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1. Introduction

1.1 Disclaimer

This Product relies on the tools of Microsoft Windows (Windows Vista, Windows 7, Windows 8) or Mac 10.6 (or more recent). 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

The Chartmaster program provides versatile tools for electrophysiological and electrochemical experiments. Pulse generation, data acquisition, storage, and analysis are among them.

Extended analysis functions for Chartmaster data are provided by the Fitmaster software. Fitmaster features analysis and fitting routines designed to fully support the extended Chartmaster 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.

Users familiar with Pulse might first read the chapter Patchmaster for Pulse Users in the Patchmaster Tutorial and then go on with specific chapters of this manual.

1.3 The Chartmaster Concept

The following section presents a rather simplified view on the overall structure of the Chartmaster program. It should help to understand the general way how to operate the program and should give the reader an idea about the capabilities of Chartmaster.

The main objects of Chartmaster are:

- Protocol scripts
- Pulse Generator sequences (stimulation and data acquisition) and experiment specific Extensions, such as:
  - Software Lock-In
  - Photometry
  - Imaging
  - (Impedance/Admittance) Spectroscopy
- Oscilloscope (display)
- Data Tree (storage)
• Analysis methods (analysis)

The new concepts in comparison to the well known PULSE software are the Protocols, Global Variables, and a powerful Analysis.

**CHARTMASTER – New Concepts**

**Protocols:** Are the top structuring layer of the program organizing the interplay between the different parts of the program. The protocol script is created and managed in the Protocol Editor.

**Global Variables:** Are a kind of glue between the program parts (see chapter Global Variables in the Chartmaster Tutorial.)

**Analysis:** For convenient representation of results during and after the experiment.

With these new concepts CHARTMASTER accomplishes two important points: **Standardization** and **Automation** of experiments.

**CHARTMASTER accomplishing Standardization and Automation**

The overall aim in good laboratory practices is to work under Reproducible Experimental Conditions. Data collected from different personnel and at different setups should have the same conditions that they can be used for Data Pooling. User errors should be prevented as much as possible.

In order to accomplish the above mentioned points you can create an experimental protocol script in the Protocol Editor. By the use of such a protocol script the experimental approach is very much standardized. The Protocol Editor gives you access to all parts of the CHARTMASTER program. You can get input from these parts, make decisions, and control other parts of the program.

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The **Protocol Editor** is the central part of **Chartmaster**, keeping the experimental cycle running. E.g. the **Protocol Editor** starts a data acquisition. Data are recorded, stored and analyzed by the **Analysis**. Results from the **Analysis** can be feed back into the **Protocol Editor**, e.g. making a decision in a conditional "If"-statement. Furthermore the **Protocol Editor** can react on input from external devices or the user. The following picture tries to visualize the work-flow of the program.
1.4 Supported Data Acquisition Interfaces

Chartmaster supports the following data acquisition interfaces:

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

1.5 Supported System Software

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

Chartmaster is supported on MacOS X version 10.6 (or more recent). Older MacOS X versions and MacOS 9 are not supported.

1.6 Naming Conventions

1.6.1 Interfaces

Throughout this manual we will address all data acquisition interface types as LIH 8+8. We will explicitly mention the particular interface if required.

1.6.2 Windows Versions

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

1.6.3 Syntax

- Chartmaster: 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.
1.7 Windows and Mac Key Conventions

In Chartmaster, all key commands are saved in the file `Chartmaster.key` and will be read at the program start (see chapter 3 on page 17).

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

Please also be aware that in case the file `Chartmaster.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.

1.8 Installing Chartmaster

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.

1.9 Starting Chartmaster

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

- Frequently used Chartmaster 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.*
1.10 Get Online Help on Keys

The option Show Keys in the drop-down menu Help (compare chapter 4.10 on page 39) 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 17.
1.11 Get Online Help Controls

The option **Show Tooltips** in the drop-down menu **Help** (chapter 4.10 on page 39) displays a short description of the control under the mouse tip.

![Oscilloscope](image)

1.12 Closing Chartmaster

To exit from **Chartmaster**, 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.

1.13 Support Hotline

If you have any questions, suggestions, or improvements, please contact HEKA’s support team. The best way is to sent 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 Dr. Schulze GmbH
A Division of Harvard Bioscience
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Germany
phone: +49 (0) 6325 9553 0
fax: +49 (0) 6325 9553 50
e-mail: support@heka.com
web: http://www.heka.com
2. User Interface

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

Dialog controls explains all types of control controls available in the Chartmaster 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 Chartmaster 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:

- 
- Box items with a drop shadow enclose changeable values, either as
  - list item or
  - pop-up menu list or
  - drag item.
- \[\text{SETUP}\] Rounded rectangles are items that perform some action.
- \[\text{496. MQ}\] Simple rectangles (without drop shadow) display a measured value.
- Plain text is for titles only.

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.

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

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

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

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.

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

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.
**Important note:** 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>
<thead>
<tr>
<th>Name</th>
<th>eng.</th>
<th>sci.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tera</td>
<td>T</td>
<td>E12</td>
</tr>
<tr>
<td>Giga</td>
<td>G</td>
<td>E9</td>
</tr>
<tr>
<td>Mega</td>
<td>M</td>
<td>E6</td>
</tr>
<tr>
<td>kilo</td>
<td>k</td>
<td>E3</td>
</tr>
<tr>
<td>milli</td>
<td>m</td>
<td>E-3</td>
</tr>
<tr>
<td>micro</td>
<td>µ / u</td>
<td>E-6</td>
</tr>
<tr>
<td>nano</td>
<td>n</td>
<td>E-9</td>
</tr>
<tr>
<td>pico</td>
<td>p</td>
<td>E-12</td>
</tr>
<tr>
<td>femto</td>
<td>f</td>
<td>E-15</td>
</tr>
</tbody>
</table>

**SI Units:** Chartmaster 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 Chartmaster 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).

**Important note:** 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.
2.2 Modifying Dialogs and Controls

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).

**Important note:** 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>
<thead>
<tr>
<th>Action</th>
<th>Mac OS</th>
<th>MS Windows</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open a configuration</td>
<td>CMD + click</td>
<td>CTRL + left-click</td>
</tr>
<tr>
<td>dialog</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Move one item</td>
<td>ALT + left-click + drag</td>
<td>ALT + right-click + drag</td>
</tr>
<tr>
<td>Move group of items</td>
<td>CMD + ALT + left-click+drag</td>
<td>CTRL + right-click + drag</td>
</tr>
</tbody>
</table>

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.

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, \(w\) 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 affected 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 Chartmaster.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.

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.

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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 CHARTMASTER 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 CHARTMASTER windows are saved in the configuration file (e.g., Chartmaster.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 CHARTMASTER 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 CHARTMASTER 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).

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.

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2.5 Copy and Paste Functions

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 (see chapter 10.4.1.12 on page 106) 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 Chartmaster 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 MacOS 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 Chartmaster.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 Chartmaster.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 1.10 on page 6.

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 Chartmaster.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 10) or by
- directly modifying the Chartmaster.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 Chartmaster.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
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 Chartmaster.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 "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
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

; 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
3.2 The Chartmaster.key File

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
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 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

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4. Menus

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

4.1 File Menu

The File menu has all options to handle CHARTMASTER experiment files. A single CHARTMASTER "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 *.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).
- 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 48), then the files *.pul, *.pgf, *.onl, *.mrk and *.dat are merged into one single *.dat file.

Raw data acquired by CHARTMASTER 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:

- 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.

**Important note:** 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.

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 CHARTMASTER 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:** CHARTMASTER 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.

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4.1 File Menu

**Convert to PPC:** Converts the raw data to PowerPC format ("big endian"). This is required if a third party program is used that cannot 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 cannot 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.

**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 Chartmaster.

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 Chartmaster.

**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.

**Important note:** 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 (MacOS 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.

**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.
4.3 Windows Menu

The Windows menu applies to the windows in Chartmaster. 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 17).

**Control Window**: Opens the Control Window or brings it to the front (see chapter 7 on page 65).

**Configuration**: Opens the Configuration window or brings it to the front (see chapter 5 on page 41).

**Oscilloscope**: Opens the Oscilloscope window or brings it to the front (see chapter 6 on page 61).

**Replay**: Opens the Replay window or brings it to the front (see chapter 8 on page 69).

**Protocol Editor**: Opens the Protocol Editor window or brings it to the front (see chapter 10 on page 99).

**Pulse Generator**: Opens the Pulse Generator window or brings it to the front (see chapter 9 on page 73).

**Analysis**: Opens the Analysis window or brings it to the front (see chapter 11 on page 119).

**Analysis Window 1, 2**: Opens the specified Analysis Window window or brings it to the front (see chapter 11.4 on page 128).

**Parameters**: Opens the Parameters window or brings it to the front (see chapter 12 on page 135).

**I/O Control**: Opens the I/O Control window or brings it to the front (see chapter 14 on page 145).

**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 5.4.5 on page 45 or chapter 5.4.4 on page 45).

**LockIn**: Opens the LockIn Control window or brings it to the front if the LockIn Extension is activated (see chapter 5.4.2 on page 44).

**Spectroscopy**: Opens the Spectroscopy Control window or brings it to the front if the Spectroscopy Extension is activated (see chapter 5.4.3 on page 45).

**Solution Base**: Opens the Solution Base window (see chapter 15 on page 147). 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 16 on page 151).

**Markers**: The Markers window allows to set sweep markers and/or trace markers during a continuous recording (see chapter 17 on page 153).

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

**Calculator**: The Calculator window allows to perform numeric calculations in Chartmaster (see chapter 18 on page 157).

**Notebook**: Opens the Notebook window or brings it to the front (see chapter 13 on page 143).

**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 9).

**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 Chartmaster user interface can be modified (see chapter 2.2 on page 10).

### 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 33) 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 corresponding analysis results.

**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.

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**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.

  *Note:* The output style, either scientific notation or engineering format, is defined in the Notebook menu, 4.7 on page 36.

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.
- **PULSE v8.6:** Exports data in PULSE format. This feature is useful when CHARTMASTER data are to be analyzed with PULSEFIT, PULSETOOLS or PULSESIM.
- **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.

- **Info File:** Sweep information are exported as Igor Info file (*.inf), e.g. labels, sample intervals, total points ....

- **Printer:** Direct output to a connected printer. The page setup magnification determines the line width; usually, 50 % gives good results. The numbers of columns and rows per page have to be entered. This determines how many items are placed on a page. In any case, a form feed is output after the selected target is output. Thus, if one prints a Group with 3 Series to a page with 2 columns and 2 rows, three quarters of the page will be filled, then the page is released from the printer. 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.

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.

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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 Chartmaster 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 Chartmaster 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 Chartmaster 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 IgorPro wave. Without this option, one wave per page is exported.

- **Igor for Windows**: If selected, the data will be exported for the MS Windows version of Igor Pro.

- **Igor for Mac OS**: If selected, the data will be exported for the Mac OS X version of Igor Pro.

- **Set PULSE v8.6 Traces (1,2)**: If Export Format is set to Pulse v8.6 (see chapter 4.4 on the preceding page) this option gets active. Specify two traces of your selected Series which will be converted as first and second trace of the Pulse file format.

**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.

- **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.

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4.4 Replay Menu

**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.
- Mac OS 9 format: Lines are terminated by carriage returns. This generates standard Mac OS 9 text files.
- Mac OS X format: Lines are terminated by line feeds, generating Mac OS X text files.
- Windows format: Lines are terminated by line feeds plus carriage returns. This generates text files that can be read by programs running under DOS or MS Windows.

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

When importing data you first have to generate a corresponding data structure (Tree) in Chartmaster. 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:

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In example "A" the With Marked Target and Children \(\rightarrow\) 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 the next page).

- 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 CHARTMASTER 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.

**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.

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4.5 Display Menu

- **Average Series**: Generates one new Series in which each Sweep is the average of all marked Sweeps 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 33.

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 50).

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

**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 9.9.2 on page 92). 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 the preceding page).

**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 33).

**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 33).

**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 overlayed.

**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.

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.

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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.

4.5.2 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
- Diamong
- 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 Chartmaster configuration file.
4.6 Buffer Menu

CHARTMASTER 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 18 on page 157 and chapter 18.2.2 on page 160.

**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 CHARTMASTER 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:

\[ \text{Buffer}(n+1) = (\text{Buffer}(n) \times n + \text{Trace})/n+1 \]

with \( n \) = number of Traces in the Buffer.

**Deaccumulate Trace:** Deaccumulates the Trace selected in the Replay window from the active Buffer:

\[ \text{Buffer}(n-1) = (\text{Buffer}(n) \times n - \text{Trace})/n-1 \]

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 29).

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.

Important note: 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.
- **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.

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4.8 Protocols Menu

The Macros menu of older CHARTMASTER 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 CHARTMASTER 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 10.1 on page 99).

**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 51).
4.9 AD-board

Enable Batch Control: Allows Chartmaster to be remotely controlled by another program (see chapter Controlling PATCHMASTER in the PATCHMASTER Tutorial).

Enable Background: Allows background process to keep running even when Chartmaster is in the background.

Initialize LIH 8+8: This will re-initialize (restart) the connected AD/DA interface.

LIH 8+8: Displays the type of AD/DA interface being used along with serial number and board version, if available.
4.10 Help Menu

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

**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 17).
**Save Keys:** Saves the actual key command settings to the default key file. The old *Chartmaster.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 Chartmaster:** Displays the *Chartmaster* software version and contact information.
5. Configuration Window

![Configuration Window](image)

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 Chartmaster.set. By means of different *.set files every user can define their individual program layout to meet specific requirements.

5.1 Individualize Chartmaster

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

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

- Rename the *.set file in your HEKA folder (e.g. "Chartmaster_User.set").
- Create a shortcut of Chartmaster.exe on the desktop.

  **Note:** The term "User" is just a placeholder for individual names.

- Rename the shortcut (e.g. "Chartmaster.exe_User").
- Right-click on the shortcut: Properties - Link - Target.
- The following command line should be written in the "Target" field, including the inverted comas and empty spaces:
"C:\Program Files (x86)\HEKA\Chartmaster\Chartmaster.exe" "C:\Program Files (x86)\HEKA\Chartmaster\Chartmaster_User.set".

*Note:* Please be aware that your installation path of your CHARTMASTER may vary on your PC.

### 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 CHARTMASTER has to be restarted upon substantial changes of the settings. Thus, in order to use another setting, quit CHARTMASTER and restart. Before you do so, make sure that there is no Chartmaster.set file available. In this case, upon start-up, Chartmaster 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 CHARTMASTER. The user interface is adapted to show only the defined number of Traces. A small number makes the dialogs and lists within CHARTMASTER 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 Values:** This number defines how many values are used by CHARTMASTER. The user interface is adapted to show only the defined number of values. A small number makes the dialogs and lists within CHARTMASTER shorter. The new setting only takes effect after saving the configuration file and upon restarting the program.

#### 5.3.2 Window Scaling

**Window Scaling:** This feature is used to scale (change size of all controls) all windows. This may be useful when there is too little space for all necessary windows on a single monitor. The new scaling only takes effect after saving the configuration file and upon restarting the program. The default Window Scaling Factor value is ‘1.2’, if you enter a ‘0’ the option is inactive.

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5.3 General

5.3.3 Memory Allocation

**Max. Sample Points**: Sets the maximum number of points that can be acquired in one Trace (see chapter 9.7 on page 78). This parameter is limited by the amount of available memory.

**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.

5.3.4 Minimum Wait Time

**Min. Wait Time**: Sets the time Chartmaster 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.

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.

5.3.5 Batch Communication

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

- Disable: If this option is checked, the Batch Communication is turned off.
- Enable as Receiver: Chartmaster is configured to serve as Receiver and will wait for command inputs of other applications.
- Enable as Sender: Chartmaster 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 send, 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.

5.3.6 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

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

**Chartmaster** supports the following AD/DA-converters:

- ITC-16
- ITC-18
- LIH 1600 / ITC-1600
- LIH 8+8
- 1...3 PCI: Additional set-up information, if there is more than one possible card slot to connect to the digitizer.
- 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.

5.4.2 LockIn

Here, the LockIn amplifier of **Chartmaster** can be activated. For a detailed description see chapter *Software LockIn Extension*, 21 on page 181, and the **ChartMaster** Tutorial "Capacitance Measurements using the LockIn Extension".

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5.4 Hardware

5.4.3 Spectroscopy

Here, the Spectroscopy Extension of CHARTMASTER can be activated. For a detailed description see chapter Spectroscopy, 22 on page 191, and the CHARTMASTER Tutorial "Using the Spectroscopy Extension".

5.4.4 Imaging

Here, the Imaging Extension of CHARTMASTER 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:

- **T.I.L.L.**: "T.I.L.L. Photonics' Polychrome", can be controlled via an analog output of the amplifier or any AD/DA converter supported by CHARTMASTER. The exposure of the camera should then be triggered via a digital output channel.

- **DG4/DG5**: 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.

- **Lamba-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 please refer to the chapter Imaging, 20 on page 169.

5.4.5 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:

- **T.I.L.L.**: "T.I.L.L. Photonics' Polychrome", can be controlled via an analog output of the amplifier or any AD/DA converter supported by CHARTMASTER.

- **DG4/DG5**: The "DG-4/DG-5" can be controlled via the digital output of the Epc 10, Itc-16 or Itc-18.

- **Lamba-10**: The "Sutter filter wheel Lambda-10" can be controlled via the digital output of the Epc 10, Itc-16 or Itc-18.

- **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, 19 on page 163.

5.4.6 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, CHARTMASTER 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, Chartmaster 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 sent 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 Chartmaster.
- 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 14 on page 145). There is also a Serial Out event available in the Protocol Editor, 10.4.3.1 on page 108.

5.4.7 Further Options

Multi-Channels: Enabling Multi-Channels has major implications on the Oscilloscope window (see chapter 6.7 on page 64) and the Analysis (see chapter 11.6 on page 133).
5.5 Files

In this section, the user has to specify the paths for several files. Chartmaster 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 (`Chartmaster.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 Chartmaster Tutorial: "Controlling Chartmaster".

**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 55). Only necessary for telegraphing amplifiers.

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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).
- CHARTMASTER 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, CHARTMASTER 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, CHARTMASTER 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, CHARTMASTER 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 15 on page 147).

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

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- [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 \rightarrow 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.

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

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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 CHARTMASTER. 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 9).

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:

- 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".

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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 31).

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.

5.7 I/O Control

Selected options in this section of the Configuration window will appear in the I/O Control window (see chapter 14 on page 145) 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.

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.

![I/O Control Window](http://www.heka.com)

**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.
- AD-Channels: Parameters are sampled via a specified AD-channel.

[Notebook] 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.

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 9.9.1 on page 88).
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.

- A label for the equation can be entered in this field.
- Already saved equations can be selected in this pop-up menu.
- 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, 18 on page 157.

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 I/O Control

5.7.2 List of I/O Parameters

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 52). 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.

**Temperature:** Temperature (from a recording device).

**Show Solutions:** If the checkbox is active you can enter/change the number of the external and internal solutions in the *I/O Control* window.

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.

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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. $I_{\text{mon}}-1$, $V_{\text{mon}}-1$, $A_{\text{de}}-0$, $A_{\text{de}}-1$, $\text{LockIn}_{\text{CM}}$, $\text{LockIn}_{\text{GM}}$, $\text{LockIn}_{\text{GS}}$ 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 $I_{\text{mon}}-1$ but the compression rate for each channel can be set differently. The benefit comes with the consistent labeling of these Traces within ChartMaster (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:

- $V_{\text{mon}}$
- $I_{\text{mon}}$

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

- $A_{\text{de}}-0...15$

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**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_CCM*  
- *LockIn_GM*  
- *LockIn_GS*  
- *LockIn_Real(Y)*  
- *LockIn_Imag(Y)*  
- *LockIn_DC*  
- *LockIn_Avg*  
- *LockIn_CV*  
- *LockIn_GP*  
- *LockIn_Admit(Y)*  
- *LockIn_Phase*  
- *LockIn_Real(Z)*  
- *LockIn_Imag(Z)*  
- *LockIn_IMP(Z)*

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

- *Chirp_Avg*  
- *Chirp_Phase*  
- *Chirp_Admit(Y)*  
- *Chirp_Real(Y)*  
- *Chirp_Imag(Y)*  
- *Chirp_Real(Z)*  
- *Chirp_Imag(Z)*  
- *Chirp_Imp(Z)*

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

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• Photo_W1
• Photo_W2
• Photo_W3
• Photo_R
• Photo_Ca

Imaging: 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

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

• I-forward
• I-reverse
• I-diff
• V-mean

Undo: The last change will be reset.
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.

Important note: 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.

Clear Comment: Clears the Comment field in the Control window after the Comment is stored with the data.
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 \(<<><><>>\). 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 \(<<><><>>\). 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

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

- **Overlay Scan**: 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 56) 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 resets the Y-scale to "1". For further information we refer to chapter 4.5 on page 31.

**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 respective Trace on the screen.

**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 12.1.5 on page 140).

**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 12.1.5 on page 140).

**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".

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6.7 Multi-Channels

If the Multi-Channel option is activated in the Configuration (see chapter 5.4.7 on page 46) 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. 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 51).

7.1 Information about the Experiment

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

- "aq: waiting": Chartmaster 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": Chartmaster 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 (PatchmasterPro 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.
7.2 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: 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.

7.3 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 51) 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.
7.4 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 51) then you will see a scroll-able 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 51) 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.

7.5 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 51) 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**.

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8. 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.

8.1 Main window functions

**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 exit. 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 26). 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.

Window Scroll Arrows: The arrow buttons on the window frame can be used to scroll/jump through the data tree.

8.1.1 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
8.1 Main window functions

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 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

[Diagram of a tree structure with labels and nodes labeled as Lock, CM, IMon-l, HEK]
9. 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.
9.1 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 settings 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.
9.2 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, Chartmaster 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 selecting a free position in the pool.

9.3 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. Chartmaster 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.

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 StimScalerelative to Vmemb
Amplifier mode: VoltageClamp, Set last Seg. Ampl.: FALSE
SEGMENTS: Voltage Duration VFact VIncr TFact Tincr

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

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Note: Copy is one way of creating a new sequence. Another way is to activate an unassigned sequence button and name the new sequence in the prompted window. The pulse pattern can be edited in the Segments section of the Pulse Generator window.

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.

UNDO: Discards all changes that had been carried out since the last opening of the Pulse Generator window within one PGF sequence.

Note: When closing the window, all changes will be saved temporarily for this session. When closing Chartmaster, you will be asked once more if you want to save the PGF pool, if it was changed but not saved.

DELETE: Removes the present sequence from the pool.

9.4 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.

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.

9.5 Timing

Timing:

- Wait before 1. Sweep: Forces Chartmaster 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.

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

Sweep Interval: The first Sweep of a sequence has a waiting period during which the stimulation template is computed and loaded to the AD/DA interface. Then the first Sweep is acquired. The next Sweep will start Sweep Interval seconds after the beginning of the first Sweep.

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 µs (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, Chartmaster asks whether the segment duration should be fixed, e.g., set to a higher value.

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9.6 Check and Execute

**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 CHARTMASTER. 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:** One trigger at the start of a Sweep.

**Use Durations / Use Scan Rates:**

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.

*Important note:* Start Seg and Start Time determine the start from where acquired data are stored. The full stimulation template is always output.

9.6 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.

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9.7 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.

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*, 5.3.3 on page 43) 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'.

9.8 Wave Parameters

Depending on the *Segment* type (see chapter *Segment Classes*, 9.10.1 on page 92) and the *Stim → DA* setting (see chapter 9.9.1 on page 88) different wave parameter buttons will appear.

9.8.1 Sine Wave Parameters

![Sine Wave](https://www.heka.com)

For segments of the *Sine* type. This button will appear between the buttons *Checking* and *Execute* and allows the specification of the wave characteristics.

9.8.1.1 Use as Simple Sinewave: Common Frequency

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

![Sine Wave Parameters](https://www.heka.com)

**Peak Ampl. [mV]:** Defines the size of the *Peak Amplitude*. The amplitude is half of the peak-to-peak amplitude.

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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.

9.8.1.2 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 9.8.1.1 on the preceding page).

9.8.1.3 Use as LockIn Sinewave

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

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**Note:** For the LockIn Sinewave to be an active option, the LockIn Extension must first be turned on in the Configuration window (see chapter 5.4.2 on page 44).

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 needed when using the Protocol Method "Impedance Spectroscopy", also know as "EIS".

- **Peak Ampl. [mV]**: See chapter 9.8.1.1 on page 78.
- **Requested Freq.**: See chapter 9.8.1.1 on page 78.
- **Actual Frequency**: See chapter 9.8.1.1 on page 78.
- **Points / Cycle**: See chapter 9.8.1.1 on page 78.
- **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\textsubscript{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.
- **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**, 21 on page 181, and the **Chartmaster Tutorial Capacitance Measurements using the LockIn Extension**.

### 9.8.2 Square Wave Parameters

For segments of the **Square** type. This button will appear above the Execute button and allows the specification of the wave characteristics.

#### 9.8.2.1 Use as Simple Squarewave: Common Frequency

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

**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.

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9.8.2.2 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 9.8.2.1 on page 80).

9.8.2.3 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 5.4.2 on page 44).

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 needed when using the Protocol Method "Impedance Spectroscopy", also know as "EIS".

Peak Ampl. [mV]: See chapter 9.8.2.1 on page 80.

Requested Freq.: See chapter 9.8.2.1 on page 80.

Actual Frequency: See chapter 9.8.2.1 on page 80.

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

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9.8 Wave Parameters

Cₘ 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 Gₘ is low.

**Note:** A value of zero is often used in the common situation where Gₘ 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, 21 on page 181, and the Chartmaster Tutorial Capacitance Measurements using the LockIn Extension.

### 9.8.3 Chirp Wave Parameters

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:

![Chirp Wave Examples](http://www.heka.com)

#### 9.8.3.1 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 Amp.. Alternatively, the Peak Ampl. can be determined by a PGF parameter. This allows to automatically change the Peak Amp. 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.

9.8.3.2 Exponential Chirp

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

All available settings have been explained before (see chapter 9.8.3.1 on the preceding page).

9.8.3.3 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 Chartmaster (see chapter 5.4.3 on page 45).
9.8 Wave Parameters

Amplitude [mV]: See chapter 9.8.3.1 on page 83.

Value or parameter: See chapter 9.8.3.1 on page 83.

Start Frequency: See chapter 9.8.3.1 on page 83.

End Frequency: See chapter 9.8.3.1 on page 83.

Min. Points / Cycle: See chapter 9.8.3.1 on page 83.

Chirps to Skip: Defines the number of skipped chirp segments.

Segment Points: See chapter 9.8.3.1 on page 83.

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, 22 on page 191 and the CHARTMASTER tutorial Using the Spectroscopy Extension.

9.8.4 Photometry Wave Parameters

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 $6 \times 3 = 18$ segments. **Trunc.** removes all segments except the initial 3 segments.

### 9.8.5 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, 20 on page 169.

The Imaging Wave parameters are usually set in the PGF Primer of the Imaging Extension and automatically integrated into the Pulse Generator sequence by the Prime function of the Imaging Extension. 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 chapter PGF Primer, 20.4.2 on page 175.
9.9 Channel Settings for DA Output and AD Input

**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.

### 9.9.1 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...3: Analog output.
- off: No output of stimulus, disables this channel.
- Dig-out (word): Output as digital word.
- Dig-0...15: Digital output channel.
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.

- relative to hold: 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 CHARTMASTER 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, 21 on page 181.
- 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 52.
- use for Spectroscopy: Has to be activated if a chirp waveform should be used for impedance or admittance analysis with the Spectroscopy Extension. For details please refer to the chapter Spectroscopy Extension, 22 on page 191.
- use for Imaging: 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, 20 on page 169.

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 CHARTMASTER.

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: \( 1 \times 10^2 + 1 \times 10^1 + 1 \times 10^0 = 4 + 2 + 1 = 7 \).

Note: For such trigger channels it is suggested to switch from Common Timing to Separate Timing. With this setting, the duration and number of segments is completely independent from the first (main) channel.
9.9.2 AD input channel settings

AD: The AD-channel for input.

- AD-0...7: AD channel.
- off: No input.
- Virtual: Virtual input. A 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 . . . .
- **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.)

**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.

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

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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 C). The Trace # is set to "5".

**Important note:** 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 11.3.2.7 on page 126), the **Buffer** menu (see chapter 4.6 on page 35) or the **Calculator** dialog (see chapter 4.3 on page 25). 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.

### 9.10 Segments

#### 9.10.1 Segment Classes

<table>
<thead>
<tr>
<th>Segment Class</th>
<th>Voltage [mV]</th>
<th>Duration [ms]</th>
<th>Mode</th>
<th>V-offset [mV]</th>
<th>Scan Rate [V/s]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Constant</td>
<td>Constant</td>
<td></td>
<td>vol -50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>val 10.00</td>
<td>val 10.00</td>
<td>vol 10.00</td>
<td>val</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Increase</td>
<td>Increase</td>
<td>Increase</td>
<td>Increase</td>
<td>Increase</td>
</tr>
<tr>
<td></td>
<td>1.00 0.00</td>
<td>1.00 0.00</td>
<td>1.00 0.00</td>
<td>1.00 0.00</td>
<td>1.00 0.00</td>
</tr>
</tbody>
</table>

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-Store:** 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 CHARTMASTER Tutorial.

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9.10 Segments

- 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.

**Segment Class:**

Segments can be the following:

*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 9.8.1 on page 78).
- **Square:** Wave segment with rectangular characteristics. Amplitude and cycle duration are defined in *Square Wave* parameters (see chapter 9.8.2 on page 80).
- **Chirp:** Wave segment with increasing sine wave frequency. Wave characteristics are defined in *Chirp Wave* parameters (see chapter 9.8.3 on page 83).

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 9.14 on page 98.
9.10.2 Scan Rates

The Scan Rate options are only available if Use Scan Rates is activated (see chapter 9.5 on page 77).

- 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.

9.10.3 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):

- 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.

9.10.3.1 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:

$$V_i = Voltage + Voltage \cdot VFactor^{i-1} + (i - 1) \cdot \Delta VIncr$$

In mode V * Factor the increment may be zero. Let Voltage be 10 mV, \(\Delta VIncr. = 0 \) mV, and \( VFactor = 2 \) then the Series 10, 20, 40, 80 mV, ... is obtained.

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**dV * Factor:** In mode \(dV * \text{Factor}\) 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:

\[ V_i = \text{Voltage} + (i - 1) \cdot dV-\text{incr} \]

For \(V\)-factor \(\neq 1\):

\[ V_1 = \text{Voltage} \]
\[ V_{i,i>1} = \text{Voltage} + dV-\text{incr} \cdot V\text{-Factor}^{i-2} \]

In mode \(dV * \text{Factor}\) the first segment may be zero and is then logarithmically incremented. Let Voltage = 0 mV, \(dV\text{-Incr} = 1\) mV, and \(V\text{-Factor} = 2\) 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 \(t * \text{Factor}\) 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:

\[ t_i = \text{Duration} + \text{Duration} \cdot t\text{-Factor}^{i-1} + (i - 1) \cdot dt\text{Incr} \]

In mode \(t * \text{Factor}\) the increment may be zero. Let Duration be 10 ms, \(dt\text{-Incr.} = 0\) ms, and \(t\text{-Factor} = 2\) then the Series 10, 20, 40, 80 ms, ... is obtained.

**dt * Factor:** In mode \(dt * \text{Factor}\) 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:

\[ t_i = \text{Duration} + (i - 1) \cdot dt\text{-incr} \]

For \(t\)-Factor \(\neq 1\):

\[ t_1 = \text{Duration} \]
\[ t_{i,i>1} = \text{Duration} + dt\text{-incr} \cdot t\text{-Factor}^{i-2} \]

In mode \(dt * \text{Factor}\) the duration of the first segment may be zero and is then logarithmically incremented. Let Duration = 0 ms, \(dt\text{-Incr} = 1\) ms, and \(t\text{-Factor} = 2\) then the Series 0, 1, 2, 4 ms, ... is obtained.
9.11 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* terminated 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.

**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 µs (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.
9.12 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.

9.13 V-membrane

**V-membrane (V):** 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.
9.14 PGF Parameters

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. Chartmaster provides 10 PGF parameters which will be stored with the Pulse Generator file. Instead of p1 to p10 you can assign use 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.

For a description of usage of the PGF parameters please we refer to the chapter Global Variables in Patchmaster in the Patchmaster Tutorial.

9.15 Error Handling

If Chartmaster 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.

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10. 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 CHARTMASTER 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.

10.1 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 47) and the Configuration File has to be saved.

**LOAD** : Loads the pool of available protocols (**.pro** file).

**SAVE** : Saves the pool of available protocols (**.pro** file).
100 Protocol Editor Window

: 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.

: Creates a new protocol in the pool.

: Removes the present protocol from the pool.

: Steps to the next line in the event list and executes the event.

: Runs the protocol through to its end.

: 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.

: Lists the present protocol in the Notebook window.

: Moves the present protocol to another position in the protocol pool.

: 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.

: 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.

10.2 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.
10.3 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. Note that the delay is executed before the events.

<table>
<thead>
<tr>
<th>Repeat Status</th>
<th>IF etc. Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>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.</td>
<td>Displays the last IF condition that has been triggered.</td>
</tr>
</tbody>
</table>

10.4 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.
10.4.1 Protocol Sequence

10.4.1.1 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.

10.4.1.2 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*.

10.4.1.3 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.

10.4.1.4 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).

### 10.4.1.5 GOTO MARK

Creates a *GOTO* Mark.

**Mark:** Enter the name of the mark for a possible destination of a GOTO jump.

### 10.4.1.6 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: 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.
• Batch Comm.: Checks if the last received batch control string contains a user-defined phrase (see Controlling CHARTMASTER in the CHARTMASTER Tutorial).
• Serial Comm.: Checks if the last received string of the serial port communication contains a user-defined phrase (see chapter 5.4.6 on page 45).
• 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).

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.

10.4.1.7 ELSEIF...THEN

Allows a secondary conditional event, e.g. a check after an IF-THEN step. Works identically to IF-THEN, see above.
10.4.1.8 ELSE

Final step of the IF condition.

10.4.1.9 Launch

The Launch event allows to start another application. The path to the application is defined relative to Chartmaster’s home path.

- Application: Enter path where the application is located.
- Arguments: Enter command line arguments if necessary.

10.4.1.10 Macro Command

Use to execute an individual Macro Command. Many controls that cannot 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 39.)

Alternatively, you can view the Macro Command for individual icons via opening the Dialog Control window (see chapter 2.2 on page 10.)

Command: Enter the name of the Macro Command you wish to include, e.g., "E Zap".

10.4.1.11 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!
10.4.1.12 Switch Window

Brings the selected window in front.

In the drop-down menu you can select the Chartmaster window you want to switch to:

10.4.1.13 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.

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10.4.2 Acquisition

10.4.2.1 Acquire Series

Used to execute an entire PGF sequence.

![Edit PGF Template](image1)

**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*.

10.4.2.2 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.

![REPEAT Each Sweep](image2)

**Duration:** Defines the total cycle time for one *Sweep*.

**Increment:** Sets a defined time increment for the *Sweeps*.

![Acquire Each Sweep](image3)

For a description of the Acquire Each Sweep event please refer to the Acquire Series event above.

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10.4.2.3 Acquire Properties

![Acquire Properties Window](image)

**Update R-membrane and I-pipette:** Compute and update the calculated \( I_{\text{pipette}} \) and \( R_{\text{membrane}} \) 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 Chartmaster 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 5.3.4 on page 43).

*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.

10.4.2.4 Set Sweep Label

Allows to put a label on the Sweep to be acquired next.

![Sweep Label Window](image)

**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.

10.4.3 Hardware

10.4.3.1 Serial Output

Use to send a string to the opened serial interface. For configuration of the Serial Output see Configuration Window, chapter 5.4.6 on page 45.
10.4 Events - Overview

**String:** Enter a string that will be sent to a device via the *Serial Output*.

Examples:
- \textit{GO} \quad The command "GO" is send.
- \textit{GO}\textbackslash r \quad The command "GO" + <Carriage Return> is send.
- \textit{GO}\textbackslash l \quad The command "GO" + <Line Feed> is send.
- \textit{GO}\textbackslash 032 \quad The command "GO" + ASCII Code 32 (<Space>) is send.

10.4.3.2 Set DAC

Channel: Select a channel for analog output.

Voltage: Enter a voltage that will be output via the given channel.

\textit{Note:} The value gets scaled as defined in the *Configuration* window.

10.4.3.3 Set Digital Bit

Channel: Select a channel for digital output.

\textit{low}: If selected, 0 Volt will be sent via the channel. If not selected, 5 Volt (standard TTL setting) will be sent.

10.4.3.4 Set Digital Word

Digital Word: The entered number will be automatically translated into the digital bits.

Digital Bits: Allows selecting the digital bits individually.

10.4.3.5 Set Solution Changer

Solution: Select a Solution (1-16) at the connected *Perfusion System*.

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Note: The output channels and their aligned solutions have to be defined first in the Solution Changer dialog (chapter 16 on page 151).

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.

10.4.4 Data/Display

10.4.4.1 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.
- 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.

10.4.4.2 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.
10.4.4.3 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 31.

**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.

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• 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 31). Note that you have to mark Show Traces for the changes to take effect.

10.4.4.4 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 26).

![Export](image)

**Filename**: Define a name for exported file according to the auto filename components described in the Configuration (see chapter 5.5.2 on page 48).

**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.
• "0,0,0,0": 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.

10.4.4.5 File Operation

![File Operation](image)

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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 21).

10.4.4.6 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).

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- **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.

### 10.4.4.7 Trace Buffer

![Trace Buffer screenshot](image)

**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.

![Scale buffer screenshot](image)

- Equation: Calculates the scaling of the Buffer via an equation. For details on the equation syntax please refer to Equation Syntax, chapter 18.1 on page 157.

![Equation buffer screenshot](image)

**Update Display**: Updates the display of the Buffer in the Oscilloscope window.

**Update Analysis**: Updates the display of the Buffer in the Analysis graphs.

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10.4.5 Value/Parameter

10.4.5.1 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 9.14 on page 98).

*Note:* Please keep in mind that the stimulus amplitude is given relative to 1 V or 1 nA in voltage clamp and current clamp mode, respectively. I.e., to obtain a stimulus amplitude of 100 pA in current clamp mode you have to enter a value of "100m" (= 0.1 * 1 nA).

10.4.5.2 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 15 on page 147.
10.4.5.3 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 Chartmaster in the CHARTMASTER Tutorial. In addition, results from the Analysis can be stored in these values (see Analysis Functions, chapter 11.3 on page 121). 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 55.

Operation: Set value to...

- manually entered values:
  - =
  - increase by
  - multiply by
  - divide by
  - modulo
  - keep: no change to the value

- an Item:
  - = item
  - inc by item
  - dec by item
  - mul by item
  - div by item

- 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

- Analysis results:
  - = online
  - inc by online
  - dec by online
  - mul by online

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- div by online

- equation results: = equation. An equation can be entered to compute a value. For details on the equation syntax please refer to Equation Syntax, chapter 18.1 on page 157.
- = 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 13.
- 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.

10.4.6 Messages

10.4.6.1 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.

10.4.6.2 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...).
10.4.6.3 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 10) – 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".

10.4.7 Extensions

10.4.7.1 Photometry/Imaging

Note that the Photometry Extension has to be active to allow the editing of the following events (see Configuration, chapter 5.4.5 on page 45 and Photometry Extension, chapter 19 on page 163).

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.

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11. 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.

Chartmaster 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 *.on1 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.
11.1 Stimulus Control

**Stimulus Control:** There are four possible options for controlling the particular *Analysis Method* of choice:

- **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 an permanent link of an *Analysis Method* to a type of *Series*.
- **No Analysis:** No *Analysis* will be performed.

11.2 Analysis Methods

An *Analysis Method* is defined by all the information stored in the areas of *Analysis Graphs* and *Analysis Functions*. *Chartmaster* provides a pool of such *Analysis Methods* and such pools of methods are stored on disk as *Analysis* files (*.onl)*.

- **1...4 38:** 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 Method* can be edited – ”IV” in this example.
- **New:** Creates a new *Analysis Method*.
- **Delete:** Deletes the active *Analysis Method*.
- **Move:** Changes the positioning of a particular *Analysis Method* within the scroll bar.

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*).
11.3 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.

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.

11.3.1 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 9.9.2 on page 91)).

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'.

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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

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11.3 Analysis Functions

**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, 10.4.5.3 on page 116). The Values-1...16 are used to exchange information between different Analysis Methods and between the Analysis and the Protocol Editor.

**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.

*Important note:* 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.

11.3.2 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.

11.3.2.1 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 50).

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.

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11.3 Analysis Functions

11.3.2.2 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.

11.3.2.3 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 Y-axis value (often called amplitude) of the threshold crossing point. Use this function e.g. if you want to know the current value for a specific (recorded) voltage 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 ($\tau$) from an exponential curve. Computes $\tau$ in two steps:

1. semi-logarithmic regression of $x$ vs $\ln(y)$
2. $\text{abs}(1/\text{slope})$

11.3.2.4 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_Phase: Returns the phase setting of the software LockIn.

LockIn_Freq: Returns the frequency of the sinusoidal wave used for LockIn measurements.

11.3.2.5 Trace Parameters

The following parameters can be retrieved for analysis from the parameter set that is stored with the data (see Parameter window, 12 on page 135).

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.

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.

11.3.2.6 Sweep Parameters

The following parameters can be retrieved for analysis from the parameter set that is stored with the data (see Parameter window, 12 on page 135).

User 1: Parameter as set by the user in the I/O Control section of the Configuration window, 5 on page 41.

User 2: Parameter as set by the user in the I/O Control section Configuration window, 5 on page 41.

Temperature: Temperature (from a recording device).


11.3.2.7 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" "$\text{-Operand}$" "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, 18.1 on page 157.

$Y(x) : y \text{ at pos} = x$: 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.

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11.3 Analysis Functions

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 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.

11.3.2.8 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, 18.1 on page 157.

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.

11.3.2.9 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.

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**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 * 10^{-25}`. The unit is in A²/Hz.

### 11.3.2.10 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:

\[
\text{bin width} = \frac{\text{Y-range}}{\text{number of bins}}
\]

*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.

### 11.4 Analysis Graphs

![Graph Display Panel](http://www.heka.com)

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 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 *Online* windows, e.g. after changing the axis scaling.

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11.4 Analysis Graphs

11.4.1 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).

Note: This setting is overruled by the setting Wipe Display at Start and Wipe at Start (see 10.4.2.3 on page 108 and 5.6.2 on page 50).

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 Chartmaster 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 10.4.4.1 on page 110).

In case Trace data are displayed in the graphs, the options Overlay and OvrlSwp are available.

- 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.
  - 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.
11.4 Analysis Graphs

- Header: Text for the graph header can be entered.

- Normalize: Select the type of normalization of the data:
  - None
  - \( y' = \frac{y}{\text{max}} \)
  - \( y' = \frac{(y - \text{min})}{(\text{max} - \text{min})} \)

- Sort: If selected, the data will be sorted 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.

11.4.2 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.

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.

### 11.5 Graph Positions

**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.
11.6 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.
12. Parameters Window

Parameters are additional values describing the experimental and measurement conditions. The Parameters are required to interpret the data correctly. In CHARTMASTER we distinguish different types of Parameters depending to which level of the data structure they relate.

**Note:** If possible CHARTMASTER 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.
12.1 Parameter Tabs

12.1.1 Root

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 Chartmaster.
- **Text**: A descriptive text can be entered and stored if the data file is opened in the Open Modify... mode.
12.1.2 Group

Level 1 of the data tree. The 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 ElProScan).
- **MatrixHeight**: Height of the acquired image in the Matrix Scan mode (only for data acquired with the ElProScan).
12.1.3 Series

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 (5.3.6 on page 43).
- **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 55).
- **Aux3...6**: Auxiliary Parameter fields only used when certain extension are switched on.
- **Comment**: Text identifier (see Control window, 7.1 on page 65).
12.1 Parameter Tabs

12.1.4 Sweep

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_A_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, 17 on page 153).
- Temperature: Temperature value from an external device (see Configuration, 5.7.2 on page 55).
- User 1/2: User-defined external values (see Configuration, 5.7.2 on page 55).
- Digital In: Status of the input bits of the digital channels (see I/O Control, 14 on page 145).
- Digital Out: Status of the output bits of the digital channels (see I/O Control, 14 on page 145).
12.1.5  Trace

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.
- **Amplifier Mode**: Recording mode of the amplifier during recording (e.g. "Whole-Cell").
- **Amplifier**: Notification field of any Amplifier event (e.g. "Clipping" - CLIP-1 = clipping, NoClip-1 = clipping).
- **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, 9.12 on page 97).
- **Y-unit**: Unit of the Y-axis.
- **X-unit**: Unit of the X-axis.
- **X-interval**: Sample Interval (see Timing, 9.5 on page 76).
- **X-start**: Indicates any delay of the X-axis, mostly a time delay when using a Start-Segment (see Timing, 9.5 on page 76). 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, 9.9.2 on page 92).
• Bandwidth: Filter frequency of the acquired Trace.

• DA Channel: DA channel number (see Channel Settings for DA Output and AD Input, 9.9 on page 87).

• AD Channel: AD channel number (see Channel Settings for DA Output and AD Input, 9.9 on page 87).

• Holding: Holding potential/current value.

• Int. Sol.: Name of the used internal solution.

• Int. Sol. Value: Value entered in the selected solution (see Solution Data Base, 15.3 on page 148).

• Ext. Sol.: Name of the used external solution.

• Ext. Sol. Value: Value entered in the selected solution (see Solution Data Base, 15.3 on page 148).

• 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.

12.1.6 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.

- ** Trace** If selected, the Parameter names will be exported together with their corresponding values. Otherwise, only the values will be exported.

- ** Trace** If selected, values and/or Parameter names will be separated with a linefeed when exported.
12.2 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.
- **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.
13. 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 Chartmaster windows (e.g. Parameters, Configuration, Online 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 24 and 4.7 on page 36).

*Note:* The Cut, Copy and Paste commands copy to and from the clipboard.
14. 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 51).

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.

### 14.1 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.

### 14.2 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 Chartmaster, i.e. as a stimulation channel in the Pulse Generator.
14.3 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 55). In addition, the names of the Values are displayed above the numeric Values. The name can be set in the Configuration dialog.

14.4 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, 5.4.6 on page 45). An example is shown in the Protocol Editor, 10.4.3.1 on page 108.

Note: This section is only visible, if the serial communication is turned on in the Configuration dialog, 5.4.6 on page 45.

14.5 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 Chartmaster.

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 10.4.3.5 on page 109) and the Solution Changer dialog (chapter 16 on page 151).

14.6 Parameters

Only the Temperature option or User Parameters can be selected in the Configuration window to be listed in the I/O Control window for display or editing.

The individual Parameters are described in the context of the Configuration window, 5.7 on page 51.
15. 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.

**Chartmaster** 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.

   You can set the entry manually or via the event Set Solutions in the Protocol Editor (10.4.5.2 on page 115). The numbers can be the entry of an external list of solutions, or the index of a specific solution in a Chartmaster Solution Data Base (see 15.1). The solution indices are stored together with the recorded data and can be reviewed in the Parameters window (12.1.5 on page 140).

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 (10.4.5.2 on page 115).

### 15.1 Activating the Solution Base

First, turn on the Solution Base in the Configuration window (5.5.2 on page 48). 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 (14.5 on the facing page) or via the Set Solutions event in the Protocol Editor (10.4.5.2 on page 115).
15.2 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.

15.3 Solution Data Base

To open the Solution Data Base you have to select Solution Base in the Windows menu of Chartmaster.

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:

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• 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 database.

**Create / Duplic. / Delete Entry:** Used to generate, copy, and remove solutions in the database.

**Next / Last Entry:** This option moves through the database 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 sizes 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.
16. Solution Changer

The Solution Changer dialog can be accessed via the Windows menu of Chartmaster. 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 Chartmaster. 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.
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.
17. 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.

17.1 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.
17.2 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.

![Notebook](http://www.heka.com)

**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.
18. Calculator Window and Equations

Equations are a very flexible way to perform complex calculations within Chartmaster. 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.

18.1 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

**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.

### 18.2 Equations - Where to Use

#### 18.2.1 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:

- \( f[i], i = \) online function index = 1...n
- \( p[i], i = \) amplifier parameter index = 1...n
- \( v[i], i = \) value index = 1...n
- icon[icon descriptor], e.g. ‘icon[E VHold]’

An example: 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:  

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• 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.

18.2.2 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*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 18.2.1 on the preceding page).

18.2.3 DA Scaling

Special Operands:

• ampl = The value for output or amplitude as defined by the PGF-stimulus. Used for equations in DA-Scaling 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 52). 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.

### 18.2.4 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 18.2.1 on page 159).
19. 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 Chartmaster is provided in the Chartmaster Tutorial.

After starting Chartmaster, open the Configuration window and select the appropriate Photometry Extension in the Hardware section of the dialog. The options are:

- T.I.L.L
- DG4 / DG5
- 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.

<table>
<thead>
<tr>
<th>R-max</th>
<th>1.0000E+00</th>
<th>R-min</th>
<th>0.0000E+00</th>
<th>Kd*Sf</th>
<th>1.0000E+00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Background</td>
<td>0.0000E+00</td>
<td>Background</td>
<td>0.0000E+00</td>
<td>Background</td>
<td>0.0000E+00</td>
</tr>
<tr>
<td>Dead Time</td>
<td>2.00 ms</td>
<td>Compute Traces of Marked Targets</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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).

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.
19.1 Photometry Configuration T.I.L.L.

Resting Wavelength: Wavelength to be set during resting periods.

Set: Only after pressing the Set button the wavelength entered in the Resting Wavelength field is output.

Excit.: Select the DA channels for the voltage output. This function is used to control the monochromator. Once it is calibrated, the output can be defined either in Volts (left) or in nm (right).

Emit-1, Emit-2: Select the AD channels for sampling the photometry responses. Emit-1 is always active whereas Emit-2 can be turned off. It may be used for dual emission fluorescence experiments.

Shutter Selection:

- No Shutter: No shutter available.
- Shutter Control: Shutter can be closed/opened via a button.
- Digital-out 7: Shutter control via digital output.

19.1.1 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.

http://www.heka.com
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.

\[
F(\lambda) = \arcsin(\lambda/1666.67 \times \cos(\pi/360 \times \beta)) \tag{19.1}
\]

\[
V = (2\pi \times F(\lambda) - \text{Offset})/\text{Slope} \tag{19.2}
\]

\[
\lambda = (1666.67 \times \cos(\pi/360 \times \beta) \times \sin((\text{Slope} \times V + \text{Offset})/2\pi) \tag{19.3}
\]

\[
\text{Slope} = 2\pi \times (F(\lambda_1) - F(\lambda_2))/(V_1 - V_2) \tag{19.4}
\]

\[
\text{Offset} = 2\pi \times F(\lambda_1) - \text{Slope} \times V_1 \tag{19.5}
\]

with

- \(\beta\) = typically 30 degree; depends on your hardware. Please refer to the manual of the monochrometer.
- \(\lambda\) = requested wavelength in [nm]; \(\lambda_1\) = 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.

19.1.1.1 Calibrating the different T.I.L.L. 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.

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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.

19.2 Photometry Configuration DG4/DG5 and Lambda-10

 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.

 Filter Position: Enter a number (Filter Position) between 0 and 15.

 Shutter Open/Close: Toggles between an open and a closed shutter.

 Shutter Selection:

- No Shutter: No shutter available.
- Shutter Control: Shutter can be closed/opened via a button.
- Digital-out 7: Shutter control via digital output.

 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.

 Speed: Enter a number between 0 and 7.
19.2 Photometry Configuration DG4/DG5 and Lambda-10

19.2.1 PGF Sequence

When using the DG4/DG5 or 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.

Note: To control the DG4/DG5 or Lambda-10 via the Chartmaster software you need a special cable connecting the Digital Outputs of the interface and the DG4/DG5 or Lambda-10. Please contact HEKA (sales@heka.com) if you need such a cable.
19.3 Photometry Configuration PTI DeltaRAM

Resting Wavelength: Wavelength to be set during resting periods.

Set: Only after pressing the Set button the wavelength entered in the Resting Wavelength field is output.

Excit.: Select the DA channels for the voltage output. This function is used to control the monochromator. Once it is calibrated, the output can be defined either in [V] (left) or in [nm] (right).

Undo: Resets all changes made since the last opening of the Photometry window.

Defaults: Resets all changes to the default entries.

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.

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.

Shutter Selection:

- No Shutter: No shutter available.
- Shutter Control: Shutter can be closed/opened via buttons.
- Digital-out 7: Shutter control via digital output.

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20. Imaging Extension

The Imaging Extension 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 Chartmaster. 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 Chartmaster’s data tree. When stepping through the data tree, the Imaging Extension 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.

The Imaging Extension consists of an Imaging window in Chartmaster, an additional Control window and an Image window.

The Image Extension supports the following hardware:

- Fluorescence Excitation light sources which can be controlled in the same way as:
  - Polychrome and Oligochrome from T.I.L.L. 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, Jenoptik, QImaging and Hamamatsu.

Image files are stored as 16-bit tiff stacks and can be opened by other programs such as ImageJ, IGOR Pro etc....
20.1 Starting the Imaging Extension

After starting Chartmaster, open the Configuration window and select the appropriate Imaging Extension in the hardware section of the dialog. The Imaging Extension has to be selected to support the fluorescence excitation light source of your setup (see above).

After activating the Imaging Extension, the Imaging window is opened in Chartmaster, the Image Control window and the Image window are opened.

20.2 Imaging Configurations

This section provides a brief overview of the settings for each of the fluorescence excitation light source used with the Imaging Extension.

Once the Imaging Extension has been activated the Imaging window can be opened from the menu Windows.

Some controls are common for all configurations describing the different fluorescence excitation light sources. These controls are described in this section.

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. The background values are stored as Series Parameters with the raw data file and are listed in the Parameters dialog.

Dead Time: Time to allow for settling of the commanded wavelength before starting the image exposure.

Traces to create: Check the Traces which should be automatically generated by the Pulse Generator or created by the Recompute function.

Compute Traces of Marked Targets: ALL: Recomputes the complete fluorescence data set starting with the calculation of the mean fluorescence values from the regions of interest (ROIs). Use this function when you have modified the set of ROIs.

R-compute Traces of Marked Targets: Ratio + Ca: Recomputes the based on the fluorescence values already stored in CHARTMASTER. Use this function when you modify the Background values or the calibration Parameters for the calculation of the calcium concentration R-min, R-max, Kd*Sf.

Compute Histogram of Marked Targets: A histogram Trace is created for every image connected with your recordings and added at the end of the Replay tree. If you have drawn a ROI in your image the histogram is created only for the ROI and not the total image. When several ROIs exist CHARTMASTER will create histogram Traces for every ROI.

Camera Trigger: Select the output channel which is used for triggering the camera.
20.2.1 Imaging Configuration T.I.L.L.

**Resting Wavelength:** Wavelength to be set during resting periods.

**Set:** Only after pressing the *Set* button the wavelength entered in the *Resting Wavelength* field is output.

**Excit.** Select the DA channels for the voltage output. This function is used to control the monochromator. Once it is calibrated, the output can be defined either in Volts (left) or in nm (right).

**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.

20.2.1.1 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.

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• 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.
• wavelength: Enter here the maximum of the bandpass filter (in nm).
• upper - Scan: Do the scan for the high-wavelength calibration filter.
• Volt: This field shows the voltage corresponding to the peak transmission.

20.2.1.2 Calibrating the different T.I.L.L. 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.

20.2.2 Imaging Configuration DG4/DG5 and Lambda-10

Filter Position: Enter a number (Filter Position) between 0 and 15.
Shutter Open/Close: Toggles between an open and a closed shutter.
Shutter Selection:

• No Shutter: No shutter available.
• Shutter Control: Shutter can be closed/opened via a button.
• Digital-out 7: Shutter control via digital output.
20.2.3 Imaging Configuration PTI DeltaRAM

**Resting Wavelength:** Wavelength to be set during resting periods.

**Set:** Only after pressing the Set button the wavelength entered in the Resting Wavelength field is output.

**Excit.:** Select the DA channels for the voltage output. This function is used to control the monochromator. Once it is calibrated, the output can be defined either in Volts (left) or in nm (right).

**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.

**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.
20.3 Image Window

The *Image* window displays the image during image acquisition or when loading an image file.

The pixel position of the mouse tip is shown as $X$- and $Y$-value on the left side in the foot line of the image. In the middle of the foot line the pixel intensity (brightness or fluorescence value) is shown. On the right side the computer’s `time` is shown.

The icons on the right side of the window are short cuts to the corresponding tabs of the *Image Control* window.

20.4 Image Control Window

20.4.1 Camera Setup

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**20.4 Image Control Window**

**Camera Reference:** Lists the identification of the camera used by the Imaging Extension.

**Binning:** Allows to set the camera binning factor. The resulting pixel dimensions of the image are shown to the right.

*Note: The binning factor is typically set once for a complete experiment.*

**Live View Parameters:** Camera parameters used during Live View mode only.

- Exposure Time [ms]: Time for image exposure during live image acquisition.

**Scaling:** Parameters determining the intensity scaling of the image.

- auto: When activated each image is scaled with respect to the darkest and brightest pixel.
- min / max: When auto is activated, min/max display the intensity values of the darkest and the brightest pixel. When auto is not active, the user can set intensity values for the darkest (black) and brightest (white) pixel manually.
- histo: Calculates an intensity histogram of all pixels and shows it in a separate graph (see below).

**Live View:** Starts and stops the live acquisition of the camera. In "Live Mode" the camera is running with its maximal speed.

**Snapshot:** A click to this button acquires a single image (YYYY-MM-DD-000.tiff). The path is defined in the Imaging Configuration (20.4.5 on page 179).

### 20.4.2 PGF Primer

Imaging related information about wavelength, exposure times and repetition rates can be automatically integrated in already existing Pulse Generator sequences. All imaging related manipulation of the sequences should be done through the PGF Primer. Once the desired imaging Parameters are found, the user can integrate them into multiple Pulse Generator sequences.

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**PGF Listing:** A list which contains all Pulse Generator sequences currently loaded in Chartmaster. You can select a specific sequence to work on. In case this sequence contains already imaging related Parameters the values are updated in the fields below.

**Prime:** Transfers the imaging related Parameters to the selected Pulse Generator sequences.

**Update List:** Reads the complete Pulse Generator pool from Chartmaster and updates the PGF Listing.

**Update from PGF:** If this option is selected the imaging related Parameters of already existing Pulse Generator sequences is read out and displayed in the fields of this dialog.

*Note: In case you want to integrate the same imaging related Parameters to multiple Pulse Generator sequences then this option has to be deselected.*

**Acquisition Parameters:**

- **Readout Time [ms]:** Displays the approximate camera read out time for one image. This setting usually depends on the binning factor.
- **Cycle Time [ms]:** Total duration of one cycle of image acquisition. One cycle can contain 1 to 3 individual image acquisitions.
- **Cycles:** Enter the number of cycles (repetitions). In case the option Adapt to length is selected, this field displays the maximal number of repetitions after the priming process.
- **Adapt to length:** This option allows to repeat the parent imaging cycle as many times until the duration of the longest sweep in the Pulse Generator sequences is reached.
- **No. of wavelengths list:** You have to specify how many different wavelengths you want to use in a single imaging cycle.
- **Wavelength:** Enter the wavelength (in nm when using a T.I.L.L. monochromator) or the filter position number (when using a filter wheel or DG-4/5).
- **Exposure [ms]:** Enter the exposure times for the different wavelength.

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20.4.3 ROI Manager

When the ROI Manager tab is selected the user can draw ROIs into the image. The ROIs are listed in the main field of the tab.

**List of ROIs:** Shows the names of all ROIs from the current set. The selected ROI from the list is shown in bright yellow color.

**Delete ROI:** Deletes the selected ROI.

**Save ROI:** Saves the current set of ROIs to a separate file with extension *.roi. In case an image acquisition is started from CHARTMASTER the ROIs, which belong to this image set, are automatically stored to a file with extension *.roi and the name of the image. When reloading this image file the corresponding set of ROIs is also loaded.

**Load ROI:** Loads a set of ROIs from file.

**Show ROIs:** When activated the ROIs are shown as overlay to the image.

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20.4.4  Image File Selector

**Filename**: Shows the name and path of the active image.

**Page Selector**: In case the image file contains several image frames you can use the left and right arrow buttons to step through the individual frames of the image stack.

**Info Text**: Shows the additional text information which is stored with the image file.

**Open Image File**: Allows to open an image file.

**Close File**: Closes the current image file.
20.4.5 Imaging Configuration

Paths:

- Snapshots: Shows the path to the folder in which all snapshots will be stored. Press the button on the right in order to change the folder.

**Note:** Images acquired with the Pulse Generator are stored in the same folder as the Chartmaster data file.

Other Settings:

- Show Tab Bar: If activated the Tab Bar is shown in the foot line.
- Keep Aspect Ratio: If activated the aspect ratio of the camera sensor is kept proportional when enlarging the window.
- Default Pixel depth: Select the dynamic range of the digitalization (8, 12, 14 or 16 bit).
20.4.6 Imaging Log

Log File Text: Protocols the communication between the Imaging Extension and Chartmaster. This information is used to facilitate the troubleshooting.
21. Software LockIn Extension

21.1 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 5.4.2 on page 44). After switching on the LockIn, the LockIn Configuration window opens, which is later on available in the Window menu.

**Important note:** In the Chartmaster 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.
- On: The LockIn mode is turned on.
- 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.

**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).

**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.

**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, 21.2).

**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.

**Default Y-ranges:** The user has to define Default Y-ranges for the resulting LockIn Traces. CHARTMASTER does not know the range of the forthcoming data because this dependents on cell size. Thus, the user has to provide a default range (e.g. 0 to 40 pF for the Cm Trace would be a reasonable estimate).

**SetDefaults:** Resets the Default Y-ranges to the default values.

**Skip:** A global number of cycles to skip can be entered.

**Important note:** The global setting Skip, when activated, overrules the setting made in the Wave Parameters of the PGF.

### 21.2 The LockIn Traces

The LockIn Extension provides several output Traces which can be selected in the AD-channel section of the Pulse Generator (9.9.2 on page 89).

The following Traces which are independent from the LockIn mode are available:

**LockIn_Phase:** The phase angle between stimulus and measured signal.

\[
\text{Phase} = \frac{180}{\pi} \cdot \arctan(B/A)
\]

**LockIn_Real(Y):** The real part of the admittance.

Voltage Clamp: \( Real(Y) = A \) (Siemens).
Current Clamp: \( Real(Y) = \frac{A}{A^2 + B^2} \) (Ohm).

**LockIn_Imag(Y):** The imaginary part of the admittance.
Voltage Clamp: $Imag(Y) = B$ (Siemens).
Current Clamp: $Imag(Y) = \frac{B}{\sqrt{A^2 + B^2}}$ (Ohm).

**LockIn_Admn**($Y$): The admittance (Siemens).

Voltage Clamp: $Admtn(Y) = \sqrt{A^2 + B^2}$.
Current Clamp: $Admtn(Y) = \frac{1}{\sqrt{A^2 + B^2}}$.

**LockIn_Real**($Z$): The real part of the impedance.

Voltage Clamp: $Real(Z) = \frac{A}{\sqrt{A^2 + B^2}}$. (Siemens)
Current Clamp: $Real(Z) = A$ (Ohm).

**LockIn_Imag**($Z$): The imaginary part of the impedance.

Voltage Clamp: $Imag(Z) = \frac{B}{\sqrt{A^2 + B^2}}$. (Siemens)
Current Clamp: $Imag(Z) = B$ (Ohm).

**LockIn_Imp**($Z$): The impedance (Ohm).

Voltage Clamp: $Imp(Z) = \frac{1}{\sqrt{A^2 + B^2}}$.
Current Clamp: $Imp(Z) = \sqrt{A^2 + B^2}$.

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").

### 21.3 Capacitance Measurements - Step by Step

**Important note:** The following section was made for Patchmaster and HEKA EPC 9 or 10 amplifier. If you are using Chartmaster or Potmaster several modifications may be necessary!

Here, we make our first capacitance measurement using a model circuit and the LockIn Extension. We used an EPC 10 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.
21.3.1 Activating the LockIn Extension

By default, the LockIn Extension of CHARTMASTER 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 CHARTMASTER configuration file **Chartmaster.set**.

21.3.2 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.

*Important note:* 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.

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21.3 Capacitance Measurements - Step by Step

**Note:** The Default Y-ranges section can be used to set reasonable display ranges according to the expected values.

21.3.3 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".

Chartmaster 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.

![Image of Pulse Generator window](http://www.heka.com)

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 ( ) 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, 9.8.1 on page 78).

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".

**Important note:** 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 \( \rightarrow \) 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.

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.

Please note, that Link is set to "1". This tells Chartmaster 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 then 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.

http://www.heka.com
21.3 Capacitance Measurements - Step by Step

21.3.4 Amplifier Settings

Now it is time for the experiment. Switch the model circuit into the "10 M" position to simulate a 10 MΩ 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 CHARTMASTER 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.

21.3.5 Running the PGF sequence

In CHARTMASTER, 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.

![Control Window](http://www.heka.com)

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.

![Oscilloscope](http://www.heka.com)

**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).
21.3.6 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.

http://www.heka.com
Analysis Window: If not visible, go to the **Windows** menu and activate **Analysis Window 1**.

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**.
22. Spectroscopy Extension

22.1 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 Chartmaster 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.

22.1.1 Chirp Wave Forms

The Pulse Generator of Chartmaster provides three different chirp wave forms:

**Linear Chirp:**

In a Linear Chirp, the frequency \( f(t) \) varies linearly with time:

\[
\text{f}(t) = f_0 + kt
\]  

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:

\[
x(t) = \sin[2\pi(f_0 + \frac{k}{2}t)t] 
\]

**Exponential Chirp:**

In an Exponential Chirp, the frequency of the signal varies exponentially as a function of time.

\[
f(t) = f_0 k^t
\]

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:

\[
x(t) = \sin[\frac{2\pi f_0}{\ln(k)}(k^t - 1)]
\]

**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.

22.1.2 Transfer Function Ratio

The transfer function (\( H \)) of two signal processing units in series is given by:
\[ H(A + B) = H(A) \otimes H(B) \quad (22.5) \]

In case one is interested the transfer function of an individual signal processing unit, the "ratio" of the transfer function can be calculated:

\[ H(B) = \frac{H(A + B)}{H(A)} \quad (22.6) \]

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.

### 22.2 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 Chartmaster.

http://www.heka.com
Correction Mode: It is possible to correct the spectra during online and offline calculation with respect to another chirp response (see Transfer Function Ratio, 22.1.2 on page 191).

- 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 MΩ 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.

22.2.1 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(°). 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:

\[ \text{Real}(Y) = \frac{\text{Admittance}}{\sqrt{1 + \tan(\text{Phase})^2}} \] (22.7)

The default unit is Siemens (S).

Chirp_Imag(Y): The imaginary part of the admittance. This is a derived frequency based Trace (X-unit is Hertz), calculated as follows:

\[ \text{Imag}(Y) = \tan(\text{Phase}) \times \text{Real}(Y) \] (22.8)

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:

\[ \text{Real}(Z) = \frac{\text{Real}(Y)}{\text{Real}(Y)^2 + \text{Imag}(Y)^2} \] (22.9)

The default unit is Ohm (Ω).

Chirp_Imag(Z): The imaginary part of the impedance. This is a derived frequency based Trace (X-unit is Hertz), calculated as follows:

\[ -\text{Imag}(Z) = \frac{\text{Imag}(Y)}{\text{Real}(Y)^2 + \text{Imag}(Y)^2} \] (22.10)

The default unit is Ohm (Ω).

Chirp_Imp|Z|: The impedance. This is a derived frequency based Trace (X-unit is Hertz), calculated as follows:

\[ \text{Imp|Z|} = \frac{1}{\sqrt{\text{Real}(Y)^2 + \text{Imag}(Y)^2}} \] (22.11)

The default unit is Ohm (Ω).

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22.3 Setting up a Spectroscopy Acquisition

*Important note:* The following section was made for Patchmaster and HEKA EPC 9 or 10 amplifier. If you are using Chartmaster or Potmaster several modifications may be necessary!

Create a new PGF sequence with one Chirp segment as stimulus. When at least one segment contains a chirp waveform then a button Chirp Wave appears in the top right section of the Pulse Generator window.

22.3.1 The Chirp Wave Dialog

A detailed description of the available chirp wave parameters can be found in Chirp Wave Parameters, 9.8.3 on page 83.

22.3.2 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.

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22.3.3 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.

22.3.4 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.

2. Use of a Pre-Chirp Segment: In order to reduce the time for swing in to a minimum, a Constant segment of fixed length can be defined before the Chirp segment. When the option Pre-Chirp is activated in the Chirpwave Parameters dialog, then this segment is automatically filled with appropriate swing-in stimulus data. Please note that the Pre-Chirp segment should not be recorded (use Start Segment "2") to ensure accurate chirp analysis.

http://www.heka.com
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.
22.4 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).

http://www.heka.com
Trace 10 (orange) and Trace 11 (green) show the phase and the admittance, respectively.
### 23. Appendix I: File Overview

#### 23.1 File Types

<table>
<thead>
<tr>
<th>file type</th>
<th>extension</th>
<th>default file</th>
<th>see also</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data file (includes the measured data)</td>
<td>.dat</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dialog setting for the dialog of windows (if no dialog file exists, the software will start with its own default values.)</td>
<td>.dia</td>
<td>-</td>
<td>Chapter User Interface, 2.3 on page 14.</td>
</tr>
<tr>
<td>Key file for key commands (includes all key commands)</td>
<td>.key</td>
<td>Chartmaster.key</td>
<td>Chapter Keys, 3 on page 17.</td>
</tr>
<tr>
<td>Macro file for macros; not supported anymore starting with version 2.40.</td>
<td>.mac</td>
<td>-</td>
<td>Now protocols are used instead. Chapter Protocol Editor Window, 10 on page 99.</td>
</tr>
<tr>
<td>Markers file for all marker information.</td>
<td>.mrk</td>
<td>-</td>
<td>Chapter Markers, 17 on page 153.</td>
</tr>
<tr>
<td>Online file for analysis settings (can include more than one analysis method).</td>
<td>.onl</td>
<td>DefAnal.onl</td>
<td>Chapter Analysis Window, 11 on page 119.</td>
</tr>
<tr>
<td>Stimulus file for the pulse generator settings.</td>
<td>.pgf</td>
<td>DefPgf.pgf</td>
<td>Chapter Pulse Generator Window, 9 on page 73.</td>
</tr>
<tr>
<td>Protocol file for a protocol pool (can include more than one protocol).</td>
<td>.pro</td>
<td>DefProt.pro</td>
<td>Chapter Protocol Editor Window, 10 on page 99.</td>
</tr>
<tr>
<td>Equation file for storing equation strings.</td>
<td>.txt</td>
<td>Equation.txt</td>
<td>Chapter Calculator Window and Equations, 18 on page 157.</td>
</tr>
<tr>
<td>Acquisition parameters file.</td>
<td>.pul</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Settings file.</td>
<td>.set</td>
<td>Chartmaster.set</td>
<td>-</td>
</tr>
<tr>
<td>Solution file for storing the entries of the solution database.</td>
<td>.sol</td>
<td>Example.sol</td>
<td>Chapter Solution Base, 15 on page 147.</td>
</tr>
<tr>
<td>Filename template file.</td>
<td>.tpl</td>
<td>-</td>
<td>Chapter &quot;Using a Recorded Waveform as Stimulus&quot; in the Chartmaster Tutorial.</td>
</tr>
<tr>
<td>Notebook file.</td>
<td>.txt</td>
<td>-</td>
<td>Chapter Notebook menu, 4.7 on page 36.</td>
</tr>
</tbody>
</table>
24. Appendix II: Data Format

In this chapter we describe the general structure of the files generated by Chartmaster.

24.1 Data Files

ChartMaster generates up to 8 files, when a data file is created:

1. The Raw Data File (*.dat).
2. The Marker File (*.mrk).
3. The Analysis File (*.onl).
4. The Stimulation Sequence File (*.pgf).
5. The Acquisition Parameters File (*.pul).

If Make Bundle Files is checked in the Configuration window (5.5.2 on page 48), 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.

24.1.1 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, Chartmaster 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.

24.1.2 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>
<thead>
<tr>
<th>Record</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root</td>
<td>Version information</td>
</tr>
<tr>
<td>Marker</td>
<td>Description of one marker event</td>
</tr>
</tbody>
</table>

24.1.3 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.
24.1.4 Analysis Methods File

It contains the assigned analysis method for every Series. The structure of the Analysis Methods File (*.onl) is defined by the file AnalysisFile_v9.txt. It has a tree structure:

<table>
<thead>
<tr>
<th>Record</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root</td>
<td>Version information</td>
</tr>
<tr>
<td>Method</td>
<td>Description of one Analysis Method</td>
</tr>
<tr>
<td>Function</td>
<td>Description of one Analysis Function</td>
</tr>
</tbody>
</table>

24.1.5 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>
<thead>
<tr>
<th>Record</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root</td>
<td>Version information</td>
</tr>
<tr>
<td>Stimulation</td>
<td>Description of an ensemble of pulse patterns; e.g., I-V curve</td>
</tr>
<tr>
<td>Channel</td>
<td>Combines the definition for one output (DAC) and one input (ADC) Trace</td>
</tr>
<tr>
<td>Segment</td>
<td>Individual segment of a pulse pattern</td>
</tr>
</tbody>
</table>

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.

24.1.6 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>
<thead>
<tr>
<th>Record</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root</td>
<td>Version information</td>
</tr>
<tr>
<td>Group</td>
<td>Larger section of an experiment; e.g., cell or patch</td>
</tr>
<tr>
<td>Series</td>
<td>Description of an ensemble of Sweeps</td>
</tr>
<tr>
<td>Sweep</td>
<td>Description of a Sweep, i.e. one collection of Traces</td>
</tr>
<tr>
<td>Trace</td>
<td>Description of an individual data Trace</td>
</tr>
</tbody>
</table>

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.

24.1.7 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:

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24.1.8 Notebook File

The Notebook File is a standard ASCII text file with line breaks.

24.2 The Tree Format

The idea of Chartmaster 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:

```
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.

**Important note:** A detailed description of the Chartmaster data file format for programmers is available for download from our FTP server: [ftp://server.heckahome.de/pub/FileFormat/Patchmaster9/](ftp://server.heckahome.de/pub/FileFormat/Patchmaster9/).

### 24.3 File Template

#### 24.3.1 Filename

In Chartmaster 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 Chartmaster 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 Chartmaster 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".

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24.3.2 Data Format of the File Template

24.3.2.1 Voltage Clamp Stimulation or plain DA output

The file should contain one voltage value per stimulus point. CHARTMASTER 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.

24.3.2.2 Current Clamp Stimulation

Template Files for stimulation can be used in Voltage Clamp and in Current Clamp mode. In Current Clamp mode, the stimulation data is defined in volts, where 1 mV corresponds with 1 pA current injection.

Example: A value of 0.1 would result in a current injection of 100 pA.
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