# “Clip detecting” with series resistance compensation using an automated patch clamp system

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## Introduction

Sodium ion channels are some of the fastest ion channels. With a high current and fast activation, it is very difficult for conventional amplifiers to correctly clamp these signals.

The main challenge in providing a perfect clamp is the error introduced by the series resistance ($R_s$), which will introduce a voltage drop as the current increases rapidly (Sigworth 1983). This leads to delays in the signal and incorrect estimation of the current magnitude.

In order to correctly compensate $R_s$ fast enough, one will have to compromise on another cell parameter; cell capacitance ($C_s$) (Sherman 1999). In the QPatch system, it is possible to conduct experiments without direct $C_s$ compensation, but still measure $C_s$ for later use.

When compensating completely for $R_s$, oscillations may occur and this is mainly due to incorrect cell parameter estimations or that the cell simply changes slightly. Oscillations can often lead to reduction in the seal resistance and terminate the whole-cell configuration. In the QPatch system, critical current oscillations are detected and the $R_s$ feedback loop is disconnected for the remaining of the sweep until new parameter estimations can be made.

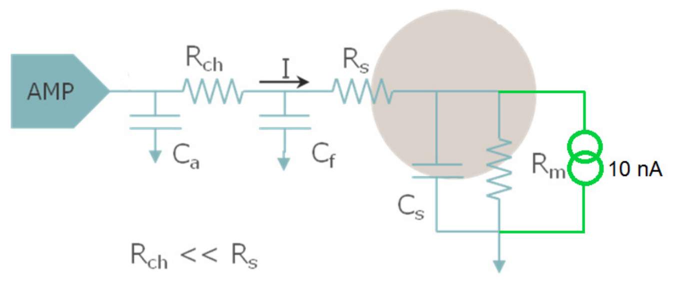



Fig. 1 Schematic of a model cell system used in the QPatch amplifiers. AMP: QPatch amplifier, $R_{ch}$: channel resistance between electrode and patch clamp orifice, $C_a$: capacitive components of interface between amplifier and QPlate, $R_s$: Series resistance, $C_f$: chip capacitance, $C_s$: cell capacitance and $R_m$: membrane resistance. $R_{ch}$ is considered to be very low, often in the kΩ range, while the $R_s$ is in the MΩ range. Green test circuit illustrates system used for injecting series resistance error current.

## Material and Methods

**Sodium channels**
Cultured HEK293 cells expressing hNav1.7. Ringer’s solutions (in mM); Intracellular: 140 CsF, 1 EGTA/5 CsOH, 10 HEPES, 10 NaCl, pH 7.3, mOsm~320. Adjusted with sucrose. Extracellular; 140 NaCl, 3 KCl, 1 $MgCl_2$, 0.1 $CdCl_2$, 20 TEA-Cl, 10 HEPES, pH 7.4, mOsm~300. Adjusted with sucrose.

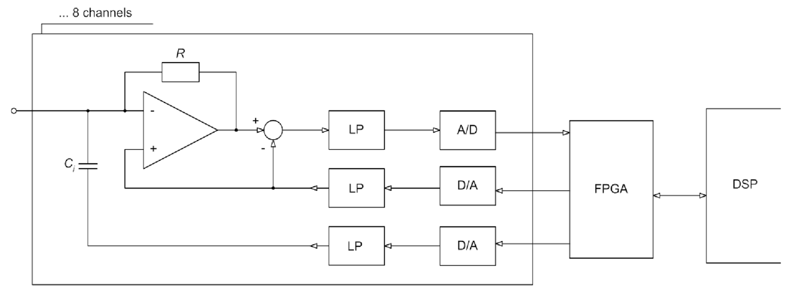
**Potassium channels**
Cultured CHO cells expressing hSK. Ringer’s solutions (in mM); Intracellular: 120 KCl, 10 HEPES, 10 NaCl, 10 EGTA/ 31.25 KOH, 1.75 $MgCl_2$, 5.374 $CaCl_2$, pH 7.2, mOsm~290. Adjusted with sucrose. Extracellular: 144 NaCl, 4 KCl, 1 $MgCl_2$, 1 $MgCl_2$, 2 $CaCl_2$, 10 HEPES. pH 7.4, mOsm~305. Adjusted with sucrose.

**Patch clamp system**
All measurements have been performed on a QPatch automated patch clamp system.

**Data analysis**
Recorded ion channel whole-cell currents were stored in an integrated database (Oracle). Data analysis was accomplished with the QPatch Assay Software.

## How is it theoretically possible to perform 100% Rs?

The big challenge of making 100% series resistance compensation is that the control of the membrane voltage is made using positive feedback from the measured current. If the $R_s$ compensation is made infinitely fast, it will induce oscillations of the membrane voltage. Using the Proportional/Integration (PI) - control loop feedback - technique patented by Adam Sherman and exclusively licensed by Sophion Bioscience, it has been possible to get stable 100% $R_s$ compensation even with large cell current passing through a significant $R_s$. The control loop technique uses a configurable time constant to reduce the speed of the positive feedback loop which makes it possible to obtain 100% $R_s$ compensation only introducing a short voltage error.




**Rs compensation: 500 kS/s**

Fig. 2 Schematic drawing of amplifier. LP: low pass filter, A/D: analog to digital converter, FPGA: field programmable gate array, DSP: digital signal processer. The analog signal passes through a low pass filter and is converted to a digital signal. The FPGA performs the fast calculations which make fast series resistance compensation possible. The end user controls the DSP, to set up sampling, filter order and filter type. The $R_s$ compensated part of the signal is made analog before it is added to the command voltage.

## Amp circuits

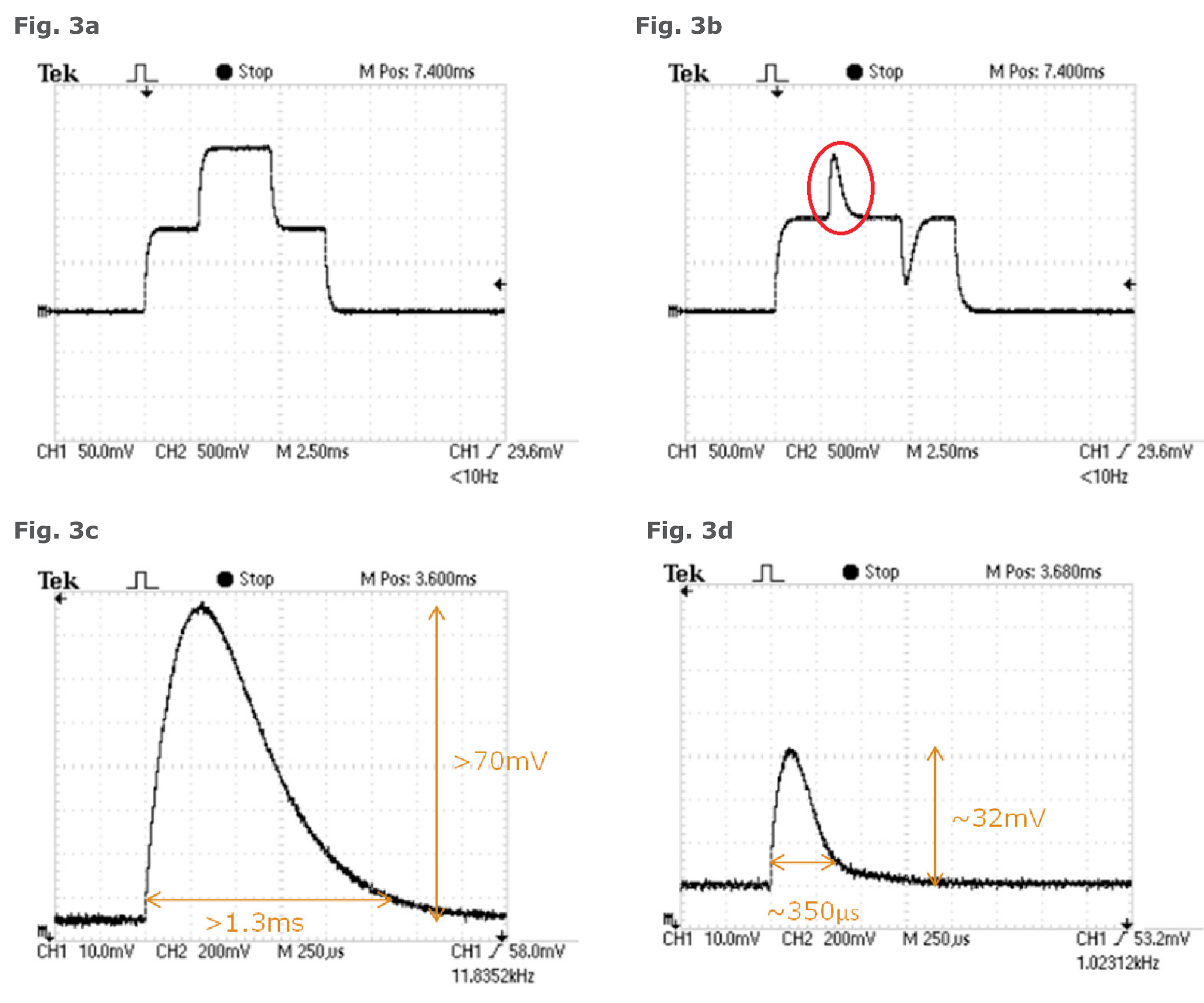



Fig. 3 Voltage traces from test circuit

a) Uncompensated voltage traces when a 10 nA current injected into the test circuit over the membrane resistance ($R_m$) with a series resistance of 10 Mohm (green part of figure 1).

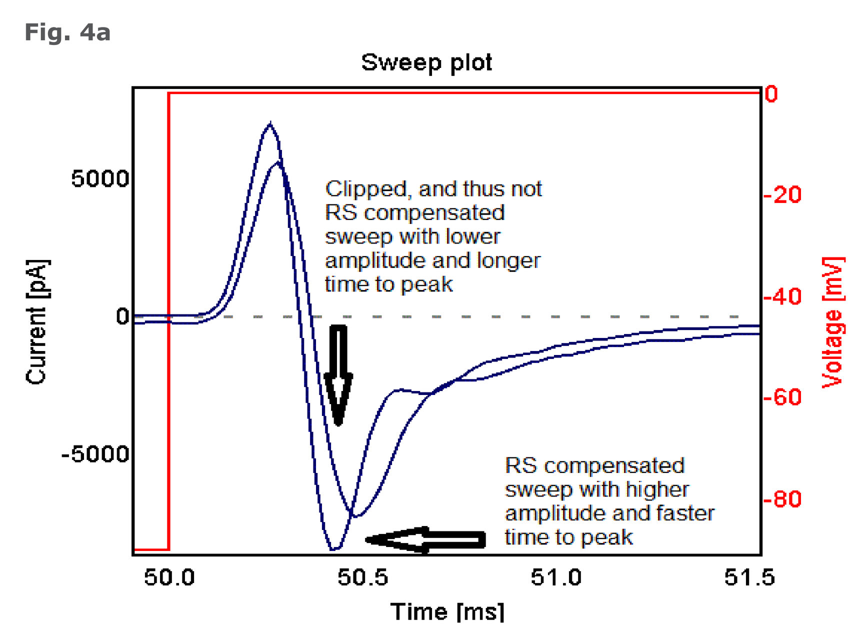
b) Regular $R_s$ compensated potential trace.

c) Zoom of figure 3b which shows the uncompensated component of $R_s$ compensation.

d) Same as figure 3c, but compensated with new fast $R_s$ compensations.

It can be seen by comparing figure 3c and 3d, that the time before the signal is stable again is almost 4 times shorter and that the transient voltage error are less than half with the “fast series resistance compensation”.

## Clip detections



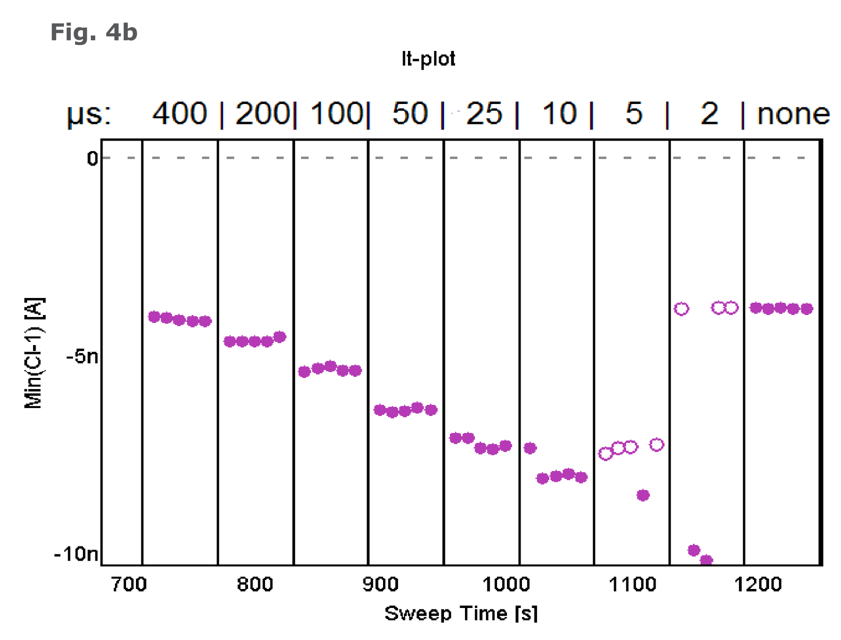




Fig. 4 Example of automatic clip detection in QPatch.

a) Two consecutive current traces from HEK293 cells expressing $Na_v1.7$ showing representative examples of a failed and passed sweep. The sodium channels are activated by a depolarizing pulse from -90 mV to 0 mV @ 0.1 Hz.

b) Current vs. time plot with increasing Voltage Clamp Time Constant (VTC) for each saline period indicated with black vertical lines. The last liquid period is an $R_s$ uncompensated period. The hollow dots indicated automatic detected clipped data which are discarded for further analysis. It is seen that the current amplitude increases with a faster (shorter) VTC, but also becomes more unstable. Recordings are made at $R_m >$ GΩ and with a $R_s$ value of 5 MΩ.

## Potassium Channels

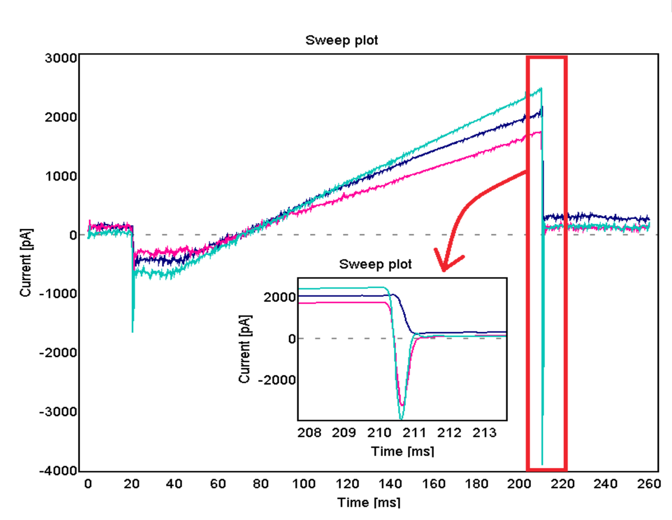



Fig. 5. hSK in CHO cells. Voltage stimulated with a 190 ms long ramp from -120 mV to +50 mV @ 0.25 Hz, from a holding potential at -90 mV. The hSK ion channels are stimulated by a voltage ramp. When comparing the $C_s$ compensated current trace (black) with the $C_s$ *un*compensated current trace (red) a large uncompensated cell capacitance spike can be seen in the two $C_s$ *un*compensated current traces (red and cyan). The 100% $R_s$ compensated and $C_s$ *un*compensated current trace (cyan) shows larger current, but also that the fast $R_s$ compensations is unsuited for tail current investigation on hSK ion channels.

## Sodium Channels

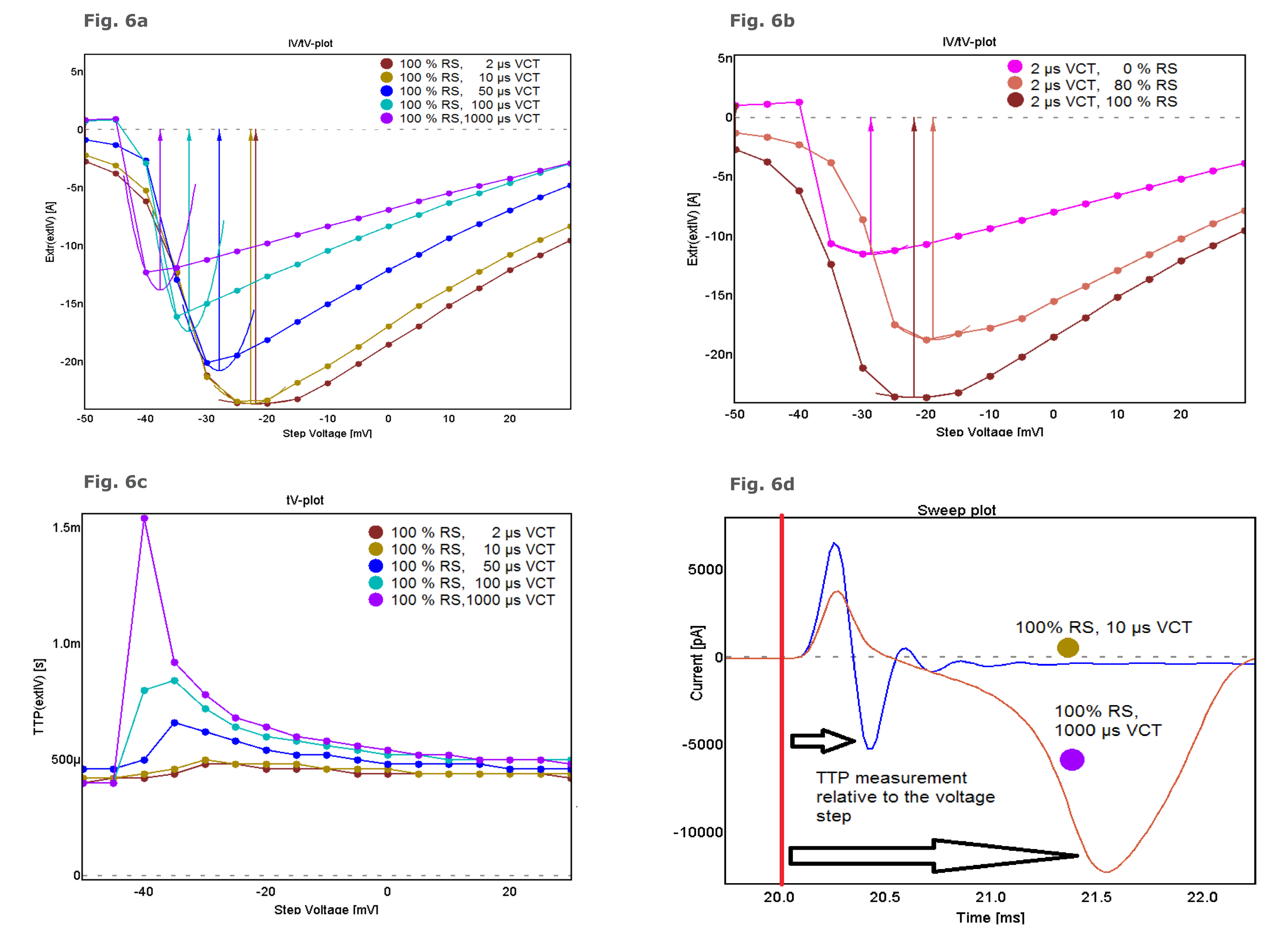



Fig. 6 Biophysics characterization of sodium channels ($Na_v1.7$) with varying settings of, $R_s$ compensations and the speed of $R_s$ loop defined by the voltage clamp time constant (VTC).

a) Current vs. voltage relations with 100% $R_s$-compensation and variable speed of the compensation loop (VTC): 2 µs (brown), 10 µs (yellow), 50 µs (blue), 200 µs (cyan) and 1000 µs (purple). The slower (larger) the VTC, the more steep the IV plots become. Furthermore the maximum currents are shifted towards more hyperpolarized potentials and the current amplitude becomes smaller. This shows that for fast currents it is necessary with a fast voltage clamp time constant in order to achieve a proper voltage clamp.

b) Three current vs. voltage plots, all with 2 µs VTC and varying $R_s$-compensation degree: 100% (brown), 80% (pale), 0% (pink). It can be seen that for especially 0% $R_s$-compensation, the current magnitude is greatly reduced and the smoothness of the curve is also reduced, exemplifying the characteristics of an improper clamp. The maximum current peak is also shifted towards more hyperloarized potentials as a consequence.

c) Time to peak measurements on raw current data at -40 mV. An improper clamp will often show a delay in the current peak after the voltage change (Sherman), as shown in fig. 6d, thus the time to peak measurement gives an evaluation of such events. If the peak is delayed, the clamp, and thus the biophysics of the ion channels in the current measurements cannot be trusted as the input variables are not properly controlled.

d) Time to peak vs. voltage. It can be seen that only with the proper clamp speed (VTC) can the true speed of the sodium current be measured.

## Conclusion

With the new fast series resistance compensation system on the QPatch, it is possible to do perfect measurements on fast ion channels with full series resistance compensation.

The QPatch system offers different kind of series resistance compensation. A regular compensation that is best suited for “slow” responding ion channels and a fast compensation which have been developed for “fast” responding ion channels. Together these two methods provided by the QPatch system allow the user to operate with different targets. Furthermore, the clip-detection system will increase the overall success rate as cells are protected against large uncontrolled voltage zaps.

## References

Sherman et al 1999. Series Resistance Compensation for Whole-Cell Patch-Clamp Studies Using a Membrane State Estimator. Biophysical Journal, 77, 2590-2601.
Sigworth, 1983, Electronic design of the patch clamp. In Single-Channel RecordingPlenum press, 3-35.
Sherman, US Patent (6,163,719)
Sherman, US Patent (6,700,427)