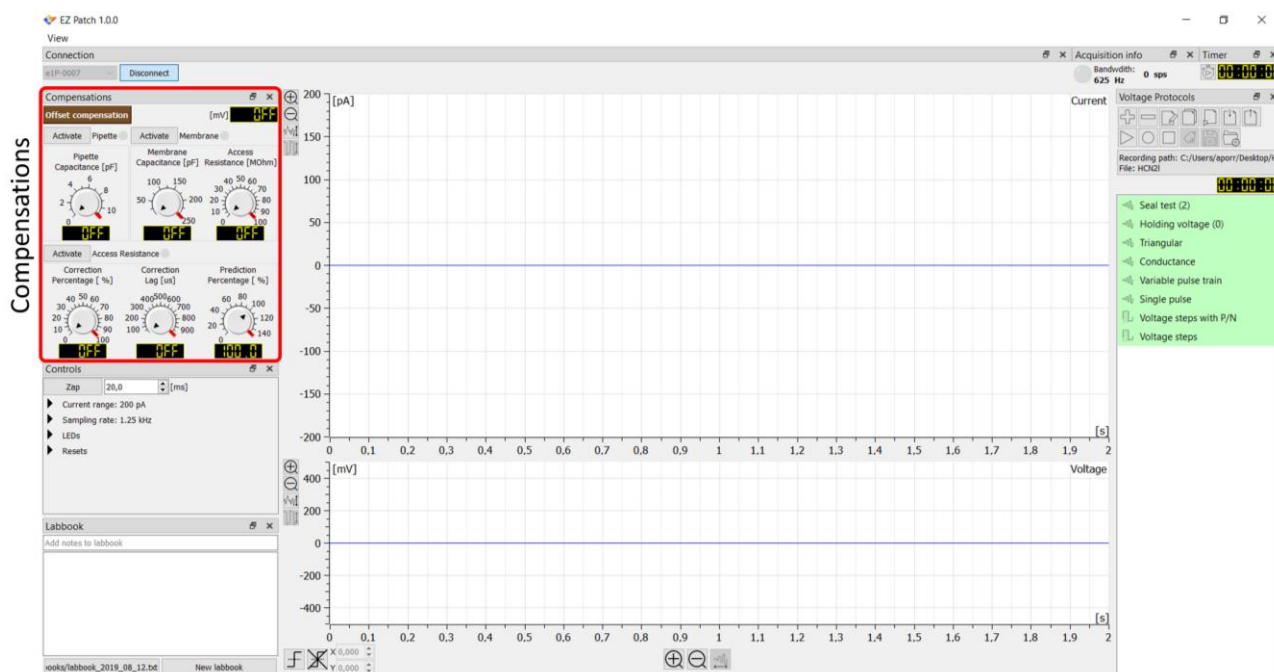


EZ Patch quick guide

Compensations



This short guide illustrates the use of the circuit compensations settings in EZ Patch. The operations will be described while using the model cell supplied with the ePatch amplifier.

The model cell

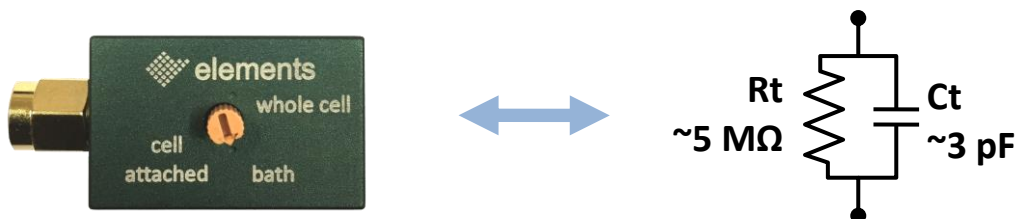
The model cell contains resistors and capacitors designed to emulate three patch clamp experimental conditions: the pipette immersed in the bath, the cell attached, and the whole cell configuration. Connect the model cell to the SMA input connector of ePatch (see image below). Use the supplied pin reference cable to connect the model cell to the reference port of the amplifier. Finally, connect ePatch to your computer and the reference wire to your Faraday cage.



Get ready for the Giga-seal and compensate the voltage offset

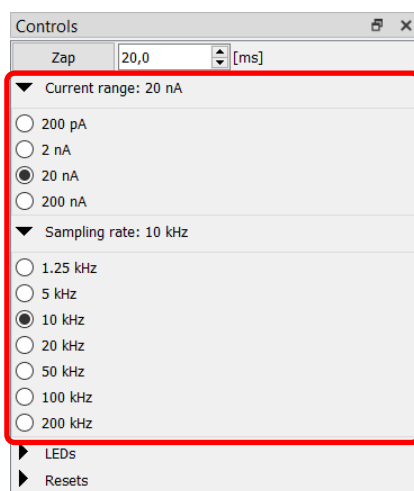
Rotate the selector of the model cell until the small arrow points the “bath” configuration.

In "**Bath**" mode, the model cell electrically simulates the experimental condition in which the pipette is immersed in the bath without touching the cell. In this configuration, offset potentials can be corrected, and the resistance of the pipette can be measured. The electrical circuit of the bath configuration of the model cell is shown in the following figure.

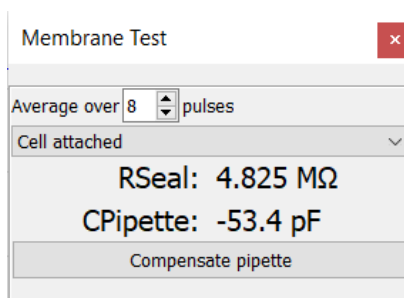


Launch EZ patch software, connect to the device and run the seal-test protocol.

Note that when the seal test protocol is applied, the current range and the sampling rate values are automatically set to 20 nA and 10 kHz respectively (see image below). These settings are the minimum required to read the large currents generated when the electrode is immersed in the bath, as well as to properly analyze the fast and slow capacitance transients that appear in cell attached and whole cell configurations. Such default settings can be adjusted by the user either using the controls widget (image below) or using the protocol editor (see related guide).

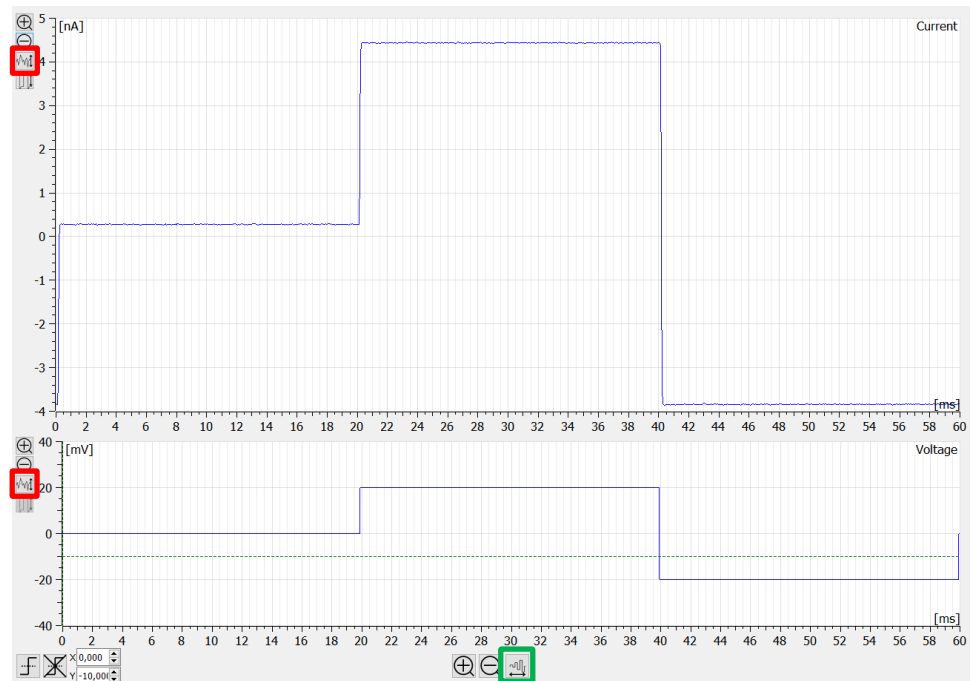


When the seal test protocol is applied, the **membrane test widget** appears below the protocols list to show the resistance and capacitance values calculated from the current response. When in bath configuration, the seal resistance value (R_{seal}) represents the electrode resistance.

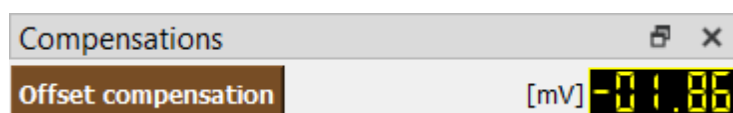


What you see in the oscilloscope is comparable to what you would see immediately following the immersion of the pipette tip in the bath solution surrounding your cells. Use the **autoscale buttons** of the Y axes to maximize the signals on the oscilloscope window. Then, click the **protocol duration zoom button** to obtain a triggered full screen view of a single square wave (see image below). This helps to better focus on the transient peaks that will arise in cell attach and whole cell configurations.

- Auto-scale
- Protocol duration zoom

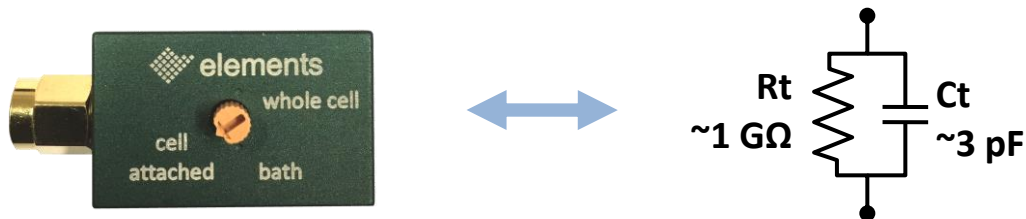


Click the **offset compensation** button in the compensations widget to eliminate any voltage offset between the bath electrode and the patch electrode. After making a brief series of steps, the software automatically finds the value that is needed to compensate the offset. During this procedure the voltage protocol widget appears locked and finally the applied value is displayed next to the offset compensation button. Once the voltage offset has been compensated, run again the seal test protocol.



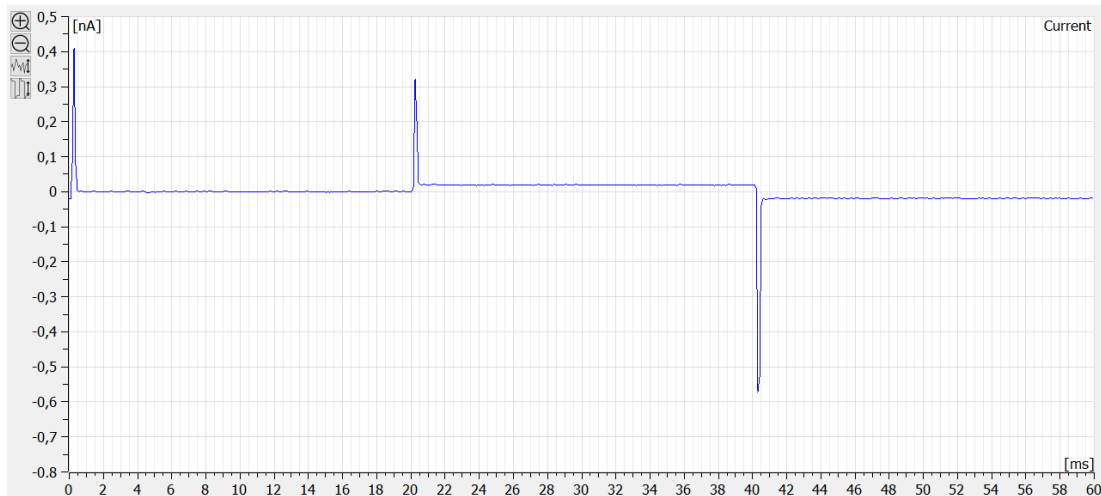
Cfast compensation

Rotate the selector of the model cell to the cell attached mode. The electrical circuit of the bath configuration is shown in the following figure.

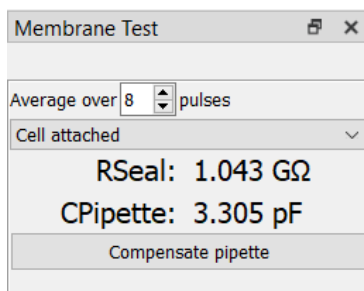


This mimics what you would see immediately following a Giga-Ohm seal between your pipette and the cell.

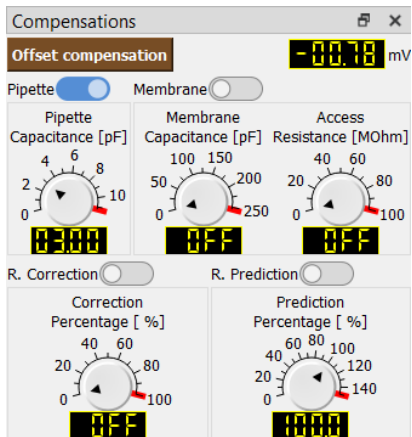
The **capacitance transients** that arise in synchrony with the rising and falling edges of the voltage stimulus, result from the charging of the **electrode capacitance** (in this case simulated by the model cell circuitry).



Current response before Cfast compensation

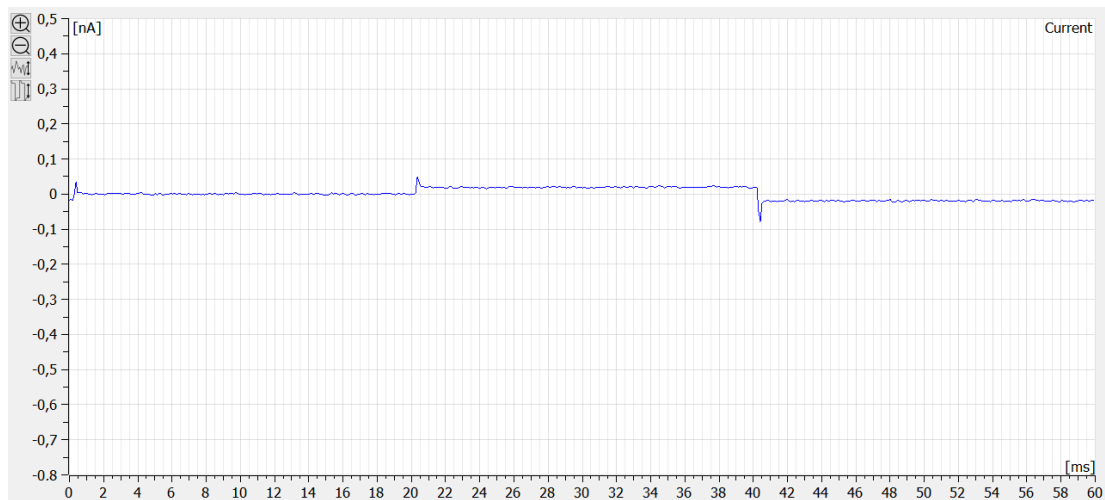


The **values of the seal resistance and the electrode capacitance** are estimated and displayed in real time within the membrane test widget.



Click on the activate button of the **pipette compensation** and adjust the value to minimize the transients. For finer adjustments use the wheel of the mouse or click on the knob and use the keyboard arrows.

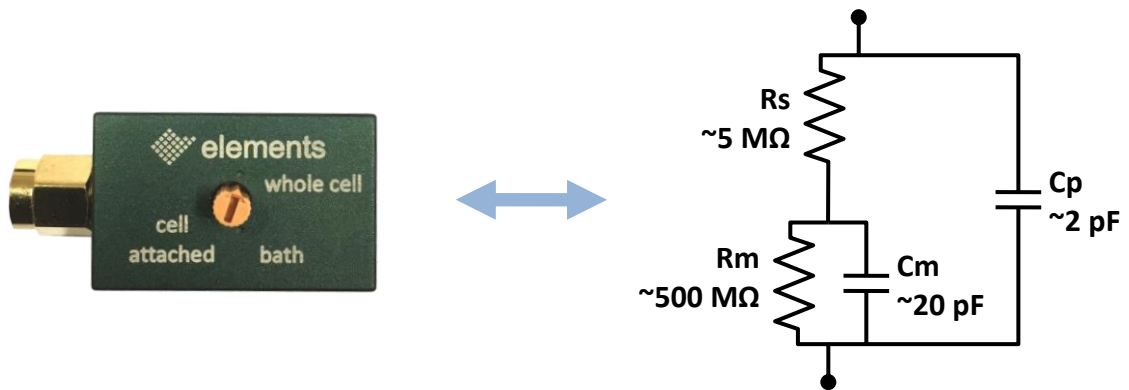
Alternatively, use the compensate pipette button (in the membrane test widget) to automatically compensate the transients. The software applies the estimated electrode capacitance value to cancel the peaks. After using the auto compensation, if needed, you can still make manual adjustments for optimum cancellation of the transients.



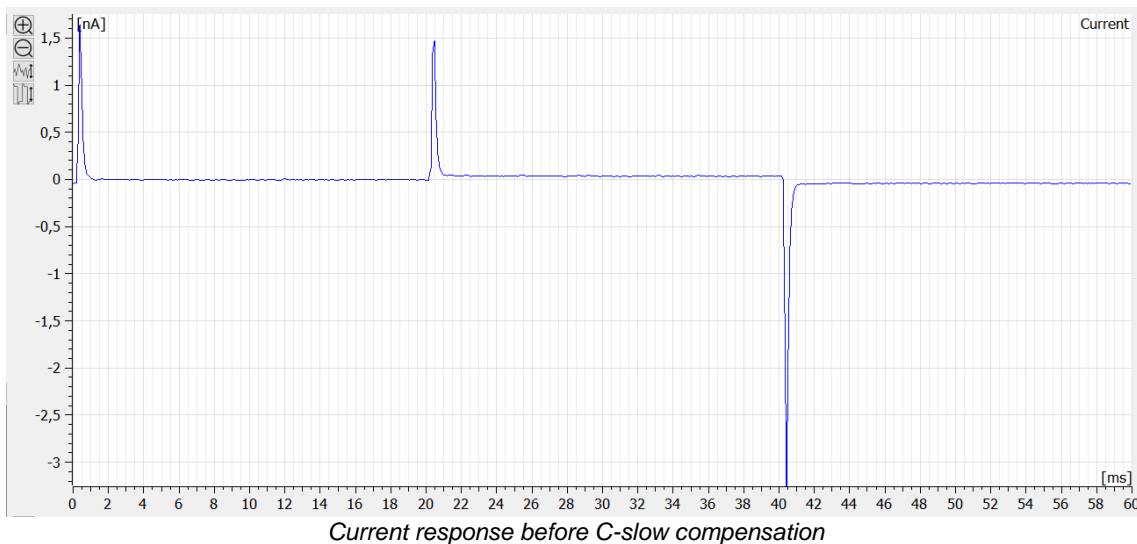
Current response after C_{fast} compensation

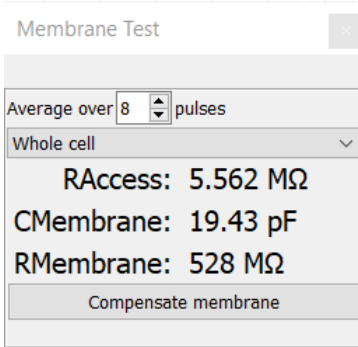
C-slow compensation

Rotate the selector of the model cell to **whole cell** mode to simulate the experimental condition in which the membrane patch is broken, and the pipette has gained access to the intracellular compartment. The electrical circuit of the bath configuration is shown in the following figure.

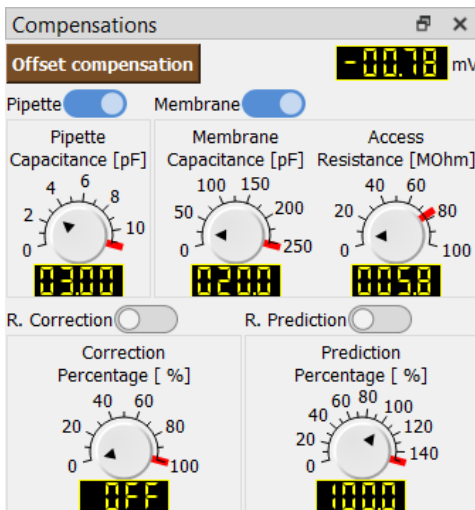


The slow capacitance transients that arise now result from the charging of the 20-pF capacitance of the model cell that simulates the membrane capacitance.





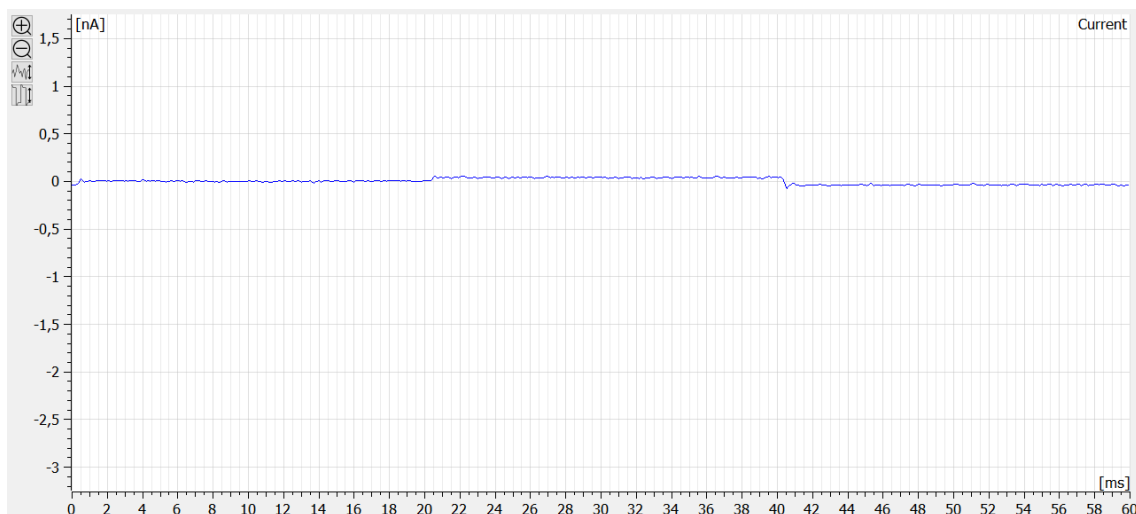
Use the drop-down menu of the membrane test widget to display the analysis of the membrane transient (whole-cell option). The real time analysis of the current response defines the values of the **membrane capacitance** (CMembrane), the **membrane resistance** (RMembrane) and the **series resistance** (RAccess).



Click on **activate membrane compensation** button and use the membrane capacitance and access resistance controls to minimize the transients.

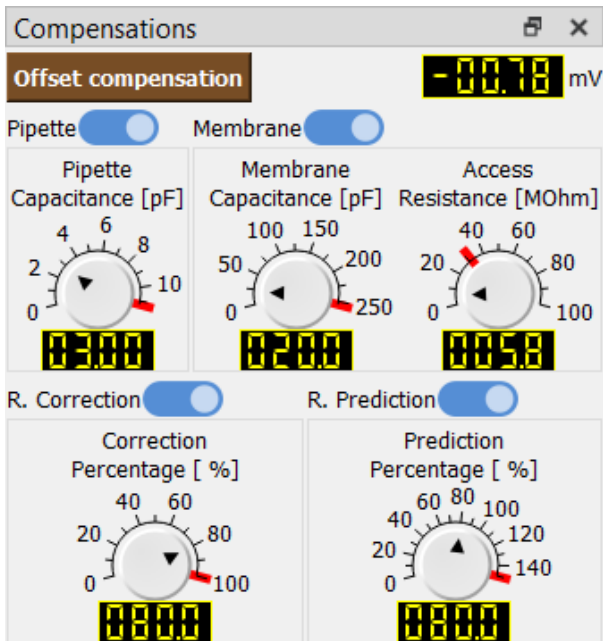
Alternatively click on the compensate membrane button (in the membrane test widget) to use the estimated values as compensation settings.

After using the **auto compensation**, you can still make manual adjustments if needed for optimum cancellation of the transients. If needed readjust the pipette compensation value to remove the small wiggle that may arise at the leading edge of the capacitive transient.



Current response before C-slow compensation

Series Resistance compensation



In a real whole cell experiment, it may be necessary to speed-up the membrane response to a step voltage command as well as to compensate for voltage drops resulting from membrane current and series resistance.

Activate the **access resistance compensation** and use the series resistance prediction and correction knobs in the compensation widget to minimize these unwanted errors.

Rs correction adds a command potential (V_{corr}) proportional to the measured current. Such increase helps to partially compensate the voltage drop across the pipette

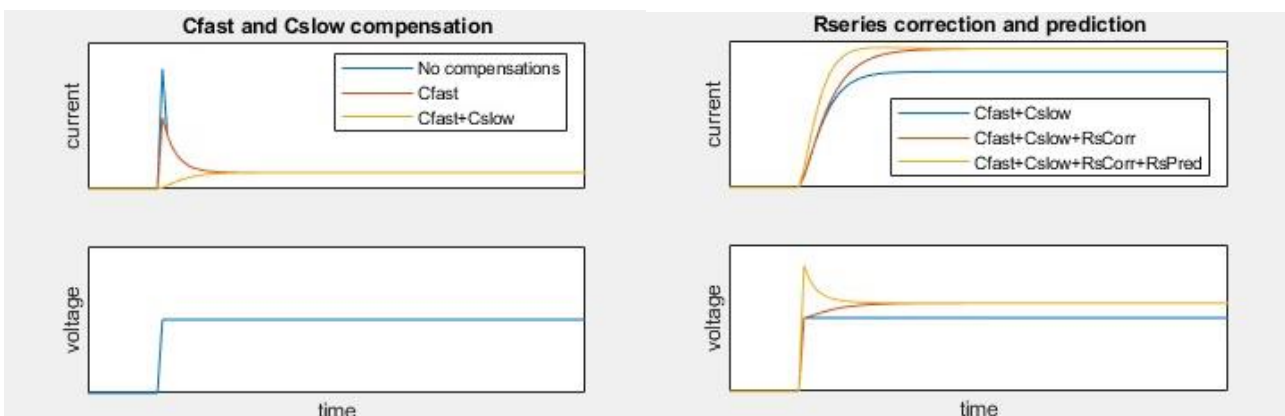
$$V_{corr} = I * R_s * R_s \text{ Correction } \%$$

Since Rs correction leads to instability of the internal feedback circuitry, the user can stabilize it:

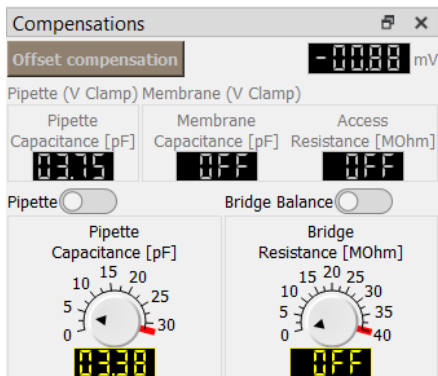
- decreasing Rs Correction %
- increasing the **Correction Lag** parameter in order to adjust the low pass filter and slow down the internal feedback circuitry stabilizing the amplifier response.

Rs prediction adds a “supercharge” voltage peak at the start and the end of the voltage command pulse to compensate the slow rise time of the current response. By increasing the Rs prediction percentage, the time constant of the “supercharge” voltage peak decay increases in turn, leading to a faster current response.

The following figure shows the effects of the described RC compensations on both the current and the voltage signals.



Pipette capacitance neutralization and bridge balance

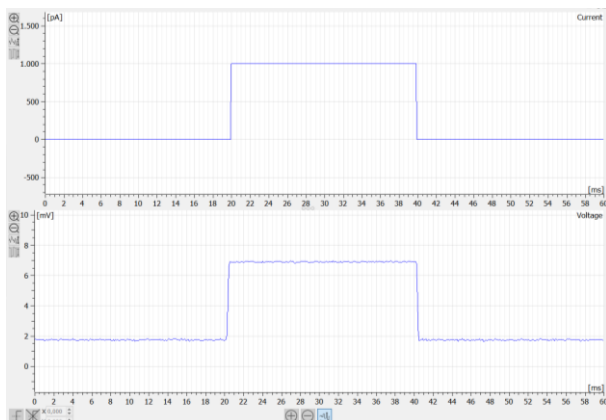


The compensation widget automatically shows the compensations commands for the current clamp mode only when the I-clamp modality is selected. In the background of the widget, the last compensation values applied in VC mode are saved for convenience.

In current clamp mode, the stray capacitance of the electrode acts as a filter to the membrane potential signal. Use the **pipette capacitance neutralization** tool to partially neutralize such undesired error. Run a squared current pulse and slightly increase the pipette neutralization value until overshoots start to appear in the step response. Beyond such value, further increase of the capacitance neutralization may cause a continuous oscillation finally leading to the cell death. To avoid undesired oscillations when switching from VC to CC (I Clamp) mode, the value of the pipette capacitance neutralization is automatically pre-set to the 90% of the Cfast compensation value set in VC mode (e.g. if the Cfast compensation was set to 3.75 pF in VC mode, as soon as the I clamp mode is selected, the pipette capacitance neutralization is set to 3.38, leaving later the user the possibility to adjust the value; see figure above).

In current clamp mode, the flow of current through the microelectrode causes a voltage drop across the electrode that is proportional to the amount of injected current and to the electrode resistance. This undesired voltage drop adds to the recorded potential. Use the **Bridge-Balance** tool to remove such drop from the recorded potential. The figure below shows how the bridge balance is done in practice: immerse the pipette in the bath solution and apply a squared current pulse. The fast voltage step that is observed at the beginning and the end of the current pulse (left) can be compensated using the bridge balance (right). The MΩ value used to correct the bridge balance indicates the resistance of the electrode.

Before bridge balance



After bridge balance

