63.

Operation: Set value to...

- manually entered values:
 - =
 - increase by
 - multiply by
 - divide by
 - modulo
 - keep: no change to the value

<input checked="" type="checkbox"/>	=
	increase by
	multiply by
	modulo
	keep
	= item
	inc by item
	dec by item
	mul by item
	div by item
	= param
	inc by param
	dec by param
	mul by param
	div by param

- an *Item*:

- = item
- inc by item
- dec by item
- mul by item
- div by item

<input checked="" type="checkbox"/>	= value
	inc by value
	dec by value
	mul by value
	div by value
	= analysis
	inc by analysis
	dec by analysis
	mul by analysis
	div by analysis
	= equation
	= input
	= batch comm. value

- *Parameters*:

- = param
- inc by param
- dec by param
- mul by param
- div by param

- Global Values:

- = value
- inc by value
- dec by value
- mul by value
- div by value

<input checked="" type="checkbox"/>	= value
	inc by value
	dec by value
	mul by value
	div by value

- Analysis results:

- = online
- inc by online
- dec by online
- mul by online
- div by online

<input checked="" type="checkbox"/>	= value
	inc by value
	dec by value
	mul by value
	div by value

- equation results: = equation. An equation can be entered to compute a value. For details on the equation syntax please refer to [Equation Syntax](#), chapter 20.1 on page 240.
- = 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.

Value:

- in case of constant value: Enter manually the constant value.
- in case of *Item* operation: Enter the item ID, e.g. "E CFastTot" to read the value of a program control (here compensated C-fast value). For a description how to get info on the control IDs please refer to [Modifying Dialogs and Controls](#), chapter 2.2.3 on page 10.

- in case of *Parameter* operation: Choose a parameter from the selection.
- in case of *Value* operation: Choose one of the 16 global *Values*.
- in case of *Analysis* operation: Choose one of 16 *Analysis* results.

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.

11.4.6 Messages

11.4.6.1 Annotation

Use this function to add comments to your protocol and improve the readability.



Text can be entered that will be shown in the event list.

11.4.6.2 Beep

Use to execute acoustic alerts.

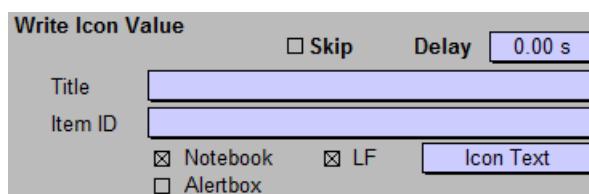


An acoustic signal will be output. This only works if your computer is properly equipped for sound (e.g. sound card, loudspeakers...).

11.4.6.3 Write Icon Value

Outputs the value of the specified icon into the Notebook like this:

[Title] [ItemID]: [value]



Title: Title for the *Item ID*, leads to an improved readability of the output but is not mandatory.

Item ID: Unique identifier of the icon. Can be inspected by opening the Icon configuration dialog (see [Modifying Dialogs and Controls, chapter 2.2 on page 6](#)) – it is given in the title of that window.

Notebook: Writes to Notebook.

Alertbox: Opens an Alertbox containing the text.

LF: When LF is on, a linefeed signal is appended.

Icon Text / Icon Value:

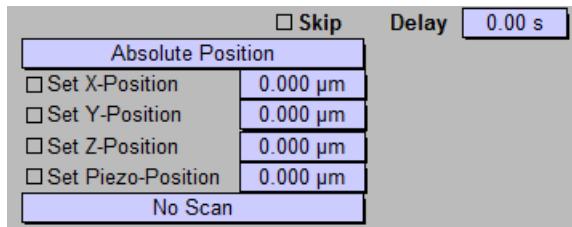
- Icon Text: Writes the text from a field. For example from the icon *E Gain* would return "1.0 mV/pA".
- Icon Value: Writes the value of a field. For example from the icon *E Gain* would return "8.0", meaning the 8th item of the *Gain* list corresponding to "1.0 mV/pA".

11.4.7 Extensions

11.4.7.1 ElProScan

Note that the ElProScan Extension has to be active to allow the editing of the following events (see Configuration, chapter 5.4.9 on page 52).

The *ElProScan* event enables users to modify the motor and scan parameters.



Position:

- Absolute Position: The motors can be moved to an absolute position by entering the destination position
- Relative Position: The motors can be moved by a certain distance.

X-Position: Enter a value into the entry field to define a *X-Position*. Enable the checkbox in front to activate the entered value.

Y-Position: Enter a value into the entry field to define a *Y-Position*. Enable the checkbox in front to activate the entered value.

Z-Position: Enter a value into the entry field to define a *Z-Position*. Enable the checkbox in front to activate the entered value.

Piezo-Position: Enter a value into the entry field to define the *Piezo-Position*. Enable the checkbox in front to activate the entered value.

Scan Mode:

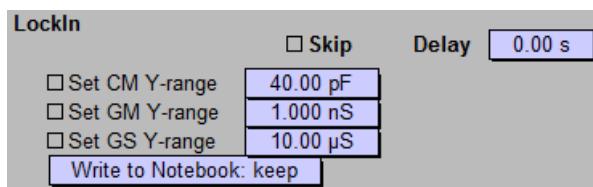
- No Scan: No scan will be performed.
- Tip-Down: Moves the electrode towards the substrate in Z-direction.
- Pull Up: retracts the electrode from the substrate in Z-direction.

- DepthScan: A two dimensional scan in the X-Z plane can be performed.
- 2D-Scan: A line scan along one of the three axis can be performed.
- 3D-Scan: A two dimensional scan in the X-Y plane with correction of the Z-axis height to compensate for a double tilted substrate can be performed.
- Matrix Scan: A *Series* will be executed at each scan point of a two dimensional plane.
- Template Scan: Like a *Matrix Scan* but using loaded template.
- Const-Dist 3D: Like a *3D-Scan* but using a constant distance.
- Const-Dist Matrix: Like a *Matrix-Scan* but using a defined constant distance.
- SF-Spectra Scan: Performs a Shear-Force scan to determine the test frequency.
- Fast 3D Scan: Can only be used if X and Y piezo motors are present. A two dimensional scan in the X-Y plane with correction of the Z-axis height to compensate for a double tilted substrate can be performed. Exclusive use of piezo motors in all directions.
- Fast Matrix Scan: Can only be used if X and Y piezo motors are present. A *Series* will be executed at each scan point of a two dimensional plane. Exclusive use of piezo motors in all directions.
- Fast C. D. 3D Scan: Like a *Fast 3D Scan* but using a constant distance mode from the ElProScan Menu.
- Fast C. D. Matrix Scan: Like a *Fast Matrix Scan* but using a constant distance mode from the ElProScan Menu.
- Surface Tracked 3D Scan: Like a *3D Scan* but keeping a constant distance to the substrate

11.4.7.2 LockIn

Note that the **LockIn** Extension has to be active to allow the editing of the following events (see Configuration, chapter 5.4.4 on page 50 and Software LockIn Extension, chapter 23 on page 257).

The **LockIn** event enables users to set some default ranges.



CM Y-range: Enter a value into the entry field to define the default CM Y-range. Enable the checkbox in front to activate the entered value.

GM Y-range: Enter a value into the entry field to define the default GM Y-range. Enable the checkbox in front to activate the entered value.

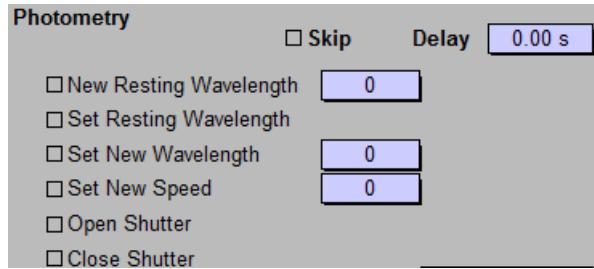
GS Y-range: Enter a value into the entry field to define the default GS Y-range. Enable the checkbox in front to activate the entered value.

Write to Notebook: This option enables to write the LockIn parameters into the Notebook.

- Write to Notebook: Keeps the status that was set before this event (either "ON" or "OFF").
- Write to Notebook: ON: Enables the writing of the LockIn parameters into the Notebook.
- Write to Notebook: OFF: Disables the writing of the LockIn parameters into the Notebook.

11.4.7.3 Photometry/Imaging

Note that the Photometry Extension has to be active to allow the editing of the following events (see Configuration, chapter 5.4.8 on page 51 and Photometry Extension, chapter 21 on page 245).



New Resting Wavelength: Sets the variable *Resting Wavelength* to the given value.

Set Resting Wavelength: Sets outputs *Resting Wavelength*. Note that this function is called "Close shutter" for those systems that have a shutter. In this case, the option *New Resting Wavelength* disappears.

Set New Wavelength: Sets a specified wavelength.

Set New Speed: Sets a new speed setting of the filter wheel.

Open Shutter / Close Shutter: Note that this function is called *New Resting Wavelength* for those systems without shutter (see above).

Note: The amount of available options for the Photometry event depend on the type of photometry device selected in the Configuration window.

11.4.8 Echem-Methods

The *Echem-Methods* of the Protocol Editor are explained in detail in chapter 12 on the facing page.

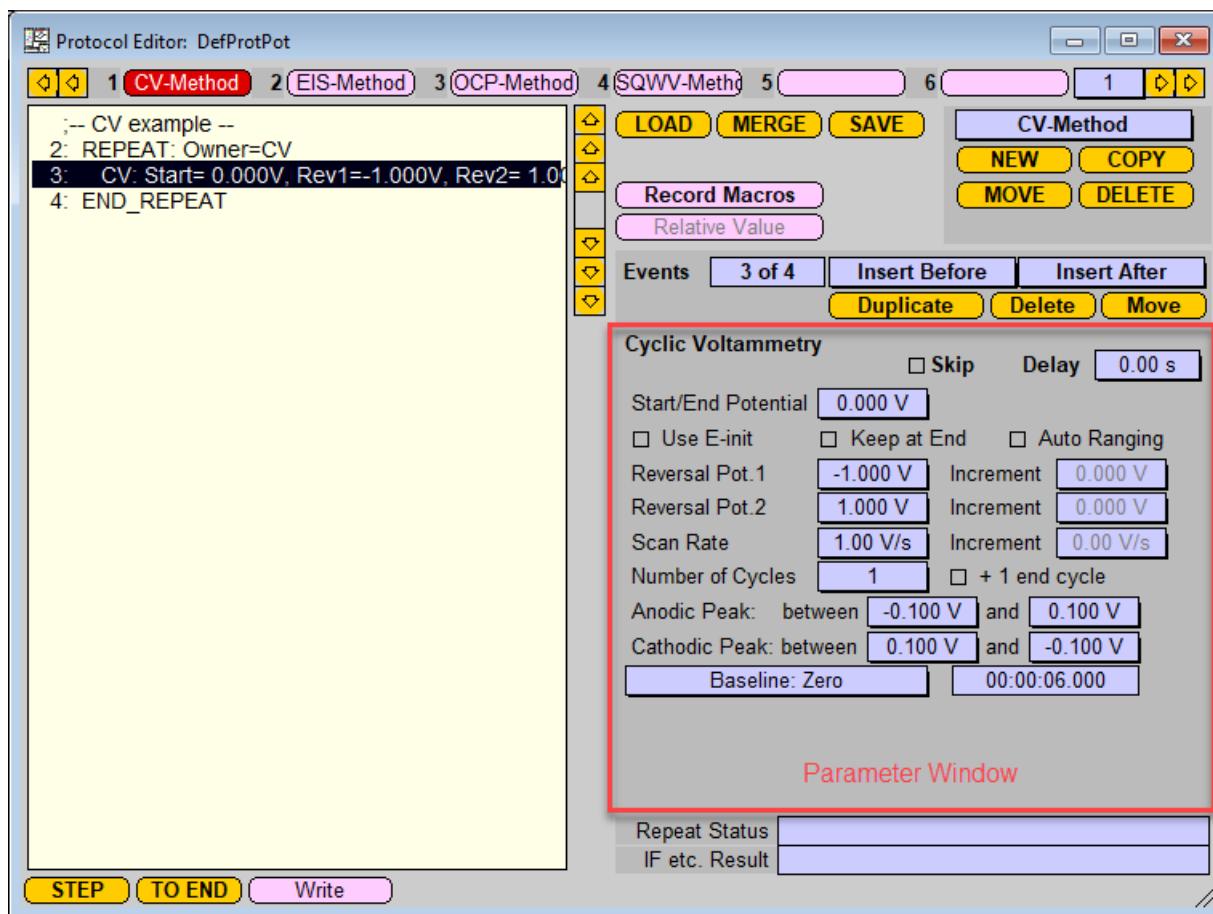
12 Protocol Editor Window - Electrochemical Methods

The electrochemical methods are a special feature of PATCHMASTER. They differ from other operations in the Protocol Editor because they automatically create a transient *Pulse Generator Sequence* and an *Analysis Method* upon execution of the protocol. Transient means that they will not be saved with other changes made in the Pulse Generator Window and Analysis Window, but instead created or overwritten when executing the protocol. Therefore, if changes should be made by the user to both the *Pulse Generator Sequence* and *Analysis Method* a copy with a new name of the created *Pulse Generator Sequence* and *Analysis Method* has to be made.

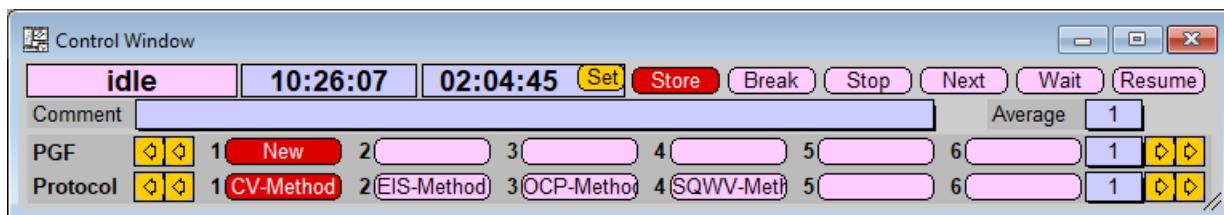
The idea of these *E-Chem Methods* for standard electrochemical techniques is that the user does not have to create a complex *Pulse Generator Sequence* with several segments and an *Analysis Method* from scratch, but instead a standardized method can be executed immediately where only measurement parameters have to be entered in the Protocol Editor.

In the following, the process of executing an *E-Chem Method* is explained for the example of the Cyclic Voltammetry Method.

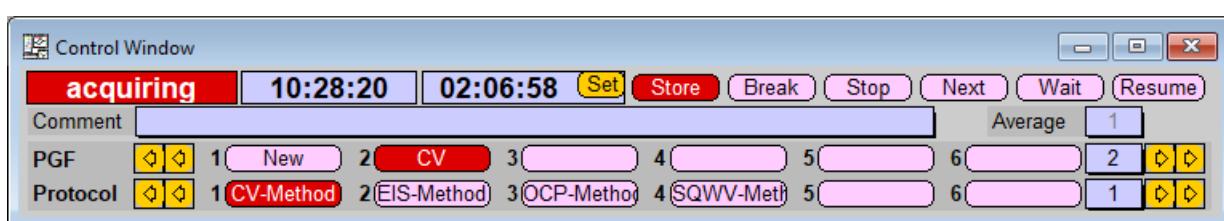
1. Create a new *Protocol* in the Protocol Editor Window and give it a meaningful name. We chose "CV-Method".
2. The operation E-Chem Methods – Cyclic Voltammetry is added to the protocol. By clicking on it in the protocol list on the left (black background when selected), a window appears on the right side where the parameters for the experiment are defined. Insert the desired parameters and close the Protocol Editor Window.



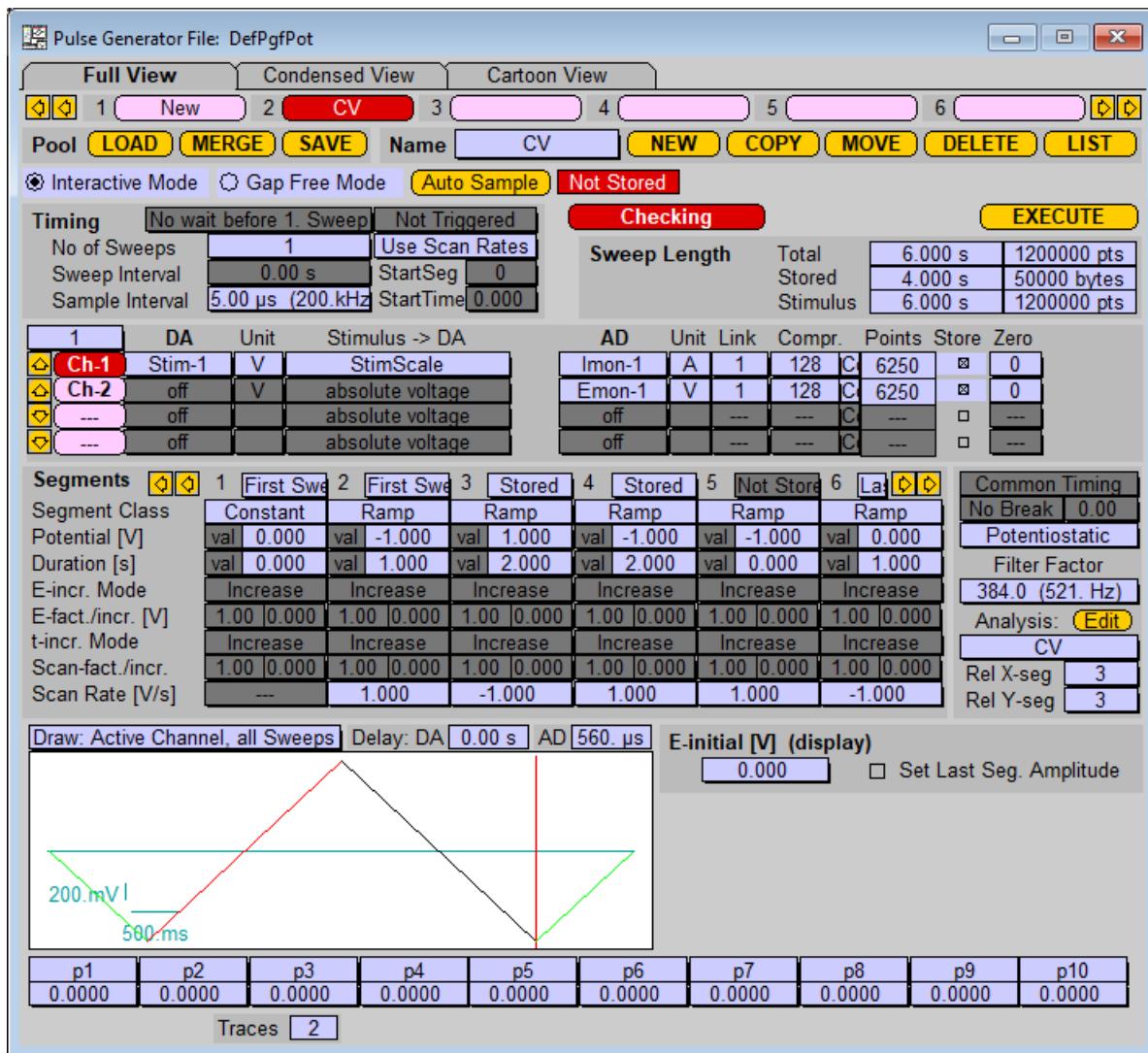
3. In the Control Window you can now see and execute the *Protocol "CV-Method"*.



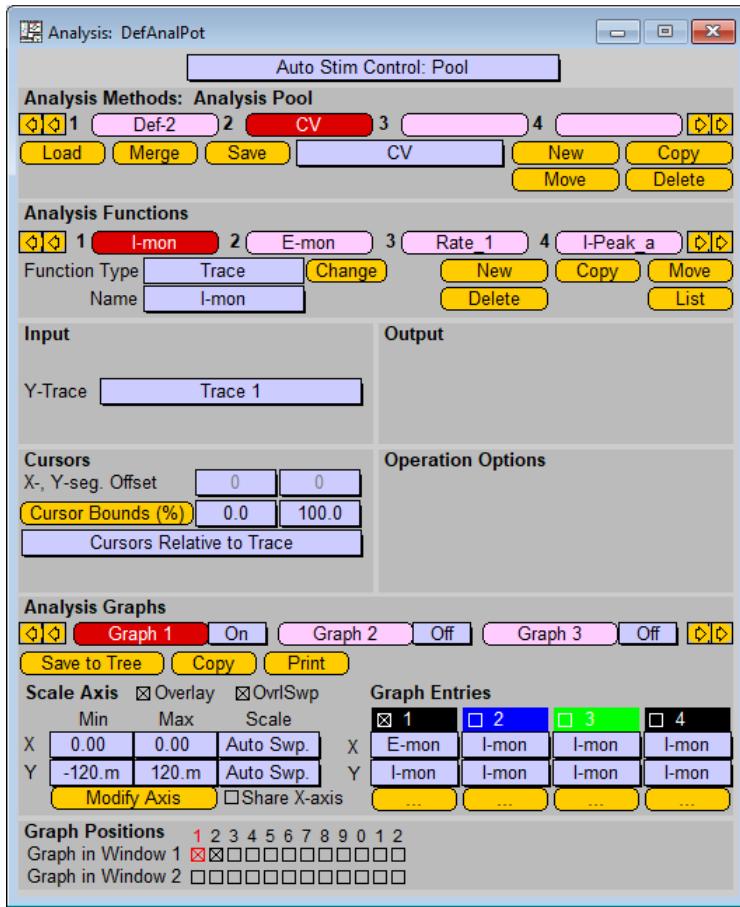
4. When executing the *Protocol* a *PGF* called "CV" is created and appears in the Control Window and an *Analysis Method* is created.



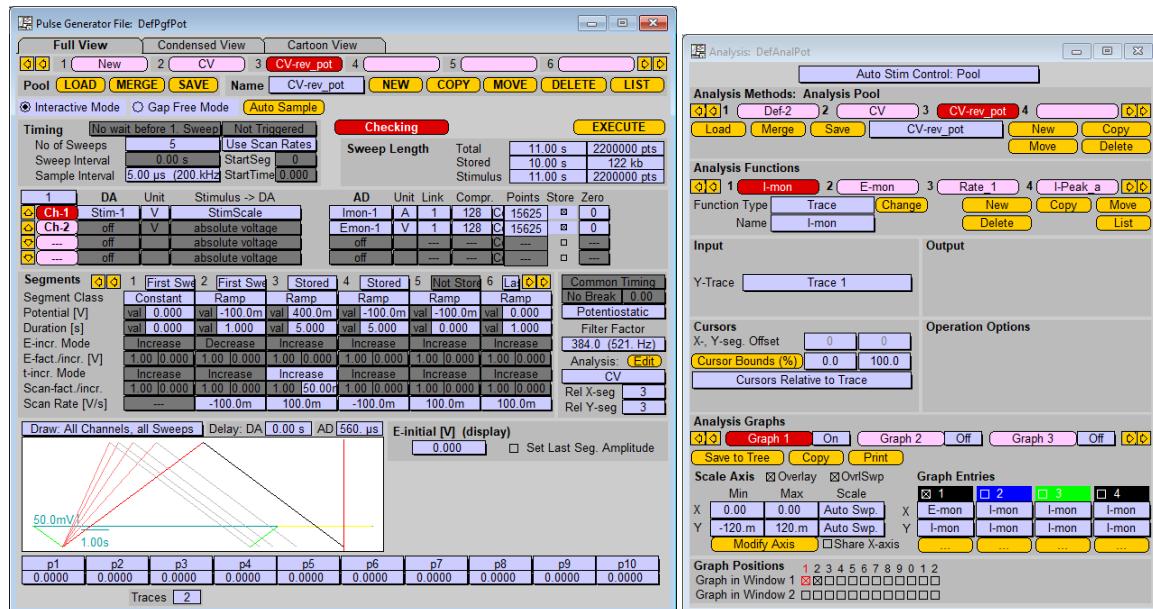
5. You can open the Pulse Generator Window to view the newly created Sequence called "CV".



6. You can open the Analysis Window to view the newly created *Analysis Method* called "CV".



7. If you want to make changes to this *Pulse Generator Sequence* or this *Analysis Method*, you have to make a copy with a different name. The automatically created *Pulse Generator Sequence* "CV" and *Analysis Method* "CV" will be overwritten upon repeated execution of all *Protocols* using the Cyclic Voltammetry Method. Also, the *Pulse Generator Sequence* "CV" and *Analysis Method* "CV" will not be saved with the other *Pulse Generator Sequences* and *Analysis Methods*.

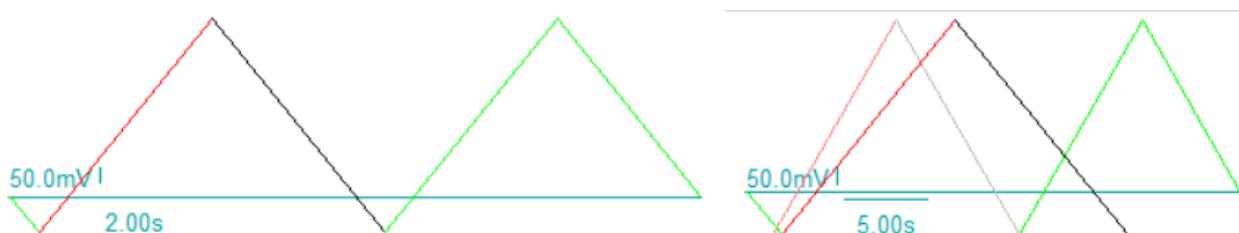


12.1 Cyclic Voltammetry (CV)

Cyclic Voltammetry (CV) is one of the most commonly used electrochemical methods. It is also called *Triangular Wave Method*, because a triangular potential time curve is applied at the working electrode by a function generator in potentiostatic mode. A redox system of interest is investigated at the working electrode by recording the current which results from the triangular stimulation. The speed of potential change during the anodic and cathodic ramps of the triangular wave is characterized by the *Scan Rate*. Cyclic Voltammetry is used as steady state method (slow *Scan Rates* <1 mV/s) and also transient method (fast *Scan Rates* >10 V/s). The method is used for analytical applications of common electrochemistry, applications for battery research, investigation of fuel cells, characterization of electrocatalysis, electrode characterization in electrolysis cells, investigation of new electrode materials, characterization of sensors, corrosion investigation, investigation of chemical following reactions and electron transfer reactions.

A cycle, i.e. in the convention of PATCHMASTER one whole *Sweep*, starts with the first reversal potential (Reversal Pot. 1). From here a linear *Sweep* with a constant scan rate reaches the second reversal potential (Reversal Pot. 2). This is the first *Segment* of the PGF Template (also defined as *Relevant Segment*). From the second reversal potential (Reversal Pot. 2) we apply a potential ramp with constant scan rate back to the first reversal potential (Reversal Pot. 1). This is the second *Segment* of the PGF Template.

The cycle is enclosed by a start phase (*Segments* of type *First Sweep*) and an end phase (*Segments* of type *Last Sweep*), which are not shown and stored. The start phase runs a linear *Sweep* from the start potential to the first reversal potential. The end phase runs a linear *Sweep* from the first reversal to the second reversal and then back to the start/end potential. This makes the entire CV stimulus symmetrical with respect to oxidation and reduction processes.



If the numbers of cycles is larger than one, additional cycles (see red and black ramps in the figure above) are executed in the *Gap Free Mode*. Note that the green parts are executed only at the beginning and at the end of the recording.

12.1.1 CV Parameters

Start/End Potential	<input type="text" value="0.000 V"/>	
<input type="checkbox"/> Use V-membra	<input type="checkbox"/> Keep at End	<input type="checkbox"/> Auto Ranging
Reversal Pot.1	<input type="text" value="-0.100 V"/>	Increment <input type="text" value="0.000 V"/>
Reversal Pot.2	<input type="text" value="0.900 V"/>	Increment <input type="text" value="0.000 V"/>
Scan Rate	<input type="text" value="50.0 mV/s"/>	Increment <input type="text" value="0.00 V/s"/>
Number of Cycles	<input type="text" value="1"/>	<input type="checkbox"/> + 1 end cycle
Anodic Peak:	between <input type="text" value="-0.100 V"/>	and <input type="text" value="0.900 V"/>
Cathodic Peak:	between <input type="text" value="0.900 V"/>	and <input type="text" value="-0.100 V"/>
Baseline:	<input type="text" value="Zero"/>	<input type="text" value="--.--"/>

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*.

12.1. Cyclic Voltammetry (CV)

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 10.5 on page 111).

Increment: If multiple cycles are executed the scan rate can be altered by a fixed increment from *Sweep* to *Sweep*.

Number of Cycles: If the number of cycles is larger than one, multiple *Sweeps* are executed in *Gap Free Mode*.

+1 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: 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.

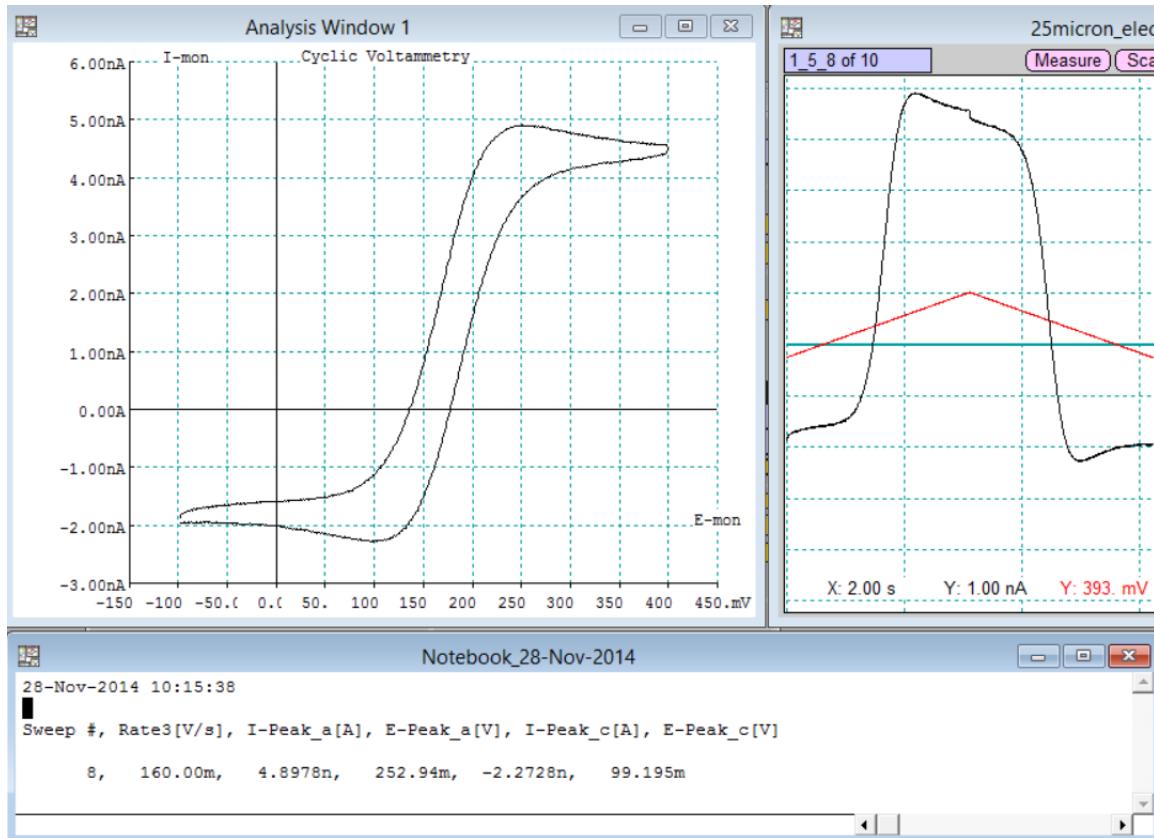
Cathodic Peak: The *Cathodic Peak* will be analyzed as *Minimum* in the segment scanning from *Reversal Pot. 2* back to *Reversal Pot. 1*. Scanning towards negative potentials is required. The voltage range for analyzing the minimum current can be defined.

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*).

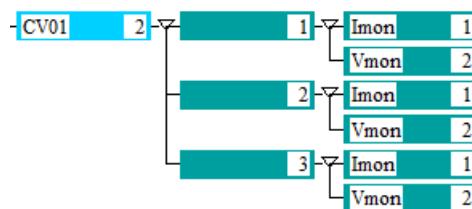
12.1.2 Acquiring a Cyclic Voltammogram

You start the electrochemical method by executing the protocol, which contains the method.



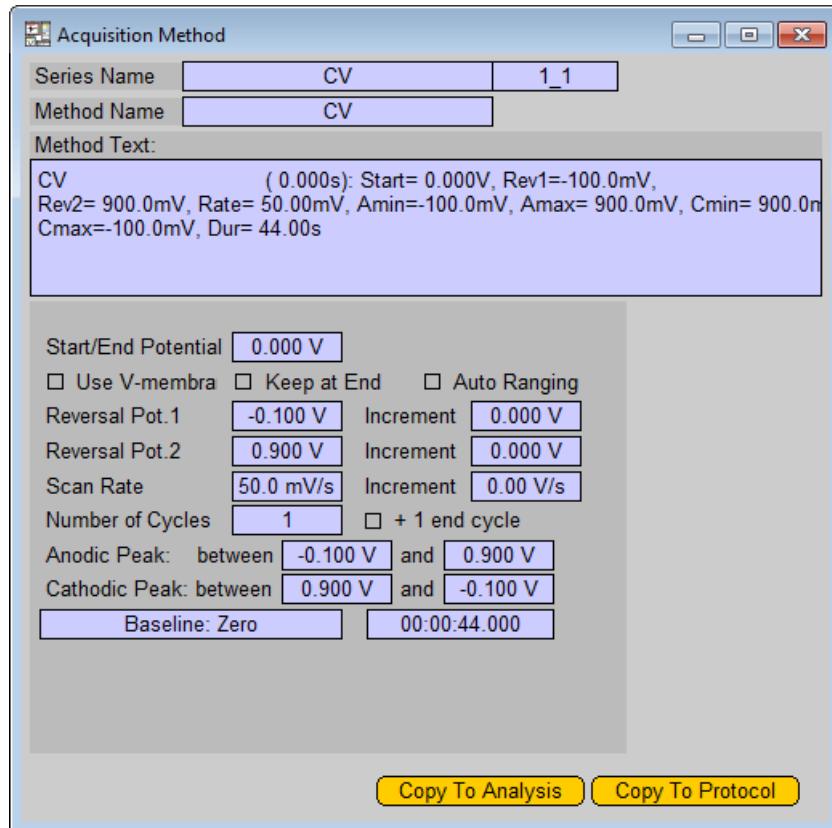
In the Oscilloscope Window the recording is shown versus the time. The method creates a standard *Analysis Method* "CV", which plots the cyclic voltammogram in *Graph 1* and analyzes anodic and cathodic current peaks and the corresponding peak potentials. The results are plotted into the Notebook.

The raw data recorded by the Cyclic Voltammetry method are stored in the data tree as *Series* with multiple *Sweeps*. Each *Sweep* containing the current and the voltage *Trace*.



The parameters of the method, which were used during the acquisition of the data, can be viewed in the *Acquisition Method* window. The window can be opened either via selecting *Show Protocol Method* in the *Replay* menu or via selecting *Protocol Methods* in the *Windows* menu.

12.1. Cyclic Voltammetry (CV)

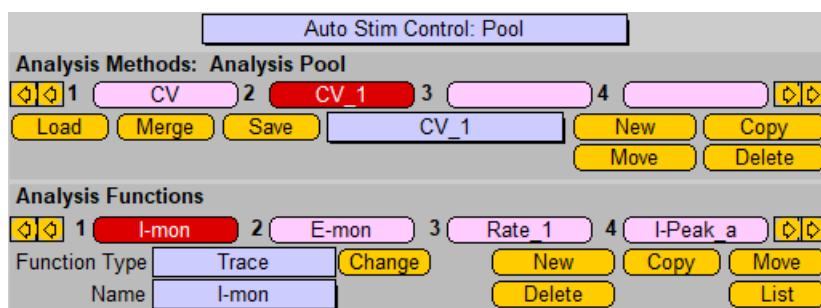


The protocol event itself can be copied in the currently active protocol or the corresponding *Analysis Method* copied to the currently opened *Analysis pool*.

12.1.3 Customizing the Analysis Method

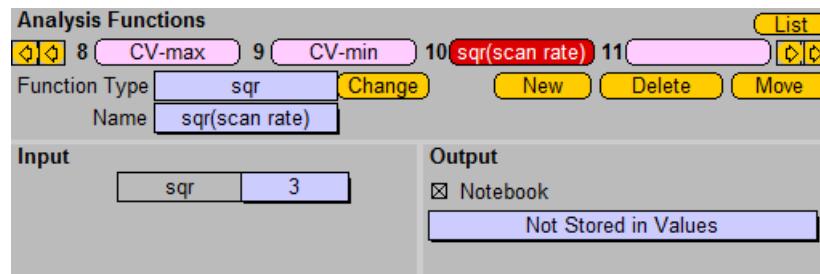
If you are acquiring multiple CVs at different *Scan Rates*, you might want to extend the standard CV analysis by a plot of *I-Peak* versus square root of the *Scan Rate*.

Replay the *Series* that contains the CV data. Then open the *Analysis* window and select the *Analysis Method* "CV". Press *Copy* to create another method (default name *CV-1*), where you can make your changes. Remember: The transient *Analysis Method* "CV" will be overwritten upon repeated execution of all *Protocols* using the Cyclic Voltammetry Method and not be saved.

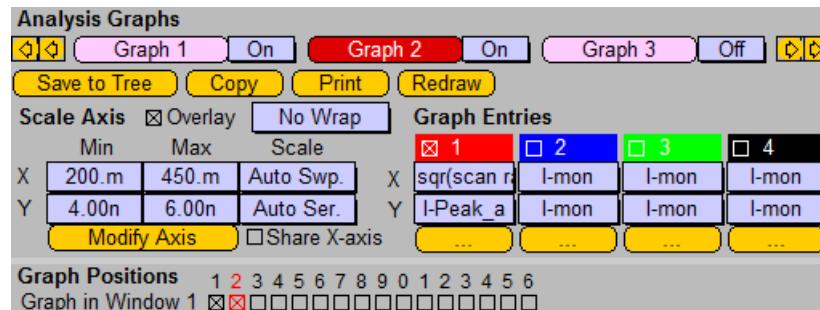


Now, you have to define a new *Analysis Function*. In the *Analysis Function* section in the lower part of the *Analysis* window press *New* and select the function *sqrt* from the *Math* group. You can assign a name to the function and have to set the index of the *Analysis Function* of the *Scan Rate* (here number 3) as input for the square root function.

12.1. Cyclic Voltammetry (CV)



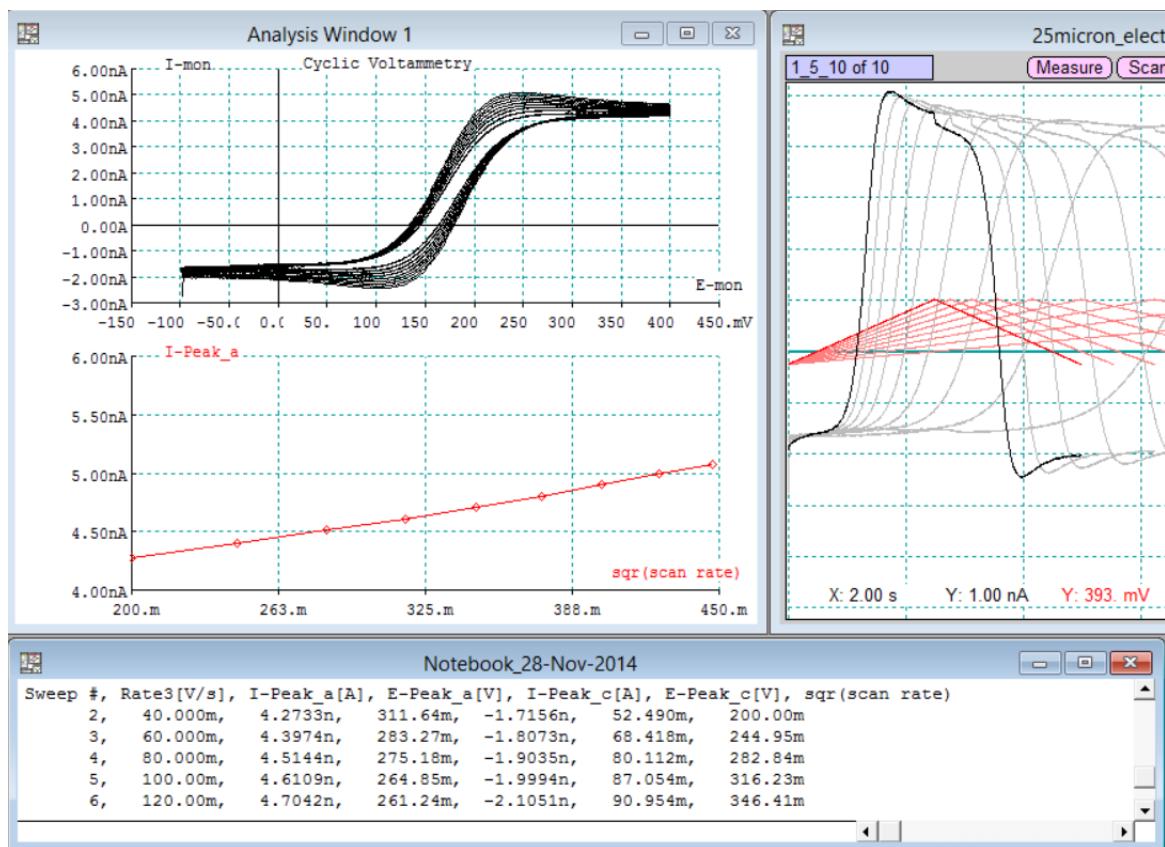
If you are replaying the data again, the square root of the Scan Rate will be written in the Notebook as additional result. Now we want to define a graph that plots *I*-peak versus *sqr(scan rate)*. In the lower section of the Analysis window switch to *Graph 2* and turn it on. Then, select *sqr(scan rate)* as 'X' and *I-Peak_a* as 'Y'. Make sure that the little box above is checked.



Finally, you have to define in which Analysis window the *Graph 2* should be shown. On the bottom of the Analysis window, in the section *Graph Positions* make a check in the second box of the upper row. This means, that the *Graph 2* is shown in Analysis Window 1.

After another replay of the *Series* you should see the second graph which plots *I*-peak versus square root of the Scan Rate in the Analysis Window 1.

12.1. Cyclic Voltammetry (CV)

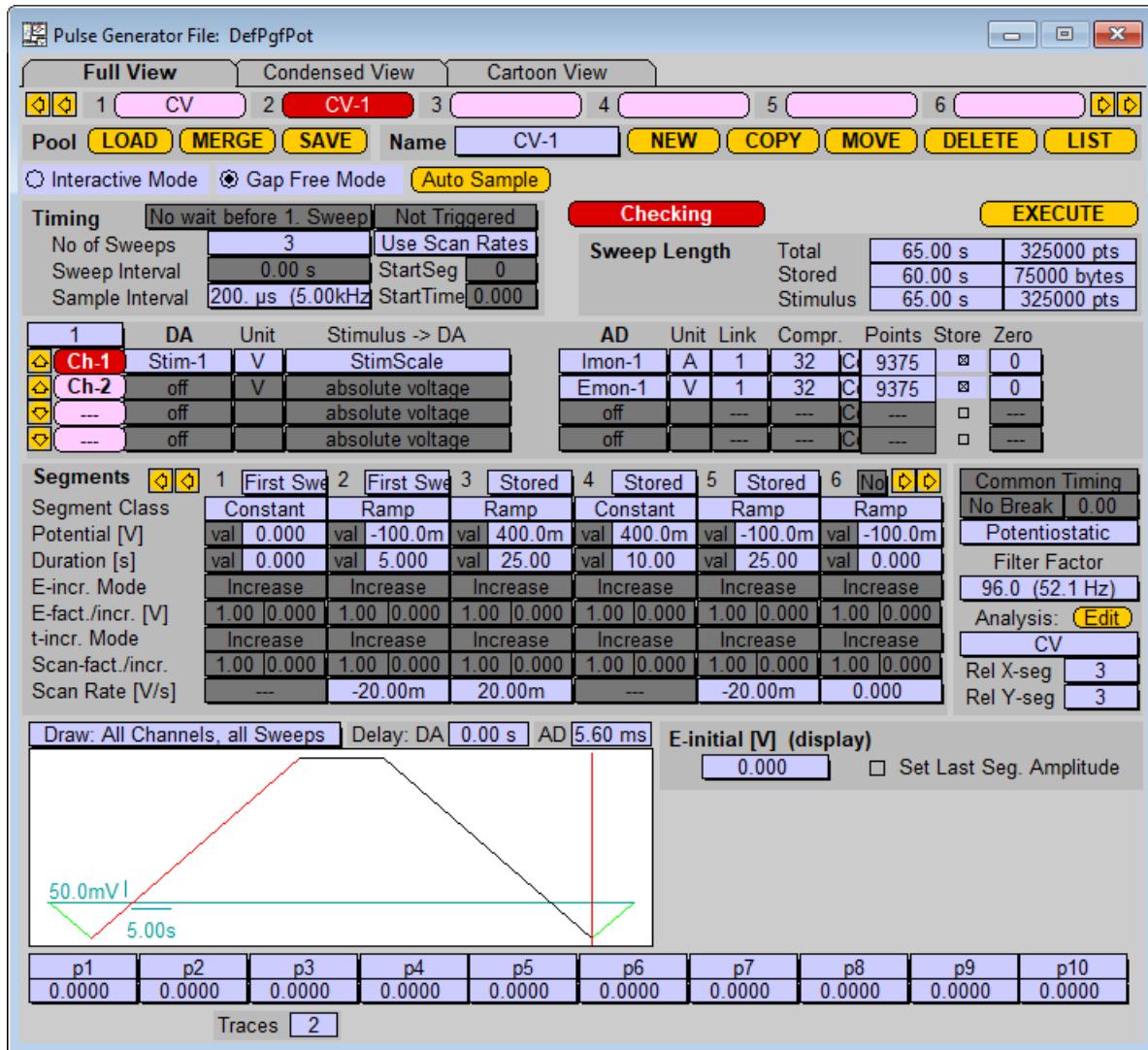


12.1.4 Customizing the CV Acquisition Sequence

Upon execution of a *Protocol* which contains the *E-Chem Method Cyclic Voltammetry* a transient *Pulse Generator Sequence* is created. This *Pulse Generator Sequence* will be overwritten by the next *E-Chem Method Cyclic Voltammetry*-containing *Protocol* and also not saved.

If you want to keep a specific configuration of the CV in your pgf pool, you should copy the *Pulse Generator Sequence* "CV" and rename it. The default name of the new sequence is CV-1. This sequence will be saved as usual.

12.1. Cyclic Voltammetry (CV)



You can now also customize your CV in a way that would not be possible using the parameter window of the *Echem Method Cyclic Voltammetry* in the Protocol Window (see page 166). The screenshot above shows a cyclic voltammogram where the upper potential is held for 10 s before starting the reverse scan. This was easily achieved by inserting a new Segment of the Class "Constant" into the existing segments. Unnumerous changes can be made to a common CV in this way.

12.2 Electrochemical Impedance Spectroscopy (EIS)

Electrochemical Impedance Spectroscopy is a characterization method where the impedance of a system depending on the frequency of an applied AC voltage is determined. Because the measured impedance is the sum of all components in the system, the data are analyzed using an equivalent circuit. Electrochemical Impedance Spectroscopy is commonly used to investigate electron transfer processes and diffusion of electrochemical reactions or the capacitance of electrode materials. Short-time behavior and fast processes correspond to high frequencies whereas long-time behavior and slow processes correspond to low frequencies. Interesting for electrochemists are commonly three processes which have different characteristic frequencies:

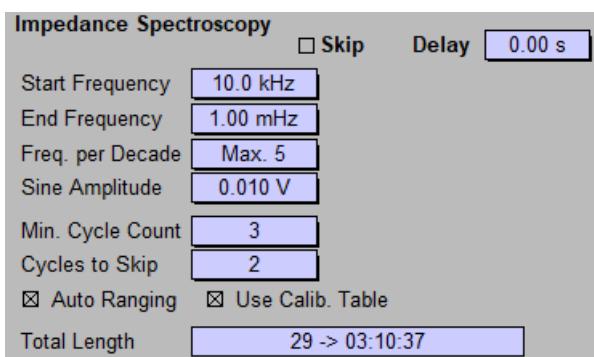
- double layer capacitance: 4.1 kHz (fast)
- faradaic reactions: 310 Hz (medium)
- diffusion of molecules: 0.4 Hz (slow)

Therefore a reasonable frequency range for studying these processes lies between some mHz and some kHz.

Before using the EIS Method you should make sure that the amplifier is calibrated for EIS. Please refer to section **Calibrated EIS in the Amplifier menu** 4.9 on page 36.

12.2.1 Parameters for EIS

The parameters for the measurement can be adjusted in the Protocol Editor Window by clicking on the *E-Chem Method - Impedance Spectroscopy* in the list of operations.



Start Frequency: Enter the first frequency of the impedance spectrum. It is advisable to go from high frequencies to low frequencies in order to be able to review the results quickly. The acquisition of data points for very low frequencies will take a long time.

End Frequency: Enter the last frequency of the impedance spectrum.

Freq. per Decade: You can set a resolution of the impedance spectrum by defining the number of frequencies which are measured per frequency decade. Note that a large number of frequencies increases the measurement time dramatically, especially when the spectrum spans into a frequency range below 1 Hz.

Sine Amplitude: Defines the voltage amplitude of the applied sine wave.

Min. Cycle Count: The number of sine cycles is adapted so that a measurement time of 1 second is filled. If the measurement time per frequency is larger than 1 second, the minimum number of cycles are executed.

Cycles to Skip: Defines the number of sine cycles that are executed but not analyzed. Commonly, the first cycle should be skipped by writing a 1 into the box.

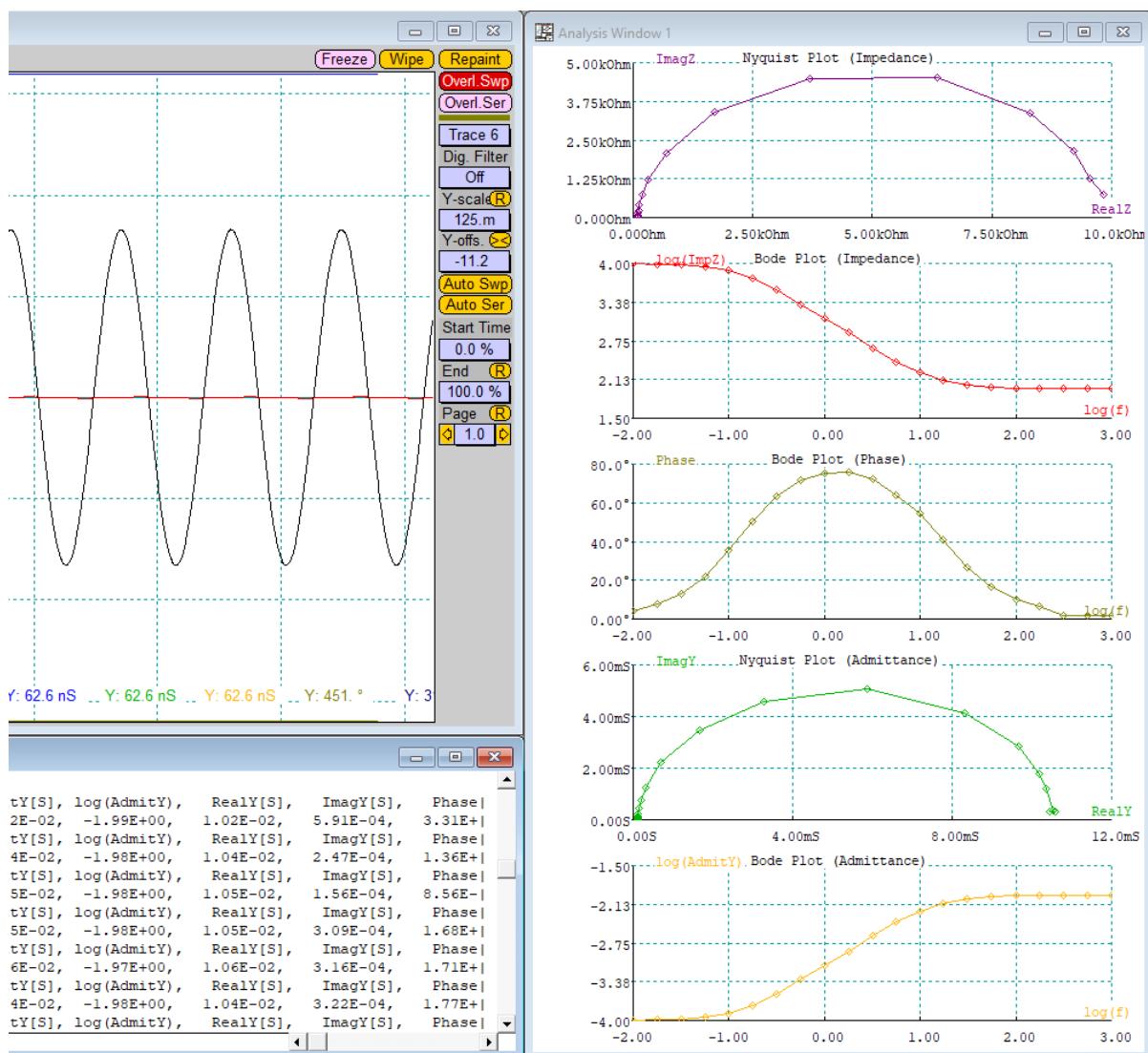
Auto Ranging: If checked the current *Gain* will be adapted so that the signal is between the *Min* and *Max* range given by the *Auto Ranging* controls of the potentiostat.

Total Length: Shows the estimated total time required for the spectrum recording.

The raw data recorded by the *Electrochemical Impedance Spectroscopy* method is stored in the data tree as *Group* with one *Series* per frequency. Each *Series* containing one *Sweep* with the current and voltage *Traces*, and a set of 7 LockIn Traces.

12.2.2 Acquiring an Electrochemical Impedance Spectrum

You start the electrochemical method by executing the protocol, which contains the method.



In the Oscilloscope Window the recording at each frequency is shown versus the time. The method creates a standard *Analysis Method* "EIS", which plots

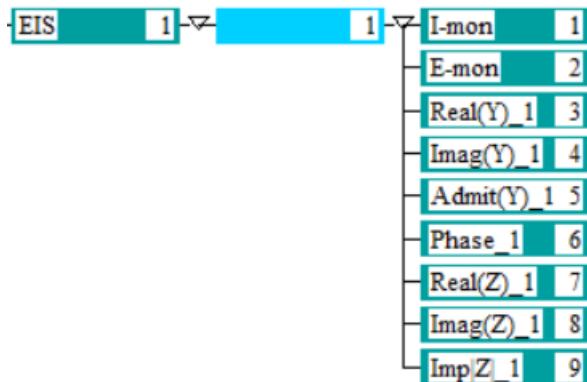
- the Nyquist Plot of the impedance: the imaginary part of the impedance is plotted vs. its real part
- the Bode Plot of the impedance: the impedance is plotted vs. the frequency, both axis have a logarithmic scale
- the Bode Plot of the phase: the phase is plotted vs. the frequency, the frequency has a logarithmic scale
- the Nyquist Plot of the admittance: the imaginary part of the admittance is plotted vs. its real part

12.2. Electrochemical Impedance Spectroscopy (EIS)

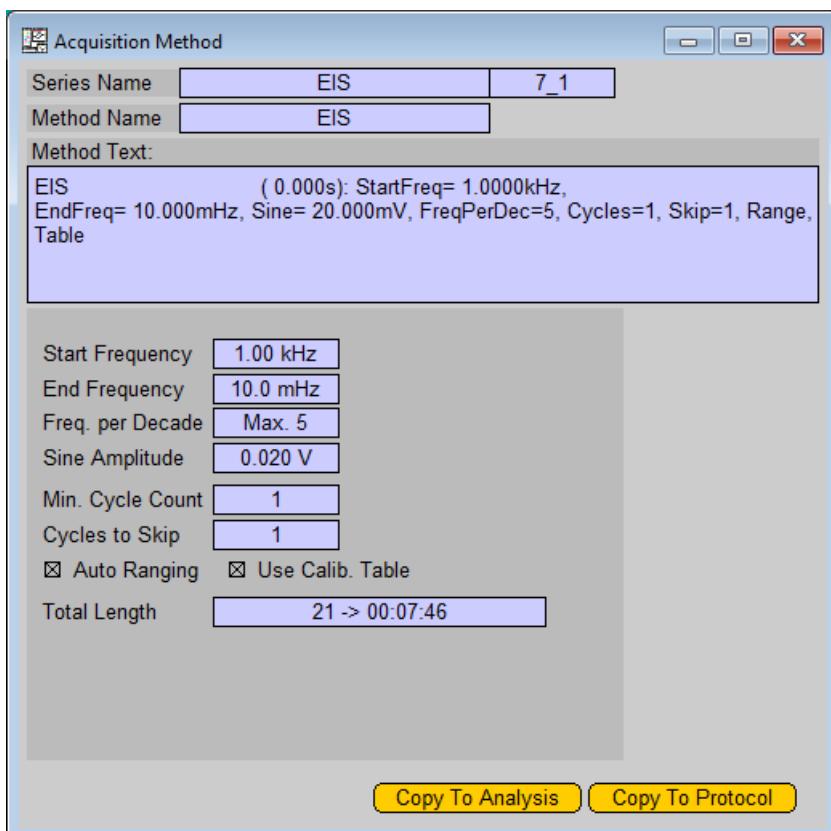
- the Bode Plot of the admittance: the impedance is plotted vs. the frequency, both axis have a logarithmic scale

The impedance format emphasizes the values at low frequency, which typically are of greatest importance for electrochemical systems that are influenced by mass transfer and reaction kinetics. Therefore, Nyquist and Bode plots working with impedance values are used.

The raw data recorded by the Impedance Spectroscopy method are stored in the data tree as *Series* with multiple *Sweeps* containing all the *Traces*.



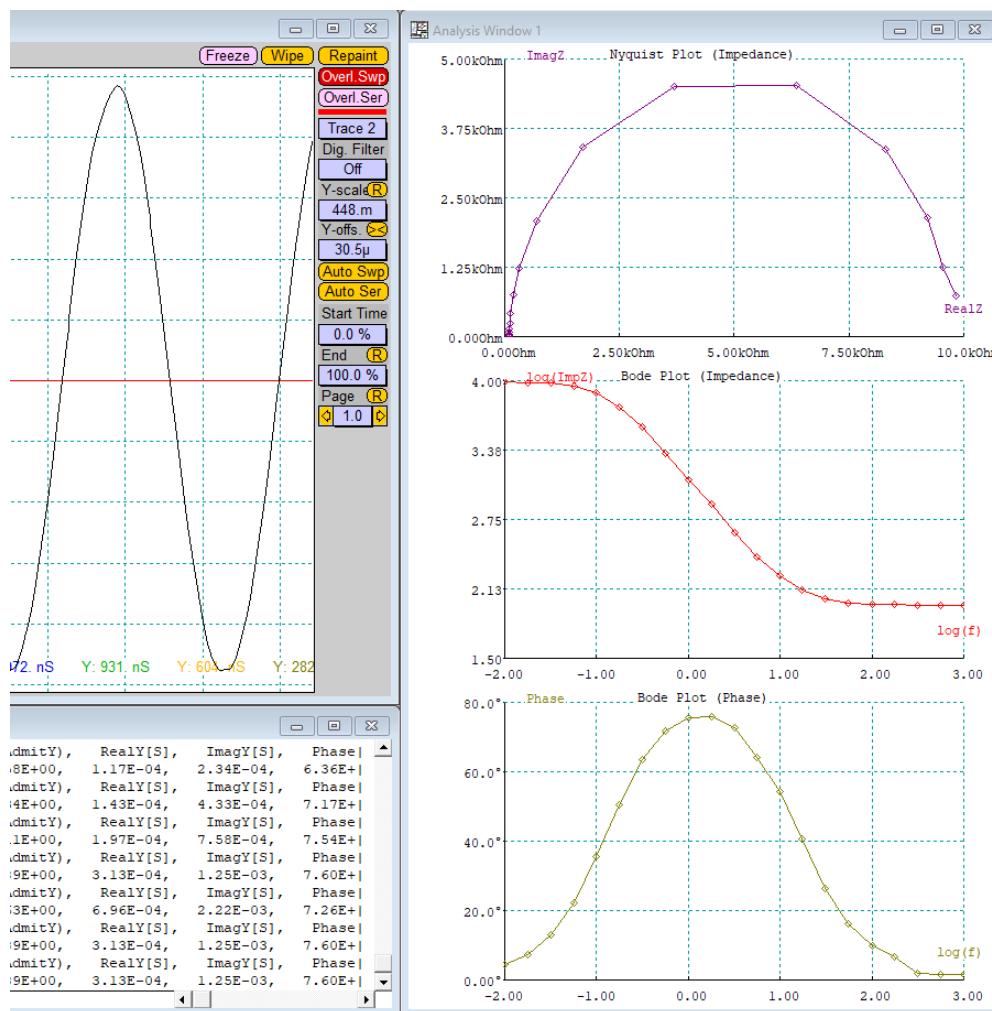
The parameters of the method, which were used during the acquisition of the data, can be viewed in the Acquisition Method window. The window can be opened either via selecting Show Protocol Method in the Replay menu or via selecting Protocol Methods in the Windows menu.



12.2.3 Customizing the Analysis Method

If you want to make changes to the *Analysis Method*, replay the *Series* that contains the EIS data. Then open the *Analysis window* and select the *Analysis Method* "EIS". Press *Copy* to create another method (default name *EIS-1*), where you can make your changes. Remember: The transient *Analysis Method* "EIS" will be overwritten upon repeated execution of all *Protocols* using the *Cyclic Voltammetry Method* and not be saved.

For example, if you are studying faradaic reactions, only plots using the impedance data are of importance. The graphs for admittance plots can be deactivated.

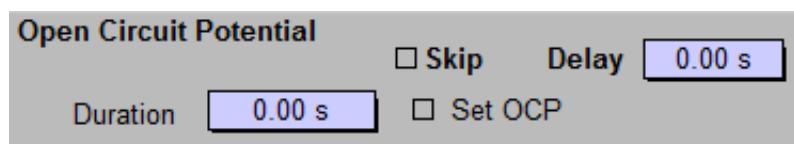


12.3 Open Circuit Potential (OCP)

The Open Cell Potential (OCP) is measured between the working electrode and the reference electrode in order to get information about the electrochemical equilibrium. The OCP during corrosion measurements is known as corrosion potential. The condition of electrochemical equilibrium is current zero in respect to the sum of the cathodic and anodic currents. But this *current less* measurement of a potential is not possible in practice. The current which flows through the instrumentation during the potential measurement has to be kept as small as possible. This is realized by a very high impedance input of the reference electrode ($100\text{ G}\Omega$) and the counter electrode is switched off during the OCP measurement. We measure the potential between two half-cells. The first half-cell is formed by the working electrode and the electrolyte, the second half-cell is formed by the reference electrode and the electrolyte.

12.3.1 OCP Parameters

The parameters for the measurement can be adjusted in the Protocol Editor Window by clicking on the *E-Chem Method - Open Circuit Potential* in the list of operations on the left.

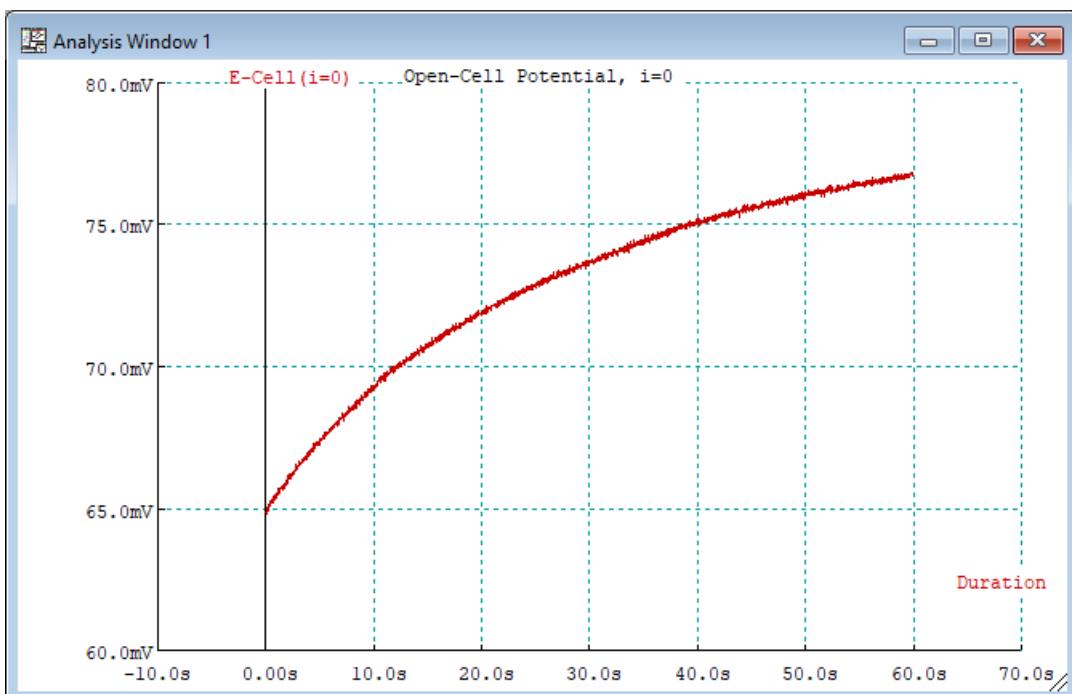


Duration: Enter the duration for the OCP measurement.

Set OCP: If this option is checked, the measured OCP will be set as initial potential (*E-init/V-membr.*). The *Cell Mode* is not changed.

12.3.2 Acquiring the OCP

You start the electrochemical method by executing the protocol, which contains the method.

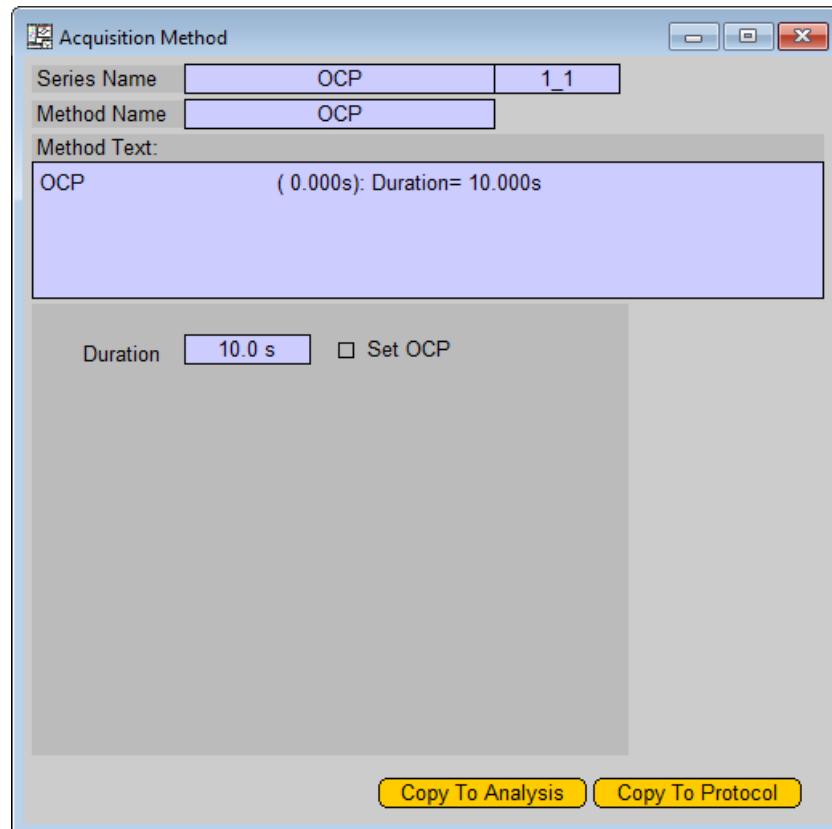


12.3. Open Circuit Potential (OCP)

The Data tree for the OCP is very simple and only shows the recorded potential E-mon as the Trace.

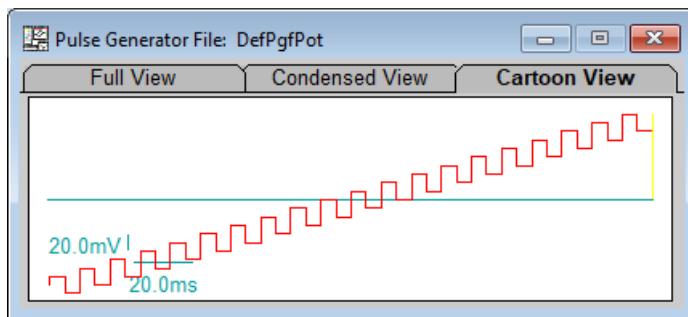


The parameters of the method, which were used during the acquisition of the data, can be viewed in the **Acquisition Method** window. The window can be opened either via selecting **Show Protocol Method** in the **Replay** menu or via selecting **Protocol Methods** in the **Windows** menu.



12.4 Square Wave Voltammetry (SWV)

Square Wave Voltammetry belongs to the pulse voltammetric techniques. In this technique, a linear potential ramp is superimposed by a square wave. The advantage of the pulse voltammetric techniques is their ability to discriminate against charging currents (capacitive currents). Therefore, they exhibit a higher sensitivity for oxidation or reduction currents (faradaic currents) than conventional DC voltammetry. Square Wave Voltammetry yields peaks for faradaic currents instead of the sigmoidal waveform obtained with DC voltammetry which results in improved resolution for multiple analyte systems and more convenient quantification. Square wave voltammetry has received growing attention as a voltammetric technique for routine quantitative analyses. The acquisition of a square wave voltammetry is very fast using scan rates of 1 V/s and higher. A typical experiment can be performed in a matter of seconds.



12.4.1 SWV Parameters

The parameters for the measurement can be adjusted in the Protocol Editor Window by clicking on the *E-Chem Method - Square Wave Voltammetry* in the list of operations on the left.

Start Potential	<input type="text" value="-0.100 V"/>	<input type="checkbox"/> Rel. to V-membr
End Potential	<input type="text" value="0.100 V"/>	<input type="checkbox"/> Keep at End
Amplitude	<input type="text" value="0.010 V"/>	Scan Rate <input type="text" value="1.00 V/s"/>
Tau	<input type="text" value="10.0 ms"/>	Frequency <input type="text" value="100. Hz"/>
		Pot. Increment <input type="text" value="0.010 V"/>
I sampled from	<input type="text" value="80 %"/>	to <input type="text" value="100 %"/>
Keep Traces	<input type="checkbox"/> I-mon <input checked="" type="checkbox"/> I-forward <input checked="" type="checkbox"/> I-diff <input type="checkbox"/> V-mon <input checked="" type="checkbox"/> I-reverse <input checked="" type="checkbox"/> V-mean	
Number of Cycles	<input type="text" value="1"/>	<input type="checkbox"/> Alternate Scan Direction
<input type="checkbox"/> 1 Peak, Given Bounds		Cursor-1 <input type="text" value="-0.100 V"/> <input type="text" value="0.100 V"/>
Total Time	<input type="text" value="200. ms"/>	<input type="checkbox"/> blocking

Start Potential: Potential at which the *Sweep* of the SWV begins. At the start of the SWV the potential steps from the initial potential to the *Start Potential*.

Rel. to V-membr: If checked the potentials will be applied relative to the V-membr..

End Potential: Potential at which the *Sweep* of the SWV ends.

Keep at End: If checked, the end potential will be set as *E-Init/V-membr.* after the *Sweep* has ended.

Amplitude: Voltage amplitude of a square half-wave.

Scan Rate: Overall *Scan Rate* of the potential increase.

Tau: Duration of a single square wave cycle.

Frequency: Frequency of the square wave, equals the inverse of *Tau*.

Pot. Increment: Potential increment from one square wave cycle to the next.

I sampled from: The current will be sampled at the end of each half cycle. The data in-between the cursor positions will be averaged. The result from the first half cycle is *I-forward* and from the second half-cycle *I-reverse*.

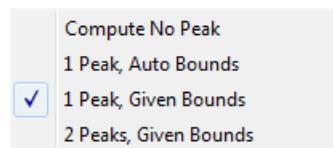
Keep Traces: The user can define which of the result *Traces* should be stored.

- I-mon: Raw data trace of the current recording.
- V-mon: Raw data trace of the voltage recording.
- I-forward: Result trace containing the analyzed current from the first half-cycle.
- I-reverse: Result trace containing the analyzed current from the second half-cycle.
- I-diff: Result trace containing *I-forward - I-reverse*.
- V-mean: Result trace containing the mean potentials each square wave cycle.

Number of Cycles: If the number of cycles is larger than one, multiple *Sweeps* are executed in *Gap Free Mode*.

Alternate Scan Direction: If checked the scan direction will be alternated when more than one *Sweep* is recorded. E.g. the second *Sweep* starts at the end of the first *Sweep* and scans back to the *Start Potential*.

Peaks: The standard analysis allows online peak analysis. The peak of *I-diff* and the corresponding potential will be analyzed.

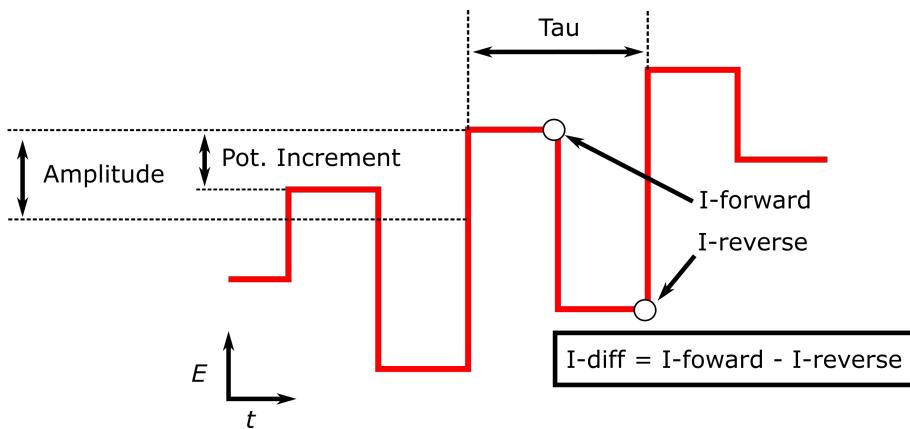


- Compute No Peak: Peak analysis is turned off.
- 1 Peak, Auto Bounds: One peak will be analyzed. The algorithm will use the derivative of *I-diff* in order to find the main peak. A noise free recording is required for this *Analysis Method*. In order to reduce the noise you might apply a digital filter to the *I-diff Trace*. The digital filter is set in the *Oscilloscope* window.
- 1 Peak, Given Bounds: One peak will be analyzed. You can set the voltage range in which the peak should be searched.
- 2 Peaks, Given Bounds: Two peaks will be analyzed. You can set the voltage ranges in which the peaks should be searched.

2 Peaks, Given Bounds	Cursor-1	-0.100 V	0.100 V
	Cursor-2	0.099 V	0.100 V

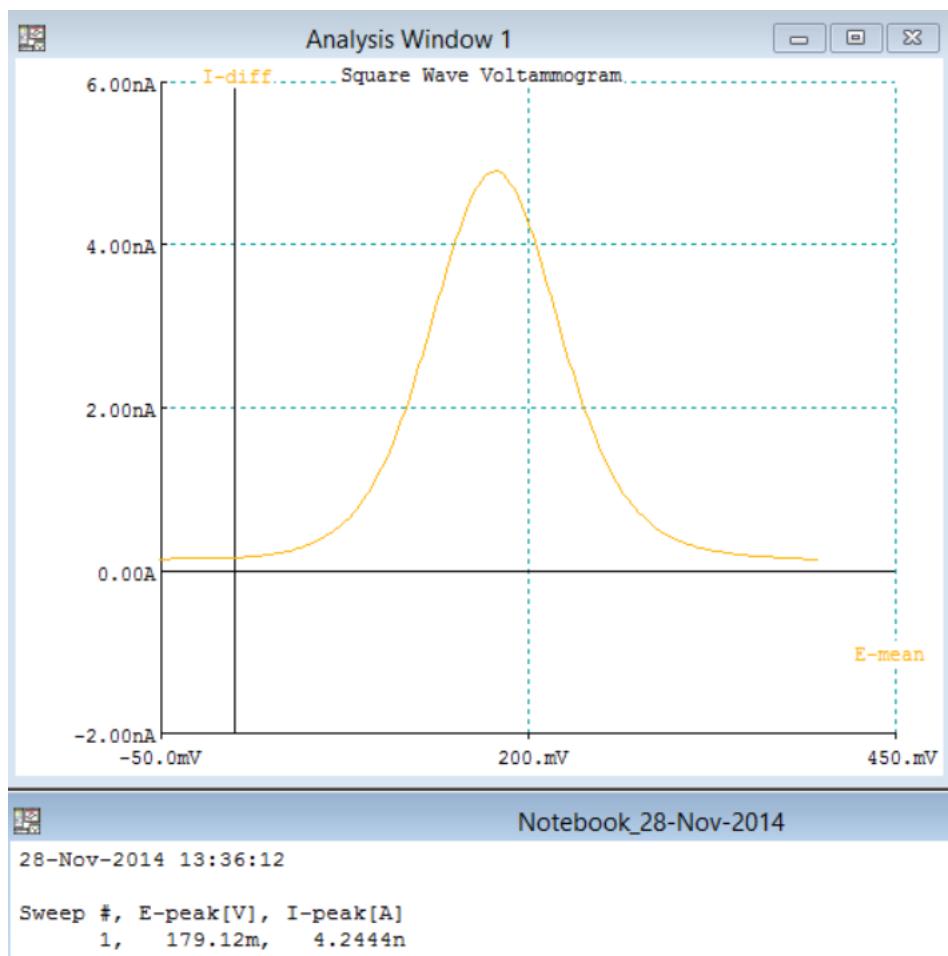
Total Time: Displays the duration of the complete Square Wave Voltammogram.

12.4. Square Wave Voltammetry (SWV)



12.4.2 Acquiring a Square Wave Voltammogram

You start the electrochemical method by executing the protocol, which contains the method.



The method creates a standard *Analysis Method* "SWV", which plots the square wave voltammogram in *Graph 1* in the form of $I\text{-diff}$ vs. $E\text{-mean}$ and analyzes the current peak and the corresponding peak potential. The results are plotted into the *Notebook*.

The raw data recorded by the Square Wave Voltammetry method are stored in the data tree as *Series* with multiple *Sweeps*. Each *Sweep* containing the recorded *Traces*.

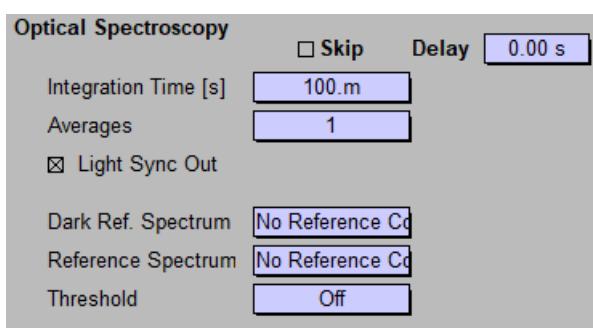


The parameters of the method, which were used during the acquisition of the data, can be viewed in the Acquisition Method window. The window can be opened either via selecting Show Protocol Method in the Replay menu or via selecting Protocol Methods in the Windows menu.

12.5 Optical Spectroscopy

The Optical Spectroscopy method can be used to study the local light absorption properties of materials with high spatial resolution by scanning the sample in the light path. The illumination and detection area are in the range of a few micrometers. It can be employed to study photoelectrocatalysts and semiconductors, where also band gaps can be determined. The spectroscopy experiments are complimentary to and can be combined with other spatially-resolved experiments which can be performed with the ElProScan. The method allows the recording of absorbance spectra between 200 nm and 1200 nm using the designated spectrometer.

Please contact us to get further information about technical requirements to use this application.



The Protocol Editor Method *Optical Spectroscopy* dialog needs certain parameters for the measurement:

Integration Time: The integration time defines the time of collecting data at the spectrometer detector. Typical integration time are between 10 ms and 1 s depending on the light intensity. The intensity of the signal will increase with increasing integration time. For a good signal quality (signal-to-noise ratio) a value of 10k should be achieved. At 80k the limit of the detector is reached and the integration time needs to be reduced.

Averages: A signal which is measured at the certain integration time can be averaged. The number of averages is used to further improve the signal-to noise ratio. Increasing this value does not increase the signal intensity. Integration time and Averages are both used to achieve a good signal quality.

Light Sync Out: This box can be checked if the illumination source should be switched off between recording spectra.

Dark Ref. Spectrum: A dark reference (with illumination switched off) has to be recorded before the spectrum acquisition. In this step the dark reference was loaded into the buffer to correct subsequent spectra. The default is Buffer 1 and has to be selected to retrieve the right data.

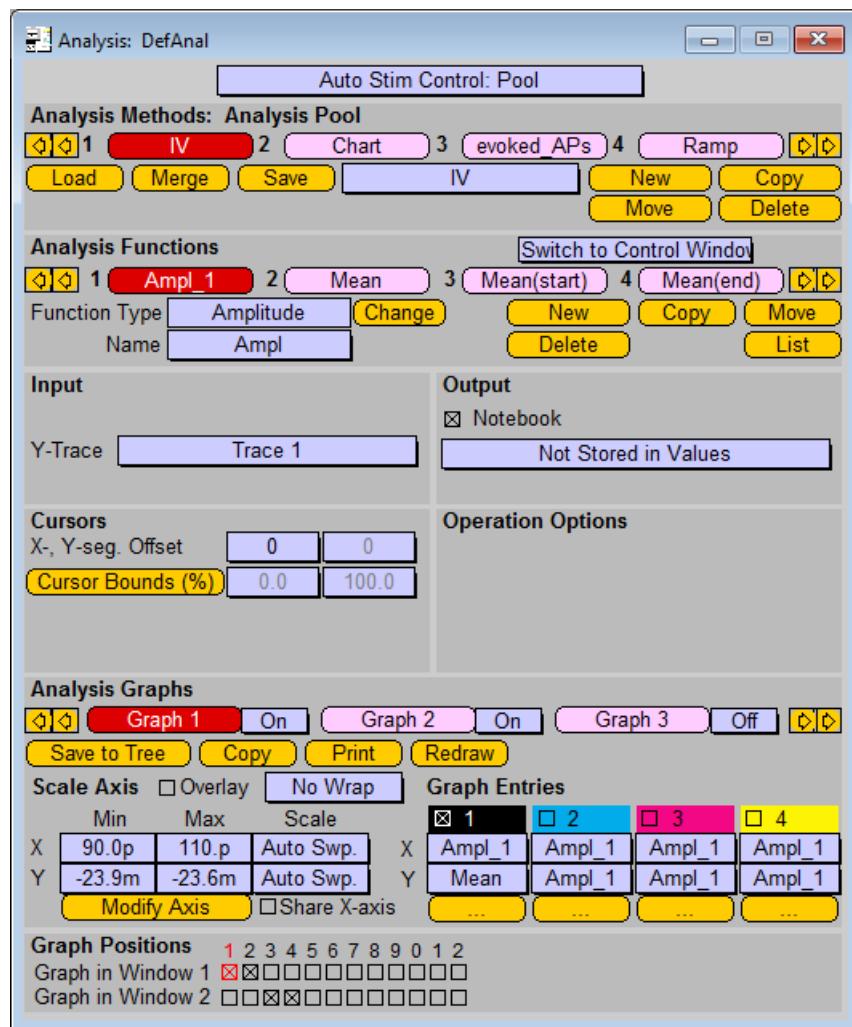
Reference Spectrum: A reference spectrum at a blank spot has to be recorded before the spectrum acquisition. In this step the reference spectrum was loaded into the buffer to correct subsequent spectra. The default is Buffer 2 and has to be selected to retrieve the right data.

Threshold: A threshold can be defined to cut data with very small intensity. Due to the detector, the spectra are automatically recorded between 200 and 1200 nm. When the light source has a smaller spectral range, intensities

12.5. Optical Spectroscopy

can be close to zero in the specific areas. This leads to not-meaningful calculation of absorbance. The threshold can be set to cut these areas from the spectrum.

13 Analysis Window



The Analysis allows you to immediately calculate and display data based on the acquired *Traces*, thus giving you a fast overview of your results.

PATCHMASTER will automatically plot the analysis to Analysis Windows 1 or 2 after or during execution of a Series (based on the settings made in the various controls inside this window).

Nomenclature: The Analysis is structured as follows. An Analysis file with the extension *.onl holds a set of *Analysis Methods*. Each method is an assembly of *Analysis Functions* and *Analysis Graphs*. 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 and/or shown in an *Online Graph* inside either Analysis Window 1 or Analysis Window 2. 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.

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.

13.1 Stimulus Control

Stimulus Control: There are four possible options for controlling the particular *Analysis Method* of choice:

Auto Stim Control: Pool

- Use Selected Method: Uses the active (selected) *Analysis Method*.
- Auto Stim Control: Pool: 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.
- Auto Stim Control: Assigned: Uses the *Analysis Method* stored in and loaded from the data file bundle. With this kind of Analysis one creates a permanent link of an *Analysis Method* to a type of *Series*.
- No Analysis: No Analysis will be performed.

13.2 Analysis Methods



An *Analysis Method* is defined by all the information stored in the areas of *Analysis Graphs* and *Analysis Functions*. PATCHMASTER provides a pool of such *Analysis Methods* and such pools of methods are stored on disk as *Analysis files* (*.onl).

⌚⌚ 1...4 ⌚⌚ : Scrolling bar of available *Analysis Methods* within this pool. The active (selected) *Analysis Method* is highlighted.

Load: Loads a pool of *Analysis Methods*.

Merge: Adds *Analysis Methods* from an *Analysis file* (*.onl) to the already loaded pool of methods.

Save: Saves the actual pool of *Analysis Methods*.

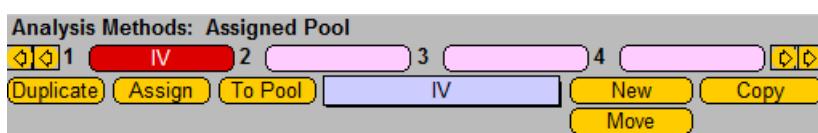
Analysis Method Name: Text field in which the name of the current *Analysis Methods* can be edited – "IV" in this example.

New: Creates a new *Analysis Method*.

Copy: Creates a copy of the active *Analysis Method* and inserts it at the end of the Analysis pool.

Move: Changes the positioning of a particular *Analysis Method* within the scroll bar.

Delete: Deletes the active *Analysis Method*.



If *Auto Stim Control: Assigned* is selected the three buttons *Load*, *Merge* and *Save* are replaced by:

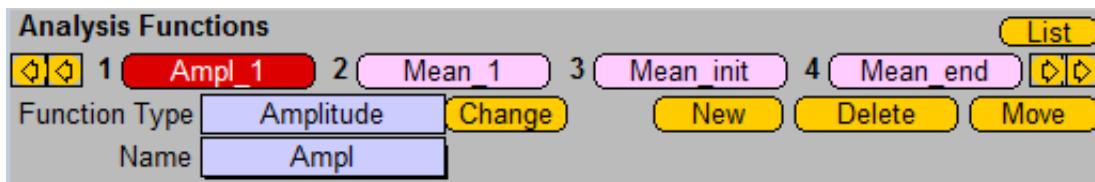
Duplicate: Makes a copy of the currently selected *Analysis Method*.

Assign: Assigns the active *Analysis Method* to the selected *Series* in the data tree. The corresponding PGF template is automatically linked to this *Analysis Method*.

To Pool: Copies the active *Analysis Method* to the standard analysis pool (*Auto Stim Control: Pool*).

13.3 Analysis Functions

In this section of the Analysis dialog, *Analysis Functions* can be added to the *Analysis Method* and parameters of already existing *Analysis Functions* can be modified.



New *Analysis Functions* can be added by clicking on an empty button in the list at the top of the section. For changing an already existing *Analysis Function* just click on the *Change* button (see below).

List: Writes a list with the name of all *Analysis Functions* into the Notebook window.

1...4: Scrolling bar of an arbitrary number of available *Analysis Functions*. The buttons contain the Name of the defined function.

Function Type: Here the *Function Type* of the presently activated *Analysis Function* is displayed, e.g. "Amplitude".

Name: Here you can rename the selected *Analysis Function Type*.

Change: Click on this button to open the *Function Type* dialog for changing the *Analysis Function*.

New: Creates a new *Analysis Function*. You can also create a function by clicking on an empty *Function* button.

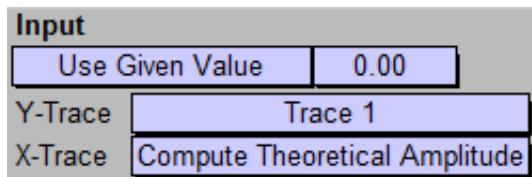
Delete: Deletes the selected *Analysis Function*.

Move: Changes the position of a particular *Analysis Function* within the scroll bar.

13.3.1 Analysis Function Settings

The Analysis provides four sections for customizing the selected *Analysis Function*. Here, we describe which settings are in general available. The availability depends of course on the selected *Analysis Function* and may vary from function to function.

Input

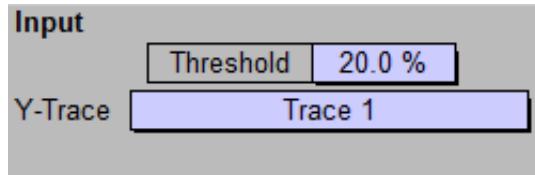


Value: Either use "Use Given Value" entered into the field beneath the function or "Get Value-1...16" which takes the value stored in *Value-1...16*.

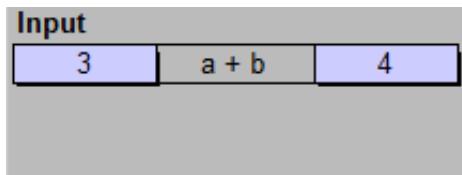
Y-Trace: Select which *Trace* (1...16) or *Buffer* (1...4) shall be analyzed using the *Analysis Functions*. The *Trace* index usually corresponds with the channel number in the PGF sequence (if the *Trace* number is not set to another value in the build instruction of the *Trace* (see 10.9.2 on page 127)).

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...16, Amplitude from Buffer 1...4*).

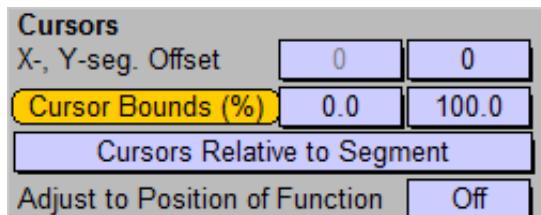
Threshold: Sets the *Threshold* value for one of the *AP Analysis* functions.



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'.



Cursors



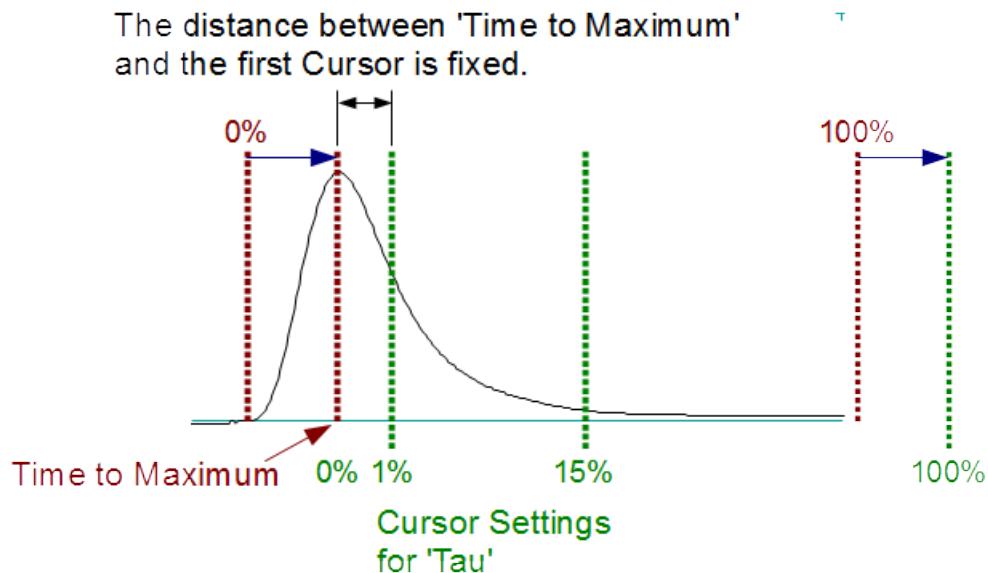
X-, Y-seg. Offset: The X- and Y-Segment offsets are set relative to the *Relevant X- and Y-Segments* as defined in the Pulse Generator window.

Cursor Bounds: Within the relevant segment, a left bound and a right bound (in %) determine the actual time period for analysis. 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 fields to the right of the button. 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:

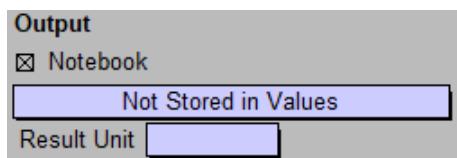
- Cursors relative to Segment
- Cursors relative to Trace
- Cursors: Copy from Function-1...16: The active function gets the cursor bounds and cursor type from the selected function.

Adjust to Position of Function: Specify an existing *Analysis Function* to set the cursor bounds relatively 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 Position of 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.



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.

Output

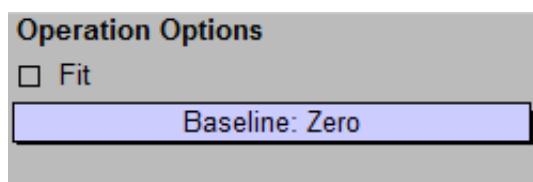


Notebook: If selected, the *Analysis Result* of this function will be written into the *Notebook* window.

Not Stored in Values / Store in Value-1...16: *Analysis Results* can be stored in one of the *Values-1...16*. They can then be retrieved and used as a *Constant* math function. In addition, they can be used in the *Protocol Editor* (see *Set Value*, 11.4.5.3 on page 159). The *Values-1...16* are used to exchange information between different *Analysis Methods* and between the *Analysis* and the *Protocol Editor*.

Result Unit: If e.g. a *Math* operation was used one can define via this entry box the unit of the output value.

Operation Options



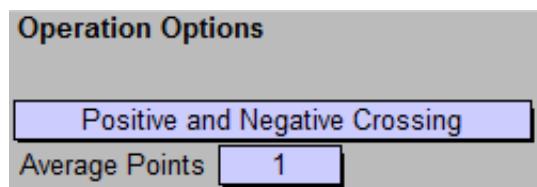
Fit: If selected, the peak value is determined by a polynomial fit around the first numeric estimate of the peak. With this function, the influence of noise can be reduced in the determination of *Peak Amplitudes*.



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 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: Define the *Baseline* 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.

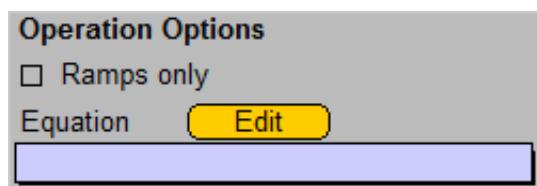


Crossings: Set the direction of the *Crossing*. There are three options available:

- 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.

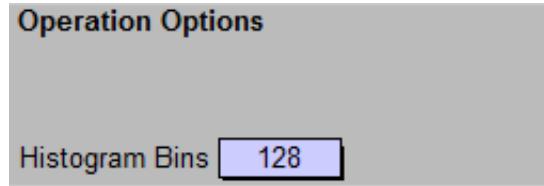
Average Points: Enter the number of points to be used for a running average to apply before extracting the voltage. The function applies to the source *Trace* as well as to the result *Trace*.

Ramps only: The *Analysis Function* does only work on *Ramp* segments if the checkbox is selected.



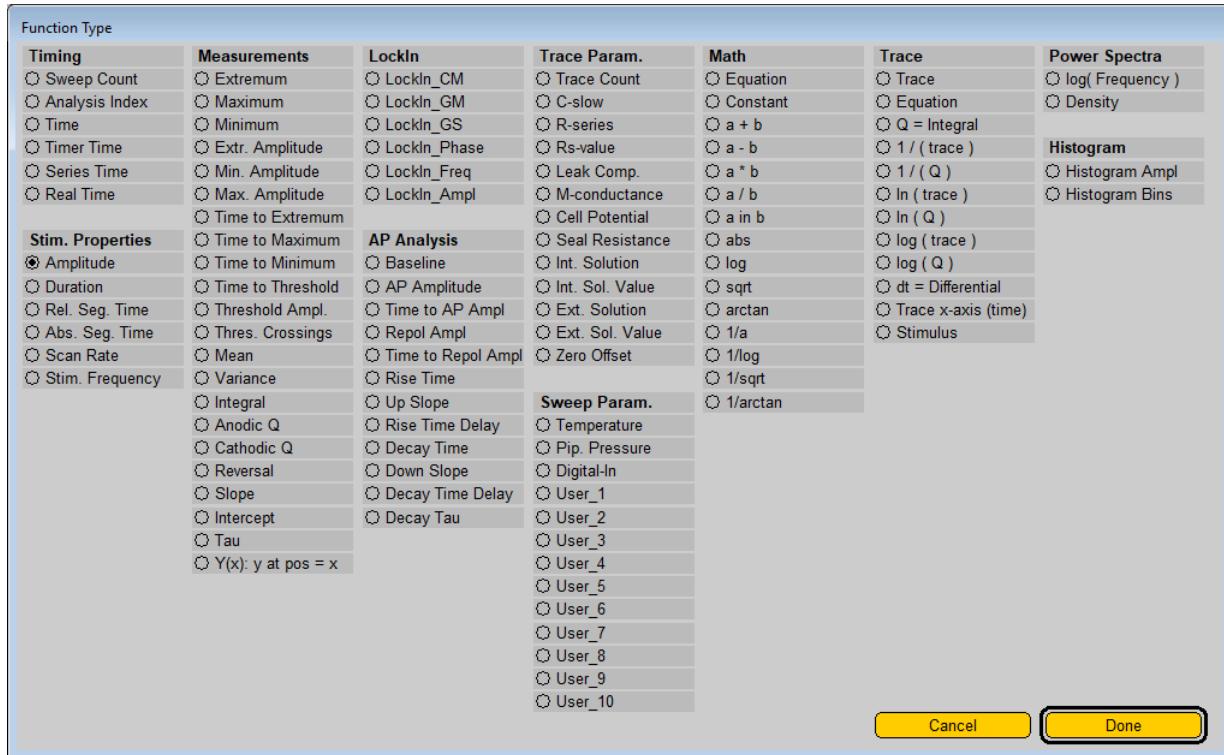
Equation: Enter an equation into the text field or modify an already existing one by clicking on the *Edit* button.

Histogram Bins: Enter the number of bins for the histogram.



13.3.2 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 in several groups.



13.3.2.1 Timing

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. The maximum number of Analysis Sweeps that can be handled, can be set in the Configuration window (see chapter 5.6.2 on page 58).

Time: Time starting with the relative "first sweep" analyzed (or created) since the last *Wipe Analysis* 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.

13.3.2.2 Stim. 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.

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. Frequency: Returns the set *Stimulation Frequency* of a corresponding *Sine Wave* or *Square Wave* segment.

13.3.2.3 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. $\text{abs}(1/\text{slope})$

13.3.2.4 Lock-In

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*.

LockIn_CM: Capacitance taken from the *LockIn* Extension.

LockIn_GM: Membrane conductance taken from the *LockIn* Extension.

LockIn_GS: Series conductance taken from 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.

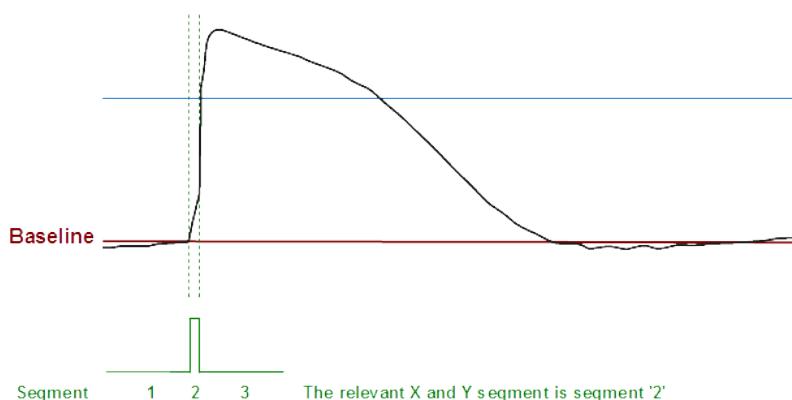
LockIn_Ampl: Returns the amplitude of the sinusoidal wave used for *LockIn* measurements.

13.3.2.5 AP Analysis

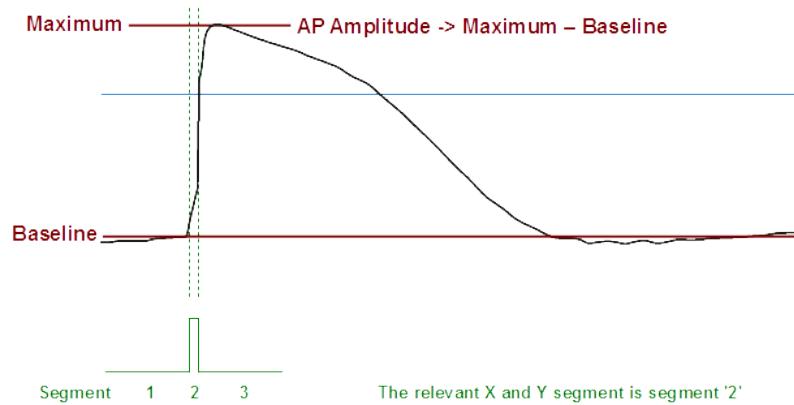
The *AP Analysis* is designed for analysis of **evoked** action potentials. The following prerequisites are essential:

- 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*.

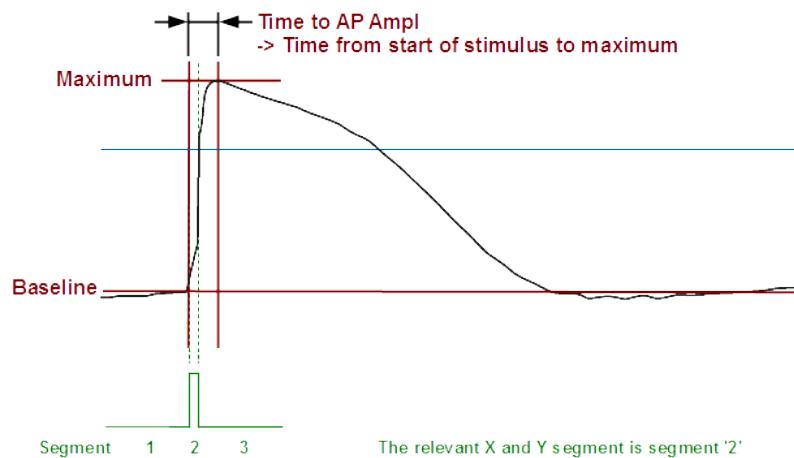
Baseline: Or resting potential, measured as the mean amplitude of the first segment (baseline segment).



AP Amplitude: Amplitude of the action potential.

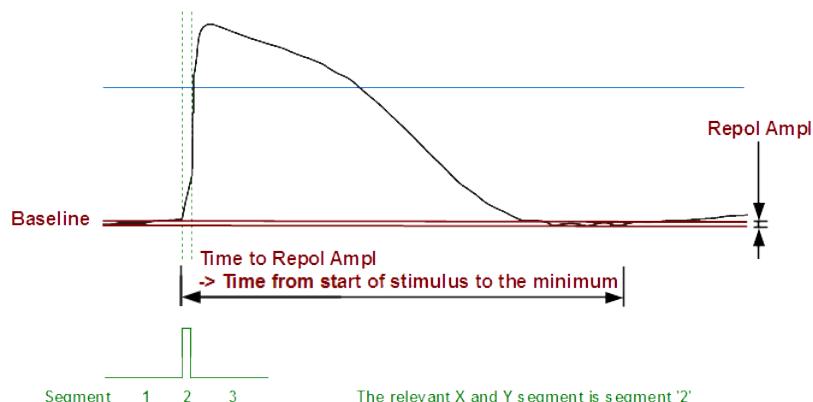


Time to AP Ampl: Time from start of the stimulation segment to the maximum of the action potential.



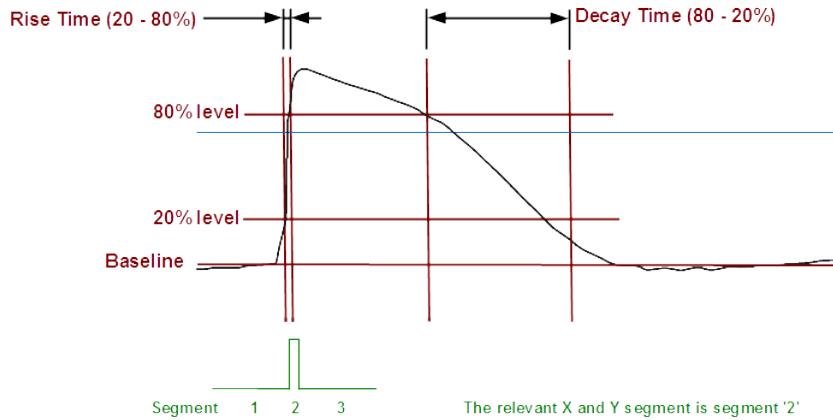
Repol Ampl: Repolarization Amplitude.

Time to Repol Ampl: Time from start of the stimulation segment to the minimum of the action potential.



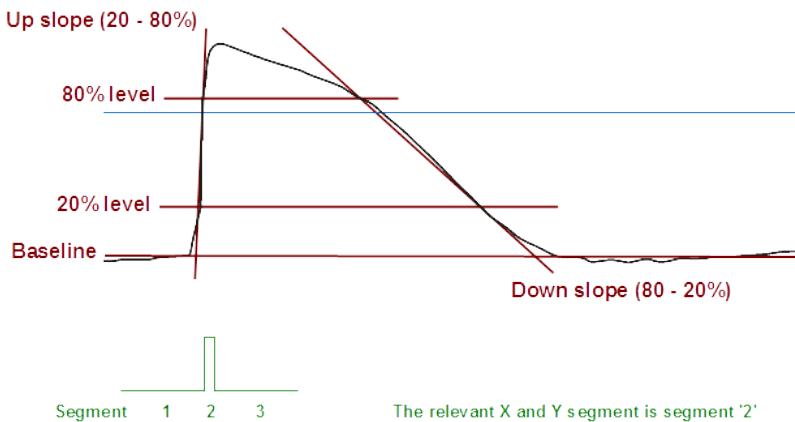
Rise Time: Time between the low level and up level on the rising edge of the action potential. The low level can be entered as parameter (*threshold*). The upper level is then given by 100 % - *threshold*.

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*.



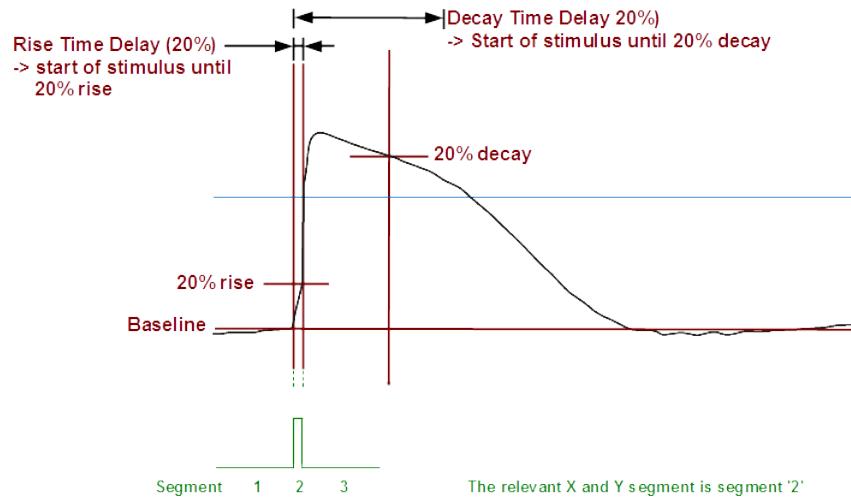
Up Slope: Slope between the two levels on the rising edge of the action potential. The low level can be entered as parameter (*threshold*). The upper level is then given by 100 % - *threshold*.

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 % - *threshold*.

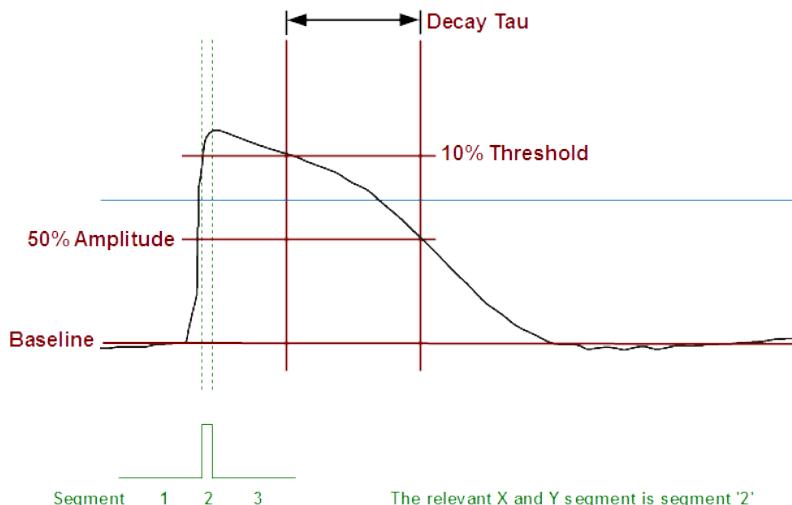


Rise Time Delay: Time between the start of the stimulation and start of the rise of the AP.

Decay Time Delay: Time between start of the stimulation and begin of the AP decay.



Decay Tau: Calculates the exponential decay time constant (τ) between the defined threshold and 50 % of the amplitude.



13.3.2.6 Trace Parameters

The following parameters can be retrieved for analysis from the parameter set that is stored with the data (see Parameter window, 14 on page 211).

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 *Sweep*.

R-series: Series resistance value taken from each *Sweep*.

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 (see EPC 10 menu, 4.9 on page 36). 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.

Pip. Pressure: Pipette pressure (from an application device).

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.

13.3.2.7 Sweep Parameters

The following parameters can be retrieved for analysis from the parameter set that is stored with the data (see Parameter window, 14 on page 211).

Temperature: Temperature (from a recording device).

Digital-In: State of the Digital-In port of EPC 10 amplifiers.

User_1 - User_10: Parameter as set by the user in the I/O Control section of the Configuration window, 5 on page 43.

13.3.2.8 Math

Math supplies various calculations on one or two function results. By combining several *Math* functions, complex expressions can be generated.

The following control shows: "Result 1" "- Operand" "Result 2".



Equation: An equation can be entered to compute a value. For details on the equation syntax please refer to the section *Equation Syntax*, 20.1 on page 240.

$Y(x) : y \text{ at } pos = x$: 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.

Constant: Sets a constant value if *Use Constant Value* is selected. 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 $Y(x) : y \text{ at } pos = x$ above.)

a + b: Calculates the sum of two Analysis results.

Note: Depending on the Math function one or two results of the Analysis 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.

13.3.2.9 Trace

This analysis is applied to a selected *Trace*. The result is again a *Trace*.

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 the section [Equation Syntax, 20.1 on page 240](#).

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 [Online Window](#).

Stimulus: The stimulus that is linked to the *Trace* is returned.

13.3.2.10 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.

log(Frequency): Returns the log(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(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 ' $1 \cdot 10^{-25}$ '. The unit is in A^2/Hz .

13.3.2.11 Histogram

The histogram functions can be used e.g. for a fast online detection of different amplitude levels.

Histogram Ampl: Returns the *Histogram Amplitude* distribution. You can select the *Trace* from which the amplitude should be calculated.

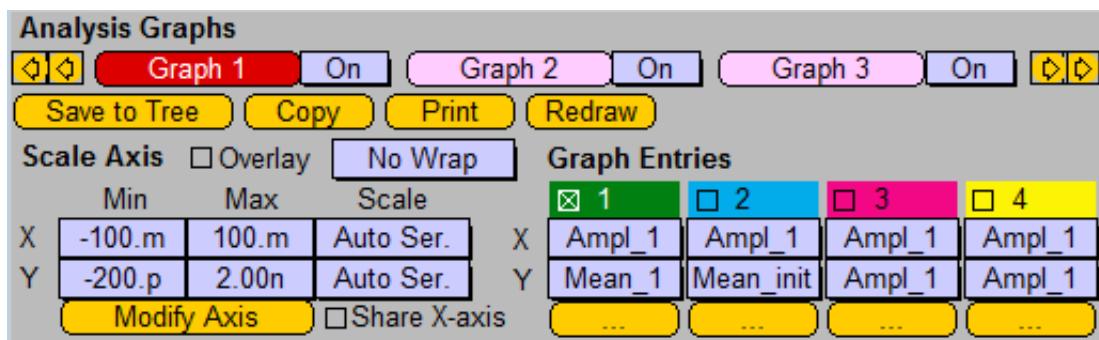
Histogram Bins: Defines the number of *Histogram Bins* for the analysis of *Histogram Amp*. The number of bins is entered manually in the *Function Type* window:

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 mode it is the largest Y-range of all analyzed Traces of the parent Series.

13.4 Analysis Graphs



Here, the properties of the graph display for the Analysis results are defined.

Graph 1, Graph 2, Graph 3 - Scrolling bar of *Analysis Graphs* (currently to a maximum of 12 graphs). All information shown in the lower part of this panel is only valid for the highlighted graph.

Graph 1 On (Graph Display Mode): A graph can either be turned *On* or *Off*.

- On: Displayed in Analysis Window 1 or Analysis Window 2.
- Off: Not displayed.

Save to Tree: Saves the active *Graph Entries* of the selected *Analysis Graphs* to the end of the data tree as a new Series.

Copy: Allows to automatically copy the settings (functions, scaling etc.) of a graph into the selected graph, rather than having to enter each entry manually. Enter the index number of the graph from which you want to copy the settings.

Print: Prints the Analysis results as displayed.

Redraw: Redraws the Analysis results displayed in the Analysis Windows, e.g. after changing the axis scaling.

13.4.1 Scale Axis

Overlay: The default setting in the Analysis is to wipe the data before a new Series is started. If you mark the *Overlay* option, the graph plot in the Online window will not be cleared when performing a new analysis (new Series acquisition).

Scale Axis			<input type="checkbox"/> Overlay	<input checked="" type="checkbox"/> No Wrap
	Min	Max	Scale	
X	-100.m	100.m	Auto Ser.	
Y	-200.p	2.00n	Auto Ser.	
	Modify Axis			<input type="checkbox"/> Share X-axis

Note: This setting is overruled by the setting *Wipe Display at Start* and *Wipe at Start* (see 11.4.2.3 on page 150 and 5.6.2 on page 58).

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.

- *No Wrap*: No adaption of the X-axis scaling when the amount of data points exceeds the X-axis scaling.
- *Wrap + wipe*: Wiping of the online graph after the wrapping around. This behavior is typically used during Chart Recording (see PATCHMASTER Tutorial).
- *Wrap, no wipe*: No wiping of the online graph after wrapping around. Old data points are overlayed by newer data points when the Series exceeds the length of the graphical display.

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.

If both *Overlay* and *Wrap* are selected, the data will wrap around at the end of the graph without clearing the graph.

More complex and custom display behavior can be controlled from a protocol, e.g. wiping individual graphs only, by using the Analysis protocol event (see 11.4.4.1 on page 153).

In case *Trace* data are displayed in the graphs, the options *Overlay* and *OvrlSwp* are available.

Scale Axis			<input checked="" type="checkbox"/> Overlay	<input checked="" type="checkbox"/> OvrlSwp
	Min	Max	Scale	
X	-3.40m	120.	Auto Swp.	
Y	-877.n	40.5μ	Auto Swp.	
	Modify Axis			<input type="checkbox"/> Share X-axis

- *No selection*: If neither *Overlay* nor *OvrlSwp* is selected the online graph is wiped after every Sweep acquisition.
- *Overlay*: General overlay, see also description above. Overlays Sweeps of different Series. Compare to *Overl. Ser* in the Oscilloscope window.
- *OvrlSwp*: *Overlay Sweep* function overlays all Sweeps within a Series. Compare to *Overl. Swp* in the Oscilloscope window.

Note: The autoscale functions *Auto Ser.* and *Auto Swp.* are incompatible with the *OvrlSwp* function. Only the last Sweep will be drawn after re-scaling the graph.

X, Y: Here you can enter the analysis display settings.

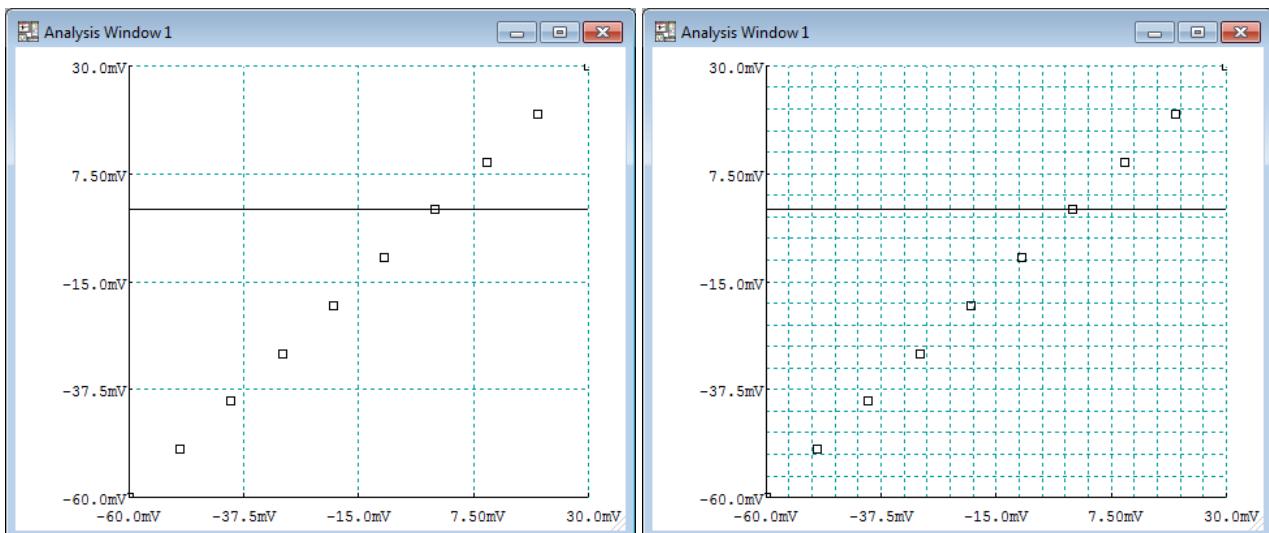
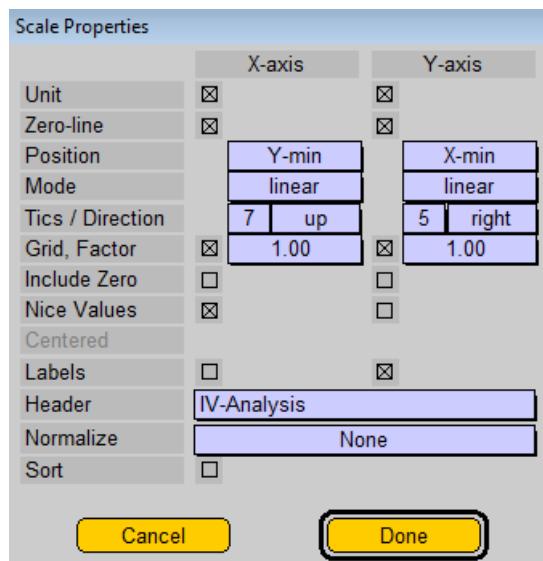
- *Min*: Minimum of axis range.
- *Max*: Maximum of axis range.
- *Scale*: There are several possible scaling options available once you click on the *Scale* field.

13.4. Analysis Graphs

- Fixed: Analysis results can be shown in the graph immediately after acquisition or replay of a *Sweep*, if the scaling is known from the beginning. Therefore, a *Fixed Scaling* option is provided and the X-, Y-scaling of the analysis graph can be specified.
- Auto after a Series: After all *Sweeps* of a *Series* have been acquired or replayed, the maximal and minimal values of the currently selected abscissa and ordinate values are determined and used to scale the graph.
- Auto after each Sweep: After every *Sweep*, the maximal and minimal values of the currently selected abscissa and ordinate values are determined and used to scale the graph.
- Fixed with last Min/Max: Uses the maximum/minimum from the last graph drawing to copy into the *Min/Max* fields. They can now be used as scaling factors when the mode is switched to *Fixed*.

Modify Axis: Clicking on this button opens the Scale Properties window.

- Unit: The units of the first graph entry are shown on the graph display.
- Zero-line: The zero-line is depicted in the online graph.
- Position: *Y-(X)-min*, *Y-(X)-zero*, *Y-(X)-max*.
Determines the position of the axis either at the minimum, at zero or at maximum of the other axis, respectively.
- Mode: Defines how the respective axis is drawn:
linear, log, inverse, sqrt, square.
- Tics / Direction: Sets the number of tics plotted on the axis. A value of zero suppresses the display of axis tics.
Direction up, down, both determines in which direction the tics point.
- Grid, Factor: A grid will be displayed in the given *Analysis Graph*. Enter a *Factor* for the grid density. If *Factor* is set to "1" then one line per *Tic* is drawn. A factor <1 increases the grid density.



- Include Zero: The Y- and X-axis always contains "0" at their origin independent of wrapping events.

- Nice Values: If selected, the axis limits will be rounded to the next possible "nice" value (e.g. 0, 1, 2, 5...).
- Centered: If selected, the first 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.

Note: This option is only available if Fixed Scaling is on and if Nice Values is off.

- Labels: If selected, the name of the *Analysis Function* is used as label for X- and/or Y-axis
- Header: Text for the graph header can be entered.
- Normalize: Select the type of normalization of the data:
 - None
 - $y' = y / \text{max}$
 - $y' = (y - \text{min}) / (\text{max} - \text{min})$
- Sort: If selected, the data will be sort the data according to ascending X-values.

Note: The Sort flag should be set for summary data and non-ordered, non-continuous Traces before performing the Save to Tree operation.

Share X-axis: If Share X-axis is checked, then the same X-axis parameters (e.g. "Min", "Max", "Scale") are used for all graphs

13.4.2 Graph Entries

1...4: Activate up to four graph entries from this list by checking the box.

Axis: Now choose from the list of available analysis result variables for the X- and the Y-axis. An example could look like that shown on the right in the diagram above.

Graph Entries			
X	1	2	3
Y	Timer	Timer	Timer
	C-slow_1	TraceX_1	Trace_1

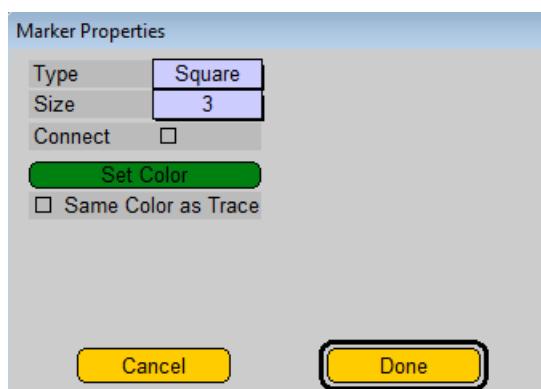
			...

The following variables are shown in the figure beneath:

- Max_1: Maximum value between two cursor of *Trace 1*.
- Timer: Time of the *Timer*.
- C-slow_1: C-slow value of *Trace 1*.
- Int_1: Integral value between two cursors of *Trace 1*.
- 5...16: Empty item.
- Select by index...: Select the variables by their index number. This is necessary when more than 16 analysis results are defined.



Clicking on opens the Marker Properties window:



Type: Choose between the available symbols (point, plus, star, diamond, cross, square).

Size: Size of the symbols.

Connect: If selected, the symbols will be connected with a line.

Set Color: Choose a color for the symbols and the lines.

Same Color as Trace: Use the *Trace* color for the symbols and the lines. The *Set Color* setting is ignored.

13.5 Graph Positions

Graph Positions	1	2	3	4	5	6	7	8	9	0	1	2
Graphs in Window 1:	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Graphs in Window 2:	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>								

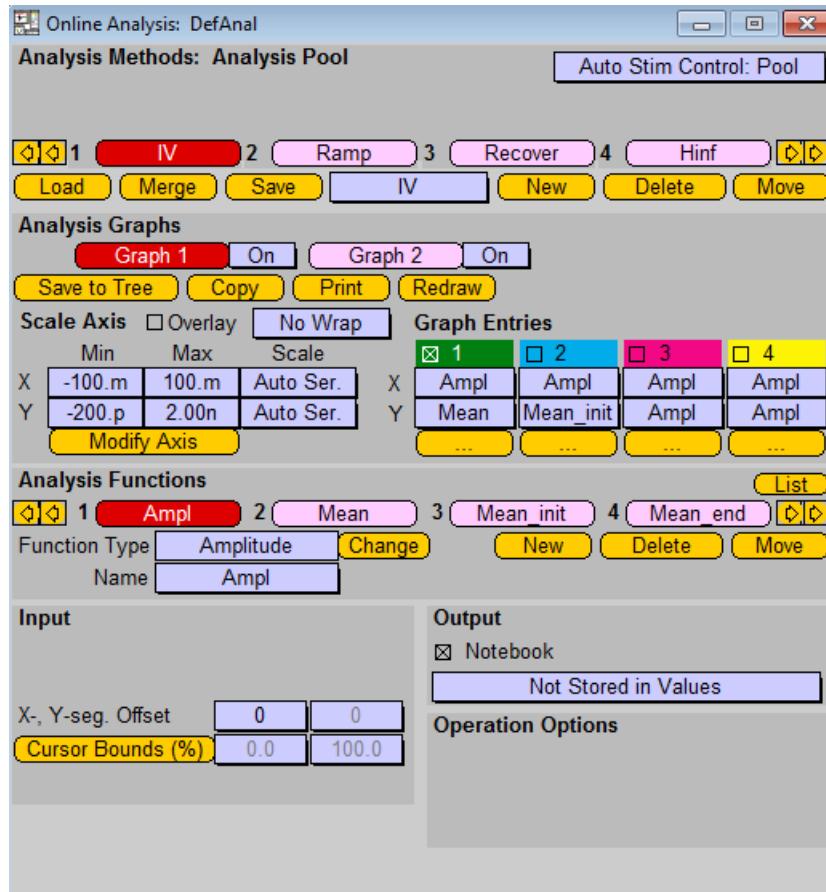
Graph Positions: In this section of the Analysis dialog, one can specify if and to what Analysis window (1 or 2) a graph is plotted. The currently selected graph (in the *Graph Entries* section) is indicated by red color.

Note: If a graph is not checked in the Graph Positions section, then it is not displayed, even if it is turned on in the Graph Entries section.

13.6 Multi-Channels Analysis

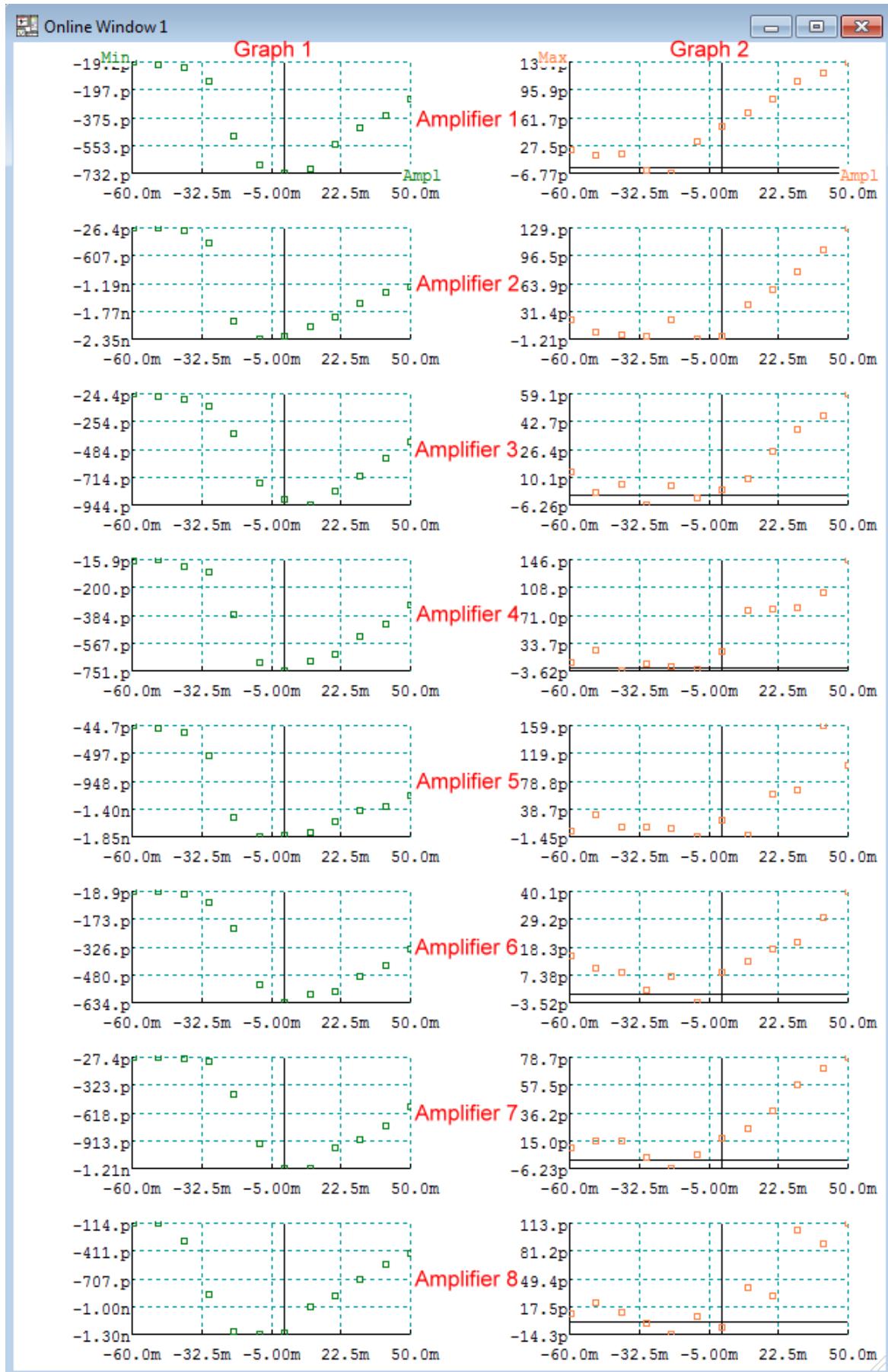
The *Multi-Channels* option facilitates the Analysis when using multiple amplifiers, e.g. EPC 10 Double, Triple, Quadro or 2x Quadro. Therefore, the user should keep in mind that the workflow is different.

Note: When using Multi-Channels only 1 AD channel (Trace) per amplifier is allowed.

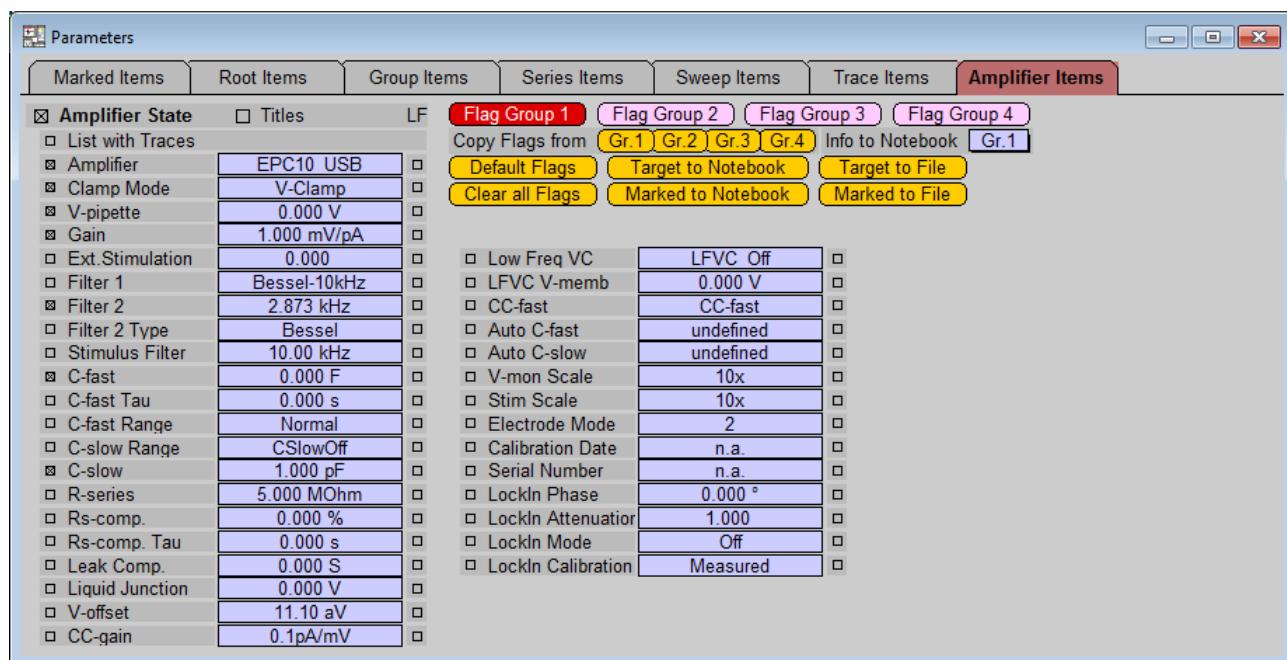


In the Analysis only 2 graphs per amplifier/*Trace* are available. Graph 1 and 2 of a *Trace* are arranged in a row, thus graphs 1 and 2 from the amplifiers are arranged in a column (see figure below).

Instead of defining every graph for every amplifier *Trace*, the user has to define the *Analysis Functions* for graph 1 and graph 2 only once. Subsequently, all graphs for all amplifiers are created automatically.



14 Parameters Window



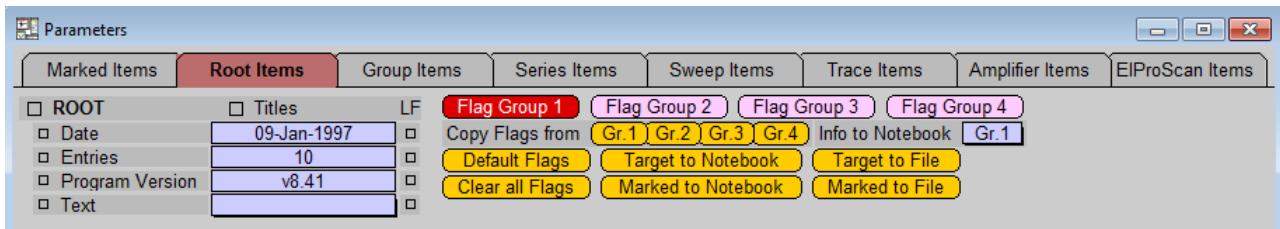
Parameters are additional values describing the experimental and measurement conditions. The *Parameters* are required to interpret the data correctly. In PATCHMASTER we distinguish different types of *Parameters* depending to which level of the data structure they relate.

Note: If possible PATCHMASTER will use internal values to set the Parameters. E.g. C-slow values will be automatically read from the amplifier and stored with the Parameters. For some Parameters it might be necessary to assign a source (e.g. AD channel or manual input) in the I/O Control section of the Configuration window. For other amplifiers than EPC 10 or EPC 9 most of the external Parameters have to be entered manually via the I/O Control window.

In the *Parameter* window either the *Parameters* that are saved with already acquired data, or the *Parameters* of the last acquisition (stored or not stored) are displayed.

14.1 Parameter Tabs

14.1.1 Root Items



Level 0 of the data tree. The *Parameters* of the *Root* level are:

- Date: Date at the time of acquisition.
- Entries: Number of *Groups* in the file.
- Program Version: Version number and date of PATCHMASTER.
- Text: A descriptive text can be entered and stored if the data file is opened in the *Open Modify...* mode.

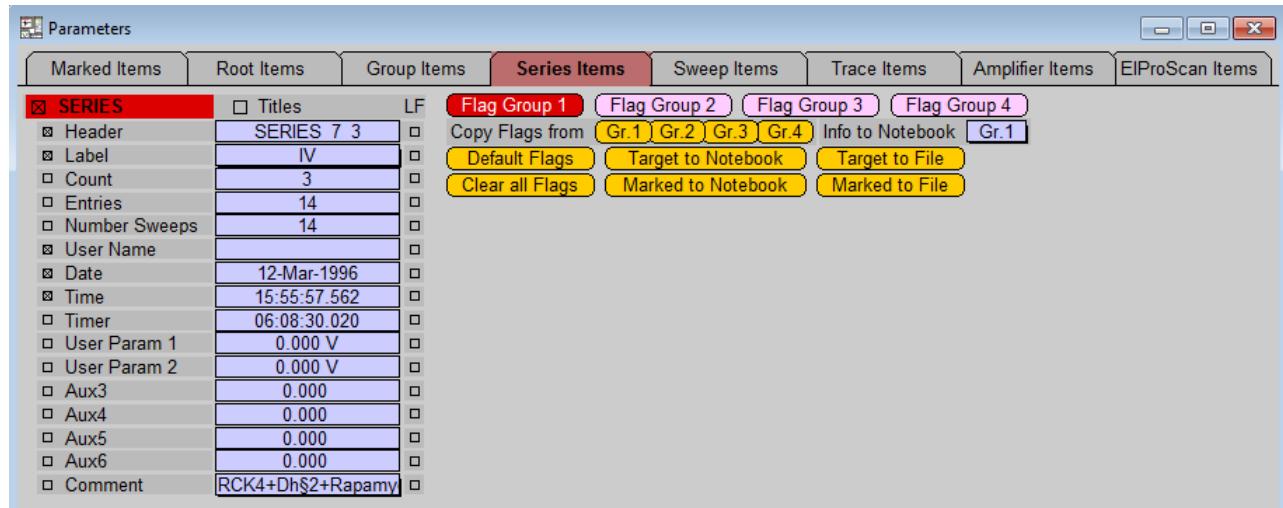
14.1.2 Group Items



Level 1 of the data tree. The *Parameters* of the *Group* level are:

- 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.
- MatrixWidth: Width of the acquired image in the *Matrix Scan* mode (only for data acquired with the EL-PROSCAN).
- MatrixHeight: Height of the acquired image in the *Matrix Scan* mode (only for data acquired with the ELPROSCAN).

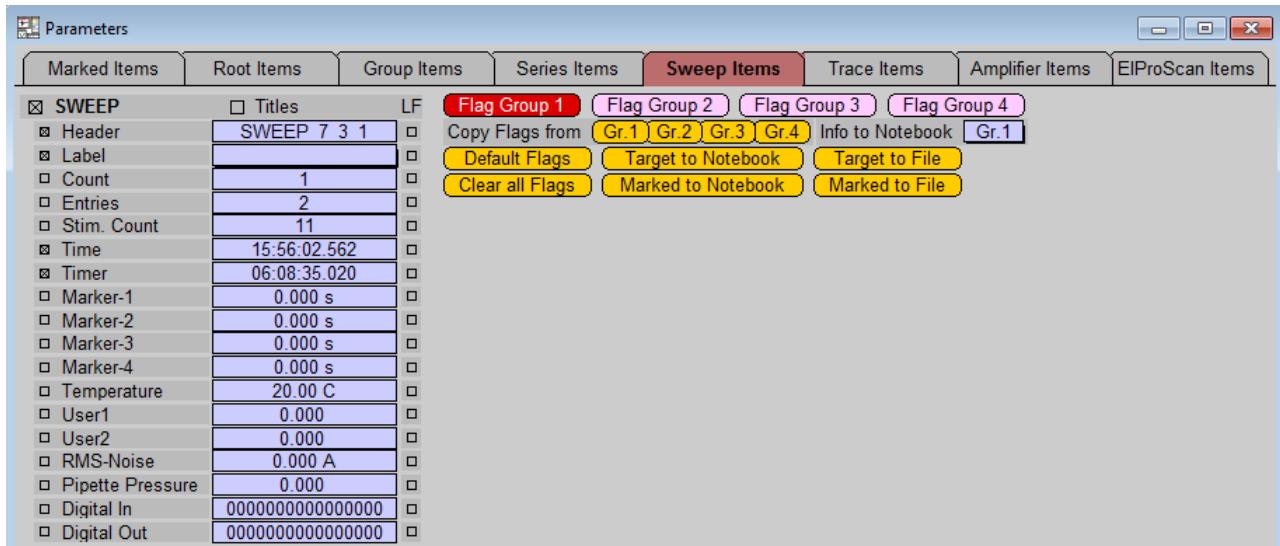
14.1.3 Series Items



Level 2 of the data tree. The *Parameters* of the *Series* level are:

- Header: Index number of the *Group* and the *Series* (e.g. SERIES_1_4).
- 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 (5.3.5 on page 47).
- Date: Date at the time of acquisition.
- Time: Time of the acquisition.
- Timer: Timer time of the acquisition.
- User Param 1/2: User-defined external values (see Configuration, 5.7.2 on page 64).
- Aux3...6: Auxiliary Parameter fields only used when certain extension are switched on.
- Comment: Text identifier (see Control window, 8.1 on page 100).
- Method: Name of the used method (see E-Chem Methods, 12 on page 165)

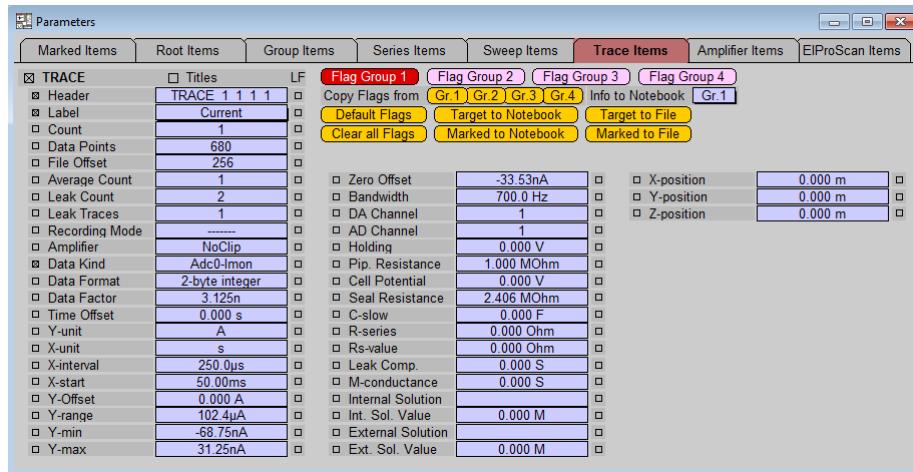
14.1.4 Sweep Items



Level 3 of the data tree. The *Parameters* of the *Sweep* level are:

- 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.
- Marker-1...4: Position of the *Sweep Marker* (see *Markers*, 19 on page 235).
- Temperature: Temperature value from an external device (see *Configuration*, 5.7.2 on page 64).
- User 1/2: User-defined external values (see *Configuration*, 5.7.2 on page 64).
- RMS-Noise: Value of RMS (=Root Mean Square; see *Test Pulse*, 5.7.2 on page 64).
- Pipette Pressure: Pressure from an external device (see *Configuration*, 5.7.2 on page 64).
- Digital In: Status of the input bits of the digital channels (see *I/O Control*, 16 on page 225).
- Digital Out: Status of the output bits of the digital channels (see *I/O Control*, 16 on page 225).

14.1.5 Trace Items



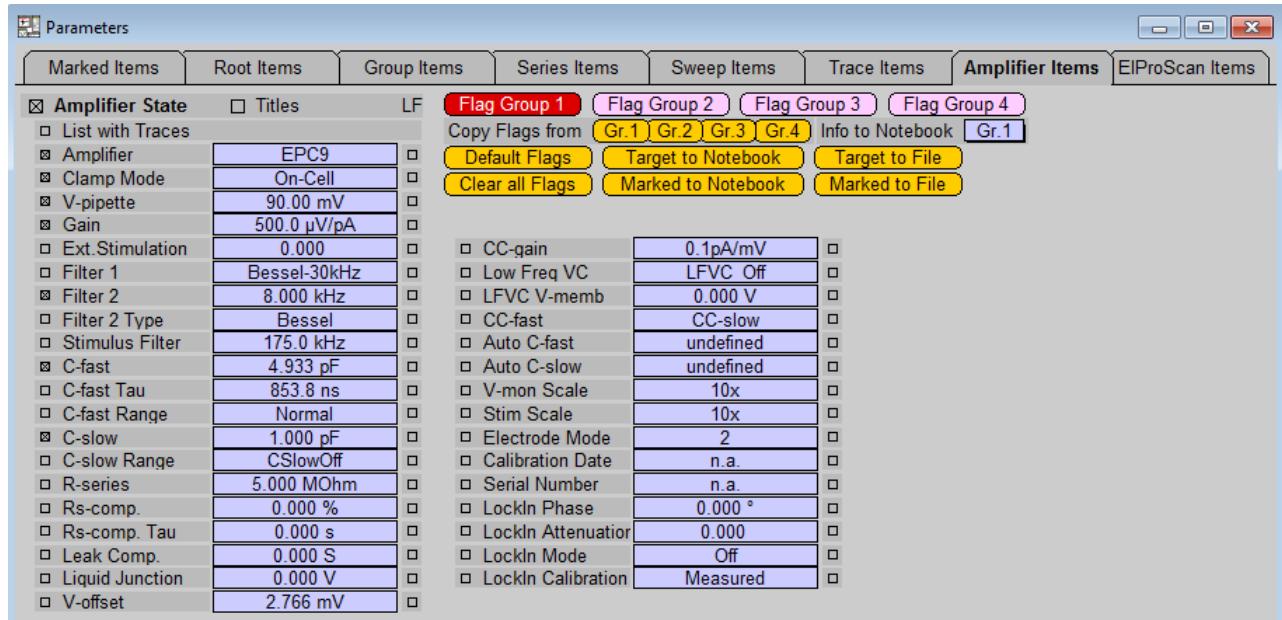
Level 4 of the data tree. The *Parameters* of the *Trace* level are:

- 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*.
- Amplifier 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 (see *Delay*, 10.12 on page 134).
- Y-unit: Unit of the Y-axis.
- X-unit: Unit of the X-axis.
- X-interval: *Sample Interval* (see *Timing*, 10.5 on page 110).
- X-start: Indicates any delay of the X-axis, mostly a time delay when using a *Start-Segment* (see *Timing*, 10.5 on page 110). 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.

- 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 AD input channel settings, 10.9.2 on page 128).
- Bandwidth: Filter frequency of the acquired *Trace*.
- DA Channel: DA channel number (see Channel Settings for DA Output and AD Input, 10.9 on page 122).
- AD Channel: AD channel number (see Channel Settings for DA Output and AD Input, 10.9 on page 122).
- 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 Monitoring, 7.1.4 on page 78).
- C-slow: Last *C-slow* value (see C-slow, 7.1.11 on page 83).
- R-series: Last *R-series* value (see Rs Comp, 7.1.12 on page 84).
- 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 7.1.14 on page 86).
- M-conductance: Membrane conductance (see Trace Parameters, 13.3.2.6 on page 199).
- Int. Sol.: Name of the used internal solution.
- Int. Sol. Value: Value entered in the selected solution (see Solution Data Base, 17.3 on page 230).
- Ext. Sol.: Name of the used external solution.
- Ext. Sol. Value: Value entered in the selected solution (see Solution Data Base, 17.3 on page 230).
- X-position: X-position value in relation to the origin.
- Y-position: Y-position value in relation to the origin.
- Z-position: Z-position value in relation to the origin.

14.1.6 Amplifier Items

Amplifier state record. Describes the amplifier settings at the beginning of the Series. Available for fully software controllable amplifiers like EPC 9 and EPC 10.



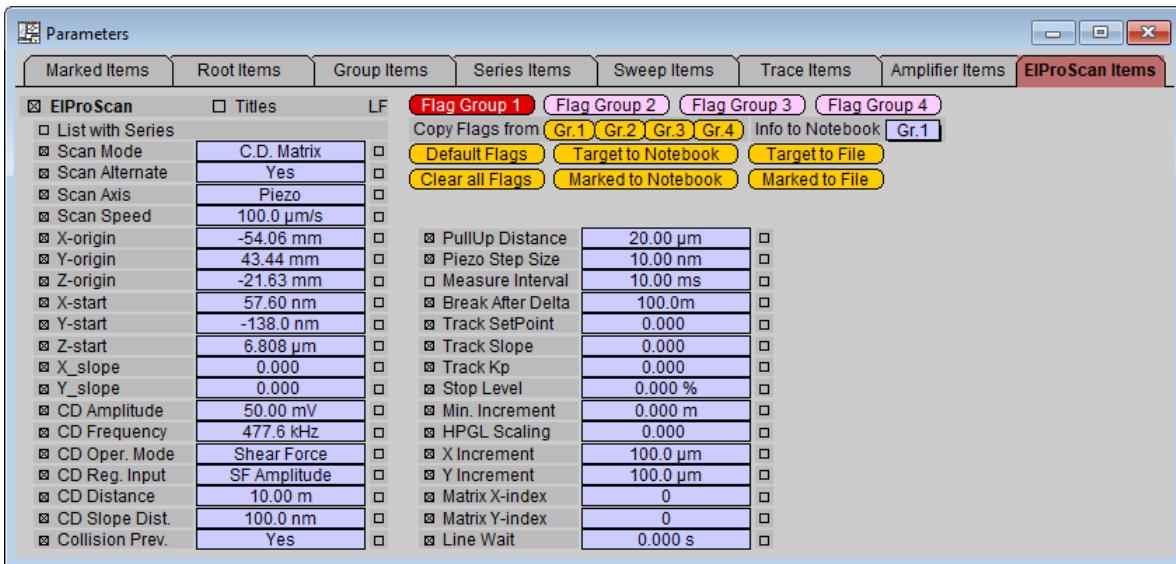
The parameters of the *Amplifier* level are:

- List with Traces: If enabled all flagged *Parameters* will be exported either to the Notebook via "Target to Notebook", "Info to Notebook" or to a file via "Target to File" (see *Flagging*, 14.2 on page 221).
- Amplifier: Type of amplifier used for data acquisition (e.g. "EPC10 USB/2-1").
- Recording Mode: Recording mode of the amplifier during recording (e.g. "Whole-Cell").
- V-pipette: Measured pipette voltage (see *Monitoring*, 7.1.4 on page 78).
- Gain: Gain setting (see *Amplifier Gain*, 7.1.1 on page 76).
- Ext. Stimulation: Scaling factor when the *External Stimulation Input* was used (see *Amplifier Selection*, 7.1.3 on page 77).
- Filter 1: Bandwidth and type of Filter 1 (see *Filter*, 7.1.13 on page 85).
- Filter 2: Bandwidth of Filter 2 (see *Filter*, 7.1.13 on page 85).
- Filter 2 Type: Filter type of the Filter 2 (see *Filter*, 7.1.13 on page 85).
- Stimulus Filter: Filter frequency of the stimulus.
- C-fast: C-fast value (see *C-fast*, 7.1.10 on page 83).
- C-fast Tau: τ value of the C-fast compensation (see *C-fast*, 7.1.10 on page 83).
- C-fast Range: Range of the C-fast compensation either "normal" or "Extended" (see *EPC 10 Menu*, 4.9 on page 36).
- C-slow: C-slow value (see *C-slow*, 7.1.11 on page 83).
- C-slow Range: Range of the C-slow compensation (see *C-slow*, 7.1.11 on page 83).

14.1. Parameter Tabs

- R-series: R-series value (see [Rs Comp, 7.1.12 on page 84](#)).
- Rs-comp.: % of R-series compensation (see [Rs Comp, 7.1.12 on page 84](#)).
- Rs-comp. Tau: Speed of feedback compensation (see [Rs Comp, 7.1.12 on page 84](#)).
- Leak Comp.: Applied leak conductance value (see [Leak Compensation, 7.1.14 on page 86](#)).
- Liquid Junction: *Liquid Junction Potential* value (see [Voltage Offsets, 7.1.9 on page 82](#)).
- V-offset: *V-offset* value (see [Voltage Offsets, 7.1.9 on page 82](#)).
- CC-gain: *CC-gain* setting (see [Current-Clamp Settings, 7.1.21 on page 88](#)).
- Low Freq VC: LFVC mode indicator (see [Low-frequency voltage-clamp, 7.1.19 on page 88](#)).
- LFVC V-memb: Membrane potential used for LFVC (see [Low-frequency voltage-clamp, 7.1.19 on page 88](#)).
- 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 [Further Options, 7.1.22 on page 89](#)).
- Stim Scale: *Stim Scale* factor (see [Input and Output channels, 5.4.2 on page 49](#)).
- Electrode Mode: Indicates if the *2- or 3-Electrode Mode* was used (see [3-Electrode Mode, 5.4.11 on page 54](#)).
- Calibration Date: Date of the last calibration.
- Serial Number: Serial number of the amplifier and revision state.
- LockIn Phase: Phase of the LockIn (see [Software LockIn Extension, 23 on page 257](#)).
- LockIn Attenuation: Attenuation value of the LockIn (see [Software LockIn Extension, 23 on page 257](#)).
- LockIn Mode: "Sine+DC", "PiecewiseLinear" or "On Cell" (see [Software LockIn Extension, 23 on page 257](#)).
- LockIn Calibration: Measured, computed or manual *LockIn Calibration* (see [Software LockIn Extension, 23 on page 257](#)).

14.1.7 ElProScan Items



The *Parameters* of the *ElProScan* level are:

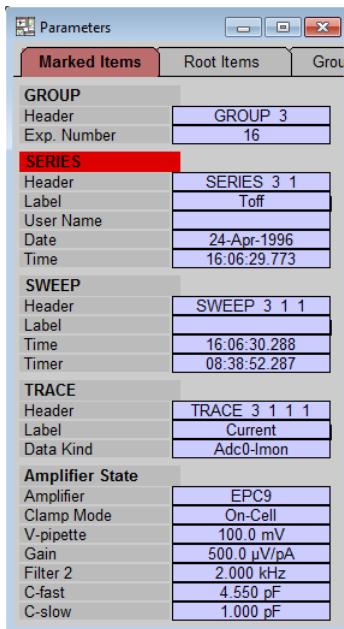
- List with Series: If enabled all flagged *Parameters* will be exported either to the Notebook via "Target to Notebook", "Info to Notebook" or to a file via "Target to File" (see *Flagging*, 14.2 on page 221).
- Scan Mode: Indicates the used *Scan Mode*, e.g. "TipDown", "2D Scan" or "3D Scan" (see *ElProScan menu* → *Scan dialog*).
- Scan Alternate: Indicates if the *Alternate* for a 3D- or Matrix Scan was used (see *ElProScan menu* → *Scan dialog*).
- Scan Axis: Indicates the scanned axis, e.g. "XX", "Piezo", or "SF Frequency" (see *ElProScan menu* → *Motors dialog*).
- Scan Speed: Speed of the performed scan (see *ElProScan menu* → *Scan dialog*).
- X-origin: Absolute value (calibrated) of the X-axis.
- Y-origin: Absolute value (calibrated) of the Y-axis.
- Z-origin: Absolute value (calibrated) of the Z-axis.
- X-start: Starting value on the X-axis in relation to the origin.
- Y-start: Starting value on the Y-axis in relation to the origin.
- Z-start: Starting value on the Z-axis in relation to the origin.
- X slope: Compensating parameter for the X-axis e.g. if the sample and the electrode are lopsided to each other (see *ElProScan menu* → *Scan dialog*).
- Y slope: Compensating parameter for the Y-axis e.g. if the sample and the electrode are lopsided to each other (see *ElProScan menu* → *Scan dialog*).
- CD Amplitude: Indicates the used Shear force amplitude in C.D. Mode (see *ElProScan menu* → *Constant Distance Modes dialog*).
- CD Frequency: Indicates the used Shear force frequency in C.D. Mode (see *ElProScan menu* → *Constant Distance Modes dialog*).

14.1. Parameter Tabs

- CD Oper. Mode: Indicates the used *constant distance mode*, e.g. "Shear Force" or "Surface Tracking" (see ElProScan menu → Constant Distance Modes dialog).
- CD Reg. Input: Indicates the used *Regulation Parameters* for the *Constant Distance Mode*, e.g. "SF Amplitude" or "Imon-2" (see ElProScan menu → Constant Distance Modes dialog).
- CD Distance: Indicates the used constant distance (see ElProScan: Scan → Const. Dist. scan modes dialogs → Distance)
- CD Slope Dist: Indicates the slope distance for the used *constant distance mode* (see ElProScan menu → Constant Distance Modes dialog).
- Collision Prev.: Indicates if the *Tip Collision Prevention* for a 3D- or Matrix Scan was used (see ElProScan menu → Scan dialog).
- PullUp Distance: Indicates the used Pull Up Distance in a Constant Distance experiment (see ElProScan menu → Constant Distance Modes dialog)
- Stop Level: Defined deviation of the measured current size in relation to the initially measured current size (see ElProScan menu → Scan dialog).
- Scan Interval: Indicates the set *Line Wait* (resting time before each line scan) (see ElProScan menu → Scan dialog).
- Min. Increment: Indicates the set minimum displacement size (template resolution).
- Matrix X-index: Indicates the X-index number in the defined grid.
- Matrix Y-index: Indicates the Y-index number in the defined grid.
- HPGL Scaling: Scaling factor of the used plotter (HPGL) file (see ElProScan menu → Settings dialog).
- Amplitude: Stimulation amplitude of the SHEARFORCE (see ElProScan menu → ShearForce dialog).
- Frequency: Stimulation frequency of the SHEARFORCE (see ElProScan menu → ShearForce dialog).
- Break After Delta: *Break Criteria* of the *Constant Distance Mode*, either in "V", "A" or "°" (see ElProScan menu → ShearForce dialog).
- 3D Y Increment: Line scan distance of a 3D Scan.
- Piezo Step Size: Defined step size of the Piezo (see ElProScan menu → ShearForce dialog).
- Measure Interval: Total time available for the electrode approximation and the measurement.
- Calib. Interval: Measuring interval during a *Constant Distance Scan* (see ElProScan menu → ShearForce dialog).
- Calib. Distance: Total distance for the measuring intervals during a *Constant Distance Scan* (see ElProScan menu → ShearForce dialog).

14.1.8 Marked Items

All marked (ticked checkboxes) *Parameters* will be displayed in this tab sorted according to their origin (*Root*, *Group*, *Series*, *Sweep*, *Trace* or *Amplifier*).



Parameter Options:

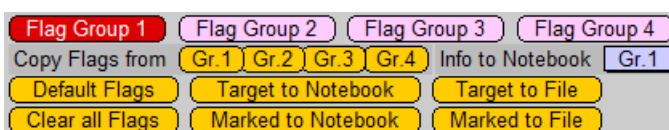
SERIES This checkbox is available in every *Parameter* tab, if there is no tick set all *Parameters* of this tab will not be displayed in the *Marked Items* tab independent how many *Parameter* checkboxes are ticked.

SERIES A highlighted red background color indicates that the display level of the tree is identical to the display tab of the *Parameters*. Here, a *Series* in the *Replay* tree and the *Series Parameters* have been displayed.

Titles If selected, the *Parameter* names will be exported together with their corresponding values. Otherwise, only the values will be exported.

LF If selected, values and/or *Parameter* names will be separated with a linefeed when exported.

14.2 Flagging



Via the checkboxes (flag options) you can select information that you want to export to the Notebook window or to a file (see section before).

Flag Groups 1...4: Four different flag groups can be defined, each containing the information for all checkboxes in this window.

By default, *Flag Group 1* contains the following flags:

- Group: Header, Exp. Number.
- Series: Header, Label, User Name, Date, Time.

14.2. Flagging

- Sweep: Header, Label, Time, Timer.
- Trace: Header, Label, Data Kind.
- Amplifier State: Amplifier, Recording Mode, V-Pipette, Gain, Filter 2, C-fast, C-slow.

Copy Flags from: Copy flag definitions from one group to another. First click on the *Flag Group* that you want information copied into, then on the copy group button of the group whose flags you want to copy to the active group.

Info to Notebook: The contents of the *Parameters* of the selected *Flag Group* are written to the **Notebook** window when the data is replayed and when **Replay → Show Tree Info** is on.

Default Flags: Set the *Flag Group 1* back to the default setting.

Clear all Flags: Reset all flags, i.e. no selected *Parameters*.

Target to Notebook: Copy all flagged information of the actual target in the **Replay** window to the **Notebook**.

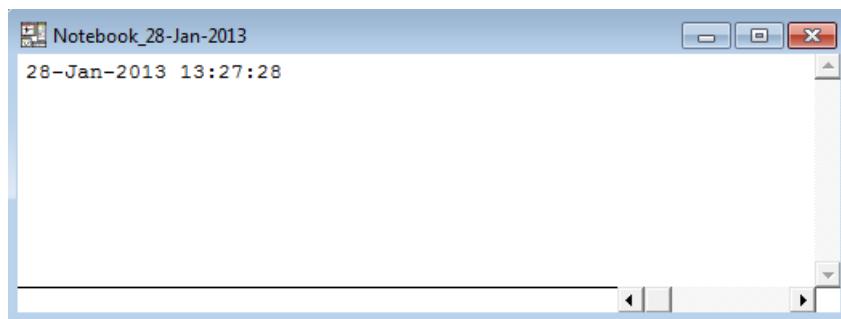
Marked to Notebook: Copy all flagged information of the marked target in the **Replay** window to the **Notebook**.

Target to File: Copy all flagged information of the actual target in the **Replay** window to a file. You will be asked for a file name.

Marked to File: Copy all flagged information of marked targets in the **Replay** window to a file. You will be asked for a file name.

15 Notebook Window

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 windows (e.g. Parameters, Configuration, Analysis), 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; its maximal size has to be specified in the drop-down menu **Notebook → Set Length**. To keep a better log of an experiment, the names of opened files and of executed series are written to the Notebook window.



When the window is activated (by clicking with the mouse pointer into it), the text editing functions are activated and applicable in the Notebook window. 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.

The applicable menu commands are described in the **Edit** and **Notebook** menus (see [Menus, 4.2 on page 21](#) and [4.7 on page 34](#)).

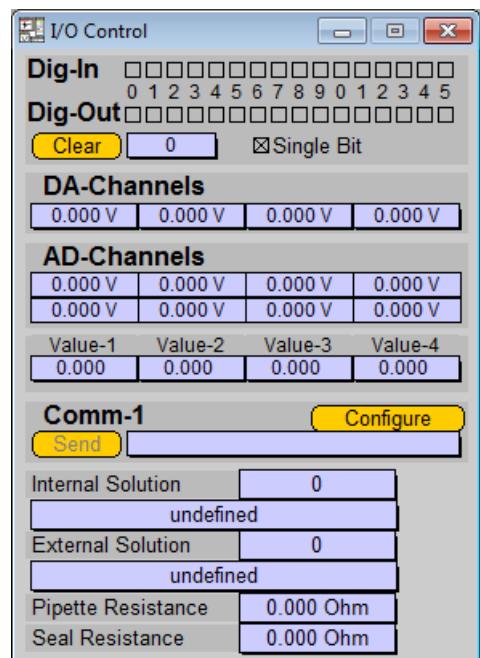
Note: The *Cut*, *Copy* and *Paste* commands copy to and from the clipboard.

16 I/O Control Window

The I/O Control window can have the following sections:

Digital In/Out, DA-Channels, AD-Channels, Values, Serial Out, and Parameters. Which sections and items are shown in the I/O Control window can be selected in the I/O Parameters section of the Configuration window (see 5.7 on page 59).

The idea of the I/O Control window is that the settings in the Configuration window ideally should be edited only during major software setups, whereas the I/O Control window should be used for Parameter editing during data acquisition. Hence, only those Parameter should be displayed in the I/O Control window that are not set internally and are subject to change during the experiment. These Parameters do not have an internal source. That means, are not defined by EPC 10 or read from a specified AD-channel. E.g. some amplifier settings such as recording bandwidth can be entered when a non-telegraphing amplifier is used.

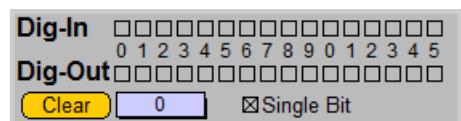


16.1 Dig-In / Out

Dig-In/Out: Control field of the digital channels. The digital input bits are only displayed. The digital output bits can be set by selecting or deselecting the checkboxes.

Clear: Clear all settings for the digital output, e.g., deselect all bits. The number in the blue field represents the output bits. It can be used to set the bits. Clear actions are written to the Notebook.

Single Bit: If selected, only one bit can be set for *Dig-Out*. The outputted digital value is set to the highest bit.



16.2 DA / AD-Channels

DA/AD-Channels: This section allows you to set the output of the DA-channels. The currently sampled voltages at the AD-channels are only displayed.

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, i.e. as a stimulation channel in the Pulse Generator.

DA-Channels			
0.000 V	0.000 V	0.000 V	0.001 V
-0.000 V	-0.001 V	-0.001 V	0.001 V
0.000 V	0.000 V	-0.003 V	0.012 V

16.3 Values

Values (1-32) are global variables which can be used for calculations in the Protocol Editor and in the Analysis. In addition, they are used to exchange information between these two program modules. In the I/O Control dialog the Values are displayed and they can be manually changed. The number of displayed Values (4, 8, 12, 16, 20, 24, 28 or 32) can be specified in the Configuration dialog (see 5.7.3 on page 64). In addition, the names of the Values are displayed above the numeric Values. The name can be set in the Configuration dialog.

Value-1	Value-2	Value-3	Value-4
0.000	0.000	0.000	0.000

16.4 Serial Output

Serial Port: A string can be sent (Send button) to a device via the serial port (RS-232) of the computer. The Configure button opens the Serial Port Configuration window (see Serial Out, 5.4.10 on page 53). An example is shown in the Protocol Editor, 11.4.3.2 on page 152.



Note: This section is only visible, if the serial communication is turned on in the Configuration dialog, 5.4.10 on page 53.

16.5 Solutions

Here, it is possible either to monitor the presently active solution or to manually change the solution index. The solution information provided here will be stored in the data file of PATCHMASTER.

Internal Solution	0
	undefined
External Solution	0
	undefined

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 11.4.3.6 on page 153) and the Solution Changer dialog (chapter 18 on page 233).

16.6 Parameters

16.6. Parameters

Various *Parameters* can be selected in the Configuration window to be listed in the I/O Control window for display or editing. E.g. if a telegraphing amplifier is used the bandwidth that is read via an AD channel can be displayed.

Pipette Resistance	0.000 Ohm
Seal Resistance	0.000 Ohm

The individual *Parameters* are described in the context of the Configuration window, 5.7 on page 59.

17 Solution Base

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.

PATCHMASTER provides two ways to handle solution changes during your experiments:

1. A simple one is to use the entries *Internal Solution* and *External Solution* in the I/O Control window. To activate this option please go to the Configuration window and active the *Show Solution* checkbox in the I/O Control tab.

Internal Sol.	1. ampl.	0
External Solution		0

You can set the entry manually or via the event *Set Solutions* in the Protocol Editor (11.4.5.2 on page 159). The numbers can be the entry of an external list of solutions, or the index of a specific solution in a PATCHMASTER Solution Data Base (see 17.1). In case you are using a multiple amplifier and you selected *Show Amplifier Solutions* in the Configuration (5.7.2 on page 64) you have to specify the used solutions for every amplifier. The solution indices are stored together with the recorded data and can be reviewed in the Parameters window (14.1.5 on page 215).

2. A more advanced method is to create a *.sol file. Such a data base is a *Tree* of solution entries ordered by the given identifying index.

This way the indices stored with the data are converted to solutions from the *Solution Data Base*. Here, it is also possible to use the event *Set Solutions* in the Protocol Editor (11.4.5.2 on page 159). In case you are using a multiple amplifier and you selected *Show Amplifier Solutions* in the Configuration (5.7.2 on page 64) you have to specify the used solutions for every amplifier.

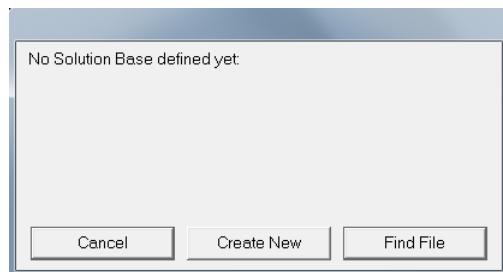
In the Parameters window (14.1.5 on page 215) the solution names are displayed. By pressing the *Show* button on the left to the solution name you can view the content of the solution.

Internal Sol.	1. ampl.	2
	2: ACSF	
External Solution		1
	1: Normal Frog Ringer	

<input type="checkbox"/> Int.Sol.	<input checked="" type="button" value="show"/>	Normal Frog Ringer
		1.800mM
<input type="checkbox"/> Ext.Sol.	<input checked="" type="button" value="show"/>	ACSF
		3.500mM

17.1 Activating the Solution Base

First, turn on the *Solution Base* in the Configuration window (5.5.2 on page 56). If no *Solution Data Base* has been loaded before and stored with the current configuration, a dialog box shows up asking you if you want to open an existing data base or creating a new data base.



During the experiment, solutions can be set either via the I/O Control window (16.5 on page 226) or via the *Set Solutions* event in the Protocol Editor (11.4.5.2 on page 159).

17.2 Using Solution Indices

The indices can be given to solutions in any arbitrary way but it certainly is of advantage if one sticks to some consistent concept in order to be able to identify solutions by their index easily. Since the solution indices are numbers between 0 and 2'147'483'648 (many more than you will find bottles in the laboratory), there is plenty of freedom to organize them.

Here is an example: Usually one has several standard solutions, which are frequently used and modified slightly for various experiments. One could assign numbers divisible by 1000 or 10000 to them. Then one has 999 or 9999 possibilities for modifications of this solution, respectively. An example would be that certain concentrations of toxin are added to the standard solution 1000 yielding numbers 1001 through 1099. Another toxin could occupy the numbers 1100 through 1199, etc..

Note: The indices given for internal and external solutions are pointing into the same Solution Data Base. It is therefore a good idea to index internal and external solutions such that they are consistent and easily identifiable. For example, you could use odd thousands (or ten thousands) for external solutions and even thousands (or ten thousands) for internal solutions.

17.3 Solution Data Base

Solution Base

1	Normal Frog Ringer		1 entry
Numeric Name	Free Ca	1.80m	Create Entry
pH	7.20	NaOH	Duplic. Entry
Osmol.	285.m		Delete Entry
Index	Ingredient	Conc.	Next Entry
1	NaCl	140. mM	Last Entry
2	KCl	2.50 mM	Export Label
3	CaCl ₂	1.80 mM	Export Listing
4	Hepes	10.0 mM	SAVE
			UNDO
			DONE

To open the Solution Data Base you have to select Solution Base in the Windows menu of PATCHMASTER.

Solution Index: Index number for the solution. By clicking into the field you are able to enter a defined index number directly which might be helpful if you have a large *Solution Data Base*.

Name: Name of the 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.).

Numeric Name Value: An editable field containing a number or value (Osmolarity, pH) which is in relation to the name entered in the field *Numeric Name*.

pH: Holds the value of the pH of the solution and the substance used to adjust it.

Osmol.: Holds the value of osmolarity of the solution.

Ingredient / Conc.: Each ingredient is defined by its index number and its concentration in the solution.

Insert, Append, Delete: By clicking into an index number of the solution ingredients options for modifying the solution content get available:

- Insert: Creates a new entry line for an ingredient before the selected index number.
- Append: Creates a new entry line for an ingredient at the end of the ingredient list.
- Delete: Deletes the selected ingredient.

Entries: Number of entries in the solution data base.

Create / Duplic. / Delete Entry: Used to generate, copy, and remove solutions in the data base.

Next / Last Entry: This option moves through the data base by selecting the next or last available solution.

Export Label: This is used to output labels of the shown solution:

- ASCII File: Exports the solution information as an ASCII text file (*.label).
- Printer: Two labels with two different size containing the solution information are created.
- Igor Text: Exports the solution information as IGOR text (*.label).
- Notebook: Exports the solution information to the Notebook.

Export Listing: This is used to output a list of the entire solution file:

- ASCII File: Exports the index number and the name of the solutions as an ASCII text file (*.list).
- Printer: Prints the index number and the name of the solutions.
- Notebook: Lists the index number and the name of the solutions in the Notebook.

SAVE: Saves the file to disk.

UNDO: Reverts the edited solution to its original form.

DONE: Exits the dialog.

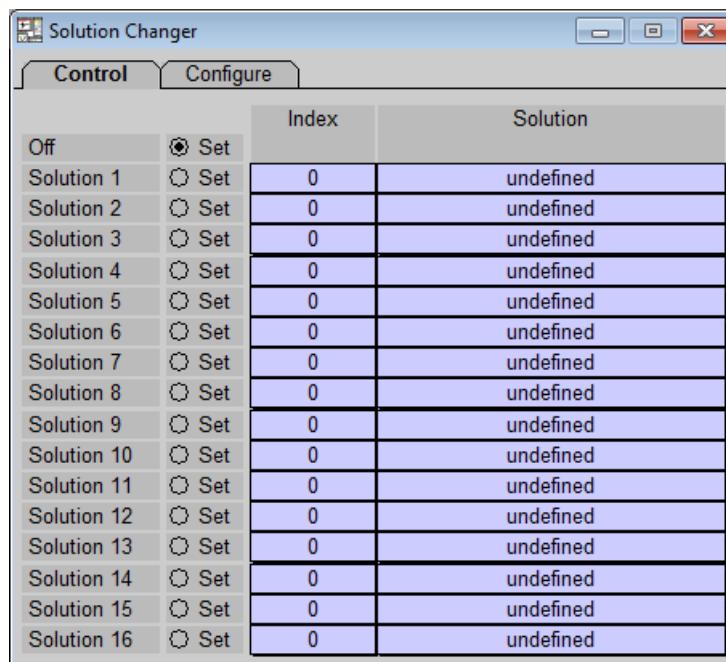
18 Solution Changer

The Solution Changer dialog can be accessed via the Windows menu of PATCHMASTER. The Solution Changer 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 Solution Changer helps to keep track with the solutions used during an perfusion experiment. The solution information is stored in the data file of PATCHMASTER. To look up this information use the Parameters dialog.

Any external perfusion system with analog or digital inputs can be used together with the Solution Changer and e.g. an EPC 10 USB amplifier.



The Solution Changer dialog has two main tabs. In the *Control* tab one sets the active perfusion channel manually ("Set"). Further the solution index and the solution name from the Solution Base is displayed here. By clicking into the selection field it is possible to change the solution index/solution name.

Note: The solution selection does only work if a Solution Base is defined and loaded.

Solution Changer

Control Configure

16 Solutions

Off		off
Solution 1	External Solution	off
Solution 2	External Solution	off
Solution 3	External Solution	off
Solution 4	External Solution	off
Solution 5	External Solution	off
Solution 6	External Solution	off
Solution 7	External Solution	off
Solution 8	External Solution	off
Solution 9	External Solution	off
Solution 10	External Solution	off
Solution 11	External Solution	off
Solution 12	External Solution	off
Solution 13	External Solution	off
Solution 14	External Solution	off
Solution 15	External Solution	off
Solution 16	External Solution	off

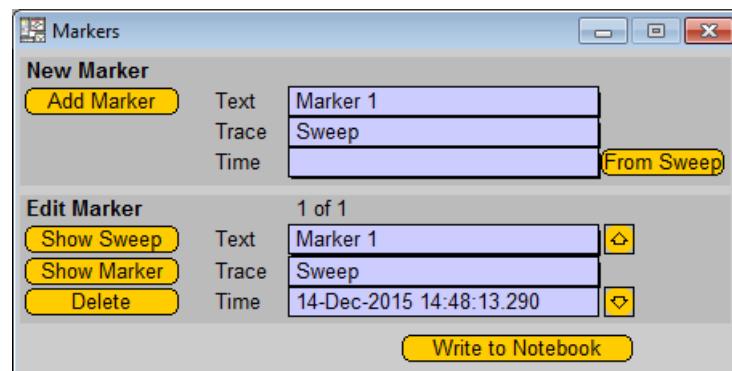
In the *Configure* tab you can define up to 16 solutions used in the perfusion experiment. Further you can define if the used solutions is an internal or an external solution. In the last column one defines the output channel e.g. analog or digital. If an analog output is selected you will have to define the voltage amplitude necessary to activate the perfusion channel.

Note: Please do not forget to define an output channel for the "Off" position.

DA-0
DA-1
DA-2
Stim-DA
DA-4
DA-5
DA-6
DA-7
<input checked="" type="checkbox"/> off
Dig-out (word)
Dig-0
Dig-1
Dig-2
Dig-3
Dig-4
Dig-5
Dig-6
Dig-7
Dig-8
Dig-9
Dig-10
Dig-11
Dig-12
Dig-13
Dig-14
Dig-15
Clear all

19 Markers

The **Markers** dialog can be opened by selecting **Markers** from the **Windows** menu.



With this dialog, **Markers** can be set at any time during a running experiment even between **Sweep** acquisitions. A **Marker** is drawn as a blue vertical line in the **Oscilloscope**.

19.1 New Marker

In this section one can add **Markers** and define the name of the label.

Add Marker: Adds a new **Marker** to the data file. The **Marker Text** and the target **Sweep/Trace** information is stored as well.

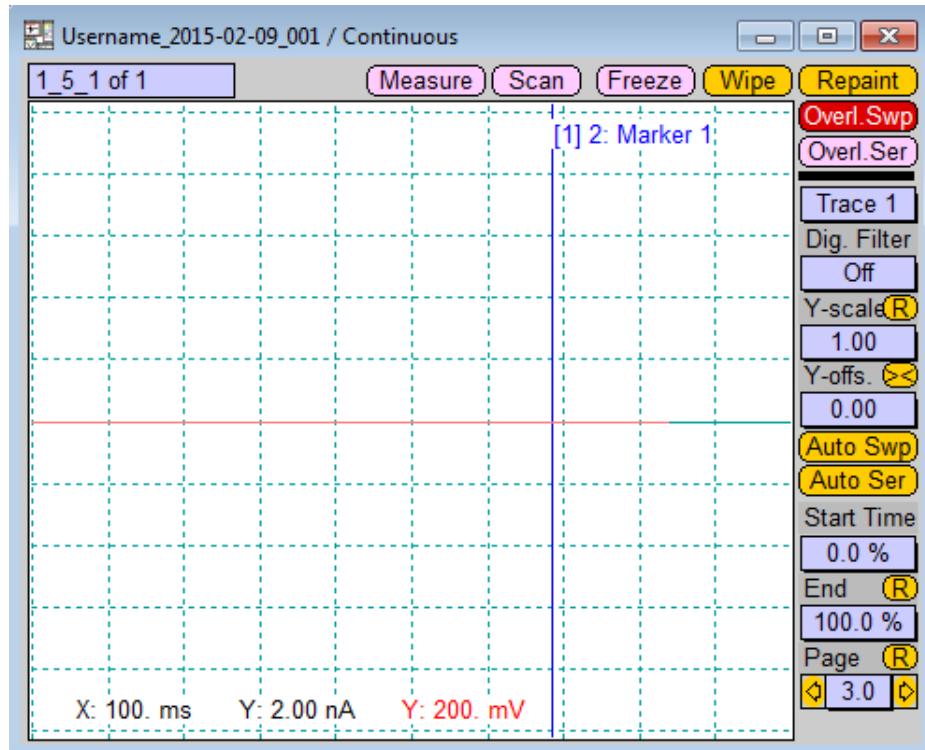
Text: Enter a label for the **Marker** you want to add.

Trace: A **Marker** can be linked either to a **Sweep** or a **Trace** which can be specified by this selection menu.

If the **Marker** is defined for a **Sweep** it is labeled "[i] Text". The i in square brackets indicates the number of the **Marker** in the list (index number) and "Text" shows the label of the **Marker**.



If the *Marker* is defined for a *Trace* it is labeled "[i] i: Text". The i in square brackets indicates the number of the *Marker* in the list (index number) whereas the index number thereafter indicates the *Trace* index number. "Text", again, shows the entered label for the *Marker*.



Time: The time of a new *Marker* is the actual time if the field is empty. It can be the given time in the time format "hh:mm:ss.mmm" e.g. if you add a *Marker* offline. Optionally the time format may be preceded by a date "yyyy/mm/dd".

From Sweep: Gets the time information of the Sweep selected in the Replay tree.

19.2 Edit Marker

Marker Index: The Marker index e.g. "1 of 7" indicates the index number of the selected Marker in the Marker list.

Show Sweep: Selects the according Sweep in the Replay tree and replays it in the Oscilloscope.

Show Marker: Shows the appropriate Marker information in the Marker list if the selected Series in the data tree owns a Marker.

Delete: Deletes the selected Marker.

Text: Modifies the label of the set Marker.

Trace: Modifies the link (Trace or Sweep) of the set Marker.

Time: Displays the time information of the selected Marker.

: The two arrow buttons enable to scroll through the list of added Markers.

Write to Notebook: Writes the time information of acquired Series and set Markers into the Notebook.

```

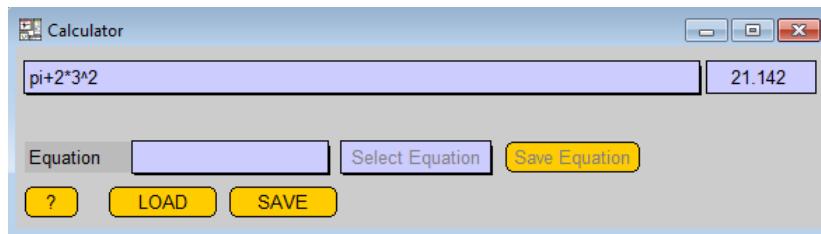
2015/02/09 08:42:24.731 series[1_1] Continuous
2015/02/09 08:42:59.707 series[1_2] Continuous
2015/02/09 08:43:15.572 series[1_3] Continuous
2015/02/09 08:43:43.091 series[1_4] Continuous
2015/02/09 08:44:34.212 series[1_5] Continuous
2015/02/09 11:06:49.682 series[1_6] IV
2015/02/09 11:11:15.726 series[1_7] Continuous
2015/02/09 11:17:49.161 series[1_8] Continuous
2015/02/09 11:18:00.471 series[1_9] Continuous
2015/02/09 11:20:27.845 series[1_10] IV
2015/02/09 11:20:32.962 marker[0] Hello
2015/02/09 11:20:37.236 marker[0] Marker 2

```

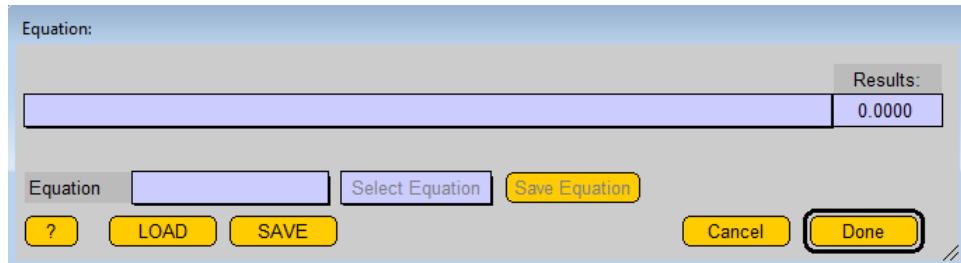
Note: In the Protocol Editor event "Set Solutions" it is possible to add Solution Markers. The Markers set there are also included into the Markers list.

20 Calculator Window and Equations

Equations are a very flexible way to perform complex calculations within PATCHMASTER. Equations can be used at various places, such as *Analysis*, *Trace Buffer*, or for *DA-Scaling*. In addition, a separate **Calculator** window allows to use the equations syntax as replacement of the calculator provided by the operating system.



In case an equation has to be edited in the *Analysis*, *Protocol Editor* or *Trace Buffer*, then the following dialog can be opened by pressing on the *Edit* button.



Command Line: Enter the equation string into this field.

Results: The result of the equation will be shown here.

Equation: You can enter an equation name which will be loaded into the *Command Line* after pressing RETURN. Alternatively if an equation was selected by the option *Select Equation* the equation name will be displayed in this field.

Select Equation: After loading an equation file you can select already stored equations.

Save Equation: For saving an equation enter a name in the *Equation* field and press the *Save Equation* button. When the entered name does not already exist in the equation file you will be asked if you want to save it as a new entry.

?: Prints a help text on the equation syntax to the Notebook.

LOAD: Brings up a file selector to open an equation file (e.g. *Equation.txt*).

SAVE: Saves the modifications made in the equation file.

Cancel: Closes the dialog without accepting the equation.

Done: Accepts the new equation string and closes the dialog.

20.1 Equation Syntax

Normal math syntax is to be used. All 3 types of brackets ('('and')','[and'],'{and}') are equivalent and can be freely used and nested. The opening and closing brackets must match.

Math functions:

- +, -, *, /, ^ (power), % (remainder)
- & (and), nor, or, xor: bit operation on integer values
- >, <, =: boolean operations: true=1, false=0
- round: rounds towards nearest value using the banking rules (-2.5 → -3.0)
- trunc: rounds towards the smaller amount (-2.5 → -2.0)
- ceil: rounds towards the positive (-2.5 → -2.0)
- floor: rounds towards the negative (-2.5 → -3.0)
- exp, sqrt, ln, log
- sin, cos, tan
- arcsin/asin, arccos/acos, arctan/atan
- abs, rad, deg
- random: returns random number between 0.0 and 1.0
- f[i], i = online function index = 1...n
- p[i], i = amplifier parameter index = 1...n
 - p[0] → IMon (Current Monitor value)
 - p[1] → VMon (Voltage Monitor value)
 - p[2] → CFast (CFast value)
 - p[3] → CSlow (CSlow value)
 - p[4] → RSeries (RSeries value)
 - p[5] → GLeak (Conductance value of the Leak Compensation)
 - p[6] → Clip (Clipping status)
 - p[7] → AutoCFast (Auto CFast Compensation status)
 - p[8] → AutoCSlow (Auto CSlow Compensation status)
 - p[9] → PipPres (Pipette Pressure)
 - p[10] → PipRes (Pipette Resistance)
 - p[11] → Temp (Temperature value)
 - p[12] → CellPot (Cell Potential acquired from an external device)
 - p[13] → User1 (User-defined Parameter 1)
 - p[14] → User2 (User-defined Parameter 1)
 - p[15] → Timer (Time of the Timer)
 - p[16] → SealRes (Seal Resistance)
 - p[17] → Time (Time)
 - p[18] → Xpos (ElProScan specific parameter)

- p[19] → Ypos (ElProScan specific parameter)
- p[20] → Zpos (ElProScan specific parameter)
- p[21] → PiezoPos (ElProScan specific parameter)
- v[i], i = value index = 1...n
- icon[icon descriptor], e.g. 'icon[E VHold]'

Constants:

- e, pi

Special case:

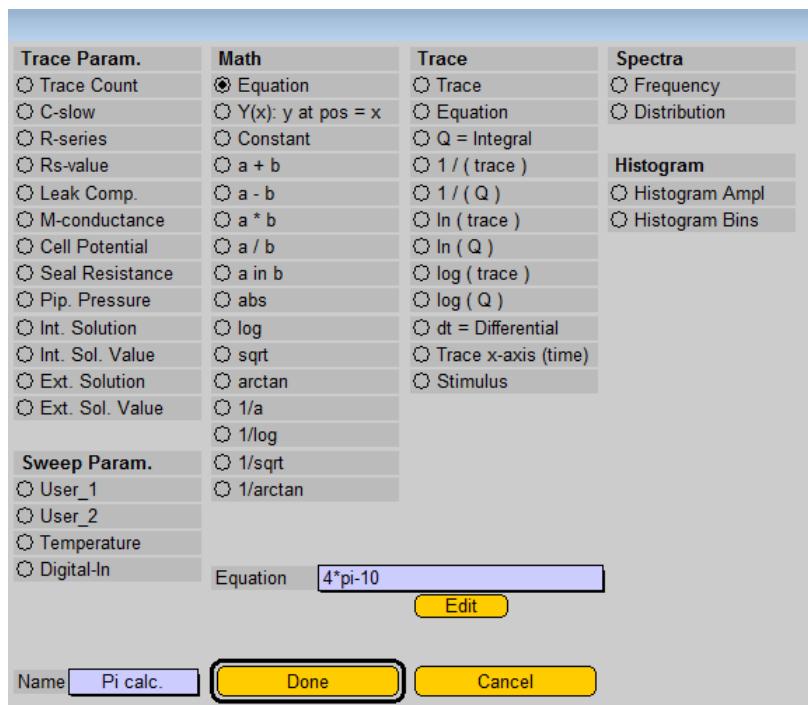
'@' as the first character will replace the given label by the formula in the equation file with that label. E.g. '@first 1+2' results in '1+2'.

Comments:

Anything after ';' is considered a comment.

20.2 Equations - Where to Use

20.2.1 Analysis



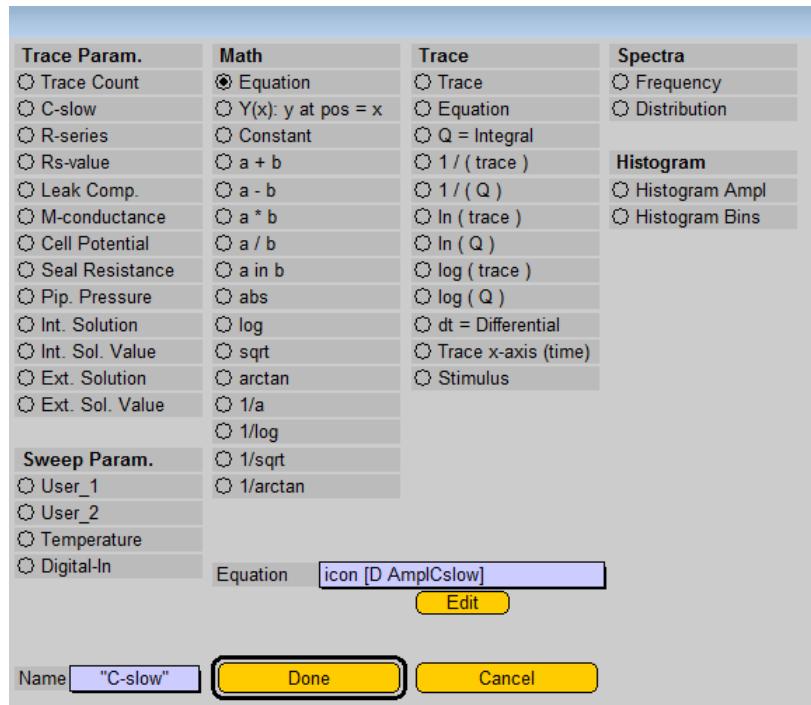
The *Equation* functions in the *Math* and *Trace* section of the *Analysis Function* dialog provide the possibility to assign the result of an equation to a function. The advantage of the equation is to perform multiple steps of a calculation with just one function. In an equation of the *Analysis* function the results of other *Analysis* functions can be easily combined.

Math - Equation

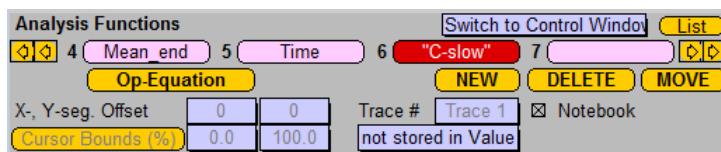
The following additional identifiers can be used in the *Math - Equation* function:

- $f[i]$, $i = \text{online function index} = 1 \dots n$
- $p[i]$, $i = \text{amplifier parameter index} = 1 \dots n$
- $v[i]$, $i = \text{value index} = 1 \dots n$
- icon[icon descriptor], e.g. 'icon[E VHold]'

Example - working with the icon descriptor: A parameter of the Parameters window can be easily plotted in the Analysis graph.



As *equation string* you choose the icon descriptor of the *C-slow* parameter from the **Parameters** window. Please note that the **Parameters** window has to stay open to access the value of this icon. However, you may minimize the window. The advantage of using the *C-slow* parameter from the **Parameters** window instead of the **Amplifier** window is that the *Parameter* is available also during offline replay and analysis of the acquired data.



Trace - Equation

The **Analysis Function Trace - Equation** provides the possibility to scale a *Trace* before plotting it in the online graph. The advantage of the *equation* is to perform multiple steps of a calculation with just one function. In an *equation* of the **Analysis function** the results of other **Analysis functions** as well as other trace data of the selected trace can be easily combined.

In addition to the identifiers of **Math - Equation** the following identifiers can be used:

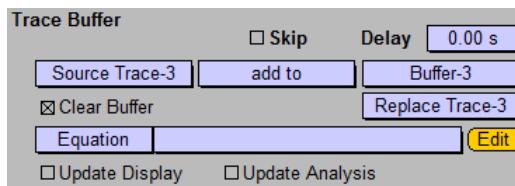
- y : The trace y -value, i.e. "amplitude"
- x : The trace x -value, i.e. " x -axis value = x -start + index * x -increment"

- Q: The integral up to the respective index, i.e. "(y[i] + y[i-1]) / 2 * dx"
- dt: The differential between present and preceding y-value, i.e. "(y[i] - y[i-1]) / dx"
- i: The index of the trace data point
- tb[i]: The y-value of equivalent sample in buffer i, i = buffer index, 1...4
- tr[i]: The y-value of equivalent sample in trace i, 1...n. One can use the trace-ID (e.g. "I-mon") to specify a trace instead of the numeric trace-count "i". The text must be identical to the text in the trace-count menu.

20.2.2 Trace Buffer

In case you would like to permanently modify (rescale) acquired data or at least let the rescaled data then run through the Analysis then you can use the equation functionality of the *Trace Buffer* menu or protocol event.

An example: As *Trace 3* we acquire the frequency dependence of the *Admittance (Gain)*. This is an output Trace of the Spectroscopy Extension. The X-axis of the *Admittance Trace* is scaled in frequency units. The *Admittance* itself is scaled in linear units. For some applications it is useful to rescale the *Admittance* in dB units.



The protocol event above shows the following: We take *Trace 3* as source, clear the destination buffer, add the source to the buffer-3, apply the equation (here we calculate $20 \cdot \log(y)$, y = values in the destination buffer after performing the operation), and then put the result back into *Trace 3*. Now we can run *Trace 3* through the Analysis again and apply the analysis function on an *Admittance (Gain) Trace*, which is now scaled in decibel. E.g. we can apply the *Time to Threshold* function with a threshold of -3 (dB) to search for the cutoff frequency.

The operands are identical to the ones used in *Trace - Equations* (see 20.2.1 on the facing page).

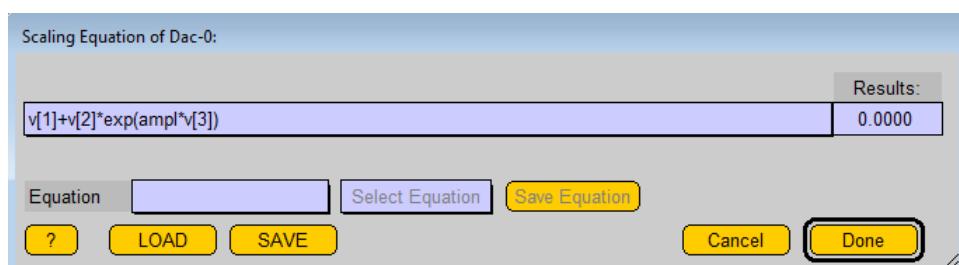
20.2.3 DA Scaling

Special Operands:

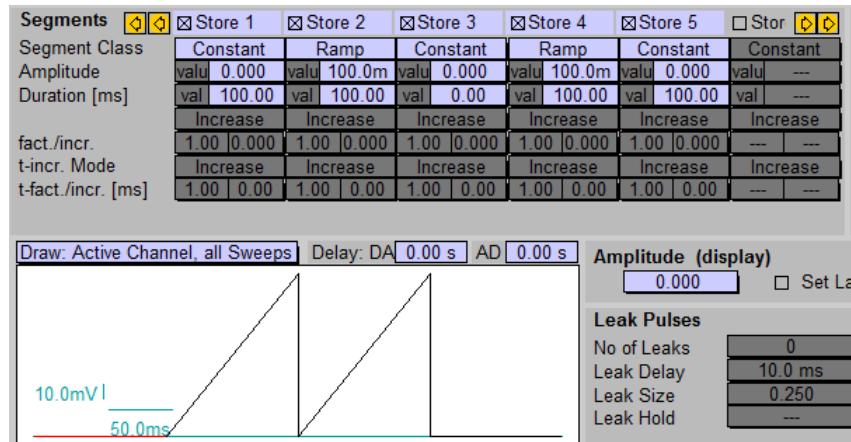
- ampl = The value for output or amplitude as defined by the PGF-stimulus. Used for equations in *DA-Scaleing* only.

In the following we will describe how to output two consecutive pulses with exponentially increasing voltages.

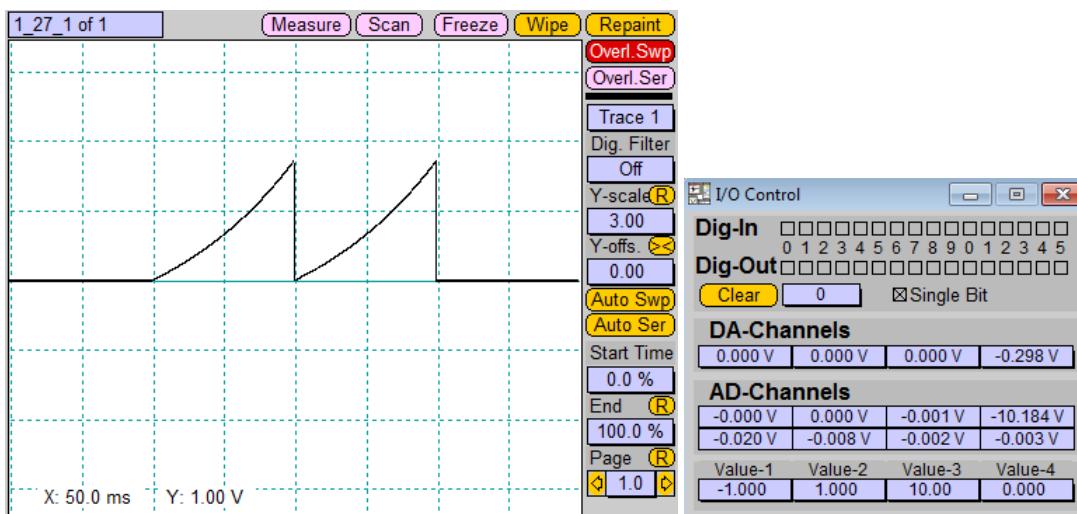
We first enter in the I/O Control pane of the Configuration window for the *DA Scaling* an equation of a single exponential (5.7.1 on page 60). As *Parameters* we use the values 1 to 3. As time or x-value we use the *Ampl Parameter* of the stimulus.



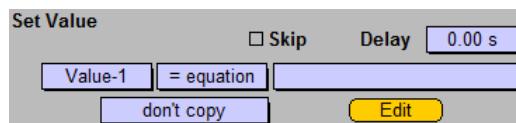
In a second step, we define the appropriate segment pattern for the DA 0 channel and select *Use DA Scaling*. In segments of amplitude 0 a constant voltage is put out. In case we want to generate the exponentially increase, we set the segment type to *Ramp* and choose for amplitude and duration the same value (1 Volt corresponds to 1 Second). In case the amplitude has to be reset to zero, we insert a constant segment with duration 0 and amplitude 0.



We now set the *Values 1 to 3* as desired via the I/O Control window and execute the sequence. The result is shown in the Oscilloscope window. By changing the values 1 to 3 the stimulus can be easily adjusted.



20.2.4 Values



The protocol event *Set Value* provides the possibility to assign the result of an equation to a value. The advantage of the equation is to perform multiple steps of a calculation with just one protocol event. In an equation of the *Set Value* event results of the *Analysis* can be easily combined. Therefore the definitions are identical to the ones in *Math - Equation* (see 20.2.1 on page 241).

21 Photometry Extension

This chapter provides a brief overview of the settings for each of the Photometry Extensions mentioned above. A complete tutorial/exercise for photometry measurements using PATCHMASTER is provided in the PATCHMASTER Tutorial.

After starting PATCHMASTER, open the Configuration window and select the appropriate Photometry Extension in the *Hardware* section of the dialog. The options are:

- TILL Photonics / Cairn OptoScan
- DG-4 / DG-5
- Lambda-10
- PTI DeltaRAM

Some controls are independent from the photometry hardware. These controls are described in this section. Hardware specific options will be described below.

R-max	1.0000E+00	R-min	0.0000E+00	Kd * Sf	1.0000E+00
Background1	0.0000E+00	Background2	0.0000E+00	Background3	0.0000E+00
Dead Time	2.00 ms	Compute Traces of Marked Targets			

R-max, R-min: Minimum and maximum ratio (F1/F2).

Kd*Sf: Effective Kd (dissociation constant) of the 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 Pulse Generator dialog.

Background 1...3: Background fluorescence for the three wavelengths F1, F2, F3.

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 **Parameters** dialog (see figure below).

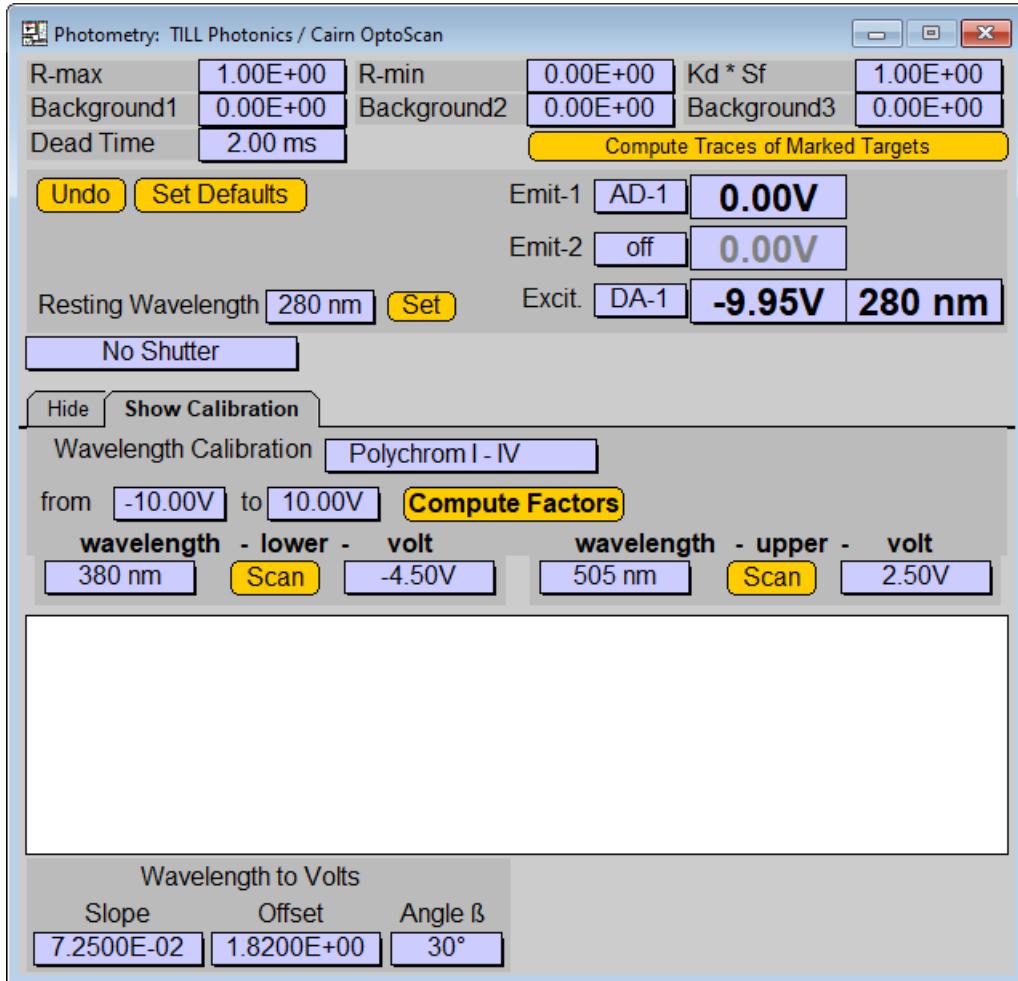
<input type="checkbox"/> Photo1 Background	0.000	<input type="checkbox"/>
<input type="checkbox"/> Photo2 Background	0.000	<input type="checkbox"/>
<input type="checkbox"/> Photo3 Background	0.000	<input type="checkbox"/>
<input type="checkbox"/> PhotoCa Kd	1.000	<input type="checkbox"/>
<input type="checkbox"/> PhotoCa Rmin	0.000	<input type="checkbox"/>
<input type="checkbox"/> PhotoCa Rmax	1.000	<input type="checkbox"/>

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.

21.1 Photometry Configuration TILL Photonics/Cairn OptoScan

This Photometry selection can either be used with the TILL Photonics Polychrome or the Cairn OptoScan monochromator.



Undo: Resets all changes made since the last opening of the Photometry window.

Defaults: Resets all changes to the default entries.

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.

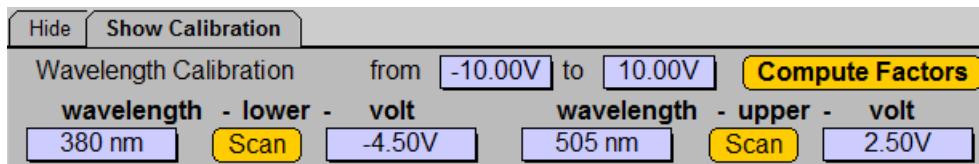
Shutter Selection:

- No Shutter: No shutter available.
- Shutter Control: Shutter can be closed/opened via a button.
- Digital-out 7: Shutter control via digital output.

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.

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 Volts (left) or in nm (right).

21.1.1 Wavelength Calibration



In this area the monochromator is calibrated, i.e. the relationship between output voltage and the wavelength is defined. To do so, voltage ramps ranging between the values specified by *from...* *to* are output when the **Scan** buttons are pressed. This is done for two bandpass filters with known transmission maxima. The corresponding wavelengths are entered in the **wavelength** fields. After both scan operations, i.e. when the voltages corresponding to the peak transmission have been determined successfully, the calibration constants for the control of the monochromator are calculated upon **Compute Factors** is pressed.

from - to: Scan range for voltage ramps. For a single bandpass filter usually the full range of ± 10 V can be scanned. However, when a single multi-band filter is used, the scan range has to be limited in order to detect only the peak of interest.

Compute Factors: Compute calibration constants based on the determined relationships of wavelength and voltage.

wavelength: Enter here the maximum of the bandpass filter (in nm).

lower - Scan: Do the scan for the low-wavelength calibration filter.

Volt: This field shows the voltage corresponding to the peak transmission.

upper - Scan: Do the scan for the high-wavelength calibration filter.

Volt: This field shows the voltage corresponding to the peak transmission.

Wavelength to Volts: *Slope*, *Offset* and *Angle* are internal calibration constants used to control the monochromator. These values are the result of the calibration procedure.

Wavelength to Volts		
Slope	Offset	Angle β
7.2511E-02	1.8235E+00	30°

The conversion between voltages and wavelength is done as follows:

$$F(\lambda) = \arcsin(\lambda/1666.67 * \cos(\pi/360^\circ * \beta)) \quad (21.1)$$

$$V = (2\pi * F(\lambda) - Offset)/Slope \quad (21.2)$$

$$\lambda = (1666.67 * \cos(\pi/360^\circ * \beta) * \sin((Slope * V + Offset)/2\pi)) \quad (21.3)$$

$$Slope = 2\pi * (F(\lambda_1) - F(\lambda_2))/(V_1 - V_2) \quad (21.4)$$

$$Offset = 2\pi * F(\lambda_1) - Slope * V_1 \quad (21.5)$$

with

- β = typically 30 degree; depends on your hardware. Please refer to the manual of the monochrometer.
- 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.

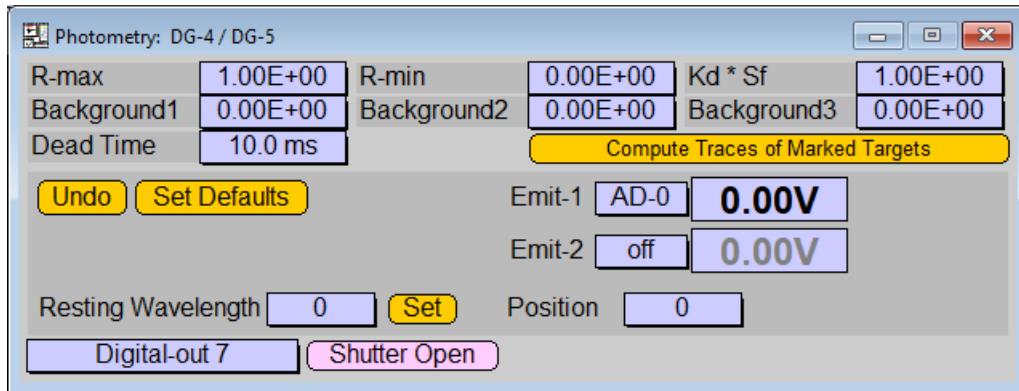
21.1.1.1 Calibrating the different TILL monochrometers

Polychrome I and Polychrome II: Two bandpass filters are provided with the Polychrome I and II. Enter the peak wavelength in the *Wavelength* fields of the *Wavelength Calibration* section, then put the first filter in place and press the left *Scan* button. The voltage of the peak intensity will be automatically detected and shown in the *Volt* field. Now, put the second filter in place and press the right *Scan* button. Finally, press *Compute Factors* to calculate all calibration parameters.

Polychrome IV: The Polychrome IV comes with a triple-band filter. When using this filter the scan range to detect the first or the last peak of the three has to be limited. Therefore, you should limit the scan range for the first peak from -10 V to -2 V and for the third peak from +2 V to +10 V. We omit the center peak in this calibration. Please enter the peak wavelengths in the corresponding *Wavelength* fields and the scan range before performing each scan in the *from – to* fields. Then, perform the two scans and finally, press *Compute Factors* to calculate all calibration parameters.

Polychrome V: The Polychrome V features an auto-calibration. Please ask T.I.L.L. Photonics for a pair of calibration values. E.g. which analog control voltages refer to the wavelength 380 nm and 505 nm. Then, please enter the wavelength and voltages in the *Wavelength* and *Volts* fields and press *Compute Factors* to calculate all calibration parameters.

21.2 Photometry Configuration DG-4/DG-5



Undo: Resets all changes made since the last opening of the Photometry window.

Defaults: Resets all changes to the default entries.

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.

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.

Position: Enter a number for the filter wheel position of the DG-4/DG-5 between 0 and 15.

Shutter Selection:

- No Shutter: No shutter available.

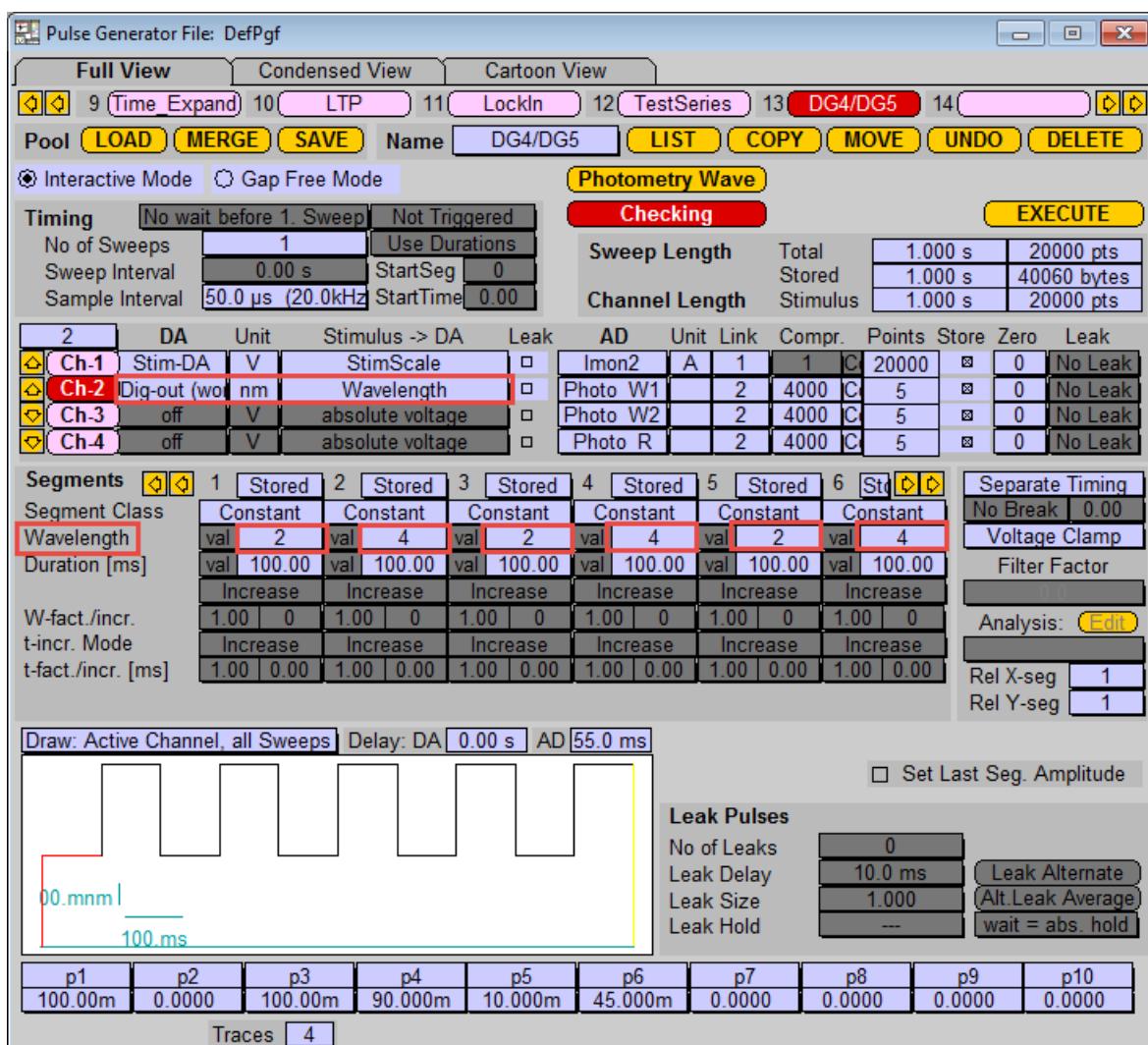
- Shutter Control: Shutter can be closed/opened via a button.
- Digital-out 7: Shutter control via digital output.

Shutter Open/Close: Toggles between an open and a closed shutter.

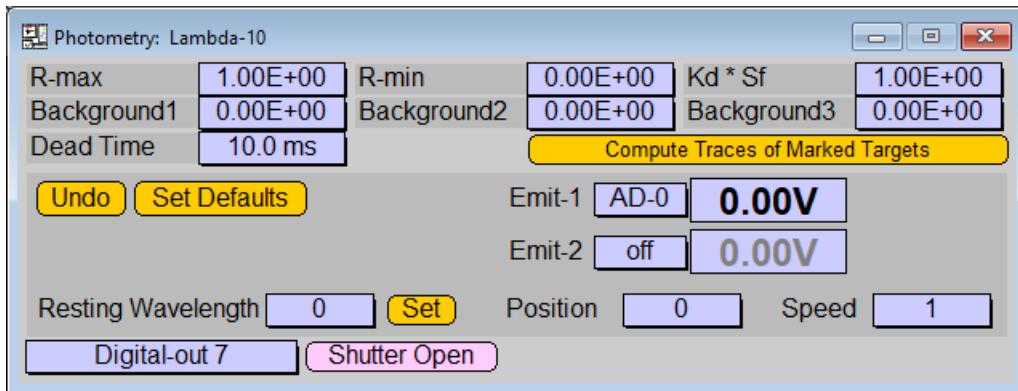
Note: To control the DG-4/DG-5 via the PATCHMASTER software you need a special cable connecting the Digital Outputs of the interface and the DG-4/DG-5. Please contact HEKA (eimeaorderscmt@harvardbioscience.com) if you need such a cable (Order Number: 895102).

21.2.0.1 PGF Setting of DG-4/DG-5

When using the DG-4/DG-5 you need to define the *Filter Position* in your PGF sequence. Therefore you have to set the DA output channel to *Dig Out (word)* and use *wavelength*. Then you can enter the *Filter Position* directly in the segments (Wavelength) of the PGF sequence.



21.3 Photometry Configuration Lambda-10



Undo: Resets all changes made since the last opening of the Photometry window.

Defaults: Resets all changes to the default entries.

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.

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.

Position: Enter a number for the filter wheel position between 0 and 15.

Speed: Enter a number between 0 and 7.

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.

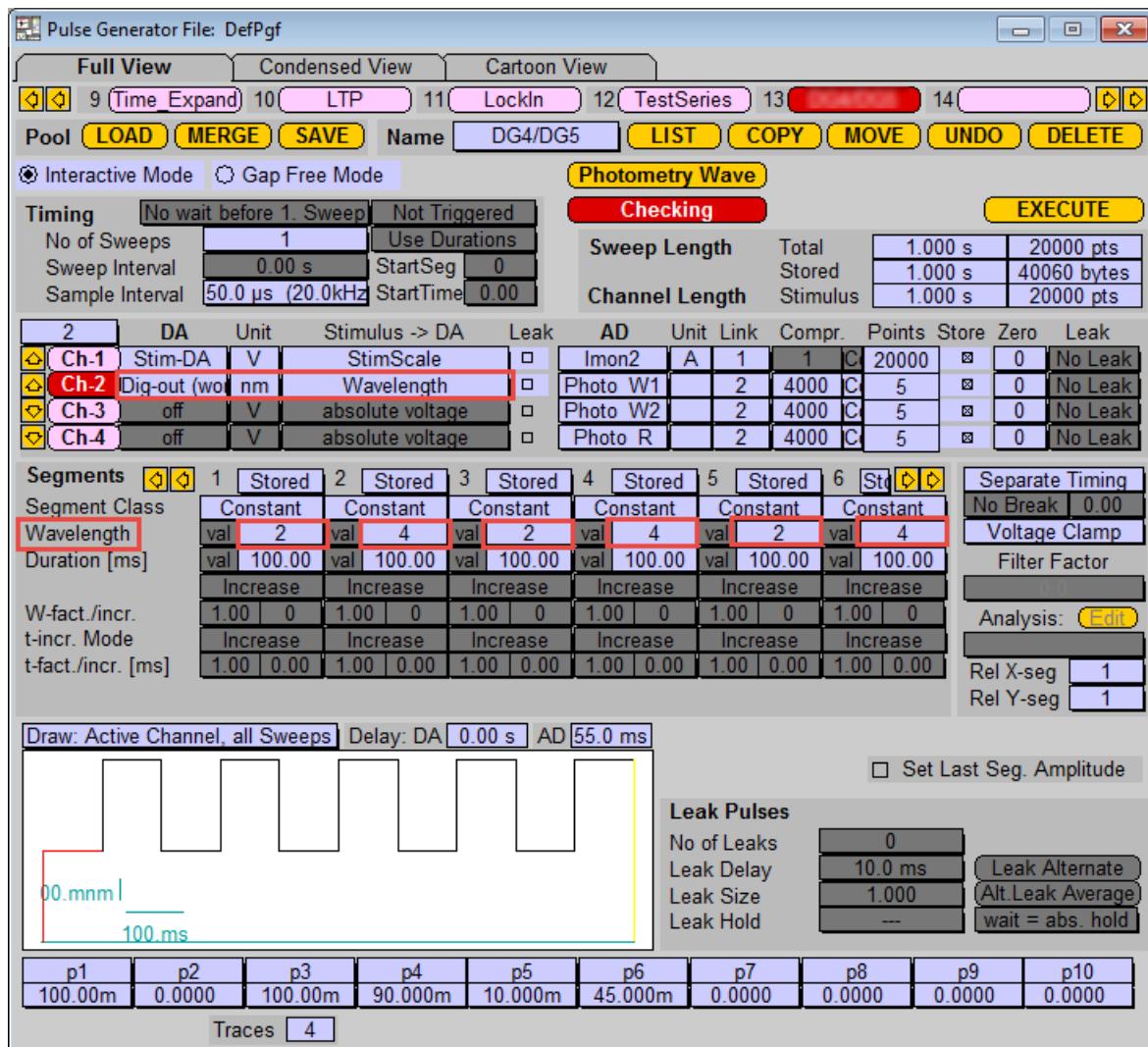
Shutter Open/Close: Toggles between an open and a closed shutter.

Note: To control the Lambda-10 via the PATCHMASTER software you need a special cable connecting the Digital Outputs of the interface and the Lambda-10. Please contact HEKA (eimeaorderscmt@harvardbioscience.com) if you need such a cable (Order Number: 895102).

21.3.0.1 PGF Setting of Lambda-10

When using the Lambda-10 you need to define the *Filter Position* in your PGF sequence. Therefore you have to set the DA output channel to *Dig Out (word)* and use for wavelength. Then you can enter the *Filter Position* directly in the segments (Wavelength) of the PGF sequence.

21.3. Photometry Configuration Lambda-10



21.3.1 Lambda 10-3

Before using the Lambda 10-3 controller for controlling the shutter and the filter wheel via the PATCHMASTER software you need to set the Lambda 10-3 controller into "Parallel Port" mode. By default the Lambda 10-3 controller is configured for "USB" mode. To set it into the "Parallel Port" mode you need to press the following keys at the controller:

- Switch on the device
- Press "Local"
- Press "Mode"
- Press "7 - Default"
- Press "1 - Set the new default"
- Press "4 - Comm Port"
- Press "3 - Parallel Port"
- Press "0 - Set no other defaults"

Once this prerequisite is fulfilled there are two reasonable ways to control the shutter via the Lambda 10-3:

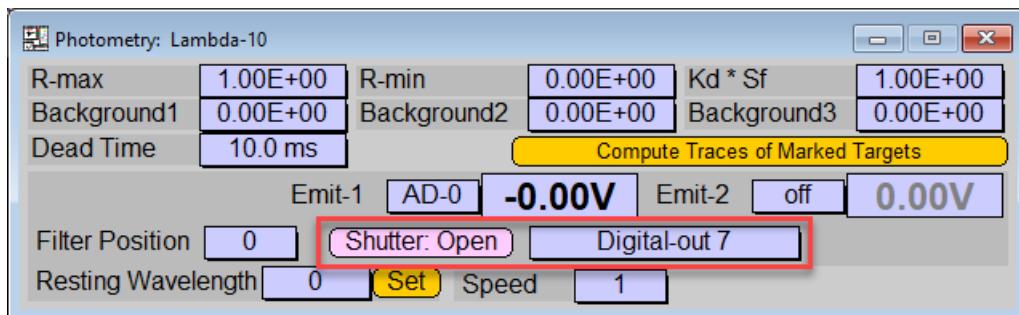
1. Using the digital output channel “Dig-7” controlled in the Photometry dialog ⇒ TTL Mode
2. Using the digital bit (word) commands either in the Pulse Generator, Protocol Editor or I/O Control Window ⇒ Conditional Mode.

TTL Mode

To control the shutter via the “Dig-7” digital port you need to enable “TTL” mode at the Lambda 10-3 controller:

- Switch on the device
- Press “Local”
- Press “Mode”
- Press “3 - TTL”
- Press “Enable TTL”

If the “TTL” mode at the Lambda 10-3 is disabled it is not possible to use the Photometry extension to control the shutter.



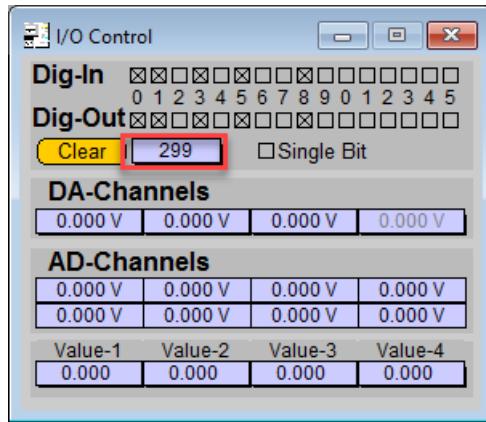
Now, one can use the *Shutter* button in the Photometry Dialog or the digital output channel “Dig-7” in the I/O Control Window or elsewhere to control the shutter.

Conditional Mode

To control the shutter in the *Conditional Mode* you need to disable “TTL” mode at the Lambda 10-3 controller:

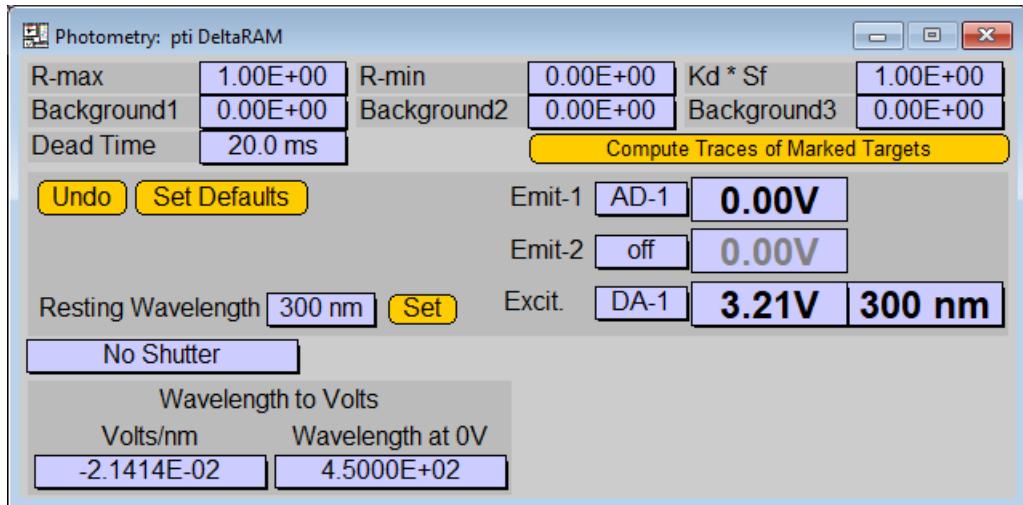
- Switch on the device
- Press “Local”
- Press “Mode”
- Press “3 - TTL”
- Press “Disable TTL”

One can activate the “Conditional Mode” of the Lambda 10-3 e.g. in the I/O Control Window and enter the bit “299” (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.



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.

21.4 Photometry Configuration pti DeltaRAM



Undo: Resets all changes made since the last opening of the Photometry window.

Defaults: Resets all changes to the default entries.

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.

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.

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).

Shutter Selection:

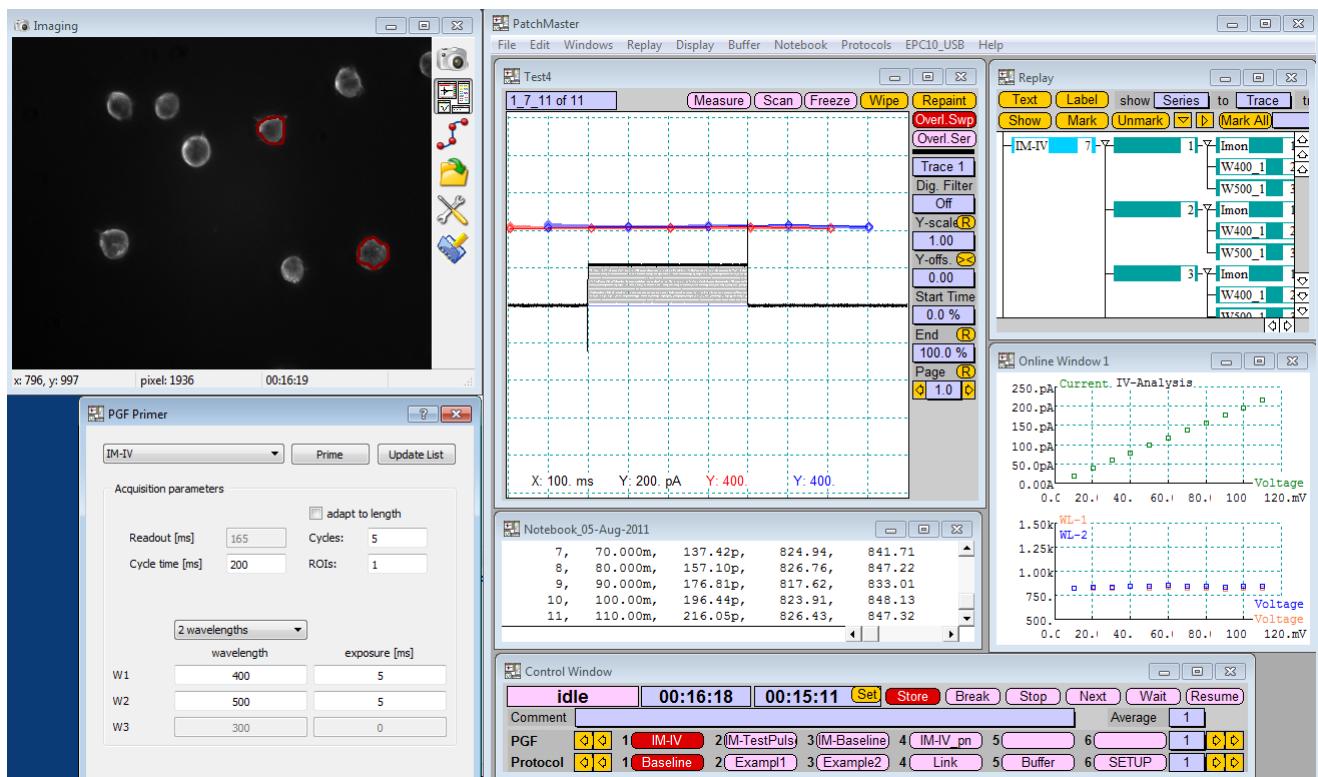
- No Shutter: No shutter available.
- Shutter Control: Shutter can be closed/opened via buttons.
- Digital-out 7: Shutter control via digital output.

21.4. Photometry Configuration pti DeltaRAM

Wavelength to Volt Volts/nm and **Wavelength at 0 V** are internal calibration parameters, which must be given as described in the specifications of your DeltaRAM system. The PTI system has a linear relationship between the applied voltage and the wavelength of the excitation light.

22 SmartLUX (Imaging Extension)

SmartLUX allows to perform simultaneous image acquisition and electrophysiological recordings. The timing of a fluorescence excitation light source, the camera and the electrophysiological command signals are synchronized by the Pulse Generator of PATCHMASTER. Fluorescence mean values from regions of interest (ROI), which can be marked by the user in the image, are plotted together with e.g. the current Trace in the Oscilloscope window and stored together with the other data in PATCHMASTER's data tree. When stepping through the data tree, SmartLUX shows the corresponding image. In case more images have been acquired during a single Sweep, the individual images are shown when stepping through the data points of the fluorescence Trace using the scan function of the Oscilloscope.



SmartLUX consists of an Imaging window in PATCHMASTER, an additional Control window and an Image window.

SmartLUX supports the following hardware:

- Fluorescence Excitation light sources which can be controlled in the same way as:
 - Polychrome and Oligochrome from TILL Photonics
 - DG-4/DG-5 wavelength switcher from Sutter Instrument Company
 - Lambda-10 filter wheels from Sutter Instrument Company

-
- DeltaRAM monochromator from pti
 - Cameras supporting the so-called "Bulb Trigger Mode" from Andor, Jenoptic, QImaging, Photometrics and Hamamatsu.

Image files are stored as 16-bit tiff stacks and can be opened by other programs such as ImageJ, IGOR Pro etc....

For further reading we refer to the SmartLUX manual.

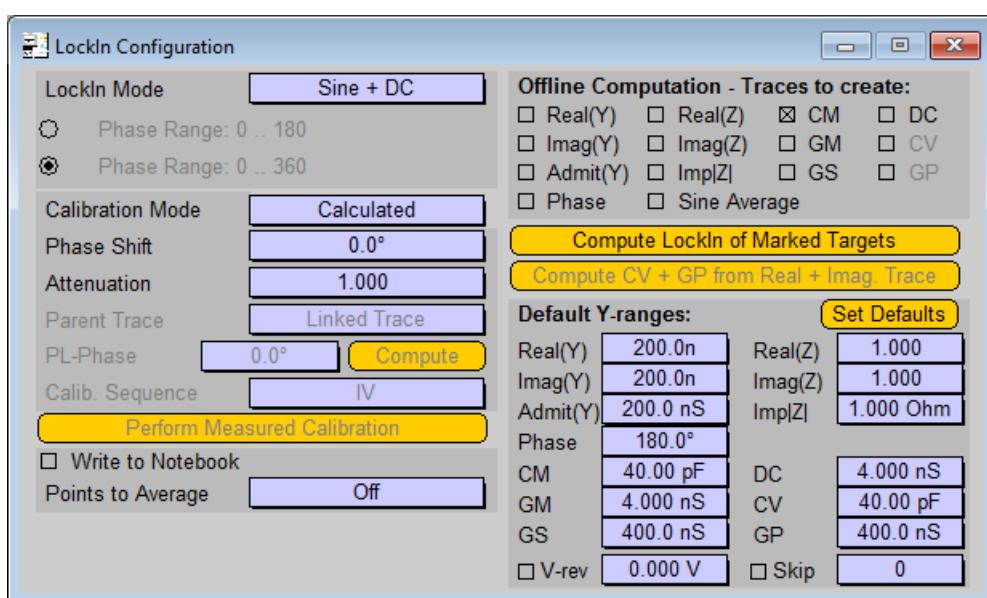
23 Software LockIn Extension

23.1 LockIn Configuration Window

For the activation and the configuration of the *LockIn Extension* you have to enable the software lock-in function in the Configuration window (see 5.4.4 on page 50). After switching on the *LockIn*, the LockIn Configuration window opens, which is later on available in the Window menu.



In the PATCHMASTER 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 PATCHMASTER Tutorial: "Capacitances Measurements using the LockIn Extension".



LockIn Mode: Set a *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 operates an EPC 9 or EPC 10.
- 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).
- 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 9 or EPC 10 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 "nulled out" 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 9 or EPC 10 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*, 23.1 on the facing 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 Tutorial: "Capacitance Measurements using the LockIn Extension".

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 PGF.

Points to Average: A global number of points to average can be entered.

Generate Traces for: Due to the full implementation of the *LockIn* software for C_m measurements, if a Double, Triple or Quadro EPC 10 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. 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.



Offline Computation - Trace to create: These sections of the *LockIn* window are used for calculating *LockIn* data from pre-recorded current *Traces* (for further details see The *LockIn* Traces, 23.3 on page 261).

Compute LockIn of Marked Targets: Computes the *LockIn* *Traces* selected in Offline Computation - Trace to create of the marked targets in the in the *Replay* 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.

Default Y-ranges: The user has to define *Default Y-ranges* for the resulting *LockIn Traces*. PATCHMASTER 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).

SetDefaults: Resets the *Default Y-ranges* to the default values.

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 PGF.

23.2 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 PGF.

A short explanation of the values printed into the *Notebook*:

- 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 conductance 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* 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*.

23.3 The LockIn Traces

The LockIn Extension provides several output *Traces* which can be selected in the *AD-channel* section of the Pulse Generator (10.9.2 on page 125).

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.

$$\text{Phase} = \frac{180}{\pi} * \arctan \text{abs}(B/A)$$

LockIn_Real(Y): The real part of the admittance.

$$\begin{aligned}\text{Voltage Clamp: } \text{Real}(Y) &= A \text{ (Siemens).} \\ \text{Current Clamp: } \text{Real}(Y) &= \frac{A}{A^2+B^2} \text{ (Ohm).}\end{aligned}$$

LockIn_Img(Y): The imaginary part of the admittance.

$$\begin{aligned}\text{Voltage Clamp: } \text{Imag}(Y) &= B \text{ (Siemens).} \\ \text{Current Clamp: } \text{Imag}(Y) &= \frac{B}{A^2+B^2} \text{ (Ohm).}\end{aligned}$$

LockIn_Admit(Y): The admittance (Siemens).

$$\begin{aligned}\text{Voltage Clamp: } \text{Admit}(Y) &= \sqrt{A^2 + B^2}. \\ \text{Current Clamp: } \text{Admit}(Y) &= \frac{1}{\sqrt{A^2 + B^2}}.\end{aligned}$$

LockIn_Real(Z): The real part of the impedance.

$$\begin{aligned}\text{Voltage Clamp: } \text{Real}(Z) &= \frac{A}{A^2+B^2}. \text{ (Siemens)} \\ \text{Current Clamp: } \text{Real}(Z) &= A \text{ (Ohm).}\end{aligned}$$

LockIn_Img(Z): The imaginary part of the impedance.

$$\begin{aligned}\text{Voltage Clamp: } \text{Imag}(Z) &= \frac{B}{A^2+B^2}. \text{ (Siemens)} \\ \text{Current Clamp: } \text{Imag}(Z) &= B \text{ (Ohm).}\end{aligned}$$

LockIn_Imp|Z|: The impedance (Ohm).

$$\begin{aligned}\text{Voltage Clamp: } \text{Imp}(Z) &= \frac{1}{\sqrt{A^2 + B^2}}. \\ \text{Current Clamp: } \text{Imp}(Z) &= \sqrt{A^2 + B^2}.\end{aligned}$$

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:

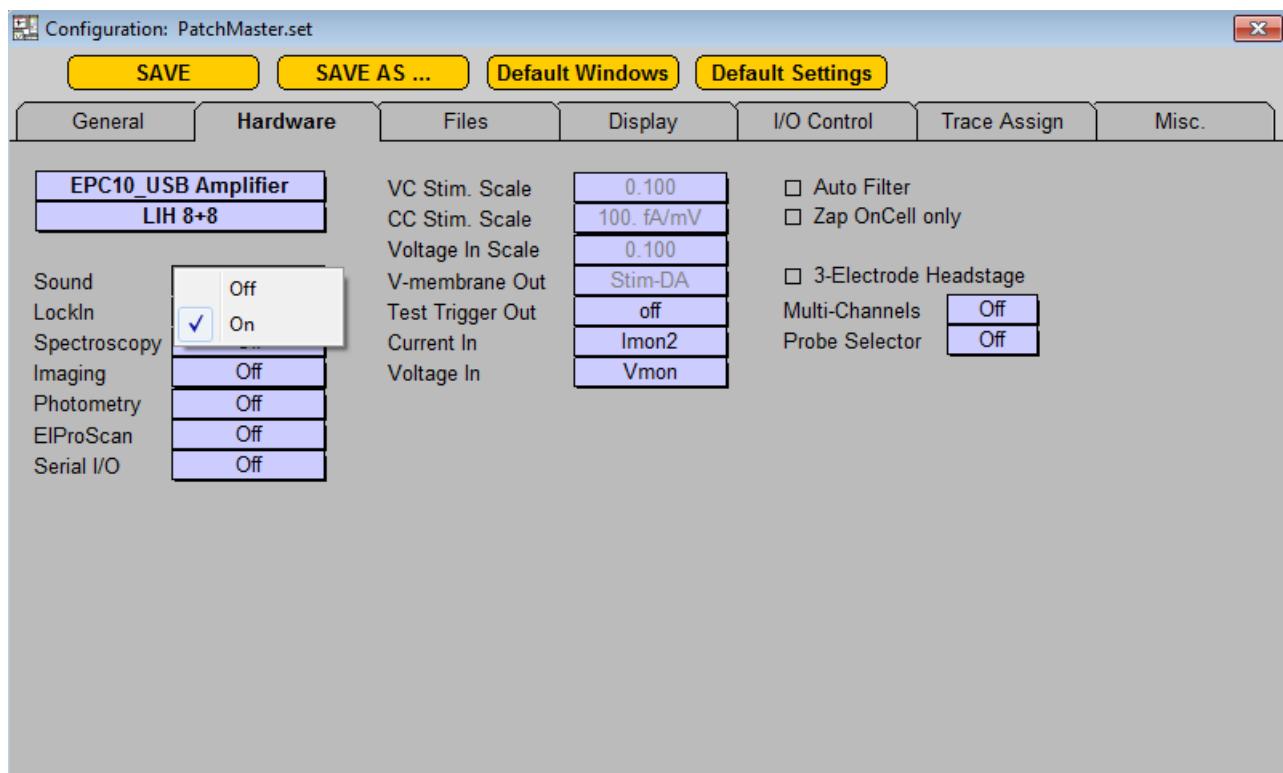
- A = Real part of the admittance.
- B = Imaginary part of the admittance.
- I_{dc} = DC current.
- E_{rev} = Reversal potential.
- V_h = Stimulus voltage.
- f_{sine} = Stimulation frequency.

A detailed description of all terms can be found in the literature (see reference list of the PATCHMASTER Tutorial: "Capacitances Measurements using the LockIn Extension").

23.4 Capacitance Measurements - Step by Step

Here, we make our first capacitance measurement using a model circuit and the LockIn Extension. We used an EPC 10 Single for this tutorial, but most settings are identical for EPC 10 Double, Triple or Quadro amplifiers and for EPC 9 Single, Double and Triple amplifiers. We will explicitly mention the particular amplifiers, where it is required.

23.4.1 Activating the LockIn Extension



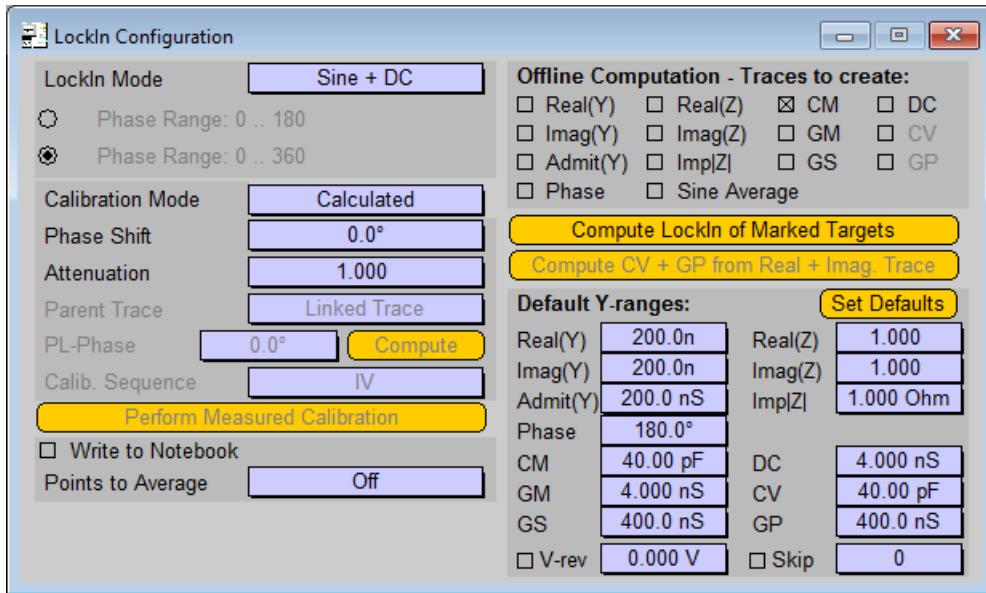
By default, the LockIn Extension of PATCHMASTER is inactive. To activate the extension, we have to open the Hardware tab of the Configuration window and turn on the *LockIn*. This step only has to be done the first time one uses *LockIn*. The setting is stored in the PATCHMASTER configuration file `Patchmaster.set`.

23.4.2 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 or EPC 9 amplifier. 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, Write to Notebook should be checked and at least one LockIn trace has to be selected as an AD input channel in the PGF.

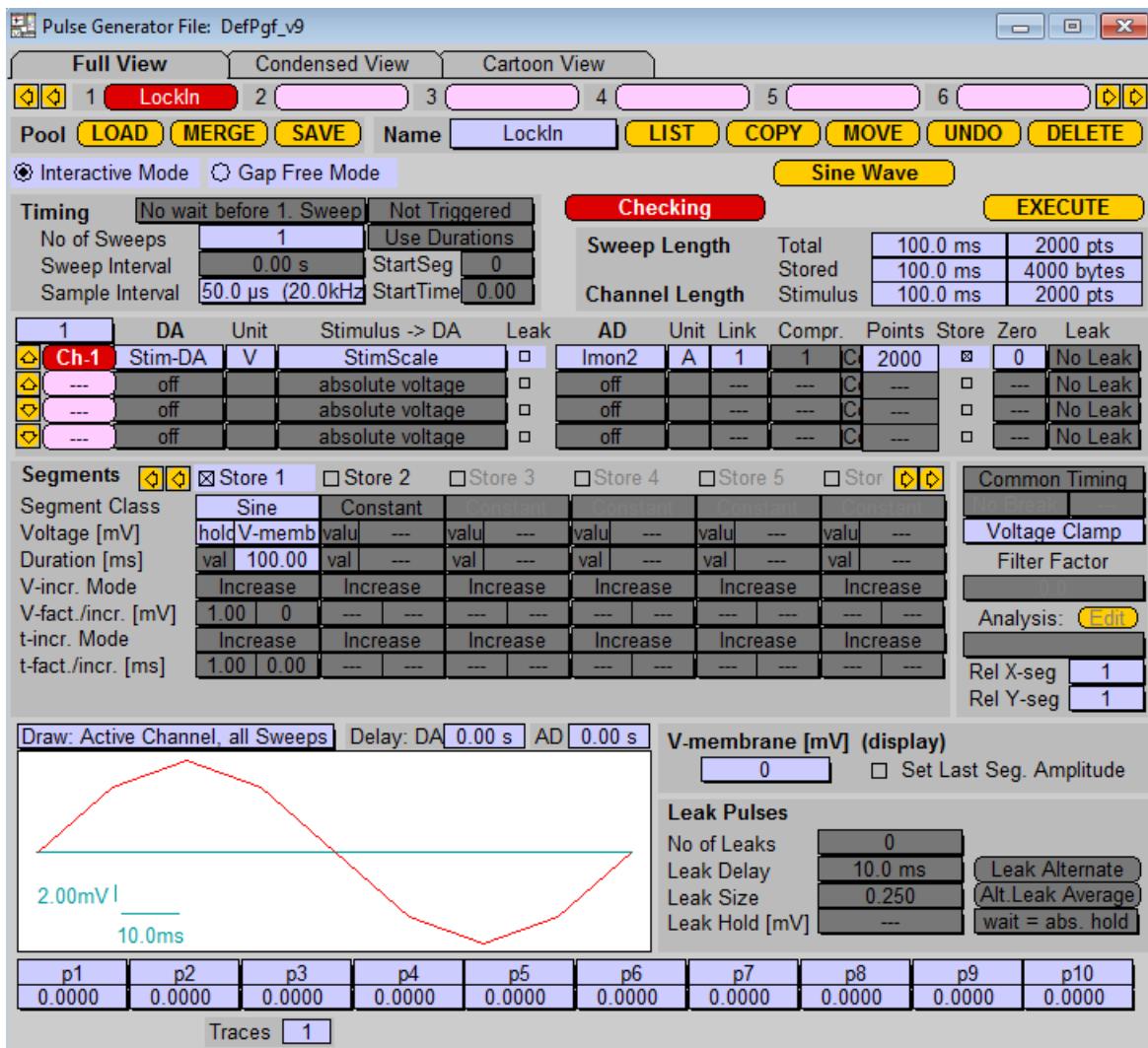


Note: The Default Y-ranges section can be used to set reasonable display ranges according to the expected values.

23.4.3 Creating a PGF sequence

The next step is to prepare a PGF sequence, that can be used for C_m measurements. Please open the Pulse Generator 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 an empty button in the sequence pool will create a new sequence and you are prompted to enter a name for the new sequence. We name the sequence we want to create "LockIn".

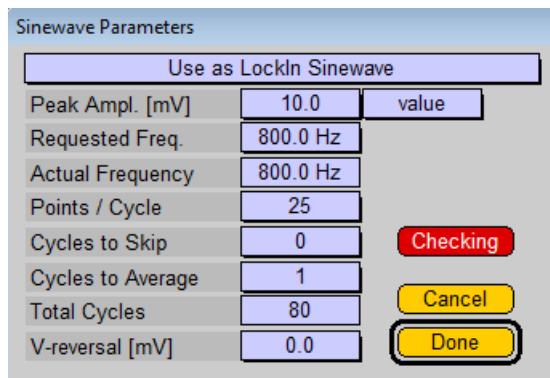
PATCHMASTER automatically creates a new and very simple protocol: it just consists of one *Constant* segment with a duration of 10 ms. 10 ms is quite short, therefore we will increase the duration to 100 ms. Furthermore, we have to change the *Segment Class* to *Sine*, since at least one sine wave segment 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.



The *Sample Interval* is set to 50 µs. 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 "hold". If *Voltage* is set to "hold", the holding potential from the Amplifier window is used.

If a *Sine* segment exists in the PGF sequence an additional button (**Sine Wave**) appears in the dialog. This button can be used to modify the parameters (frequency, amplitude,...) of the sine wave segments in a protocol (see *Sine Wave Parameters*, 10.8.1 on page 112).

In this dialog, you can decide, if the *Sine* segments in the sequence should be used for *LockIn* measurements (Use as LockIn Sinewave) or just as a simple sine wave stimulus (Use as 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 durations 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.

Please note, that the *Sample Interval* in the PGF 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 *V-reversal* of 0 mV is also correct for our model circuit. The *V-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. Now, we can go back to the PGF sequence.

Since we decided to use the *Sine* segment in the sequence for *LockIn* measurements, the entry *Stimulus → DA* in the PGF sequence has automatically been changed from "StimScale" to "StimScale, LockIn" upon closing the Sinewave Parameters dialog. This *LockIn* flag is important. Without that flag, no C_m data can be calculated.

1	DA	Unit	Stimulus → DA	Leak
Ch-1	Stim-DA	V	StimScale, LockIn	<input type="checkbox"/>
---	off		absolute voltage	<input type="checkbox"/>
---	off		absolute voltage	<input type="checkbox"/>
---	off		absolute voltage	<input type="checkbox"/>

The *AD input* is set to "Imon-2", the current input of your amplifier. This is undoubtedly a good idea, however, we need an additional *Trace* to store the C_m value. A click in an empty AD field opens a long list of available inputs. What we need is a *LockIn_CM Trace*. You will see, that the compression factor is automatically set to "25" (the value, we have set for *Points/Cycle* in the Sinewave Parameters 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*.

AD	Unit	Link	Compr.	Points	Store	Zero	Leak
Imon2	A	1	1	C 2000	<input checked="" type="checkbox"/>	0	No Leak
LockIn CM	F	1	25	C 80	<input checked="" type="checkbox"/>	0	No Leak
off		---	---	C ---	<input type="checkbox"/>	---	No Leak
off		---	---	C ---	<input type="checkbox"/>	---	No Leak

Please note, that *Link* is set to "1". This tells PATCHMASTER to use the first *Trace* (the current *Trace*) 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 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*.

23.4.4 Amplifier Settings

Now it is time for the experiment. Switch the model circuit into the "10 M" position to simulate a $10\text{ M}\Omega$ pipette that is open to the bath solution.

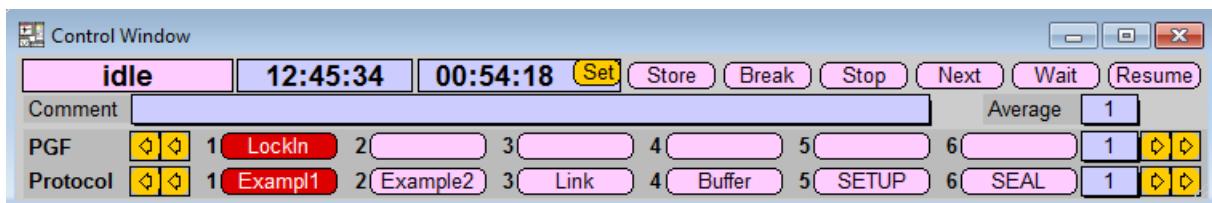
In the Amplifier window you can correct pipette offset potentials by adjusting the V_0 value or you can alternatively click on the *Auto V_0* button to let PATCHMASTER do this correction automatically for you. The same is done by calling the protocol "SETUP".

Now 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.

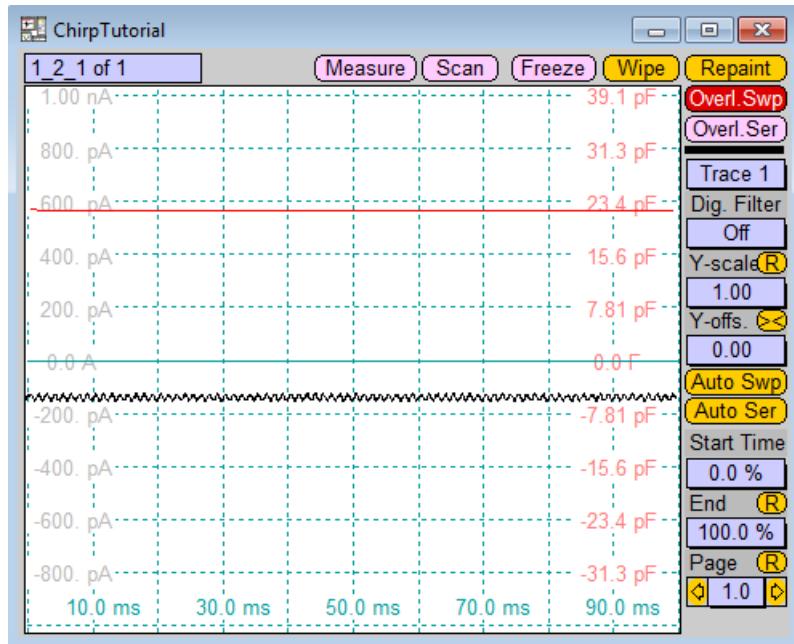
To break into the cell, set the switch of the model circuit to the "0.5 G" position. Make an automatic slow capacitance cancellation by clicking on the *Auto C-slow* or "WHOLE-CELL" protocol button. With the *V-membrane* control change the pipette holding potential to "-70 mV". Now we are ready to run the PGF sequence we defined before.

23.4.5 Running the PGF sequence

In PATCHMASTER, PGF sequences can be run from the Pulse Generator directly (*Execute* button) or from the Control window. We click on the *LockIn* button to start the sequence.



The results are displayed in the Oscilloscope. As defined in the PGF sequence, you can see the current *Trace* (black) and a second *Trace* (red) with the *LockIn* data.



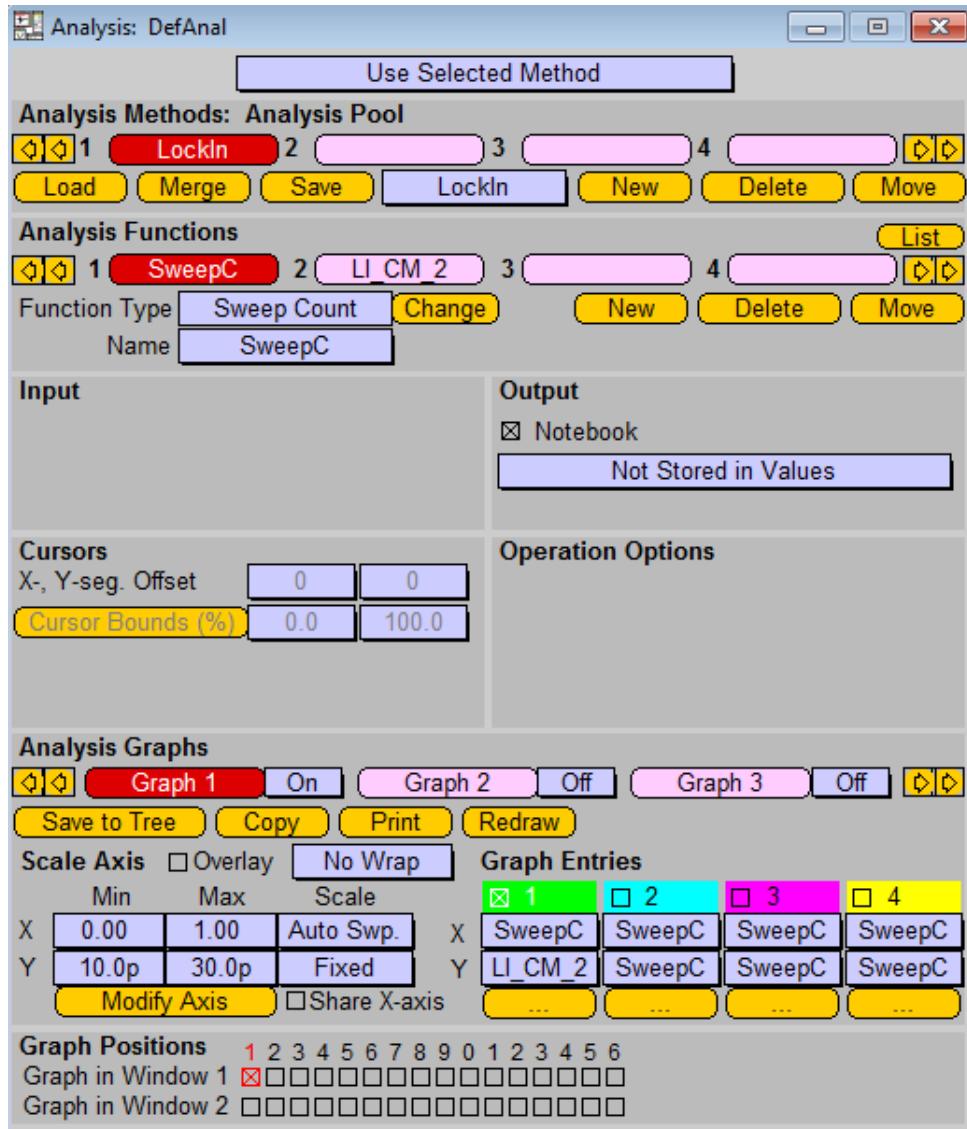
Note: Change the display labeling to Grid + Values in order to display the axis scaling.

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 *Display* menu. 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*).

23.4.6 Analysis

In the *Oscilloscope*, the *LockIn* information is displayed with a high time resolution. Even fast membrane capacitance changes can be observed in that way.

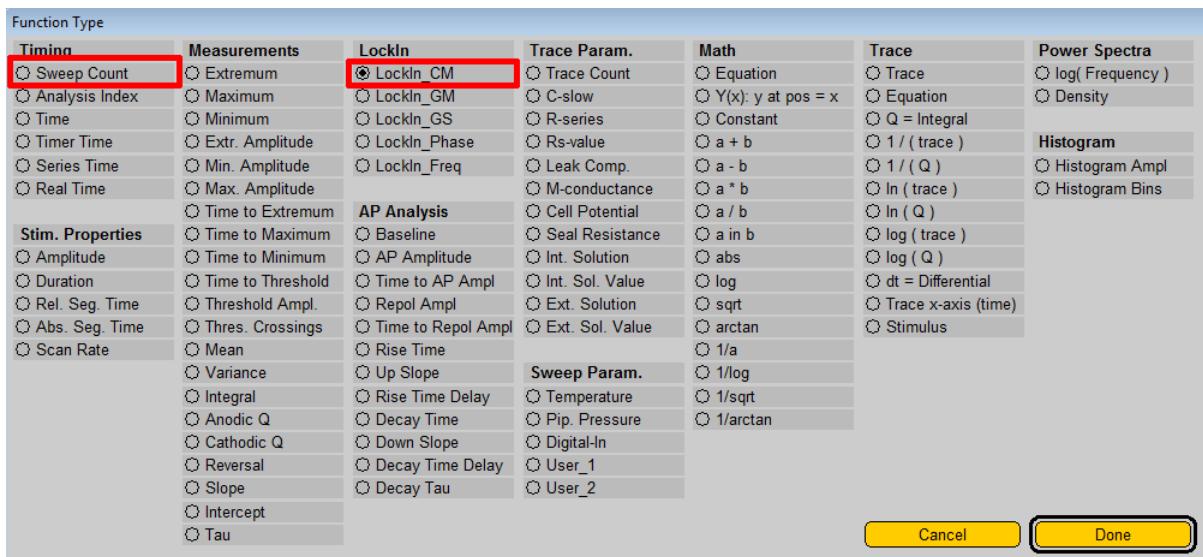
However, in some experiments, the expected C_m changes are in the range of several seconds or minutes. In that case, we should use the *Analysis* to monitor the changes in membrane capacitance. For that purpose, it is necessary to modify the *LockIn PGF* sequence. We will increase the *No of Sweeps* from 1 to 100. That is all we have to do for the moment.



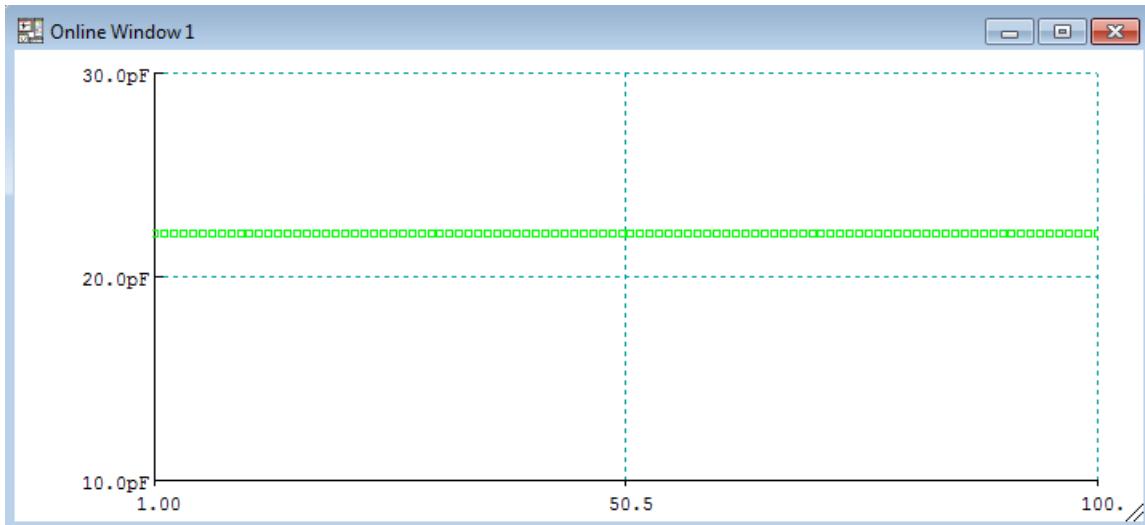
Click on an empty button to define a new *Analysis Method*. We name the new analysis "LockIn". We decline the question if we want to copy the settings from an previous method. Again we will start from the scratch. Further, you have to do the following modifications:

- **Graph Positions:** Activate checkbox "1" in the row "Graphs in Window 1". This enables the display of the *Graph 1* (which will be defined below) in the *Analysis Window 1*. Enable "Use Selected Method" to make sure that the now specified *Analysis Method* is used.
- **Analysis Functions:** Click on an empty field and the Function Type dialog will open. First, select *Sweep Count* for the X-axis analysis then click again on an empty button field and select *LockIn_CM* for Y-axis analysis. Please take care that Trace # for the proper C_m values has to be Trace 2.
- **Analysis Graphs:** Switch on the *Graph 1*.
- **Graph Entries:** Activate the checkbox "1" and select *SweepC* (Sweep Count) for X-axis and *LI_CM_2* (CM for trace 2) for Y-axis.
- **Scale Axis:** Select *Auto Swp.* for X-axis and *Fixed* for Y-axis. 10 to 30 pF (10 p / 30 p) are sufficient for our model cell.
- **Analysis Window:** If not visible, go to the Windows menu and activate *Analysis Window 1*.

23.4. Capacitance Measurements - Step by Step



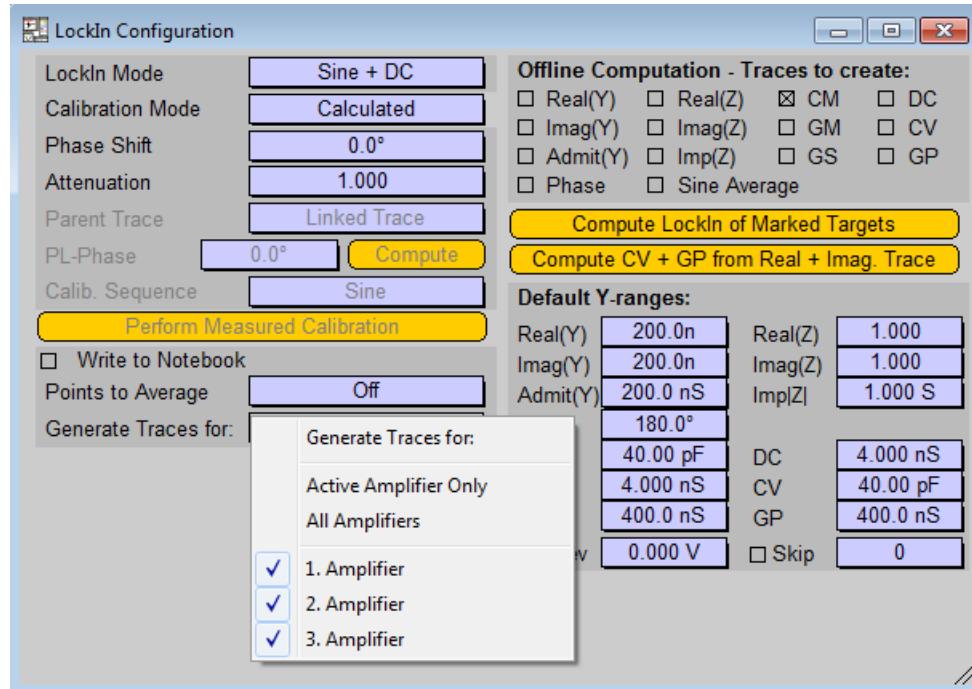
If we now run the *LockIn* PGF sequence, one *LockIn* data point per *Sweep* is plotted in the Analysis window. This single point is the mean value from all C_m values within a *Sweep*.



23.5 C_m measurements with Double or Triple amplifiers

We recommend to read the section Capacitance Measurements – Step by Step before you go on with this section.

If a multiple amplifier configuration is used, you can specify which amplifiers should be used for *LockIn* measurements. By default, all available amplifiers are selected and you should keep that setting as long as you do not have a good reason to change it.



For *LockIn* measurements with multiple amplifiers, we have to add additional *Traces* to our PGF sequence. For stimulation of the second amplifier, we add the DA output channel *Stim-2*, which is the stimulus output of the second amplifier board. Furthermore, we have to set the *LockIn* flag for that DA channel.

	DA	Unit	Stimulus -> DA	Leak	AD	Unit	Link	Compr.	Points	Store	Zero	Leak
1	Ch-1	V	StimScale, LockIn	<input type="checkbox"/>	Imon-1	A	1	1	C	2000	<input checked="" type="checkbox"/>	0 No Leak
	Ch-2	V	StimScale, LockIn	<input type="checkbox"/>	LockIn CM	F	1	25	C	80	<input checked="" type="checkbox"/>	0 No Leak
	Ch-3	V	absolute voltage	<input type="checkbox"/>	Imon-2	A	2	1	C	2000	<input checked="" type="checkbox"/>	0 No Leak
	Ch-4	V	absolute voltage	<input type="checkbox"/>	LockIn CM	F	3	25	C	80	<input checked="" type="checkbox"/>	0 No Leak

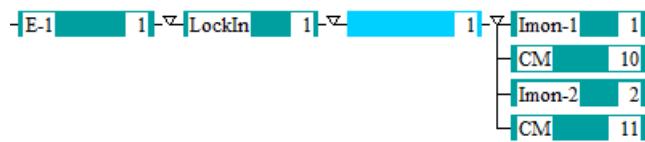
Channel 3 is set to "Imon-2", the current input of the second amplifier board. Finally, channel 4 is set to *LockIn Cm*. Let's have a look on the *Link* column:

1. Ch-1: AD channels are always linked to a DA output channel. Therefore "Imon-1" is linked to the corresponding *Stim-1* DA output.
2. Ch-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* channels uses Ch-1 as data input, therefore the *Link* is set to "1".
3. Ch-3: This current channel is linked to *Stim-2*, the stimulus DA output of the second amplifier.
4. Ch-4: This is a "virtual" channel and therefore is linked to "its" current channel.

In case you are working with many different Pulse Generator sequences that record a different number of channel, you should use the *Trace Assignment* which makes it easier to keep track with them.

- Close your data file.
- Select the *Trace Assign* tab of the Configuration window.
- Assign your *Traces*, e.g.:
 - Trace 1: Amplifier – Imon-1
 - Trace 2: Amplifier – Imon-2
 - Trace 10: LockIn – LockIn_CM
 - Trace 11: LockIn – LockIn_CM
- Save your changes.
- Restart PATCHMASTER.
- Execute the PGF "LockIn".

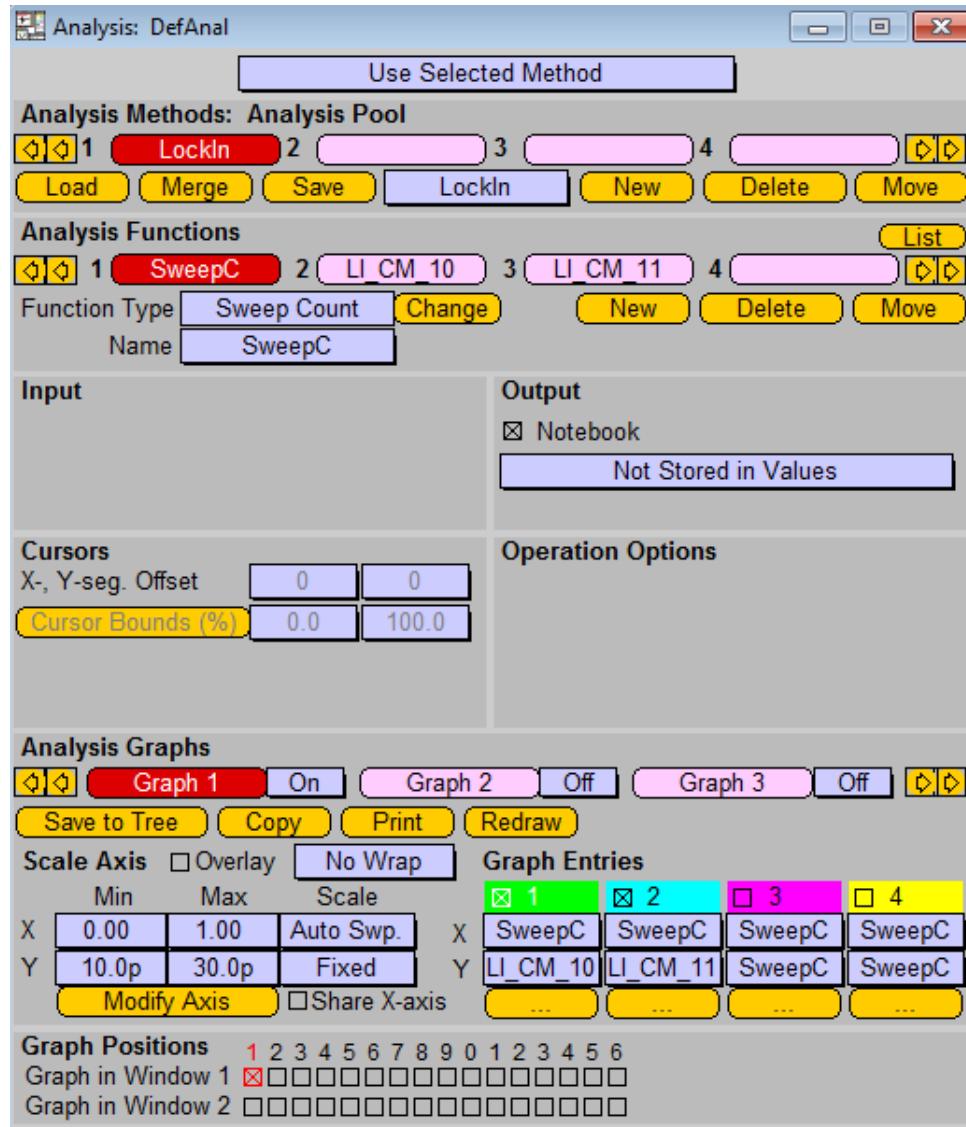
The number of the *Traces* in the *Replay* window is now:



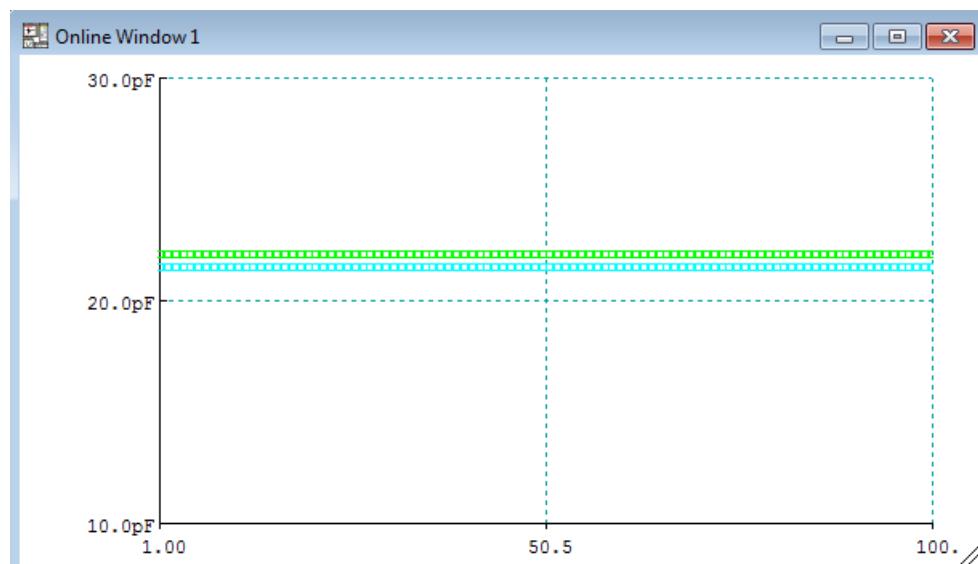
Of course, we want to monitor the measures C_m values in the *Analysis*. Therefore, we have to modify the *Analysis* settings. However, the required changes are quite moderate.

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*. The X-axis for that entry can also be set to *Sweep Count*, but for the Y-axis, we need a new *Analysis Function*. So, click on an empty entry in the *Analysis Functions* section and select *LockIn_CM*. It is now important to set the *Trace #* of the *Analysis Function 2* and *3* to *LockIn_CM*. The *Analysis Functions* 2 and 3 are now named *LI_CM_10* and *LI_CM_11*, respectively.

23.5. C_m measurements with Double or Triple amplifiers



Before starting the PGF sequence, you should make all required settings for both amplifiers as described in Amplifier Settings, 23.4.4 on page 267 and Running the PGF sequence, 23.4.5 on page 267.



We used two model circuits with slightly different capacitance values. One is slightly below, the other slightly above 20 pF. Therefore, we use again the fixed scaling range of "10 p" and "30 p" in the Analysis.

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.

23.6 Using LockIn with amplifiers other than EPC 9/10

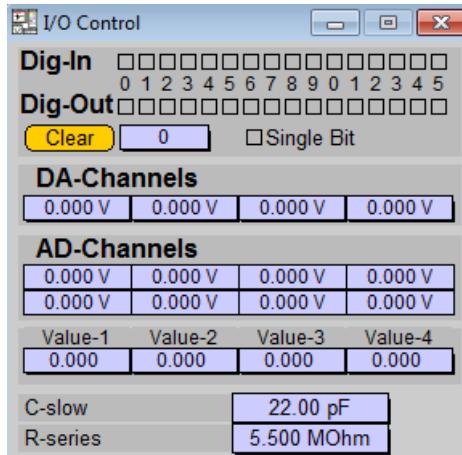
The following are important points to keep in mind when using *LockIn* with an amplifier other than an EPC 9 or EPC 10.

LockIn Configuration: Measured Calibration must be selected as the Calibration Mode in the LockIn Configuration window. Capacitance compensation must be switched Off and the C-slow parameter must be set to "0.0" in the Configuration window when a Measured Calibration is performed.

Note: As discussed in *LockIn Configuration*, 23.1 on page 257, 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 Configuration window as default Parameter values or via the I/O Control window.



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.

24 Spectroscopy Extension

24.1 Introduction

The Spectroscopy 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 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 Spectroscopy Extension, however, uses a stimulus containing a set of multiple frequencies, allowing to calculate a complete spectrum from a single measurement.

24.1.1 Chirp Wave Forms

The Pulse Generator of PATCHMASTER provides three different chirp wave forms:

Linear Chirp:

In a *Linear Chirp*, the frequency $f(t)$ varies linearly with time:

$$f(t) = f_0 + kt \quad (24.1)$$

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:

$$x(t) = \sin[2\pi(f_0 + \frac{k}{2}t)t] \quad (24.2)$$

Exponential Chirp:

In an *Exponential Chirp*, the frequency of the signal varies exponentially as a function of time.

$$f(t) = f_0 k^t \quad (24.3)$$

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:

$$x(t) = \sin[\frac{2\pi f_0}{\ln(k)}(k^t - 1)] \quad (24.4)$$

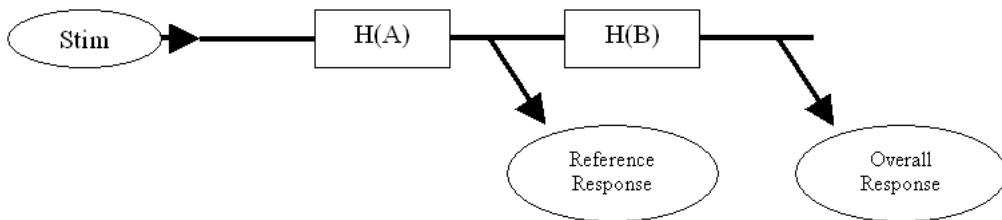
Spectroscopy Chirp:

A chirp wave form created by inverse *Fourier Transform* from a spectrum with frequencies increasing with t^2 and constant amplitude. The Spectroscopy Extension uses this stimulus wave form as input only.

24.1.2 Transfer Function Ratio

The transfer function (H) of two signal processing units in series is given by:

$$H(A + B) = H(A) \otimes H(B) \quad (24.5)$$



In case one is interested the transfer function of an individual signal processing unit, the "ratio" of the transfer function can be calculated:

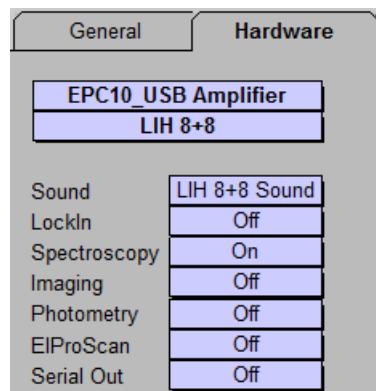
$$H(B) = \frac{H(A + B)}{H(A)} \quad (24.6)$$

The Spectroscopy Extension provides a so-called *Correction Mode* to calculate the *Transfer Function Ratio* of the recorded response (nominator) and the reference response (denominator).

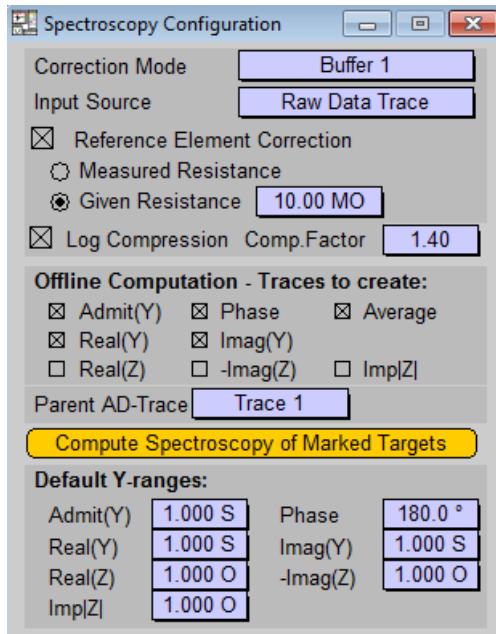
In some application a reference element (e.g. calibrated resistor) is used for a calibration measurement of the system. In this case the value of the reference element has to be taken into account when calculating the transfer function of the measuring system without reference element. We offer the option to use the measured resistance or a given resistance for this correction.

24.2 Activating the Spectroscopy Extension

The Spectroscopy Extension is activated in the Configuration window on the tab *Hardware*.



Once the extension is turned *On*, the Spectroscopy window can be opened via the Windows menu list in PATCHMASTER.



Correction Mode: It is possible to correct the spectra during online and offline calculation with respect to another chirp response (see Transfer Function Ratio, 24.1.2 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 calibrated with respect to a reference element (e.g. a $10\text{ M}\Omega$ 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 unscaled raw 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.

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 spectroscopy, either *All* or any other *Trace* (Trace 1...16).

Compute Spectroscopy of Marked Targets: Recalculates and generates the selected *Traces* (select and mark the target (*Sweeps* or *Series*) first).

Default Ranges: For the various spectroscopy *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 shows a range from -1 S to +1 S.

24.2.1 The Spectroscopy 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($^{\circ}$). 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:

$$\text{Real}(Y) = \frac{\text{Admittance}}{\sqrt{1 + \tan(\text{Phase})^2}} \quad (24.7)$$

The default unit is Siemens (S).

Chirp_Img(Y): The imaginary part of the admittance. This is a derived frequency based *Trace* (X-unit is Hertz), calculated as follows:

$$\text{Imag}(Y) = \tan(\text{Phase}) * \text{Real}(Y) \quad (24.8)$$

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:

$$\text{Real}(Z) = \frac{\text{Real}(Y)}{\text{Real}(Y)^2 + \text{Imag}(Y)^2} \quad (24.9)$$

The default unit is Ohm (Ω).

Chirp_Img(Z): The imaginary part of the impedance. This is a derived frequency based *Trace* (X-unit is Hertz), calculated as follows:

$$-\text{Imag}(Z) = \frac{\text{Imag}(Y)}{\text{Real}(Y)^2 + \text{Imag}(Y)^2} \quad (24.10)$$

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:

$$Imp|Z| = \frac{1}{\sqrt{Real(Y)^2 + Imag(Y)^2}} \quad (24.11)$$

The default unit is Ohm (Ω).

24.3 Setting up a Spectroscopy Acquisition

Create a new PGF 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 top right section of the Pulse Generator window.

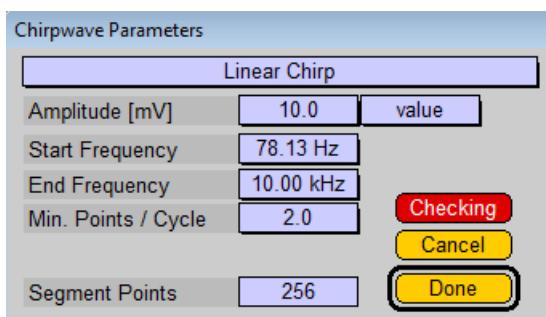
24.3.1 The Chirp Wave Dialog

A detailed description of the available chirp wave parameters can be found in *Chirp Wave Parameters*, 10.8.3 on page 118.

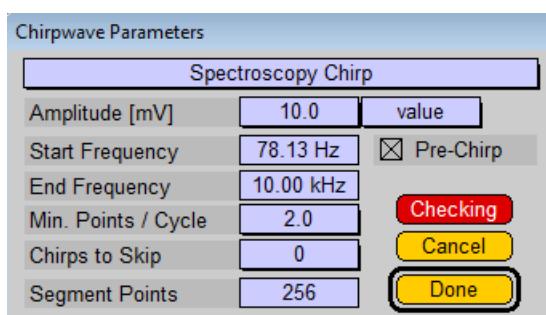
24.3.2 Parametrization of the Chirp Stimuli

Possible Frequency Range: The maximal possible frequency in a chirp wave is half the sampling frequency (*Min. Points / 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.



- 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.



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.

24.3.3 Adding Chirp Analysis Traces

Once you have configured the chirp stimulus you can add multiple chirp analysis *Trace* to the AD selection in the Pulse Generator.

Dig-15
Chirp_Avg
Chirp_Phase
Chirp_Admit(Y)
Chirp_Real(Y)
Chirp_Imag(Y)
Chirp_Real(Z)
Chirp_Imag(Z)
Chirp_Imp Z
LockIn_Avg

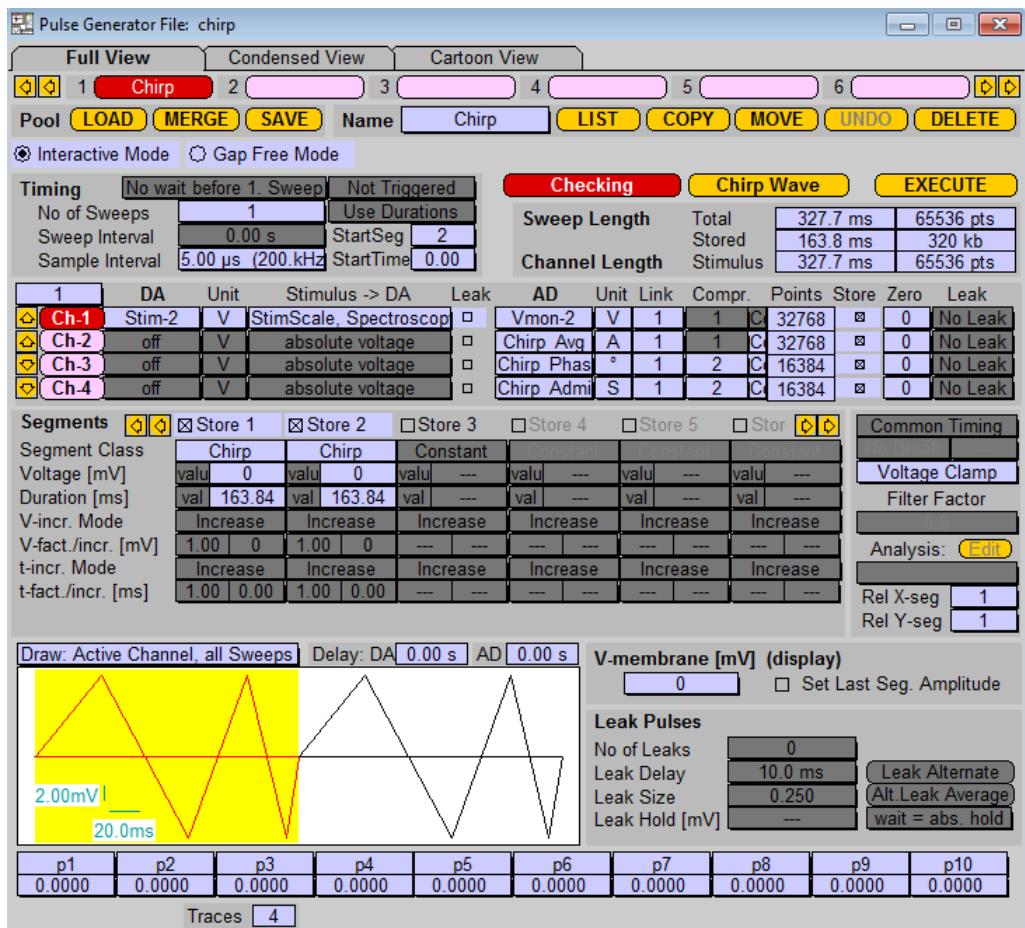
24.3.4 Minimizing Swing-In Effects

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 *StartSeg* to "2". In case of very long chirp stimuli this method is very time consuming.

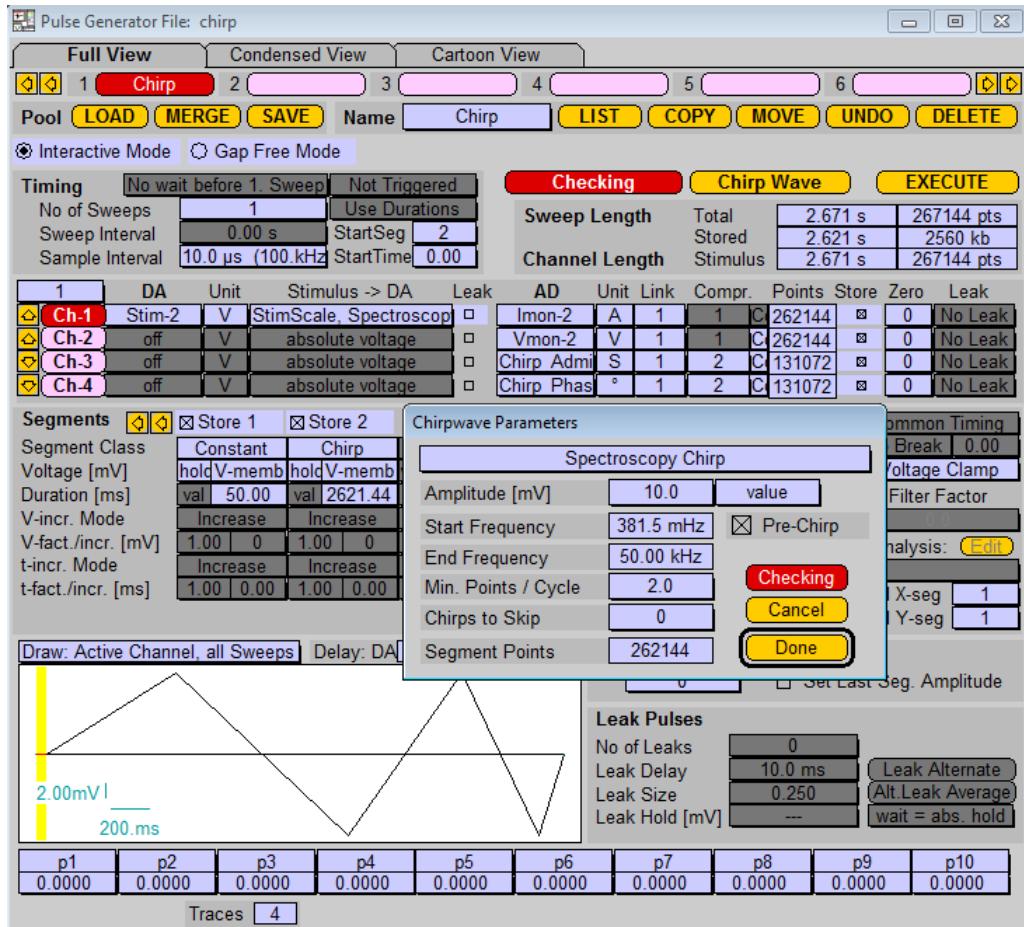
Note: When you have more than one Chirp segment, please deactivate the checking routines during editing the parameters of the segments. Once you have finished the edition, turn the Checking on again.

In the following screenshot you see a sample Pulse Generator sequence for a chirp acquisition.

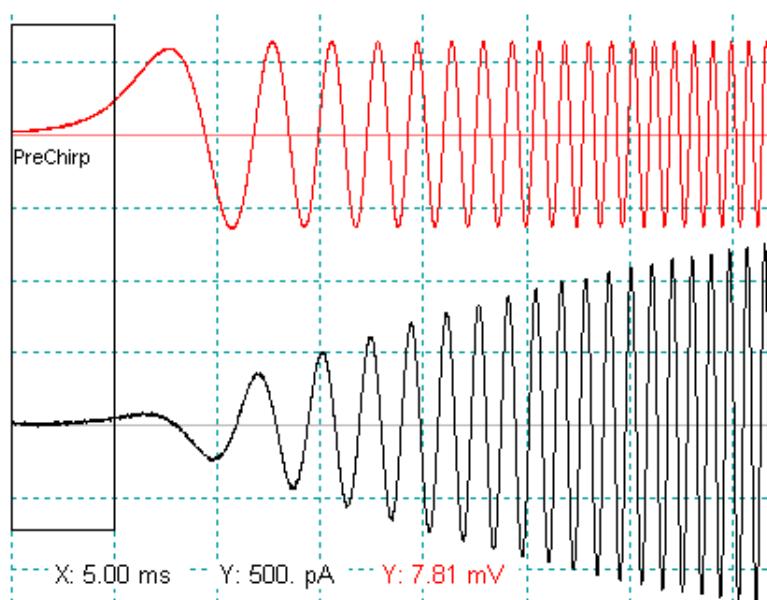


- Use of a Pre-Chirp Segment: In order to reduce the time for swing in to a minimum, a *Constant* segment of fixed length can be defined before the *Chirp* segment. When the option *Pre-Chirp* is activated in the Chirpwave Parameters dialog, then this segment is automatically filled with appropriate swing-in stimulus data. Please note that the *Pre-Chirp* segment should not be recorded (use *Start Segment "2"*) to ensure accurate chirp analysis.

24.3. Setting up a Spectroscopy Acquisition

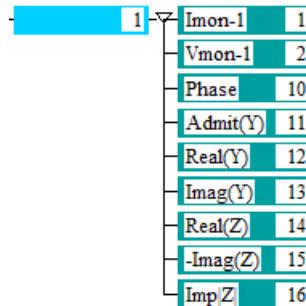


In the following screenshot the swing in signal of a *Pre-Chirp* segment is shown. The first 5 ms of the stimulus are the *Pre-Chirp* segment which provides an optimal swing-in of the chirp segment.

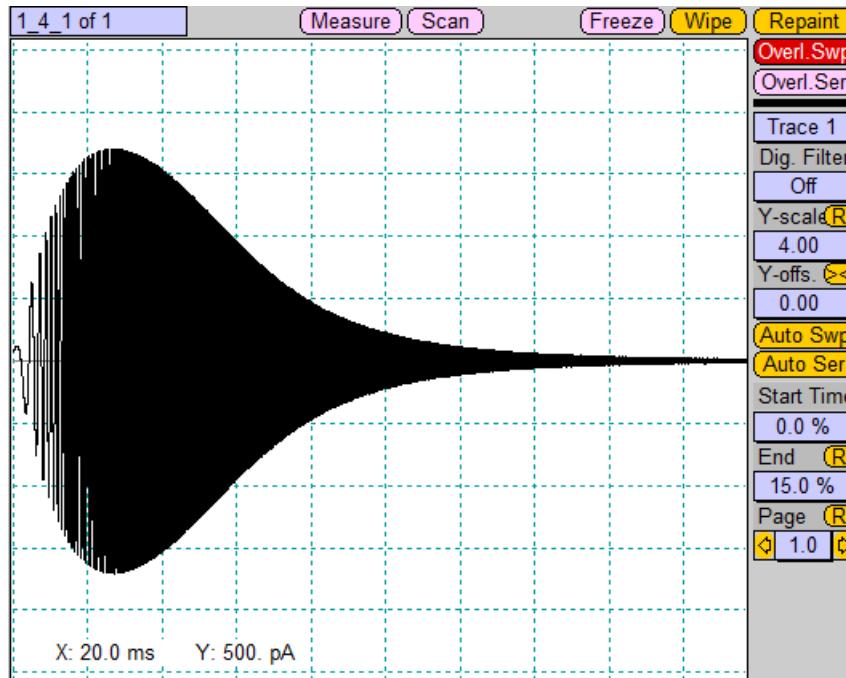


24.4 Display of Spectra in the Oscilloscope Window

The chirp analysis *Traces* are stored in the *Replay* window.



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, which supports only one X-scaling. By default you will see the raw data *Trace* in the Oscilloscope, since the Oscilloscope takes its X-scaling from the first *Trace* in the *Sweep*.



In order to show also the chirp analysis *Traces* in the Oscilloscope, please select *Fixed X-width* from the *Display* menu and enter as *X-max* (panel to the right in the Oscilloscope window) the maximum frequency in the spectrum (e.g. 12k, what stands for 12 kHz).

24.4. Display of Spectra in the Oscilloscope Window



Trace 10 (orange) and Trace 11 (green) show the phase and the admittance, respectively.

25 Appendix I: File Overview

25.1 File Types

Figure 25.1: The different file types of PATCHMASTER, POTMASTER AND CHARTMASTER

File Type	Extension	Default File	Note
Amplifier file (only generated when multiple amplifiers are active (EPC 10 USB Double, Triple and Quadro))	*.amp		Chapter 26.1 on page 287
Data file (includes the measured data)	*.dat		
Dialog setting for the dialog of windows (if no dialog file exists, the software will start with its own default values.)	*.dia		Chapter 2.3 on page 10; (*.dia files are always named after the window that is saved, e.g., Oscilloscope.dia.)
EPC files for amplifier-dependent calibration parameters; SCALE for amplifier calibration; CFAST for Cfast calibration. Starting with EPC 10 amplifiers of revision "N" the scale file is stored on the EEPROM of the amplifier.	*.epc	SCALE-XXXXXX.epc	
Key file for key commands (includes all key commands)	*.key	Patchmaster.key	Chapter 3 on page 13
Macro file for macros; not supported anymore starting with version 2.40	*.mac		Chapter 11 on page 139
Markers file for all marker information	*.mrk		Chapter 19 on page 235
Method file for Protocol Editor Methods	*.mth		Chapter 12 on page 165
Online file for Analysis settings (can include more than one Analysis Method)	*.onl	DefAnal.onl	Chapter 13 on page 187
PG3 files for amplifier-dependent calibration parameters SCALE for potentiostat calibration	*.pg3	SCALE-XXXXXX.pg3	Only for Potentiostats (PG Series)
Stimulus file for the Pulse Generator settings	*.pgf	DefPgf.pgf	Chapter 10 on page 107
Protocol file for a protocol pool (can include more than one protocol)	*.pro	DefProt.pro	Chapter 11 on page 139
Equation file for storing equation strings	*.txt	Equation.txt	Chapter 20 on page 239
Acquisition parameters file	*.pul		

Continues on next page

25.1. File Types

File Type	Extension	Default File	Note
Settings file	*.set	Patchmaster.set	
Solution file for storing the entries of the Solution Database	*.sol	DefSolutionBase.sol	Chapter 17 on page 229
Filename template file	*.tpl		Chapter Using a Recorded Waveform as Stimulus in the PATCHMASTER Tutorial
Notebook file	*.txt		Chapter 4.7 on page 34

26 Appendix II: Data Format

In this chapter we describe the general structure of the files generated by PATCHMASTER.

26.1 Data Files

PATCHMASTER generates up to 9 files, when a data file is created:

1. The *Amplifier File* (*.amp).
2. The *Raw Data File* (*.dat).
3. The *Marker File* (*.mrk).
4. The *Method File* (*.mth).
5. The *Analysis File* (*.onl).
6. The *Stimulation Sequence File* (*.pgf).
7. The *Acquisition Parameters File* (*.pul).
8. The *Solution File* (*.sol).
9. The *Notebook File* (*.txt).

If *Make Bundle Files* is checked in the Configuration window (5.5.2 on page 56), then all files will be merged into one single bundle file with the file extension *.dat.

Except for the *Raw Data File* and the *Notebook File*, all other files have a "Tree" structure. The entire trees are kept in memory, whereas the raw data Traces are always loaded from disk, when needed.

26.1.1 Amplifiers File

This file is only generated when multiple amplifiers are active (EPC 10 USB Double, Triple and Quadro). It contains the amplifier state records of each amplifier for every Series. The structure of the *Amplifier File* (*.amp) is defined by the file AmpTreeFile_v9.txt. It has a tree structure:

Table 26.1: Amplifier File Description

Record	Description
Root	Version information
Series	Collection of all amplifier state records of one Series
Amplifier State	The amplifier state record

26.1.2 Raw Data File

This *Raw Data File* has an optional header, followed by a continuous data stream. Each data point is a 16-bit signed integer or 32-bit IEEE real as defined in the respective PGF template. When a *Sweep* is stored, PATCHMASTER stores the various *Traces* (if available) sequentially as defined in the respective PGF template. *Traces* with leak pulses are stored leak subtracted. The leak *Traces* are normally stored after the *Traces* themselves.

Very long traces with "continuous" segments may be stored as interleaved blocks. The size of such a block is given in the "InterleaveSize" field (in bytes) of the *Trace* record, the distance to the next block in the "InterleaveSkip" field (in bytes).

The structure of the *Raw Data File* (*.dat) is defined by the file DataFile_v9.txt.

26.1.3 Markers File

It contains the *Marker* records. The structure of the *Markers File* (*.mrk) is defined by the file MarkerFile_v9.txt. It has a tree structure:

Table 26.2: Markers File Description

Record	Description
Root	Version information
Marker	Description of one marker event

26.1.4 Protocol Methods File

It contains possibly used protocol method records. The structure of the *Protocol Methods File* (*.mth) is defined by the file MethodFile_v9.txt.

26.1.5 Analysis Methods File

It contains the assigned analysis method for every *Series*. The structure of the *Analysis Methods File* (*.on1) is defined by the file AnalysisFile_v9.txt. It has a tree structure:

Table 26.3: Analysis Methods File Description

Record	Description
Root	Version information
Method	Description of one <i>Analysis Method</i>
Function	Description of one <i>Analysis Function</i>

26.1.6 Stimulation Template File

Stores the stimulation protocol. The structure of the *Stimulation File* (*.pgf) is defined by the file StimFile_v9.txt. It has a tree structure:

Table 26.4: Stimulus Template File Description

Record	Description
Root	Version information
Stimulation	Description of an ensemble of pulse patterns; e.g., I-V curve
Channel	Combines the definition for one output (DAC) and one input (ADC) Trace
Segment	Individual segment of a pulse pattern

Stimulation Files can be loaded into the **Pulse Generator**. In fact, the *Pulse Generator Files* for the stimulation protocols used during the experiments have the same data structure as the PGF files, which belong to the recorded data. In this way it is possible to exactly repeat an experiment by using a copy of a PGF file as *Pulse Generator File*.

26.1.7 Acquisition Parameters File

Stores parameters, such as e.g. *Gain*, *Capacitance*.... The pointer to the data stored in the *Raw Data File* is also contained in this file. The structure of the *Acquisition Parameters File* (*.pul) is defined by the file *PulsedFile_v9.txt*. It has a tree structure:

Table 26.5: Acquisition Parameters File Description

Record	Description
Root	Version information
Group	Larger section of an experiment; e.g., cell or patch
Series	Description of an ensemble of <i>Sweeps</i>
Sweep	Description of a <i>Sweep</i> , i.e. one collection of <i>Traces</i>
Trace	Description of an individual data <i>Trace</i>

A graphical template of the *Pulsed File* (Tree) is shown in the **Replay** window. It contains information necessary to reconstruct the experimental conditions as the data were recorded.

26.1.8 Solutions File

The file is only generated, when the option *Solution Base* is activated. It contains the solution record for every *Series*. The structure of the *Solutions File* (*.sol) is defined by the file *SolutionsFile_v9.txt*. It has a tree structure:

Table 26.6: Solutions File Description

Record	Description
Root	Version information
Solution	Description of one solution
Chemical	Description of one chemical compound

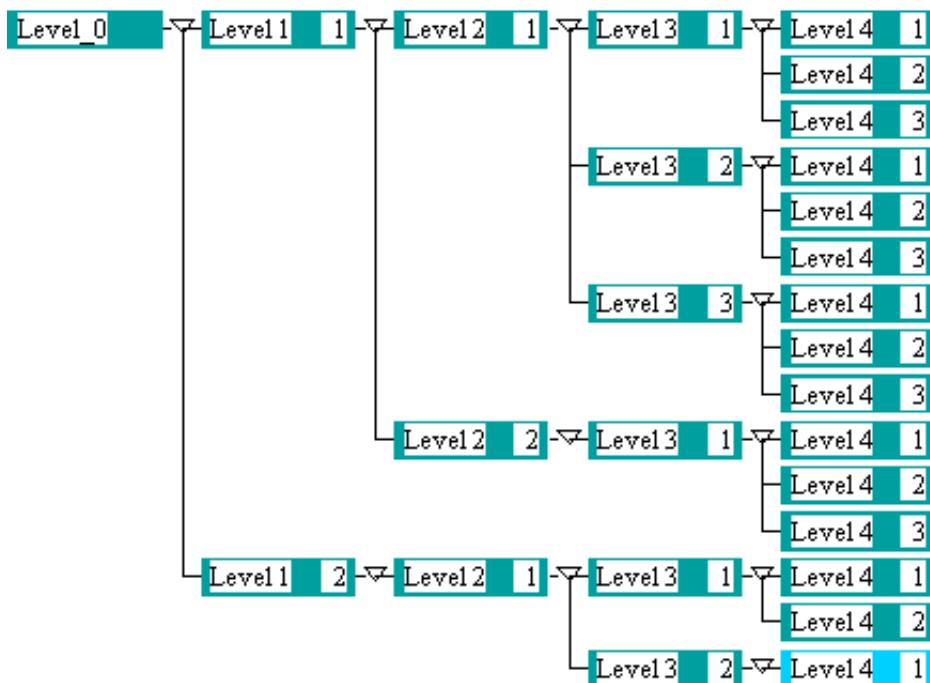
26.1.9 Notebook File

The *Notebook File* is a standard ASCII text file with line breaks.

26.2 The Tree Format

The idea of PATCHMASTER is to order the data of an experiment in "Trees". The trunk of the tree (*Root*) is the main descriptor of a data file (it could, for example, correspond to one cell or the entire experiments of one day). The next level is the *Group*. This level can be defined by the user to identify data that belong together. An example would be to open a new *Group* for each patch. The *Group* may contain several families of records. Such a family (e.g., records of a current–voltage relationship) is called *Series*. The individual records of a family are called *Sweeps*. Finally, each *Sweep* may be composed of *Traces*. A copy of this data tree is accessible to the user throughout the experiment (so one has an overview of what was recorded, and one can immediately edit the entries (e.g., discard bad records).

The following is a description of the *Tree* format. An example tree with 5 levels can be diagrammed as follows:



The above tree has five levels:

- 0 : Root
- 1 : Group
- 2 : Series
- 3 : Sweep
- 4 : Trace

The format of a tree stored to a file is as follows:

1. Magic number: 054726565 (hex) = "Tree"

2. Number of levels
3. Level sizes, one per level
4. Tree records, top down, left-to-right. Each record has the format:
 - Record contents
 - Number of children

All of the values (except the record contents) are INT32 values, i.e., 32-bit (4 bytes) values.

Note: Check the record sizes in the file headers. The record sizes may differ from what you are expecting, e.g., because the file has been created by an older program version which used fewer fields than it is currently using, or a newer version with additional fields. You must use the record sizes stored in the files themselves.

Note: The "Magic2 number will be 065657254 (hex) = "eerT", if the file was written on an operating system with opposite byte ordering (e.g. written under Mac OS, read under MS Windows). In that case, appropriate byte swapping has to be performed (highest → lowest, etc.), when the data is read in.

A detailed description of the PATCHMASTER data file format for programmers is available for download from our FTP server: <ftp://server.hekahome.de/pub/FileFormat/Patchmasterv9/>.

26.3 File Template

26.3.1 Filename

In PATCHMASTER you can use so-called template files for stimulation.

You have the following options how to use the file templates:

Option 1: One template per DA channel common to all Sweeps of a Series

In this case, the name of the template file would be:

[stimulus name]_[channel number].tpl

E.g., if the stimulus name is "IV", then PATCHMASTER looks for the *Template File* IV_1.tpl to be used as *Template File* for the first DA channel of all *Sweeps*.

These *Template Files* must be in a sub-folder of the folder with the pgf files. The sub-folder must be named identically to the stimulus, e.g., "IV".

Option 2: A different template per DA channel and Sweep

In this case, the name of the *Template File* would be:

[stimulus name]_[sweep index]_[channel number].tpl

E.g., if the stimulus name is "IV", then PATCHMASTER looks for the *Template File* IV_1_1.tpl to be used as *Template File* for the first DA channel of the first *Sweep*, IV_1_2.tpl for the first DA channel of the second *Sweep* and so on.

These *Template Files* must be in a sub-folder of the folder with the pgf files. The sub-folder must be named identically to the stimulus, e.g., "IV".

26.3.2 Data Format of the File Template

26.3.2.1 Voltage Clamp Stimulation or plain DA output

The file should contain one voltage value per stimulus point. PATCHMASTER fills missing samples with the holding value, if *Apply StimScale* is active, or alternatively with zero. Excessive samples are ignored.

The voltage must be a *short* (4 byte), binary IEEE-floating point format number. All values must be in Volt, i.e., if a voltage of -80 mV has to be output, the required value is -0.080. The total number of samples should be equal to the total number of input samples.

26.3.2.2 Current Clamp Stimulation

Template Files for stimulation can be used in *Voltage Clamp* and in *Current Clamp* mode. In *Current Clamp* mode the setting of the *CC-Gain* setting in the *Amplifier* window needs to be taken into account.

Example: A value of 0.1 would result in a current injection of 100 pA.

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