

High Resolution On-Cell Capacitance Measurements with a Software Lock-In System

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Introduction

Since the introduction of the tight-seal patch clamp technique lock-in amplifiers have been used to measure the capacitance of the cell membrane in a small membrane patch (Neher and Marty 1982). While the software controlled methods have long been used for capacitance measurements in the whole cell mode, low noise, on-cell recordings are still done with hardware lock-in amplifiers.

We used a EPC-10 patch-clamp amplifier (version I with increased band-width) controlled by patchmaster software (both Heka elektronik, Lambrecht, Germany) to obtain simultaneous recordings of the patch current, capacitance and conductance without an extra lock-in amplifier. Here we present a comparison between the “amplifier-only” system and the standard setup with external lock-in amplifier (Stanford Research SR830).

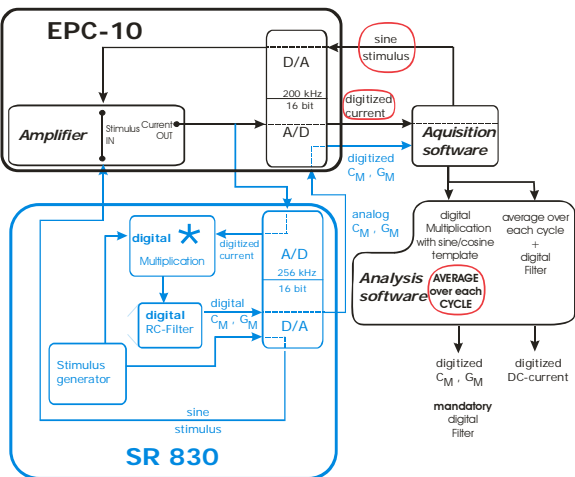


Figure 1: Data Processing in the software lock-In (black) and with external hardware Lock-In (blue). The most important differences are marked red (see also Table 1).

	Software only	External Lock-In
Stimulus Frequency	restricted to fractions of 200kHz (10, 20, 25, 33, 40, 50 kHz)	Any frequency up to 100 kHz
Primary data	Current	Low-pass filtered C_M , G_M
G, C - extraction	Multiplication with sine/cosine template average over each cycle	Multiplication with sine/cosine

Table 1: The most important differences between the systems

Which system to choose?

Both systems are **equally capable of low-noise recordings**, simultaneous recording of current, G_M and C_M as well as post-hoc phase calibrations. However having full control over the G and C calculation and filtering makes the **software approach more versatile** (Figure 3a/b) while at the same time **less instrumentation is needed**. The software approach is less costly and allows to study G_M and C_M with unprecedented temporal resolution (Figure 4).

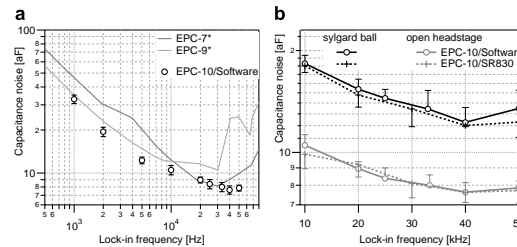


Figure 2: The noise performance of both systems is indistinguishable (b) and better than combinations of older amplifiers with a hardware lock-in (adapted from Debus & Lindau 2000).

	Software only	External Lock-In
PRO	<ul style="list-style-type: none"> •Identical performance with less instrumentation •Greater flexibility for post-experiment manipulations plus higher bandwidth for C_M, G_M •Fast semi-automatic calibration 	<ul style="list-style-type: none"> •Flexible frequency up to 100 kHz •Less datapoints have to be recorded
CONTRA	<ul style="list-style-type: none"> •Large datafiles (200kHz current) •At least at the time of recording •Limited range of stimulus frequencies 	<ul style="list-style-type: none"> •Slightly more effort for phase and attenuation calibration

Table 2: Experimental (dis-) advantages of the systems

High temporal resolution reveals rapid fission events

With the software system the C_M trace is averaged over a whole cycle. Therefore the “unfiltered” trace does not contain signal components at the stimulus frequency in contrast to the weakly filtered output of the external lock-in (Figure 3c). Additional filtering can be omitted allowing very high temporal resolution. This allowed for the first time to study the fusion and fission kinetic in detail. It turned out that in bovine chromaffin cells fission events of large vesicles are completed within less than 100 μ s (Figure 4b & c).

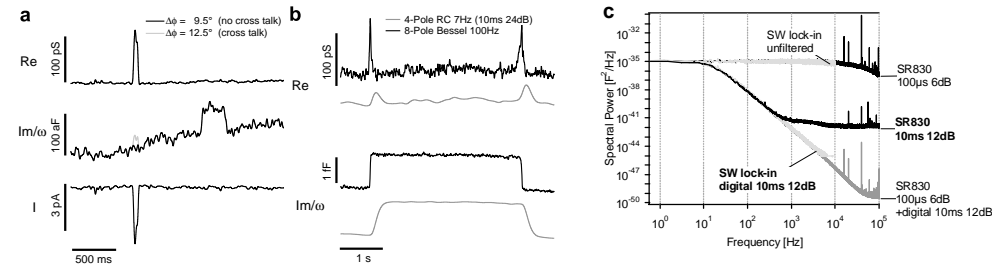


Figure 3: Traces from a recording with bovine chromaffin cells: **a** Post-hoc phase correction: if the channel flicker (bottom panel), is only reflected in the conductance (top) the phase is correctly set. In this example the phase can be set with an accuracy of app. 1° . The capacitance flicker (app. 100 aF) is too small to be reflected in the conductance trace for a phase error of 3° and is less useful for phase calibration. (all 20 Hz cut-off). **b** This example illustrates the flexible choice of post-recording filtering. **c** Spectral densities of capacitance traces obtained with both systems. Note the strong component at the stimulus frequency for the external lock-in.

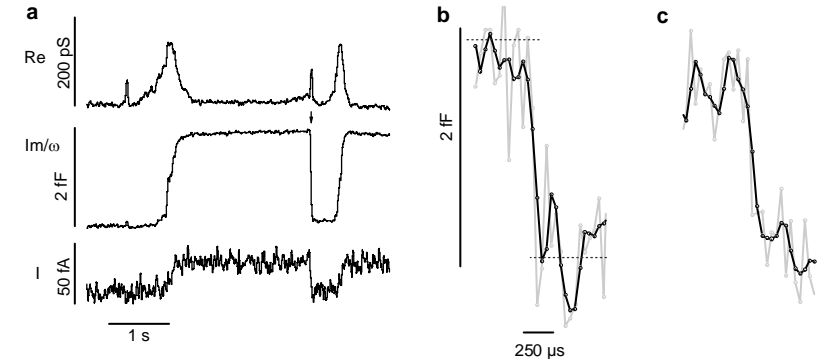


Figure 4: Traces from a recording with bovine chromaffin cells: **a** Transient changes in apparent conductance (top panel) accompany the spontaneous fusion and fission events revealed by abrupt increments of the apparent capacitance (middle trace). Bottom panel displays minute changes in the low-pass filtered patch current accompanying exocytosis and endocytosis. (20 Hz cut-off). **b** High bandwidth is needed to study endocytosis kinetics: the fission (arrow in a) is so rapid, (90 % – 10 % fall time < 100 μ s) that the 25 kHz sampling with a 5 kHz filtering cannot resolve temporal details. Even in the 25kHz raw capacitance trace (grey) the jump appears instantaneous. **c** Example from a different bovine chromaffin cell.

References

Description of the Method, Patchmaster files and Igor Pro Macros for offline analysis:
Neef A, Heinemann C, Moser T. 2007 “Measurements of membrane patch capacitance using a software-based lock-in system”, Pflugers Arch [Epub ahead of print]

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Citations:

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Debus K, Lindau M. 2000. Biophys J 78(6):2983-97.