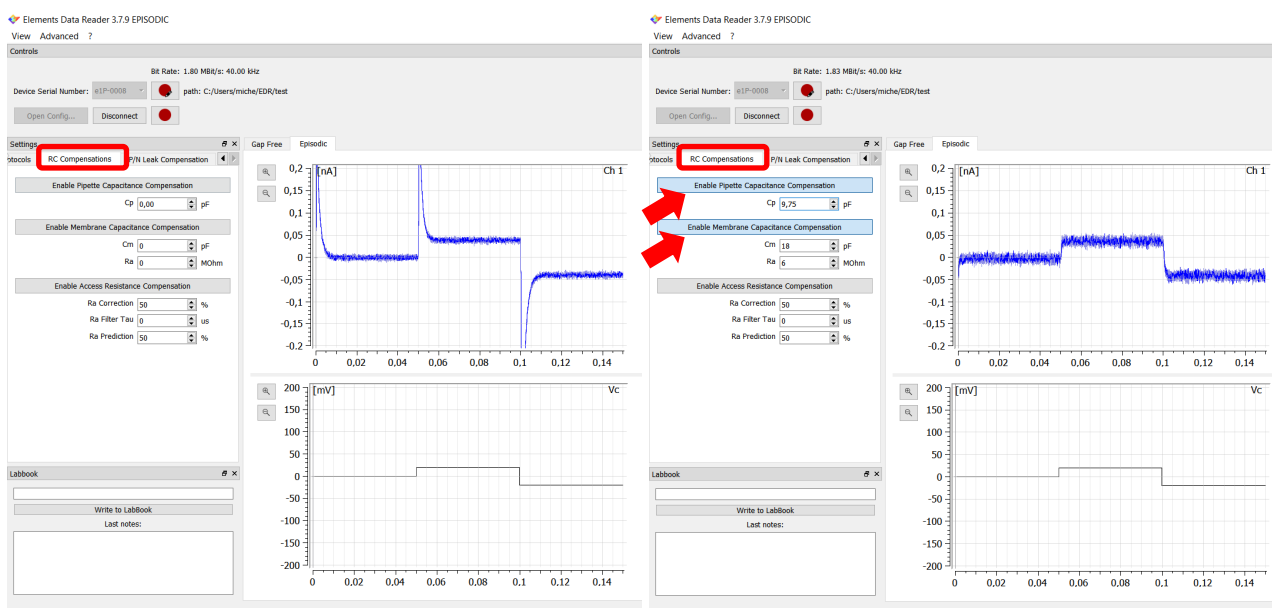




# ePatch

## Elements patch clamp Miniaturized Amplifier

# COMPENSATIONS GUIDE



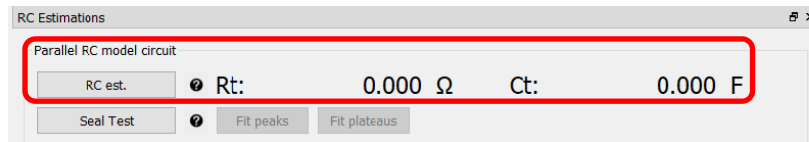
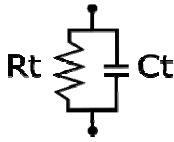
June 2018



# elements

enabling technologies for Life Science

## RC Estimations



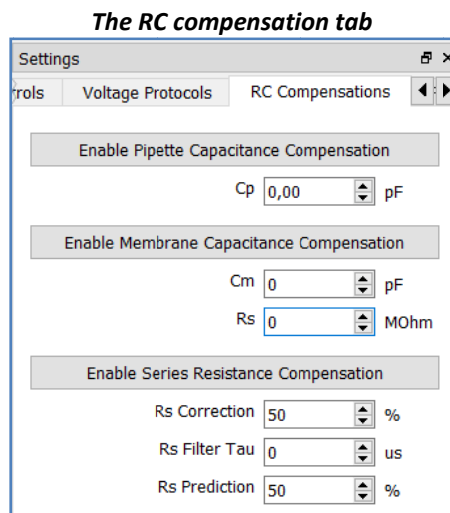
The RC estimation widget assists the user in estimating both  $C_t$  or  $R_t$ , respectively, the total resistance and capacitance considering a simple parallel RC model circuit.

The estimation is automatically enabled when the triangular wave (protocol 1) or the seal test (protocol 2) voltage stimuli are applied.

Click on “RC est” button to perform a continuous RC estimation. By enabling this option, the software starts a continuous and alternating application of the triangular wave and seal test stimuli, needed for a better estimation.

## RC Compensations

All the capacitance compensation tools can be found in the “RC compensation tab” of the “Settings” widget.



### Pipette capacitance compensation (Cfast)

When the voltage stimulus is stepped from a potential to another, fast current transient due to the pipette capacitance ( $C_p$ ) arise. The Cfast compensation tool allows the user to cancel out most of these current transients.

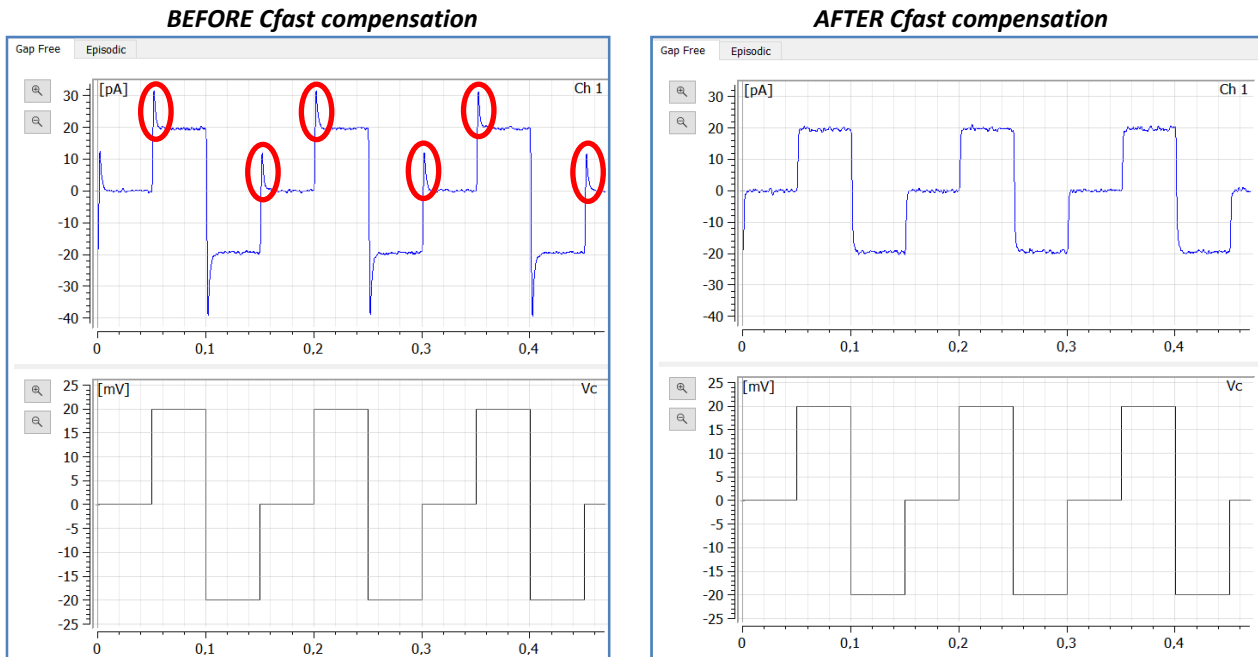
Click on the “Enable pipette capacitance compensation” button in the RC Compensations tab and properly adjust the controller of the  $C_p$  value (by either clicking on the arrows or scrolling the mouse wheel over the numerical control or by directly editing the numerical value) in order to cancel out the transients.

Enable Pipette Capacitance Compensation

Cp 6,75 pF

**Note:** Cp can be compensated in the range of [0 - 11,25 pF] with a resolution of 0,75 pF.

The quality of the pipette capacitance compensation can be verified applying the seal test protocol: when Cfast is properly compensated, the current trace does not show capacitive pulses.



## Cell capacitance compensation (Cslow)

After going into whole-cell configuration, it is possible to progressively compensate the membrane cell capacitive transients similarly to the Cfast compensation.

To compensate the whole-cell capacitance (Cslow compensation) click on the “Enable membrane capacitance compensation” button and manually adjust both the Cm and Rs parameters to achieve the most squared response to the seal test pulse as possible, without any slow capacitive transient (as shown in the cover figures).

Enable Membrane Capacitance Compensation

Cm 20 pF

Rs 6 MOhm

- Cm can be compensated in the range [0 - 255 pF] with a resolution of 1 pF.
- Rs can be compensated in the range [0 - 100 MΩ] with a resolution of 1 MΩ.

**NOTE:** the maximum time constant is 3300 μs, so if the product of Rs and Cm exceeds this value the actual compensated values will be lower.

Enable Membrane Capacitance Compensation

Cm  pF

Rs  MOhm

Enable Series Resistance Compensation

Rs Correction  %

Rs Filter Tau  us

Rs Prediction  %

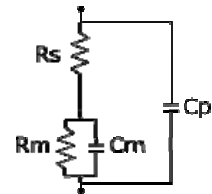
Trying to compensate for 3600us C Slow time constant  
Max tau is 3300us

## Automatic whole cell compensation tool

Auto whole cell compensation

Rs: -
Cp: -

Rm: -
Cm: -



Once in whole cell configuration the software assist the users to automatically estimate and properly compensate both pipette and cell capacitive contributions in order to have a flat response without any slow decay transients. To automatically compensate just click on “Start” button and wait the end of the automatic compensation procedure.

For best results of the whole cell estimation and auto compensation procedure the software automatically set 10 kHz bandwidth when the button “Auto whole cell compensation” is pressed.

After the automatic procedure ends, users can manually adjust RC compensations (as previously described) to obtain the optimal compensation values.

## Series resistance compensation (Rs)

Rs correction and prediction can be enabled by clicking on “Enable series resistance compensation” button. It adds a “supercharge” voltage pulse that allows the compensation of the voltage drop on the pipette access resistance both in terms of amplitude and rise time speed of current response.

Enable Series Resistance Compensation

Rs Correction  %

Rs Filter Tau  us

Rs Prediction  %

Rs correction adds a command potential ( $V_{corr}$ ) proportional to the read current

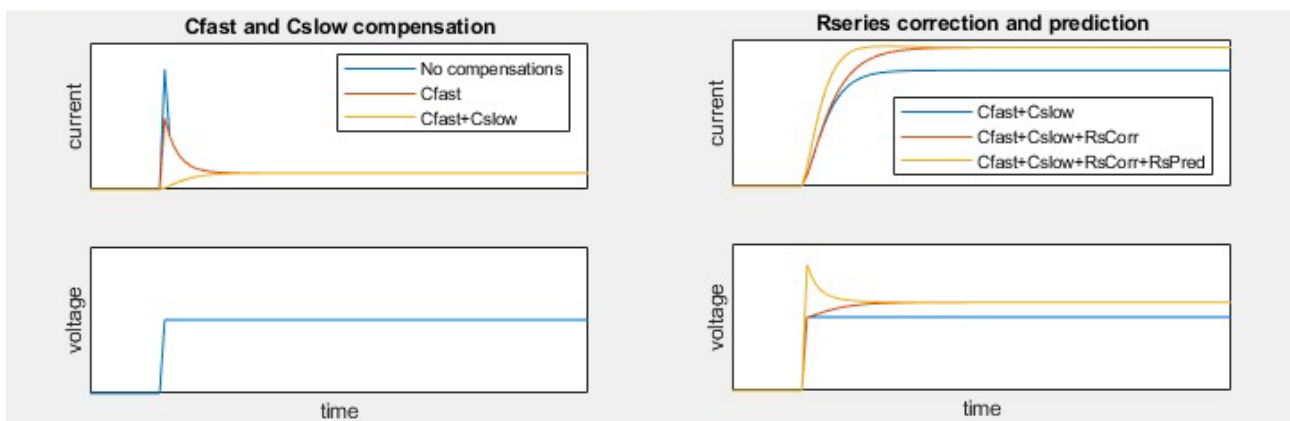
$$V_{corr} = I * R_s * \text{Rs Correction \%}$$

Since  $R_s$  correction can lead to instability of the internal feedback circuitry, the user can stabilize it:

- decreasing  $R_s$  Correction %
- increasing the  $R_s$  Filter Tau parameter in order to adjust the low pass filter and slow down the internal feedback circuitry stabilizing the amplifier response.

$R_s$  prediction adds a “supercharge” voltage peak to compensate the slow rise time of the current response. By increasing the  $R_s$  prediction percentage, the time constant of the “supercharge” voltage peak decay increases in turn allowing a faster current response.

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



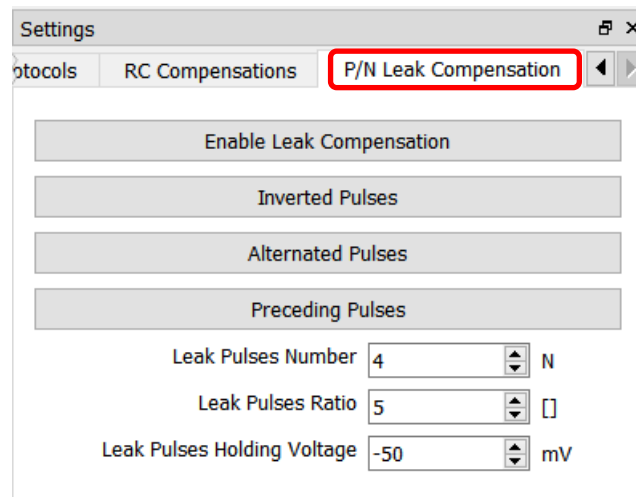
## P/n Leak Compensation

Leak currents can be removed with P/n on-line leak compensation tool, located in the “P/n Leak Compensation” tab of the “Setting” widget.

When “Enable Leak Compensation” button is enabled, the applied episodic voltage protocol (protocols 4 or 5) is followed by a series of scaled replicas that the software uses to mathematically subtract the currents not due to ion channels activation.

**Note:** For a proper P/n compensation, prevent the amplifier saturation by selecting the required current range.

- When the “Inverted Pulses” button is enabled, inverted polarized replicas are applied.
- When the “Alternated Pulses” button is checked, replicas with alternating polarization are applied. This option can be useful to reduce biases in current responses.
- When the “Preceding Pulses” button is checked, replicas are applied before the voltage protocol.



- The “Leak Pulses Number” field allows to set the number of scaled replicas. The higher is the Leak Pulses Number, the smaller is the noise but longer the total duration of the episode.
- The “Leak Pulses Ratio” field allows to set the scaling ratio of scaled replicas. E.g. leak pulses ratio=5 (default value) applies replicas 5 times smaller in amplitude than the voltage protocol pulses
- The “Leak Pulses Holding Voltage” field allows to set the holding potential of the scaled replicas. For a proper functioning this potential should be set in a region of ion channel inactivation