Contents

1. The First Experiment

This chapter will guide you briefly through the main features of the PATCHMASTER program and should take you a maximum of about 2 hours to read it. It briefly describes how a very simple first experiment with PATCHMASTER could look like. Of course, you will not have to do a *real* experiment, instead you should use the model circuit to *simulate* the conditions of a patch-clamp recording. The reader should not worry about options that are unclear, because more detailed descriptions of all of the mentioned steps are to follow. This section is designed for users that can't wait to get something done with PATCHMASTER. The basic requirements for starting the program and for performing a simple experiment are outlined. For more detailed descriptions of the features, refer to the PATCHMASTER reference manual.

> ***Note:*** *In the following it is assumed that the hard- and software have already been set up correctly. Please refer to the chapter "Software Installation" in the* PATCHMASTER *reference manual for the installation of* PATCHMASTER*. If you plan to use the EPC 10 Double or Triple you should also first read the chapter "Setting up the EPC 10" in the* PATCHMASTER *reference manual to get an idea of the basic amplifier operation.*

1.1 Starting PATCHMASTER

Turn on the interface and the computer and start PATCHMASTER either via Programs/Heka/Patchmaster (Windows) or by clicking on the icon PATCHMASTER (Mac). While starting the program, these things can happen:

- You may not have the hardware key correctly installed. PATCHMASTER will continue to run in Demo mode, with a stimulus simulation of the AD board. For installation of the hardware key, please refer to

the Installation Guide. Note that in the Demo mode it is *not* possible to save files!

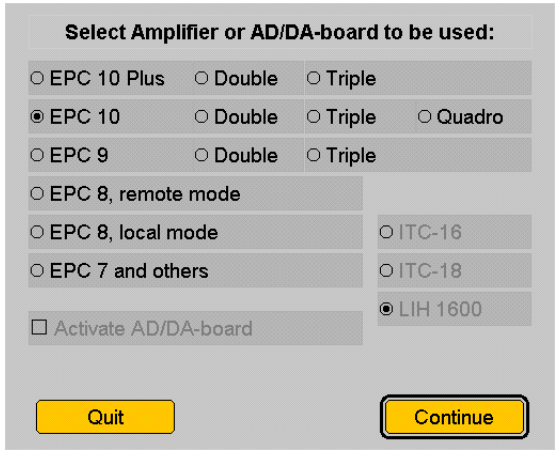
- You may not have connected any AD/DA hardware. PATCHMASTER will recognize this and will ask you to abort, to continue, or to try again. If you just forgot to turn on the power of the EPC 10 or the ITC-16, do so and select Retry. If you don't have either of them, you still can select Continue, i.e., PATCHMASTER will continue to run. Data acquisition will be disabled but opening, saving and printing files will be possible.

- You may not yet have a so-called "Configuration File" (i.e., a file with the extension ".set" that contains all of your individual program settings). PATCHMASTER will then come up with "Cannot find configuration file: use defaults or find file". If you do not have a configuration file, select Use Defaults. In this case, PATCHMASTER will generate default settings and will come up with a selection of windows.

Based on your configuration file, PATCHMASTER will now look for file paths and a default Pulse Generator File (PGF) that contains your stimulus protocols. The factory default is the file `DefPGF_v9.pgf` in the Data folder inside the HEKA folder.

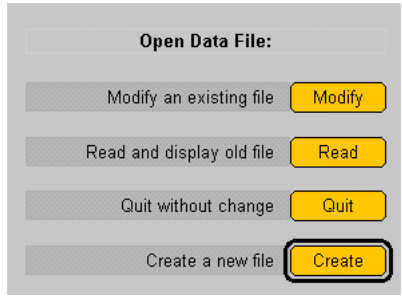
If PATCHMASTER cannot find your .pgf file, it will write a message into the `Notebook` window and will create a default file with only one entry called `Test`. There may be other paths missing and PATCHMASTER will put up an alert to that effect. You can safely ignore that error message, we will setup these paths next in the `Configuration` window (see below).

After loading its configuration and pulse generator file, PATCHMASTER will ask for your amplifier or AD/DA board. Please choose your connected device.



Note: *In case* PATCHMASTER *does not find the "Scale file" that includes the calibration of your amplifier, you will be asked to enter the correct path.*

Then PATCHMASTER will ask whether you wish to create a new experiment or just want to analyze some data:



There are four possibilities:

- Modify opens an existing experiment for modification, i.e. you can delete or add further experimental data to a file.

- Read opens and displays an existing experiment. The file will be write protected, so that modification (or loss) of data is prevented.

- Quit cancels the dialog.

- Create allows you to create a new experiment file.

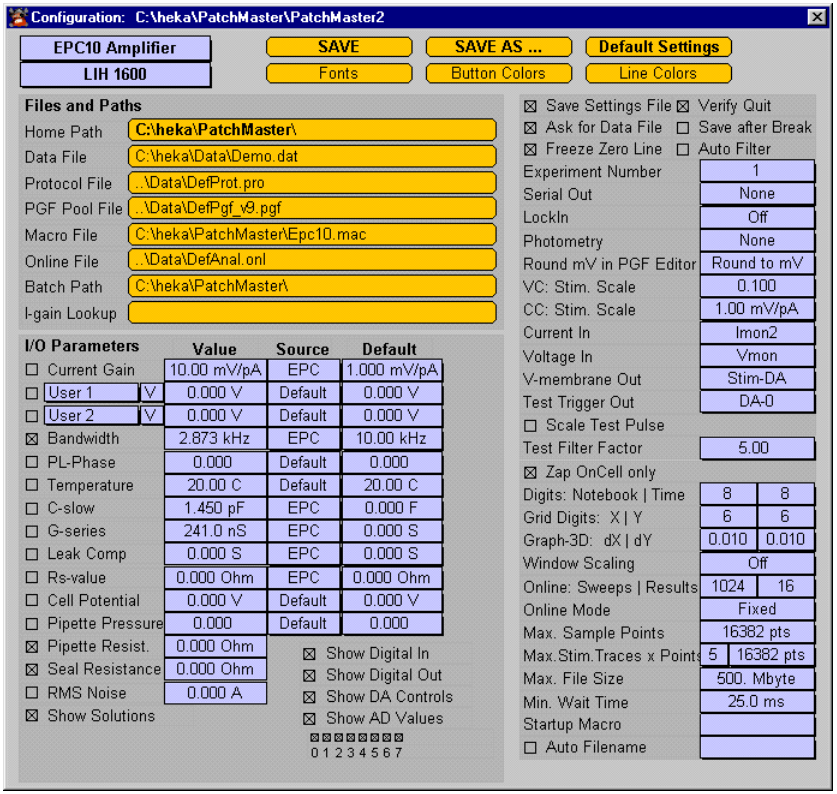
Select the Create option to start with a new experiment. You can call the file whatever you like, e.g. Tutorial.dat.

> ***Note:*** *A* Patchmaster *experiment consists of at least 3 files, the raw data (file name extension: *.dat), the pulse protocols used (*.pgf) and a file that contains the amplifier settings and structure of your experiment (*.pul). You don't have to create all files by yourself and you can also ignore the file extensions. If you create a new experiment, simply type the name of the experiment, e.g. "Tutorial". For more information, see* Patchmaster *reference manual, Appendix: File Overview.*

1.2 Configuring the Hardware

Patchmaster will open some windows: the most obvious one is the `Oscilloscope` window. We will deal with that window soon; however, first we have to make sure that the hardware is connected properly and that the software settings meet the requirements. The most important hardware settings are defined in the `Configuration` window.

To open it, select Configuration from the drop-down menu Windows.



1.2.1 Files and Paths

The `Configuration` window provides a variety of parameters that can be adjusted. First of all, in order to tell PATCHMASTER where to look for the relevant files and where to store your data, you need to set up the paths and files. Usually, if you install PATCHMASTER with its default settings, you can leave these entries untouched.

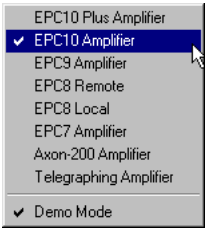
In case you install into other directories, though, please adapt the paths, e.g., Home Path, Data File, Protocol File, PGF Pool File etc., in the Files and Paths section of the `Configuration` window according to your local

installation settings.

For more information on the contents of the various files, see the PATCHMASTER manual, *Appendix: File Overview*.

1.2.2 AD/DA Channels

PATCHMASTER has to know whether to use an EPC 10 patch-clamp amplifier or another amplifier: use the control in the top left of the screen.



- If your particular amplifier is not in the list, select the `EPC 7`.
- If you have no amplifier at all, use the `Demo` mode instead.
- If your amplifier is not an EPC 10 (nor EPC 10/2 or EPC 10/3) you will also have to define the AD/DA converter you use (`ITC-16`, `ITC-18`, or `LIH 1600`) in the lower popup control (3). In this example, you cannot select an AD/DA-board (the selections are disabled), since the EPC 10 uses its built-in AD/DA converter. In case you are running PATCHMASTER with multiple PCI boards, the active one has to be specified as well.

The next step will be to define the AD and DA channels to be used for stimulation and acquisition of data in the sections DA channels and AD channels. For users of the EPC 10, some of these channels are predefined:

- the voltage stimulus is always expected to go via Stim-DA (V-membrane Out).
- the current input is sampled via Imon2 (Current In) and
- the voltage is sampled from Vmon (Voltage In).

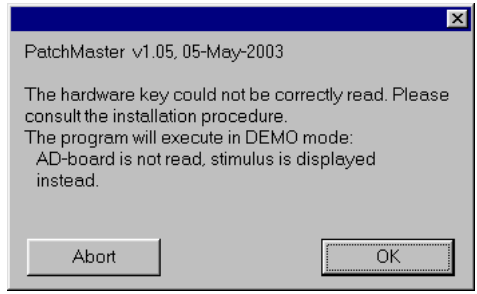
Note: *The EPC 10 has 4 DA output channels (0...3) and 8 AD input channels (0...7). For the EPC 10 Single, the channels DA 0..2 and AD 0..4 are available.*
For the EPC 10 Double, the channels DA 0..1 and AD 0..2 are available.
For the EPC 10 Triple, the channels DA-0 and AD-0 are available. The other channels are internally hardwired to the current and voltage output of the respective amplifiers.

1.2.3 Using the Software without Connected AD/DA Hardware

In general you have two possibilities for using the software without connected amplifier hardware: the Demo mode and the Stand-alone mode.

1.2.3.1 Demo mode

When no dongle is connected to the computer, the software will start in the true Demo mode. In this case, you will see the following warning:



The Demo mode can be used to inspect and analyze data on an extra computer that is not connected to the setup and to evaluate the software package. In this mode, no data can be stored to disk, but replay and export functions are working.

Note that any output that is created is taken as input; i.e., if a Stim.Scaling

of 0.1 is selected, the system now behaves as if an amplifier is connected with a pipette having a resistance of 10 MΩ.

1.2.3.2 Stand-alone mode

When a dongle is connected to the computer, the software will start in the Stand-alone mode. In this case PATCHMASTER will come up with the message "AD/DA initialization failed: check power!" or "EPC 10 initialization failed! Please, check power and connections".

The Stand-alone mode can be used to inspect and analyze data on an extra computer that is not connected to the setup. In this mode, data can be edited and saved to disk.

Note that any output that is created is taken as input; i.e., if a Stim.Scaling of 0.1 is selected, the system now behaves as if an amplifier is connected with a pipette having a resistance of 10 MΩ.

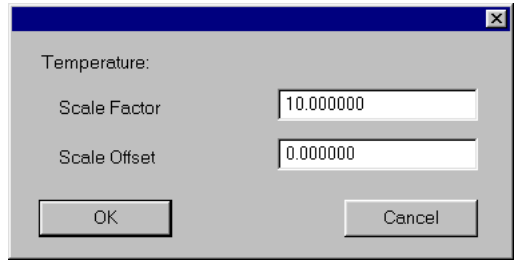
1.2.4 Entering the Parameters

So far, we specified the most important settings. On the left-hand side of the `Configuration` window - the Parameters section - there is a list of further values that are acquired and stored together with the experiment. These parameters can be input via different means:

- they can either be sampled through a free AD channel (`Source` = AD-0 ... AD-4)
- they can be derived directly from the Amplifier (`Source` = EPC) like the settings of the `C-slow` compensation (C-slow and G-series) or
- they can be typed in by the user during the running experiment (`Source` = Default).

The checkboxes in the parameter list left to each parameter determine whether the parameter is displayed in the `I/O-Control` window.

Note: *The checkboxes to the left of each parameter only define if the corresponding setting will be visible within the* PATCHMASTER *session. Irregardless of this setting,* PATCHMASTER *will always store every parameter with every block of data acquired. A separate block of data is called a "sweep" in the* PATCHMASTER *terminology.*



Go through all of these parameters, select the input source and maybe new default values. If an AD channel is used as an input source, then the scaling can be specified. Let us suppose the temperature is read from a temperature control unit via AD channel 4. Let's further assume that the control unit delivers an analog signal of 100 mV/ ˚C and 0 V at 0 ˚C. Then the scaling factor to be entered is 10 (1 V corresponds to 10 ˚C) and the offset is 0. Click the scale button next to the `Temperature Parameter` and fill in these settings.

If you are sampling from a so-called "telegraphing" amplifier, the determination of the encoded amplifier gain and filter bandwidth settings is much more involved and is done via lookup tables that are provided as ASCII files. You can select the corresponding gain or bandwidth table with the button table.

1.2.5 Saving the Configuration

Finally, you can save the configuration:

- click SAVE if you want to save the file under the default name Patchmaster.set or
- click SAVE AS if you want to save the file under another name. This is simply for your personal use, since PATCHMASTER will always start with the default file Patchmaster.set.

1.3 Controlling the Amplifier

The `EPC 10 Amplifier` window provides the amplifier control functions when an EPC 10 amplifier is used (the picture is for an EPC 10 Single). More detailed descriptions of the functions of the EPC 10 versions and their control windows are given in the corresponding amplifier manuals.

If you have an amplifier other than the EPC 10, you have to make sure that:

- the command potential at the amplifier is set to zero,
- the stimulus scaling is set correctly,
- the DA channel for Stimulus Out is connected to the amplifier's stimulus input,
- the amplifier's current monitor is connected to the Current In AD channel,
- if the amplifier has telegraphing capabilities for gain and/or bandwidth, ensure that the corresponding analog outputs are connected to the assigned AD channels. Gain and bandwidth lookup tables in ASCII format, which translate the voltage output to the setting of the corresponding switch, can be used for telegraphing amplifiers (see Chapter "Configuration" in the PATCHMASTER reference manual).

1.3.1 The Default Macros for Cell Set-Up

In the first row of the `EPC 10 Amplifier` window, you can find three controls. These are predefined macros.

SET-UP: Resets all parameters (with the exception of LJ and V_0), and defines the parameters of the test pulse.

ON-CELL: Switches the Gain range to a typical setting for a cell-attached patch recording, sets initial C-fast estimates, and invokes an Auto C-fast compensation.

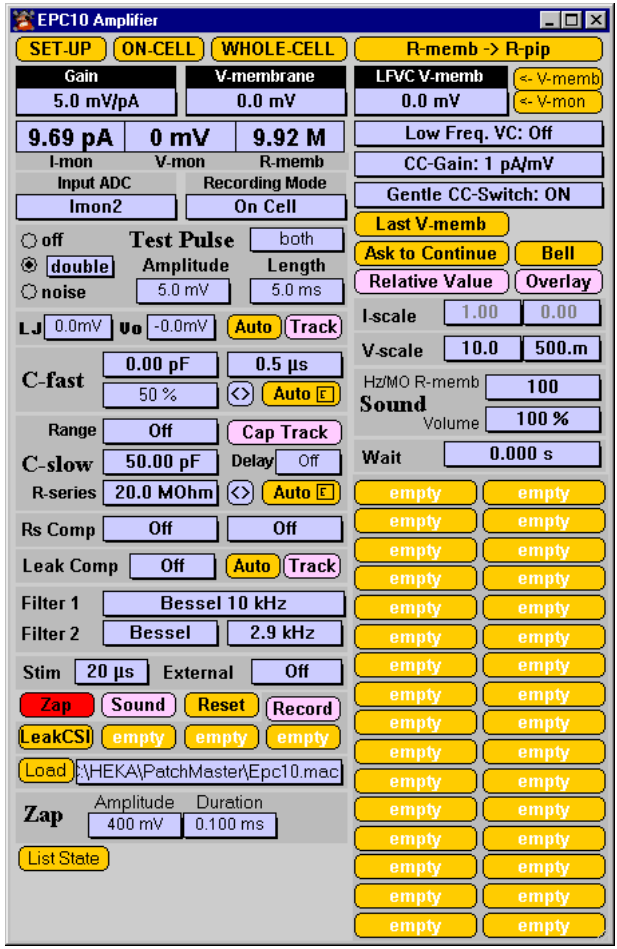


Figure 1.1: The Amplifier Window

WHOLE-CELL: Switches the Gain range to a typical setting for a whole-cell recording, sets initial C-slow estimates, and invokes an Auto C-slow compensation.

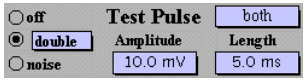
These macros can be redefined and other macros can be recorded using the macro record function, which you can find in the Macros menu. Since the `Protocol Editor` (see Chapter 1.8.1, on page 41) allows for more complex automation, we will not discuss macros further in this manual.

1.3.2 Test Pulse

The Test Pulse is applied to the pipette whenever you activate the amplifier by bringing the `Amplifier` window to the front. Test pulses are added to the holding potential and applied to the pipette; the current responses are sampled and displayed. Test pulses are applied at maximal rates depending on the pulse length specified.

The Test Pulse is defined in two different windows:

- in the `Amplifier` window you set the **Test Pulse Sequence** that shall be used and all other parameters for the Test Pulse, like amplitude, duration and pulse type. Current means that the current trace is displayed in the `Oscilloscope` every time the test pulse runs, while Both displays both the current and the voltage trace. Use this if you frequently want to apply test pulses in the `Current Clamp` configuration.



- in the `Configuration` window you can set whether the Test Pulse shall be scaled.

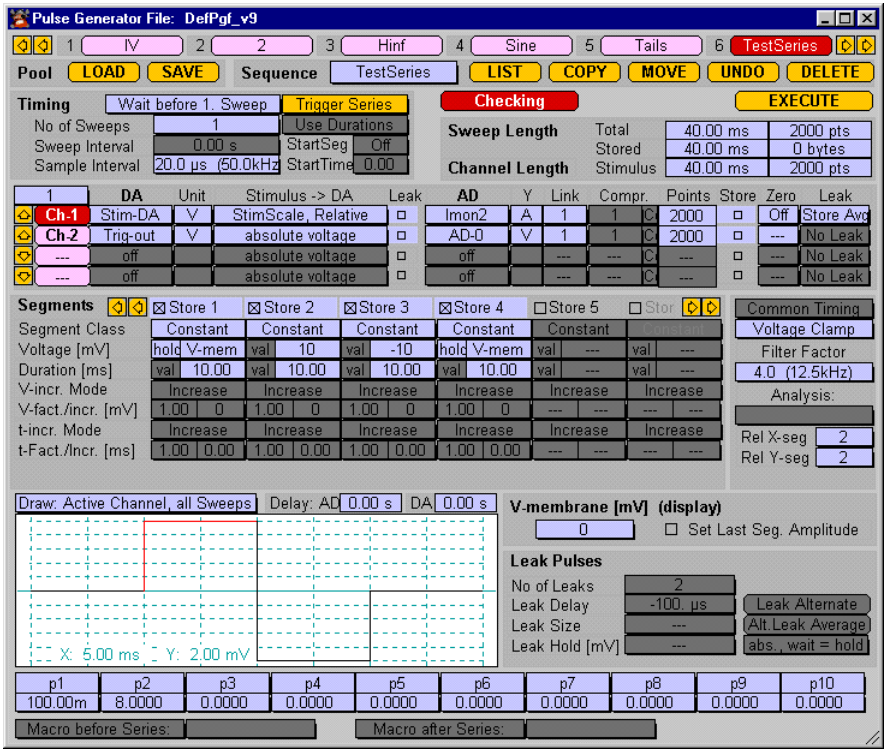
1.4 Setting up a Simple Pulse Protocol

The PATCHMASTER software allows you to stimulate with protocols that range from simple rectangular pulses to highly complicated stimulation

patterns. Stimulus templates are edited in the `Pulse Generator` window.

To open it, select Pulse Generator from the Windows drop-down menu.

A pulse pattern (called "Stimulation") consists of an arbitrary number of pulse `Segments` that have a constant, ramp, or sinusoidal voltage. In the default PULSE GENERATOR FILE, only one sequence is created. The file `DefPGF_v9.pgf`, distributed with the software release, is usually installed into the Data folder inside the HEKA folder and contains several useful pulse protocols which are a good starting point to create your own ones.



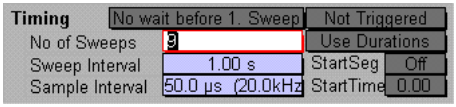
1.4.1 Creating a New Sequence

Click a free position in the PGF pool. If there is no free position, click the right arrow unless you reach the end of the pool. PATCHMASTER will ask you for a new Entry Name: type "IversusV".

1.4.2 Setting up the Timing

We want to create a protocol that gives us a current-voltage relationship. The response to 9 depolarizing pulses in steps of 10 mV given at an interval of 1 s has to be studied.

In the Timing section set No of Sweeps to9and the Sweep Interval to 1. Choose the sampling interval: here 50 μs.



To edit the fields, double-click the corresponding field. Then you can enter the number.

Usually PATCHMASTER will wait the time defined in Sweep Interval before starting the pulse sequence. However, right now we want the sequence to start immediately after activating it, so please select No wait before 1. Sweep from the popup next to Timing.

1.4.3 Defining the Segments

The section Segments of the `Pulse Generator` defines the actual pulse protocol to be applied. It will consist of three parts: the cell will be held at the actual holding potential in the beginning and the end of the protocol, the middle part has the depolarizing step. The individual parts of the pulse protocol are called `Segments`.

At the beginning, the protocol has only one segment of 10 ms duration. To add the additional two segments, mark the Store option for the second and the third segment. The result should look like the following:

Segments	Store 1	Store 2	Store 3
Segment Class	Constant	Constant	Constant
Voltage [mV]	val 0	val 0	val 0
Duration [ms]	val 10.00	val 10.00	val 10.00
V-incr. Mode	Increase	Increase	Increase
V-fact./incr. [mV]	1.00 0	1.00 0	1.00 0
t-incr. Mode	Increase	Increase	Increase
t-Fact./Incr. [ms]	1.00 0.00	1.00 0.00	1.00 0.00

Although you can edit the segments in any order, it is often advisable to start by defining the length of the individual segments. Since we want to give all three segments the same length, we can use the PGF parameter function.

At the bottom of the window you will find this row:

p1	p2	p3	p4	p5	p6	p7	p8	p9	p10
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

p1 to p10 are called PGF parameters. You can use them as variables in the segment settings for Voltage or Duration. This allows you to change multiple settings with changing only one parameter. Proceed as follows:

1. Select p2 instead of val from the drop-down menu right before the Duration value in the first segment.
2. Click on the number under the p2 entry and enter "0.1".
3. Now you can see that the value in the Segments section has changed.

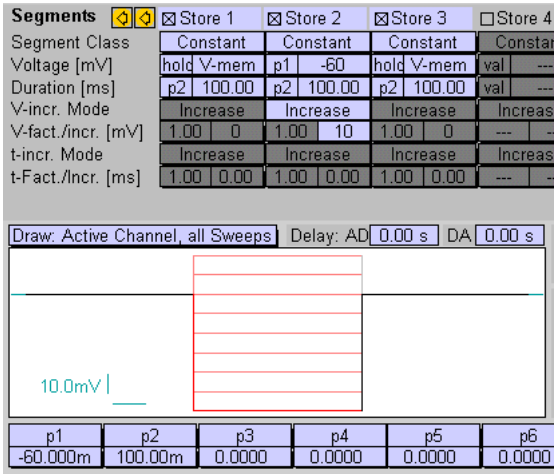
Segments	Store 1	Store 2	Store 3
Segment Class	Constant	Constant	Constant
Voltage [mV]	val 0	val 0	val 0
Duration [ms]	p2 100.00	val 0.00	val 0.00
V-incr. Mode	Increase	Increase	Increase
V-fact./incr. [mV]	1.00 0	1.00 10	1.00 0
t-incr. Mode	Increase	Increase	Increase
t-Fact./Incr. [ms]	1.00 0.00	1.00 0.00	1.00 0.00

4. Choose p2 for the other two segments too. All segments are set to "p2=100" [ms] now.

The first and last segment should be at holding potential, so select hold instead of val from the drop-down menu right before the Voltage value. The value in Voltage [mV] changes to V-membr. (i.e. the actual pipette holding potential at the time of executing the protocol).

Change the value in the second segment to p1 and set the PGF parameter p1 to − − 60.

Then set the Delta V-incr. [mV] field to 10. This will instruct PATCHMASTER to jump to -60 mV when it first executes the protocol and then always increment this segment by 10 mV for the following 8 repeats (-50, -40, -30, ..., +20 mV). The segments and their preview should look like the following:



If a segment is set to `V-membr.`, PATCHMASTER will fill in the actual holding voltage at time of data acquisition. In the sequence cartoon of the `Pulse Generator` the segments are filled in with the value entered under V-membrane [mV] (display). Thus, to make the cartoon look realistic, you may want to enter a typical holding voltage (e.g. to -80 mV) into the field.

> ***Note:*** *This value does not affect your measurement – it is only used for previewing the sweep!*

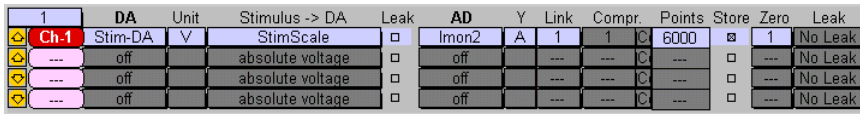
1.4.4 Defining the Segment for Online Analysis

Maybe you wondered why one segment is drawn in red color in the preview while the rest is black. PATCHMASTER can perform an `Online`

`Analysis` whenever you run or replay an experiment (see below). This is done by analyzing one segment (Rel Y Seg), e.g. determining its peak or mean current, and plotting it against another parameter like the duration or potential of any other (or the same) segment (Rel X Seg). You can define which segment has to be analyzed by setting the so-called Relevant Segment. This is done separately for the segment that delivers the x- and the y-value. Set both values to "2".

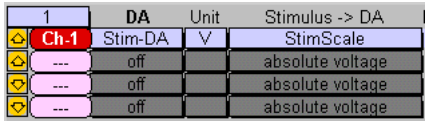
Your later analysis will of course not be restricted to the segments you define here. In the Analysis Functions section of the `Online Analysis` window you can set a positive or negative `Segment Offset` that will be added to the relevant segment, thus allowing you to analyze other segments. For more information, see Chapter 1.7.3, on page 37.

1.4.5 Setting the Output Channel and the AD Input



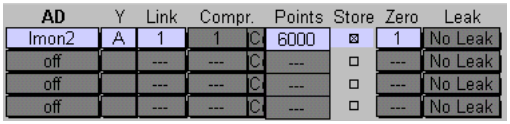
In the rows `Ch-1...` you set the parameters for each channel. The default channel is 1, the other channels (Channels = 2...) may be used to simultaneously record other data such as the potential, an amperometric signal, or a fluorescence.

DA and AD settings are **independent** from each other. Their reference is only given by the variable Link in the AD settings! So to prevent confusion here, we will split the above picture for a closer look.



The **DA** section on the left allows you to set the properties of the DA

output, e.g., the stimulus signal. Note that the expression "channel" is used **exclusively** for the DA stimulus output! The output via Stim-DA and the Unit V are the default entries for EPC 10.



The **AD** section on the right allows you to set the properties of the AD input, e.g., the acquired data. The input via Imon2 and the Y-unit A are the default entries for EPC 10. The variable Link defines with which DA stimulation this AD input is associated, in our case to channel 1.

Remember that this Link variable allows you to associate several AD inputs to the same DA stimulation! The rationale behind the Link variable is that during analysis one has to know which stimulus was applied for a given data trace.

The option Store will make sure that the acquired data can be stored to disk. For some protocols it might not be required to save the data (such as a Test Series), so you can disable this feature in these cases.

1.4.6 Other Settings in the Pulse Generator

There are a few more options in the right and bottom part of the `Pulse Generator` window that did not have to be changed in our case. Nevertheless, it is still important to know what they do: the setting `Voltage Clamp` will restrict the execution of the pulse protocol to the voltage-clamp modes only. Thus, PATCHMASTER will refuse to start this sequence if you are in the current-clamp mode and instead will produce an error message.

> ***Note:*** *A given pulse protocol only makes sense for Voltage- or Current-Clamp conditions, never for both modes. The option Any Mode in the* `Pulse Generator` *window is only there for special applications like Photometry. If you want to be able to run a Current-Clamp sequence while you are in a Voltage-Clamp mode, you should create a macro or a protocol that switches to the CC mode, and associate it with the pulse proto-*

col.

The section Sweep/Channel Length gives you some important information about the pulse protocol.

Sweep Length	Total	300.0 ms	6000 pts
	Stored	300.0 ms	12000 bytes
Channel Length	Stimulus	300.0 ms	6000 pts

Sweep Length: Maximal possible length of a sweep, determined by the timing settings.

- **Total:** Denotes the total time needed for one sweep of the given sequence in ms and points.
- **Stored:** Denotes the total time stored for one sweep of the given sequence in ms and bytes. Total and Stored durations may be different when a Start Seg. and Start Time were set or when conditioning segments were used (e.g. segments with the Store button off).

Channel Length: Length of the actual DA stimulation. This can be shorter than the sweep length, e.g., a short trigger pulse.

Stimulus: Denotes the time for the stimulus signal in ms and points.

For our example, the value Total as the total length of stimulation calculates like this: Each sweep has a duration of 100 + 100 + 100 = 300 ms sampled at an interval of 50 μs or a frequency of 20 kHz. This makes a total number of 6000data points.

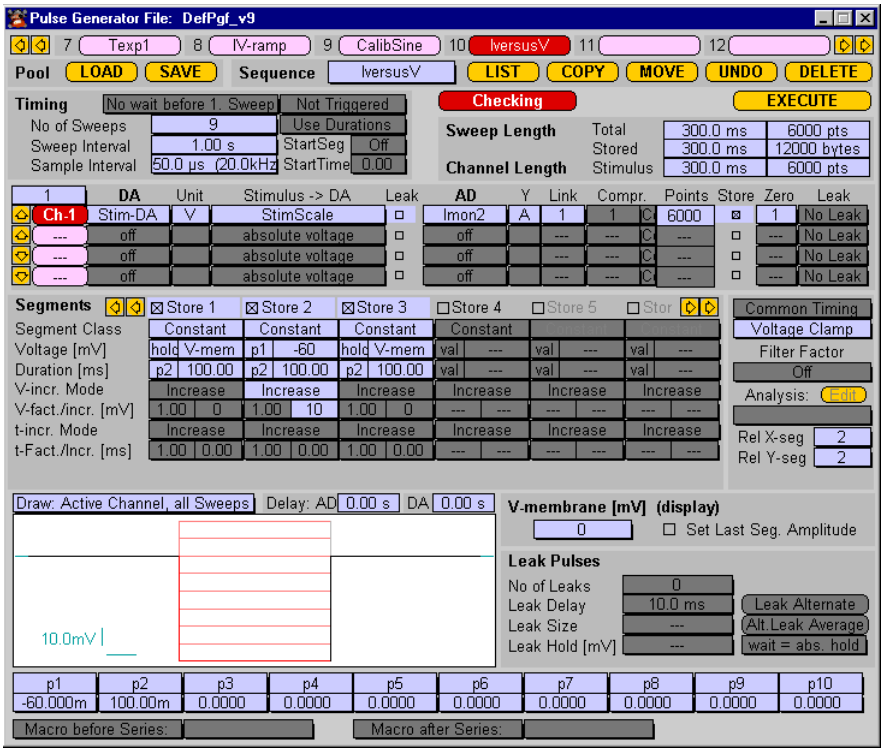
If, for example, there was a StartSegment 1 and a StartTime 5 ms, the first 5 ms (or 100 data points) would not be saved. But since we want to store the whole sweep to disk, we did not specify a StartSegment.

> ***Note:*** PATCHMASTER *allows you per default a maximum of 5 channels with 16382 points each. These parameters can be adjusted in the* `Configuration` *window, provided your computer has enough RAM.*

This new, modified `Pulse Generator File` should now be stored to disk by clicking on SAVE and entering a name. The default file extension is

".pgf". Note that on program start PATCHMASTER will always load the file defined in the `Configuration` window; the default is DefPGF_v9.pgf. If another 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.

The resulting `Pulse Generator` window should look like this:



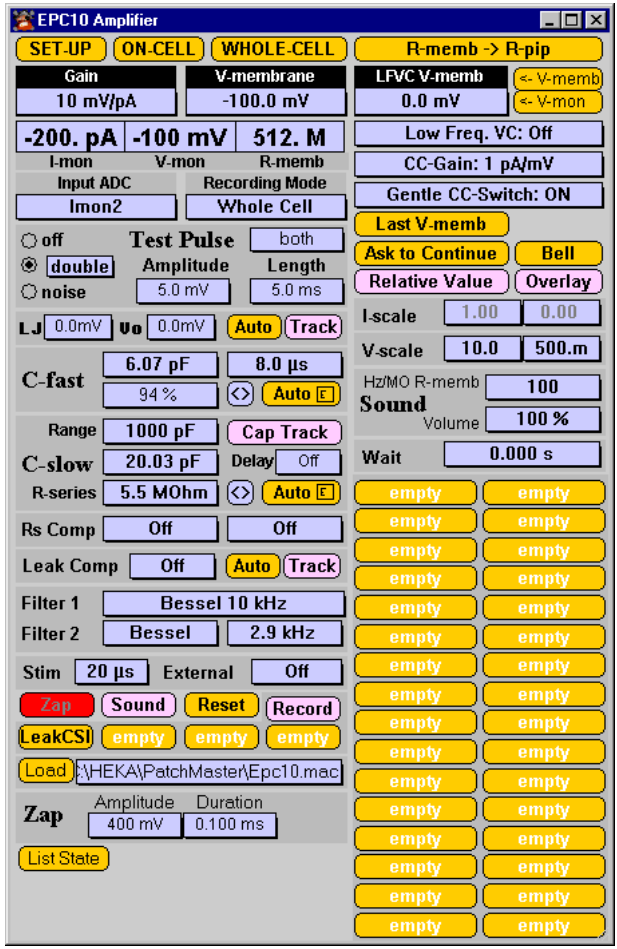


Figure 1.2: The Amplifier Window

1.5 Starting the Experiment

1.5.1 Making a Seal

Now it is time for the experiment. Switch the model circuit into the "10 MOhm" setting to simulate a 10-MΩ pipette that is open to the bath solution.

Hit the space bar in the main dialog to activate the `Amplifier` window - if the `Oscilloscope` is not in front, hit the space bar twice, the space bar toggles between the `Oscilloscope` and the `Amplifier` window.

As long as the `Amplifier` window is on top, the program will generate test pulses according to the settings in the Test Pulse section: a double pulse of 5 mV amplitude and a duration of 5 ms per pulse will be output. The sampled current responses will be shown in the `Oscilloscope` window. The resistance of the pipette is calculated from the responses and displayed in R-memb.

Besides the fast test pulses (single or double) you can select the third entry in the Test Pulse pop-up list, which requires to specify a sequence from the `Pulse Generator File`. Instead of the fast test pulses, this sequence is then repeated continuously providing an alternative and quite flexible `Test Pulse` mode.

> ***Note:*** *The currently measured resistance of the pipette is always called Rmemb because the program cannot distinguish between an open and a sealed pipette. As long as the pipette is open to the bath, R-memb corresponds to the pipette resistance.*

The command potential is controlled by the program via the control V-membrane. This variable always displays the physiological membrane potential, i.e., the Recording Mode is already taken into account reverting the polarity of the applied potential in On Cell and Inside Out modes.

> ***Note:*** *Most functions, such as canceling the offset current, setting the amplifier gain or the holding potential, etc. should be obvious, but make sure that the Recording Mode is always set*

properly, because this setting will automatically determine the actual polarity of the voltage at the patch pipette!

You can correct pipette offset potentials by adjusting the Vo value or you can alternatively click on the Auto Vo button to let PATCHMASTER do this correction automatically for you. The same is done by calling the macro SET-UP, in this case, PATCHMASTER will also adjust the amplifier gain and the test pulse. When the pipette potential is adjusted and you are ready to form a seal, store the value of the Pipette Resistance - which is the actual R-memb value that will be overwritten after forming the seal. This is done by clicking on R.memb → R-pip. This value is not changed any further, unless you click on R.memb → R-pip again.

Note: *R-memb is updated as long as the test pulses are active, i.e. every time the* `Amplifier` *window is in front, and stored as variable Seal Resistance with every acquired sweep. The Pipette Resistance will be stored together with every acquired series of sweeps. This value is updated every time you click on R.memb → R-pip.*

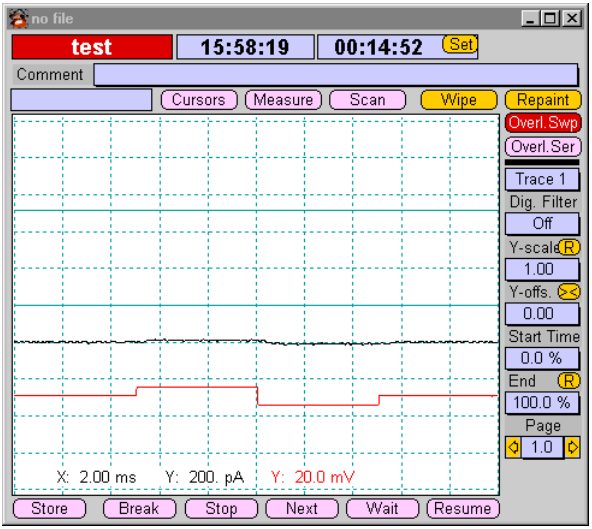
Now simulate a pipette sealed to the membrane by switching the model circuit into the middle position. If you have an EPC 10, make an automatic fast capacitance cancellation by clicking on the Auto C-fast or ON-CELL macro button. Otherwise, compensate your amplifier for the pipette capacitance of about 6 pF.

To break into the cell, set the switch of the model circuit to its bottom or "0.5 GOhm" position. If you have an EPC 10 make an automatic slow capacitance cancellation by clicking on the Auto C-slow or `WHOLE-CELL` macro button. Otherwise, compensate your amplifier for the cell capacitance of about 20 pF. Watch the R-memb display that now shows 500 M instead of 10 M. With the V-membrane control change the pipette holding potential to -100 mV. Now we are ready to run the pulse protocol we defined before.

1.5.2 Setting up the Display

Bring the `Oscilloscope` window to the front. If the button Store is not highlighted, click on it to make it active; otherwise, PATCHMASTER will

show the data but not write them to disk! If you did not create a file yet, PATCHMASTER will ask you to do this now.



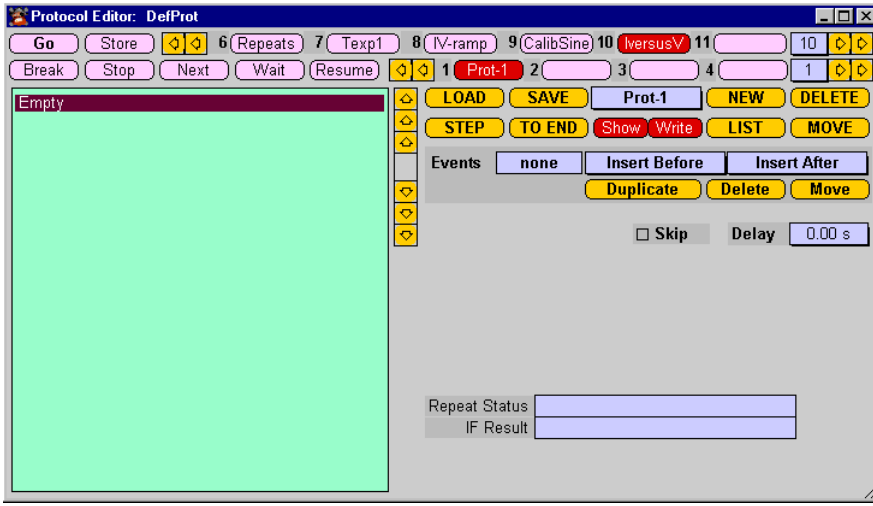
The bottom of the `Oscilloscope` window shows the buttons used to control the execution of sequences or protocols.

To see all sweeps from one series, activate the Overlay Sweep button in the `Oscilloscope` window; otherwise, the display will be erased before every sweep.

Before we execute the `IversusV` sequence (or "series" in PATCHMASTER terminology, which describes a number of individual sweeps based on the same `Pulse Generator Protocol`) we will set up the display. Usually you can use the default settings of a new PATCHMASTER installation, but let us have a look at the Display menu. The following options should be activated: Show Zero Line, Show Potential, Dimmed Overlay, Labeling → Grid and Labels.

1.5.3 Starting the Sequence from the Protocol Editor Window

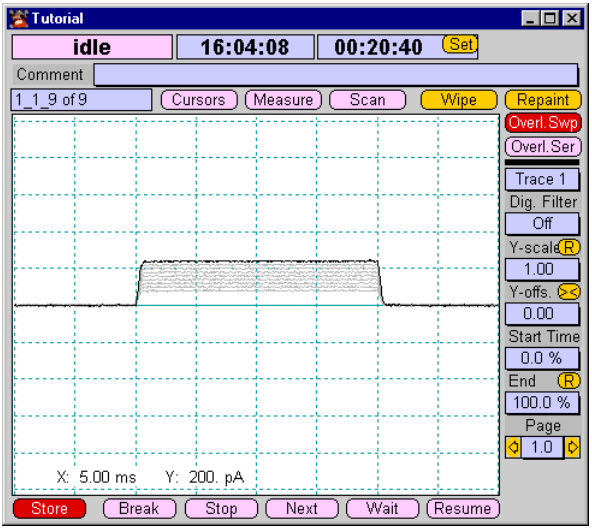
Now open the `Protocol Editor` window. In the top right sequence pool you can see all available sequences/pulse patterns. (For now, we are ignoring all other possibilities of this window, but we will return to it in the Chapter 1.8, on page 41.)



To start data acquisition directly, click on the `IversusV` button or type "10" into the blue entry field, if `IversusV` is the sequence in the tenth position.

1.5.4 Displaying the Data

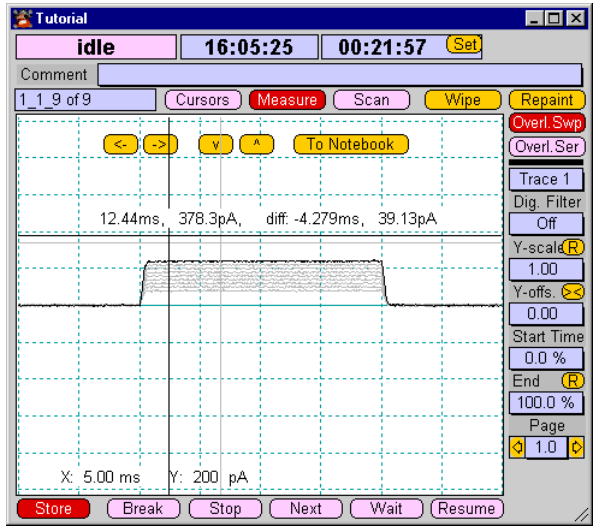
The pulse pattern we defined above is output via the specified DA channel and the response is shown in the `Oscilloscope` window. The last sweep of the series is shown in black color, the other sweeps are gray since we activated Dimmed Overlay. The grid is drawn in green color and scaling values are given in the lower left side of the `Oscilloscope`.



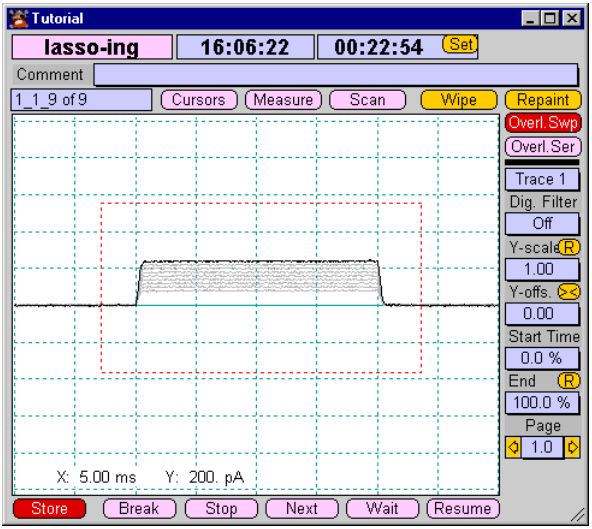
1.5.5 Changing the Display Settings

In case you want to have a closer look at your displayed data, you have various possibilities to change the display settings.

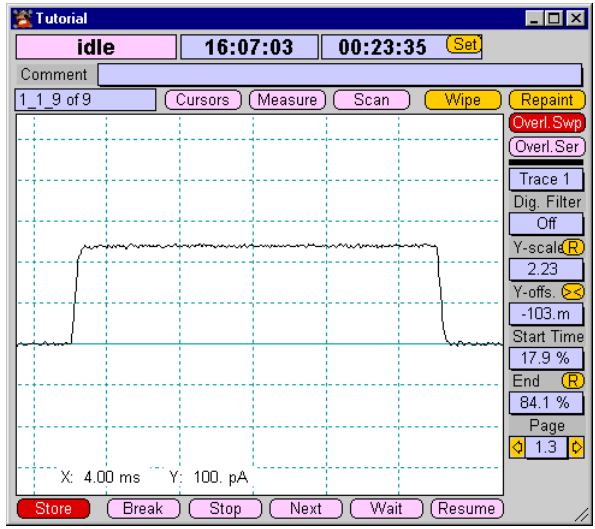
For example, you can do a quick checkup on the measured values. When you click on the button Measure, a cursor with two connected lines will be displayed in the `Oscilloscope` window. The data of the actual point will be displayed, and you can copy them into the `Notebook` via the To Notebook option.



In case the data is too small on your display, you can use the "lasso-ing" function. Start in the top left corner and press the left mouse button. Pull the opening red square to the appropriate size and release the mouse button.



The marked area will be set to fill the oscilloscope screen. Note that the scaling has to be done for each trace separately, even when you have selected the Overlay option! So the result looks like the following:



1.6 Handling Data

1.6.1 Saving Data

To write the recorded data to disk, select File → Update File or close the experiment with File → Close. The latter will automatically store all files associated with the experiment.

To create a new file for data acquisition, select File → New... . PATCHMASTER will close the running experiment and open a new, empty one.

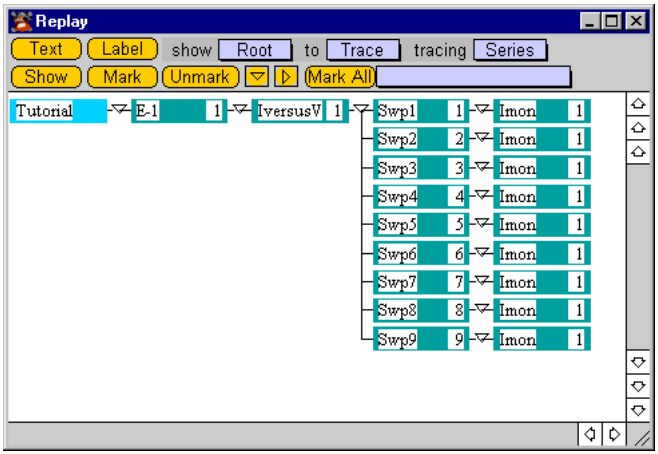
Remember: Recorded data can only be saved and/or replayed if the Store button was active during acquisition!

1.6.2 Replaying Data

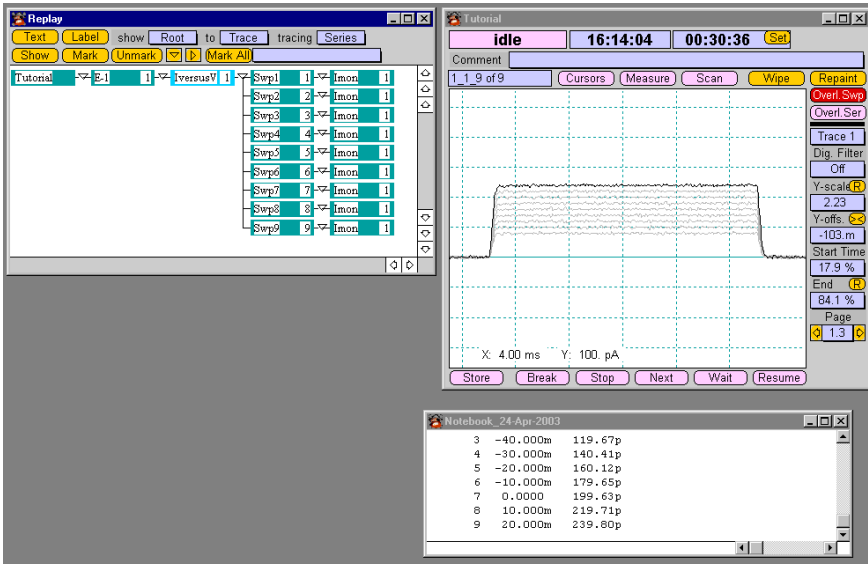
If – and only then! – the Store button was active, the structure of the stored data will be shown in the `Data Tree` of the `Replay` window. This is also the basis for the replay of data.

To open the `Replay` window select Replay.

Double-click the `IversusV 1` entry to replay the just recorded sequence; double-click a single sweep to inspect it in the `Oscilloscope` window. You might use the cursor keys (`up´, `down´, `left´ and `right´) to walk through the data tree.



If you press `Return´ or double-click on the currently active group, series, sweep or trace, it will be displayed in the `Oscilloscope`. This may look like the following example:



While replaying the data, the Online Analysis will be calculated. For more information on the Analysis options, see Chapter 1.7, on page 35.

The Replay menu provides functions for modification of the tree entries. E.g., a single sweep, a series, or a whole group of series can be removed by activating the item and then selecting Replay → Delete.

1.6.3 Exporting Data

You can export the data into various other file types via the export options in the Replay menu. PATCHMASTER cannot only export to plain text files, but also to formats for software like Igor Pro or MatLab.

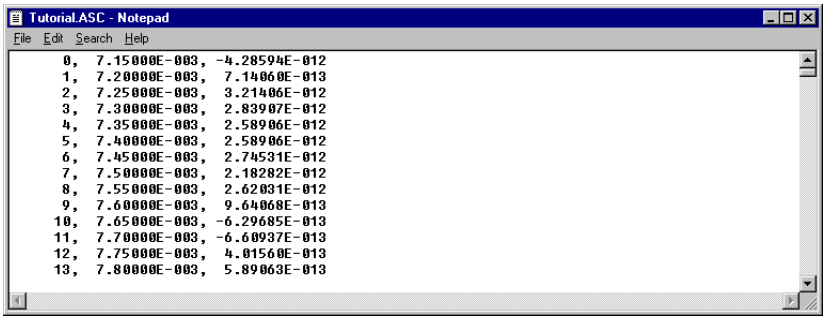
For example: to export raw data to comma-separated ASCII format, you would have to set the following options:

- Export Format to ASCII
- Export Mode to Traces

- ASCII option to comma-separated and the linefeed type that would fit your operating system.

Then you are asked for a file name. The pre-set data extension for the output is .asc (as for ASCII).

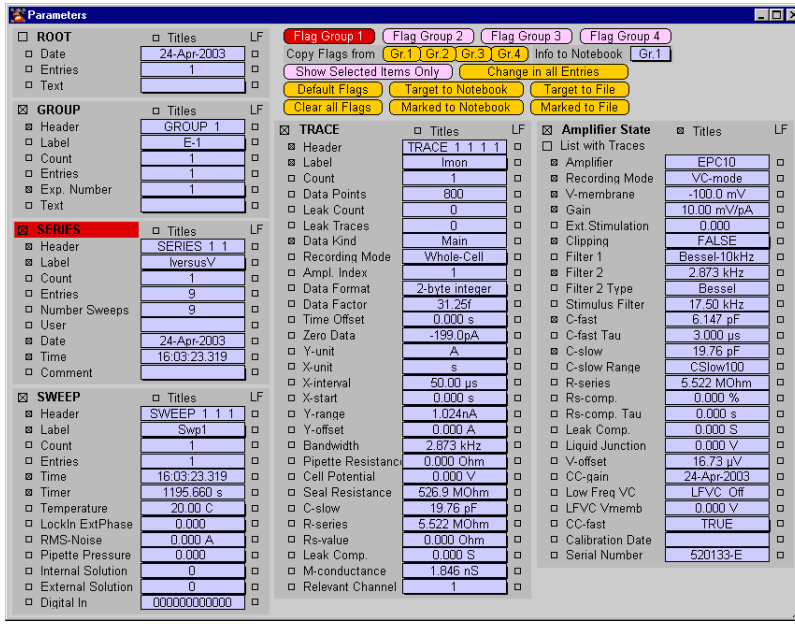
The resulting file would look like this in a ASCII viewer, e.g., Notepad:



1.6.4 Exporting Parameters

For long series of data, you may want to get an overview of the settings and parameters with which the data were acquired.

For this, open the `Parameter` window. Here all information concerning replayed or actual data is displayed. Via the checkboxes (flag options) you can select information that you want to export to the `Notebook` window or to a file.



The window is structured as follows:

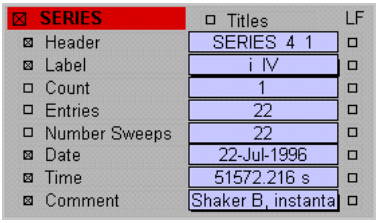
- On the top right side you can find the flag management and the export features.
- On the left and middle part of the window you can find the data tree entries Root, Group, Series, Sweep and Traces, together with their parameters.
- On the right, you can find information about the amplifier settings.

Select the data you want to export by checking the small checkboxes in front of the relevant entries. Note that you have to check the main entries, e.g., Root, Series etc., to export the other parameters of that group!

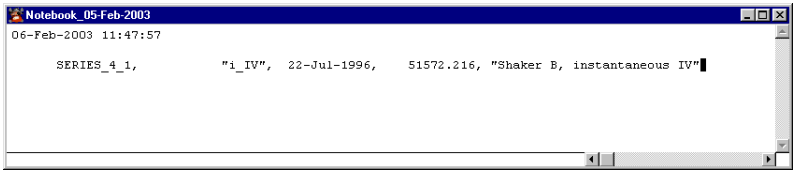
To test if you have selected all necessary parameters, click on Show Selected Items only. This will close the `Parameter` window and give you only the selected parameters in a new window.

If you want to export the parameter names together with their corresponding values, check the option Titles above the parameter values. Otherwise, only the values will be exported. Then click on Target to Notebook to export the parameters into the `Notebook` window.

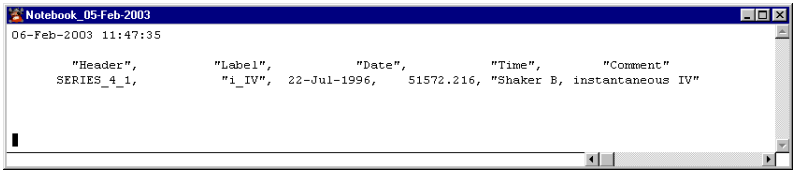
For a setting like the this:



where the parameters of the series - that is the Target in the `Replay` window – are exported with Header, Label, Date, Time and Comment (but no Titles option), the result in the `Notebook` window may look like this:



With the Titles option, which will export also the parameter names, the result would look like this:

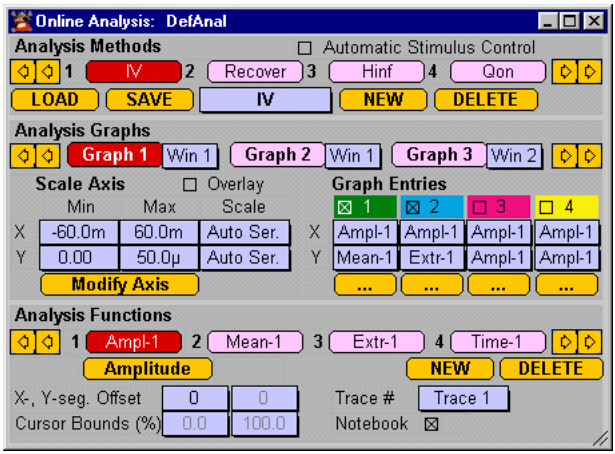


When you click on Target to File, the program will ask for a file name. The pre-set data extension for the output is .asc (as for ASCII).

1.7 Analyzing the Results

1.7.1 Using the Online Analysis

The online analysis allows you to immediately calculate and display data that are based on the acquired traces, thus giving you a fast overview over your results.



The highlighted Analysis Method is the one that will automatically be executed when you acquire or replay data.

PATCHMASTER can show such analysis results as columns in the `Notebook` window or plot them in the `Online Analysis` windows 1 or 2 after or during execution of a series (based on the settings made in the various controls inside this window).

The `Online Analysis` is structured as follows:

1. Based on incoming data, a number of `Analysis Functions` are defined.
2. These functions produce `Analysis Results` based on the relevant segments of the sequence.

3. These results are then displayed in the `Notebook` (if the Notebook option is checked) and/or shown in an `Online Graph` inside either `Online Window 1` or `Online Window 2`.

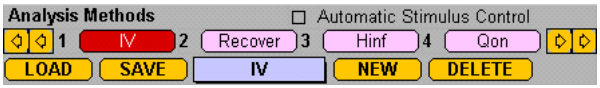
Elements of a Graph are `Graph Entries`, i.e. couples of Analysis Results to be used as X- and Y-reference. Up to 4 Graph Entries fit into one Graph; multiple Graphs fit into `Online Window` 1 or 2.

The entire setting of the Online Analysis is called `Analysis Method`. An arbitrary number of such Analysis Methods can be saved in Online Analysis files (*.onl).

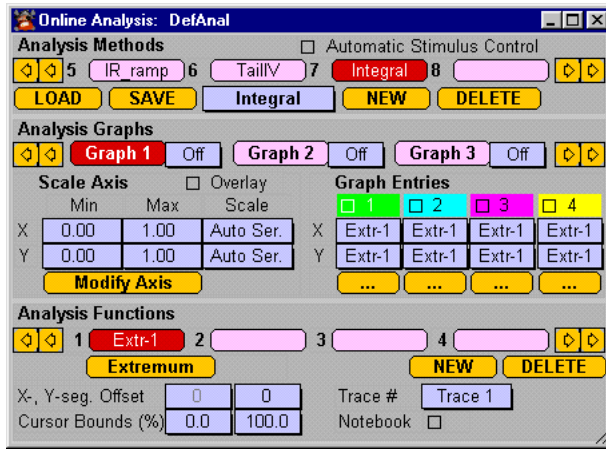
Thus, the first thing to do is to define `Analysis Functions`. Only then, the respective `Analysis Results` are placed as `Graph Entries` in Graphs and Windows.

1.7.2 Entering a New Analysis Method

Usually, you can set up a new Analysis Method by copying the data from one method to the other. However, for the purpose of this tutorial we will start from scratch.



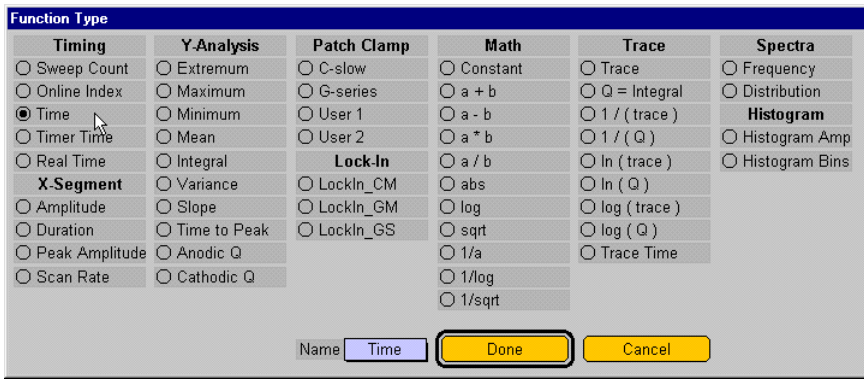
Click on `New` and enter the name "Integral" for the new Analysis Method.



It will be created and placed on the next free entry number.

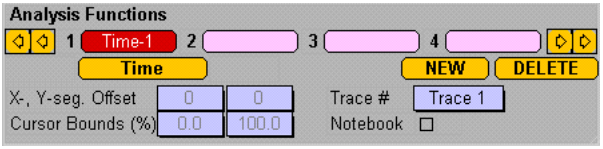
1.7.3 Entering the Analysis Functions

`Extr-1` is given as default Analysis Function. Now we need to customize our method. Click on `Extremum` to open the `Function Type` window.

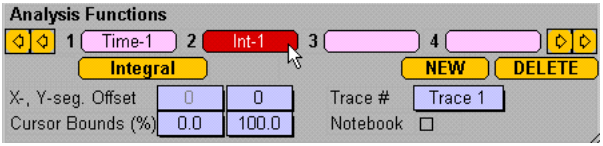


Choose the entry Time and click Done.

The first entry in the section Analysis Functions of the `Online Analysis` window has changed to `Time-1`. The "1" tells you that you analyze trace 1 here, as you can see on the right. Later we will use this result for an x-axis variable.



Now we need some other function to provide a variable for the y-axis. We simply want time at data acquisition there, so click on New to set up a new function, choose Integral from the `Function Type` window and click Done.



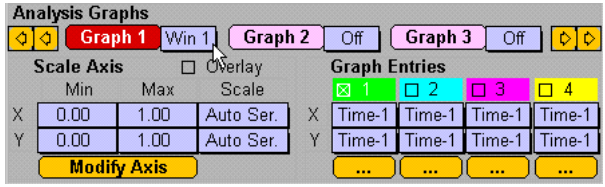
Now we have two functions that analyze Trace 1. We also want both results to be copied into the Notebook, so check the Notebook option for both.

Remember that all analyses will be performed on the relevant segment of the sequence as it is set in the `Pulse Generator` window (see Chapter 1.4.4, on page 16).

Also note that the order in which the data is displayed in the `Notebook` window later on depends on the order of the functions in the Analysis Function section. This means, if you prefer a certain order, you have to select them accordingly at the very start.

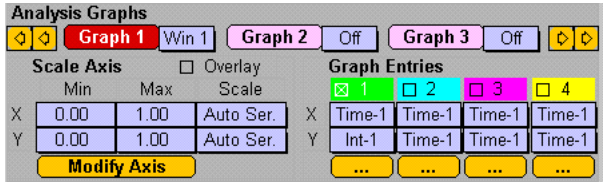
1.7.4 Setting up the Analysis Graph

To set up the graph, you first have to define in which `Online Window` (1 or 2) the graph shall be displayed. We want to display Graph 1 in Win 1.

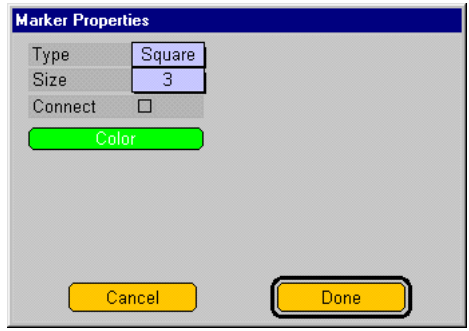


Then we have to define the Graph Entries. Up to four Graph Entries can be in one Graph, but we need only one entry here.

To define the entry, check the first entry (light green in our example) and then choose the x- and the y- axis.



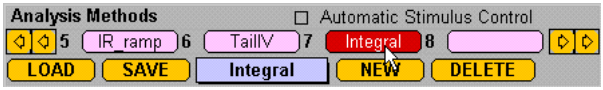
The color of the graph field will be the display color in the future graph. Light green is not very handy – let us change this to dark blue. For this, click on ... to open the following window:



Change the color by clicking on the Color button and choosing from the possible colors. Click Done to save your selection.

1.7.5 Performing an Online Analysis

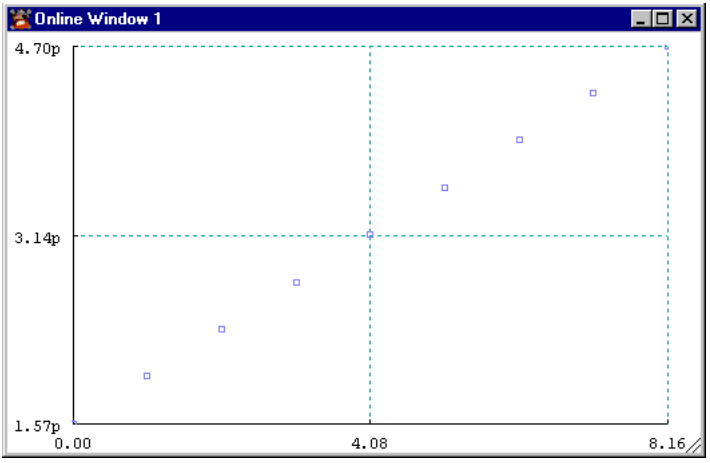
First, make sure that the new method "Integral" is highlighted.



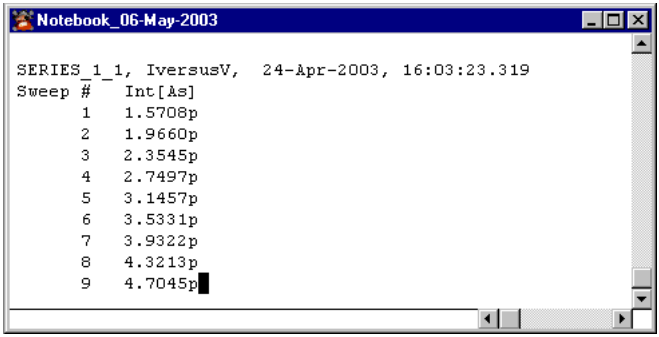
Second, open the `Online Window 1` by selecting Windows → Online Window 1.

Third, acquire data or replay data by double-clicking on the series in the `Replay` window. The analysis results will be displayed in the `Online Window 1` and in the `Notebook` window.

The result in the `Online Window` should look like the following:



If you bring the `Notebook` window to the front (e.g. by selecting Windows → Notebook), you should see something like this:

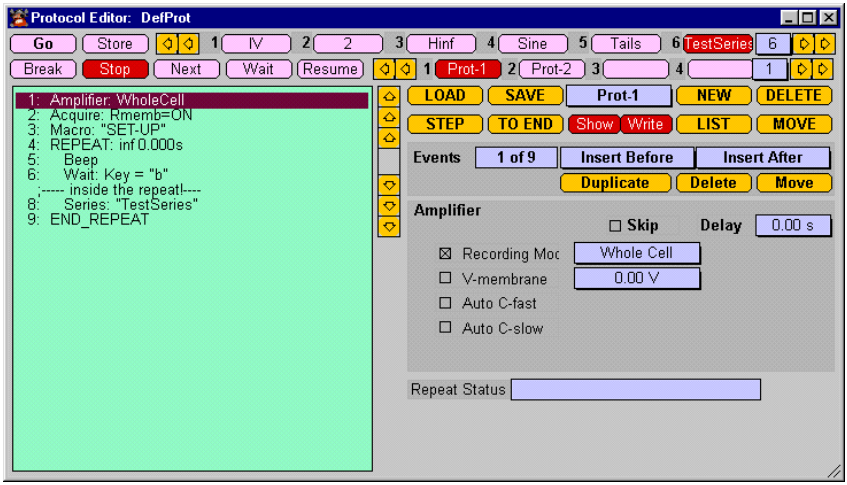


In case not all data are listed here, check the Notebook checkbox for each function!

1.8 Automating the Data Acquisition

1.8.1 Getting to Know the Protocol Editor

In a former chapter, we used the `Protocol Editor` window solely to start the sequence. However, the `Protocol Editor` has much more possibilities – in this window you can assemble complex experimental arrangements by combining PGF-templates with other operations (e.g. breaks, IF-THEN loops, setting changes). This window is the heart of the PATCHMASTER software concerning the automation of experiments.

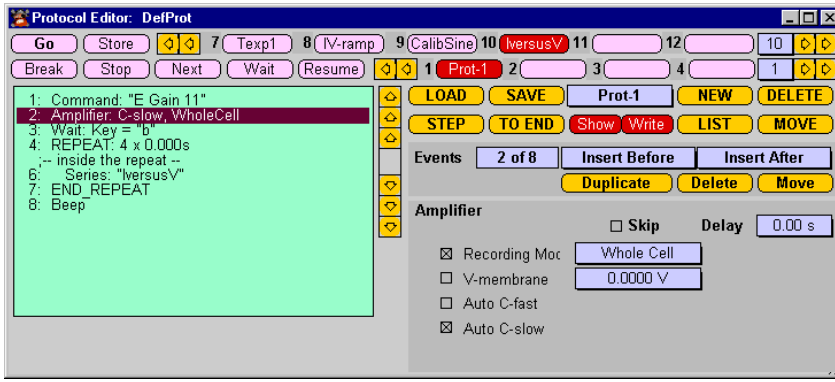


Note that in the `Protocol Editor` window there are two different kinds of pools:

The sequence pool on top right is the direct connection to the `Pulse Generator` window. When you click on an entry, the respective sequence will be started and executed as often as it is set in the No. of Sweeps variable in the `Pulse Generator` window. To stop its execution, click on Stop. Note that the buttons on the left of the sequence pool correspond to the buttons in the `Oscilloscope` window.

Below the sequence pool, there is the protocol pool. Here you can find all protocols that have been set up until now.

The protocol that we use in this tutorial looks like the following:

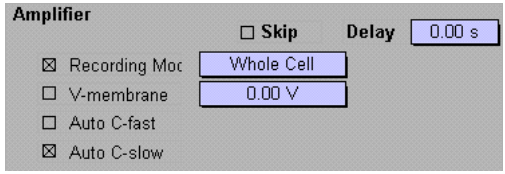


As you can see, each entry – or as it is called, event – has its own index number. You can use these numbers if you want to move an entry to another position.

After the index number, the event name is displayed, e.g., `Amplifier`. When you click on an event, the corresponding input fields will be opened on the right.

1. In the first event – `Macro Command`– the command "E Gain 11" will be executed. This command sets the Gain value in the `Amplifier` window, in our case to 10 mV/pA. Although there are some amplifier settings that can be set in the `Protocol Editor` (see below), others have to be set via a macro command. You can find out these commands by recording a macro with the necessary steps and then analyzing the macro content. For further information, please read the Chapter `Macros` in the PATCHMASTER reference manual.

2. In the second event – `Amplifier` – nothing more is done than to set the `Recording Mode` to `Whole Cell` and to mark `Auto C-slow` correction.



3. This is followed by a `Wait` event. This event is useful if you want to be alerted during the protocol execution, perhaps because you want to change some external settings before the actual data acquisition takes place. Only when you press the key "b", the protocol execution will proceed.

4. Up to now, these settings could have been set manually by the user. However, the following event `Repeat` is the start of a loop, in this case a `Repeat Counts` with "4". This way, the loop will be repeated four times.

5. "– inside the repeat –" is a text entered in the so-called `Annotation` event. This annotation is only readable in the `Protocol Editor`, i.e., will not be displayed in the `Notebook` or elsewhere.

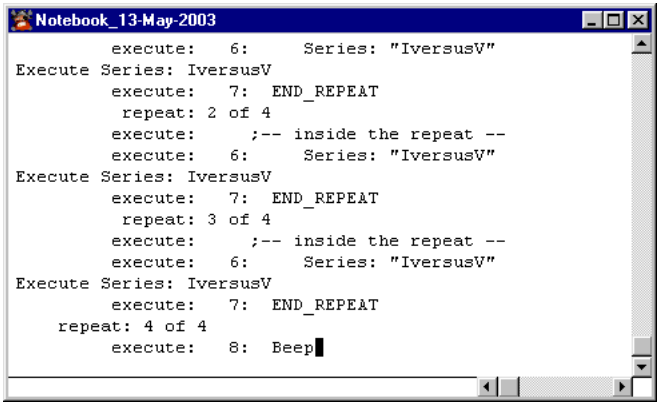
6. `Series` shows that the `Acquire Series` event is called, in this case our PGF Sequence "IversusV". This starts the data acquisition. Note that you can directly open the PGF Template from the event menu to edit this sequence.

7. The next event is `End REPEAT`, which marks the end of the loop. This event is automatically inserted when you insert a `REPEAT` event.

8. When the loop has finished, the `Beep` event is called.

To start this protocol, click on the Go button.

Since in our example the options `Show and Write` are activated, the respective event will be highlighted in the event list and also written into the `Notebook`; the latter should read like the following:



Just as in normal data acquisitions, the data will be automatically analyzed by the activated `Analysis Method`. But be aware that as long as the protocol is executed, you cannot change entries in the `Online Analysis` window. If you try it anyway, the message "Cannot modify online: still executing or acquiring..." will be displayed in the `Notebook` window.

The same holds for the `Protocol Editor` itself – if you click on an entry in the event list during execution, the message "Cannot run: protocol is already executing..." will be displayed in the `Notebook` window.

In this case, you have to click `Stop` or `Break` to halt protocol execution. Then you are able to modify parameters again.

1.8.1.1 On the Difference between Macros and Protocols

In general, automation can also be achieved with macros. The differences between macros and protocols are:

- With macros, you can record sequences that are actually performed. All buttons and settings in all windows can be included into a macro. While recording the macro, though, you have to jump between the necessary windows. This will also happen during macro execution, thus slowing-down the process.

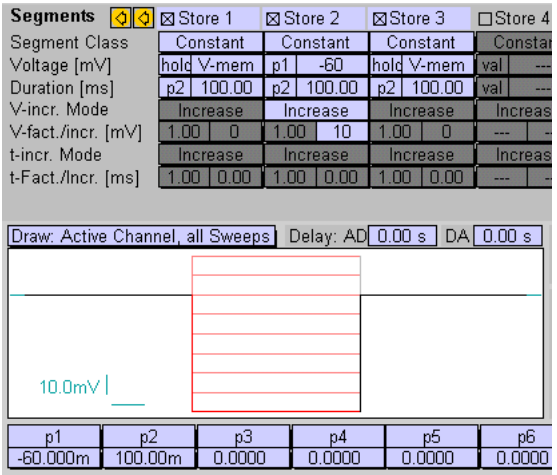
- With protocols, very complex sequences can be edited. During protocol execution no opening or closing of windows is necessary, thus the execution is faster than for macros. Another important advantage of protocols over macros is the possibility of conditional events, depending on e.g., analog or digital input, analysis results or key commands. But note: Only a limited number of settings can directly be set in the events. For all others you have to include a macro command via the `Macro Command` event.

We highly recommend the use of protocols.

1.8.2 Changing PGF Parameters via a Protocol

You also have the possibility to manipulate the PGF parameters of the `Pulse Generator` window in a protocol.

Remember: We used the PGF parameters p1 and p2 in our segment definitions, see Chapter 1.4.3, on page 14.

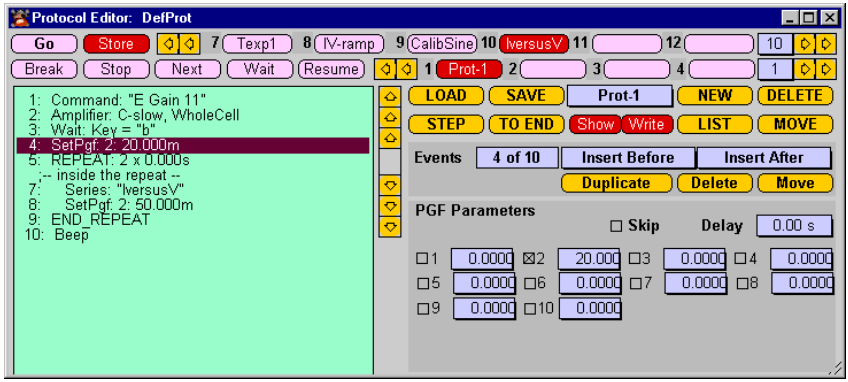


With the event `PGF Parameters` you can set a value for the parameter. Do as follows:

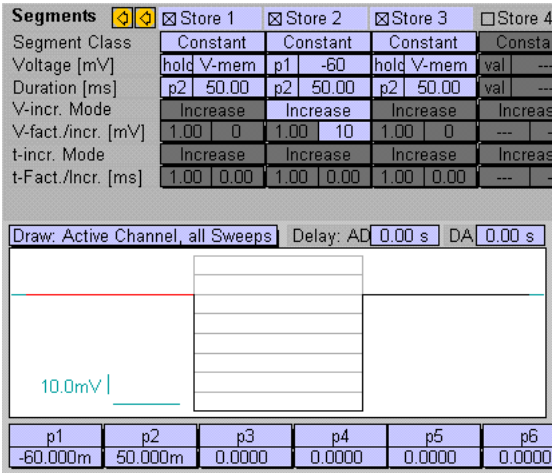
1. Mark line 3 in your event list and Insert After the event `PGF Parameters`. Set p2 to 20 mV.

2. Then mark the new line 7 and Insert After the event `PGF Parameters` again. Here, set p2 to 50 mV.

This way, you will get a first execution of "IversusV" with a lower p2, and a second execution with a higher p2.



When the protocol has been executed, you can see the resulting PGF values also in the `Pulse Generator` window.



Note that when you execute a series the next time, the value 50.000m will be used as start value for p2!

1.9 Customizing the Front-End

1.9.1 Customizing the Keys

In PATCHMASTER, all key commands are saved in the file `PatchMaster.key` and will be read at program start. In case the file `PatchMaster.key` is not available at program start, no key commands are available!

Please take also in consideration that you can customize all commands, so the settings in your working version of PATCHMASTER might differ from these default settings.

To display the key assignments in the various windows, choose Help → Show Keys.

To list the keys, choose Help → List Keys.

To save the keys, choose Help → Save Keys. The keys are saved in the file PatchMaster.key. Old keyboard assignments will be automatically saved

with an incrementing extension, e.g., .k00, .k01, .k02 ...

You can freely customize the key commands by

- editing the keys via the dialog control and saving them or by
- directly modifying the key file, e.g., in a text editor.

1.9.2 Customizing the Windows

To modify dialog and control items in the PATCHMASTER user interface, you have to select Enable Icon Configuration from the Windows menu and then press certain controls, depending on your intended action and your operating system (Windows, Mac OS). For further information, please refer to the Chapter "Modifying Dialogs and Controls" in the PATCHMASTER reference manual.

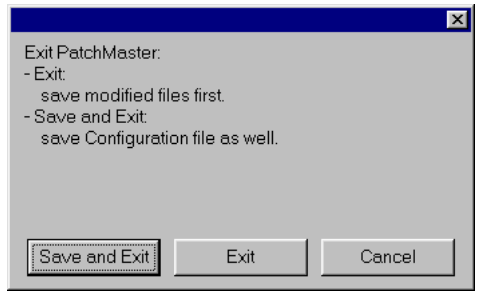
1.10 Closing PATCHMASTER

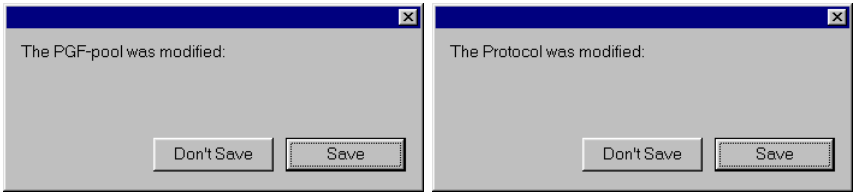
To exit from PATCHMASTER, do this:

 Choose Quit from the drop-down menu File or press `CTRL´ + `Q´.

 Press `CMD´ + `Q´.

The following window will appear:





You have three possibilities:

- **Save + Exit:** Saves data files and configuration and quits the program. At least the first few times of running PATCHMASTER, after tuning the system, you should do that, since this file contains all of the settings that were adjusted as outlined above. Once you have a stable system, which you don't want to modify anymore, you can safely ignore this question.
- **Exit:** Saves data files and quits the program.
- **Cancel:** Aborts the exit process, you return to the program. This is the right button if you accidentally pressed the shortcut combination for exiting.

As you can see, data files will always be saved. If you lose data files, you might verify if you checked the option Store in the PGF editor or the option Store in the `Oscilloscope` window during your experiment.

If you changed the pools in the `Protocol Editor` or the `Pulse Generator`, you will be asked independently if you want to save them.

The default is Save for each, so just press `Return´ twice to save them and exit the program.

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