

## **Guide to low noise on-cell capacitance measurements using the EPC 10**

In this step-by-step protocol we describe how to practically obtain low-noise on-cell capacitance measurements using an EPC 10 and PATCHMASTER software.

### **General**

#### **Hardware Set-up:**

For optimal noise performance an EPC 10 with increased bandwidth in high gain range should be used (EPC 10 with revision of at least I or K). For recording of high resolution sweeps of several seconds make sure that your PC has enough RAM (1 GB, 2 GB recommended). Recalibrate the EPC 10 with PATCHMASTER software.

#### **Software Set-up:**

Along with this guide we provide a set of default files for OnCell capacitance measurements.

*Using the default OnCell\_Cm example*

The default configuration is adapted to PATCHMASTER version 2.15 (available for the HEKA web site: <http://www.heka.com/download.html>) and requires a PATCHMASTER installation of this version in the path C:\HEKA\Patchmaster. Furthermore the configuration is adapted for an EPC 10 Single patch clamp amplifier and a computer monitor resolution of 1024 x 768. Memory requirements have been adapted to a Pentium IV with 512 MB RAM running under Windows XP.

Please copy the folder "OnCell\_Cm" and the configuration file "OnCell\_Cm.set" in your PATCHMASTER folder.

**NOTE:** *In order to start your PATCHMASTER software with the provided configuration, drag the configuration file "OnCell\_Cm.set" over the PATCHMASTER application.*

If you are not using the "OnCell\_Cm.set" file, please perform the most important settings in the configuration manually:

Turn on LockIn configuration and set the

- LockIn Mode: On Cell
- Calibration Mode: Manual
- Phase Shift: 0
- Attenuation: 1.0

- Write to Notebook: should be off during routine measurements. Turn it on for inspection.
- Default Y Ranges: CM: 10 fF; GM: 1.0 nS

In the Configuration Window on pane General increase "Max. Sample Points" to the maximum allowed by the RAM in your computer. ( If you exceed the RAM the following message appears: "There seems to be insufficient memory for the present Configuration settings...." Press "Undo Changes" and try to increase the "Max. Sample Points" by a smaller amount.

In the Macros menu turn off "Protocol to Notebook".

Load the following files:

- OnCell\_Cm.pgf (Pulse Generator File)
- OnCell\_Cm.pro (Protocol Editor File)
- OnCell\_Cm.onl (Online Analysis File)

### **Description of the content of the OnCell\_Cm files**

In our configuration we use the following convention: AD channel 1 is the MUX channel (output of the active amplifier after Filter 1 prior to Filter 2), AD-channel 2 is the capacitance trace (named "LockIn\_CM") and AD-channel 3 is the conductance trace (named "LockIn\_GM"). It is important to keep this order of channels for the protocols and the online analysis to work correctly.

#### ***The Pulse Generator File: OnCell\_Cm.pgf***

It contains the sequences SineTest and Rec20kHz.

##### *SineTest:*

Used for calibration of the LockIn.

##### *Rec20kHz:*

Used for the recording of capacitance changes.

In general the same as SineTest but with longer duration a smaller sine wave amplitude and (if required) more input and output channels.

Please make sure that your recording sequence and your calibration sequence “SineTest” are identical with respect to the following critical parameters of the pulse generator:

- Sampling Frequency
- Sine Wave Frequency
- Points per Sine Wave Cycle
- Current Recording Channel (AD)

***The Protocol File: OnCell\_Cm.pro***

It contains 6 default protocols. For calibration of the LockIn we use the protocols "ScanPhase", "AdjustPhase", and "GetAttenuation".

*ScanPhase:*

Varies the LockIn Phase setting and measures the change in Gm and Cm. Is used to quickly find the correct range of the phase setting.

*AdjustPhase:*

The LockIn Phase can be manually adjusted and the change in Gm is displayed. Is used to fine tune the LockIn Phase setting.

*GetAttenuation:*

Changes the C-Fast compensation of the amplifier and measures the CM with the LockIn at a given phase setting. Is used to calibrate the amplitude of the LockIn (Attenuation) via linear regression of the plot measured CM versus decompensated C-Fast.

*Adjust\_Osci:*

Is a tool that helps setting the oscilloscope scaling offsets appropriately.

*Conf\_1:*

Is a protocol that sets a custom configuration of the amplifier. Should be used to maintain the critical parameters of the amplifier unchanged between measurements. The critical parameters of the amplifier that might change the LockIn calibration are:

- Recording Mode
- Gain
- Filter 1

- Filter 2 (if the current is measured on the Imon-2 channel)
- Stim Filter

*LinReg:*

Performs a linear regression. Called from protocol "GetAttenuation".

***The Online Analysis File: OnCell\_Cm.onl***

Four default analysis methods are provided. The methods "ScanPhase", "AdjustPhase" and "GetAttn" are used to analyse and display the results during the calibration procedure of the LockIn. The fourth method "MeasureCm" is used to measure the mean values of Cm and Gm and the standard deviation of Cm at the beginning (cursor position 2-15%) of the trace.

**The LockIn Calibration Procedure**

*Phase Calibration:*

It is not obligatory to perform calibration only after the on-cell recording configuration has been established. Identical results are obtained with calibrations prior to patching, with the pipette above the solution or with a closed pipette in the solution. If you perform the calibration in the actual recording configuration, the seal has to be stable to allow for a correct and fast calibration. In any case it is crucial, that the amplifier settings and Pulse Generator settings are identical for calibration and recording (see above, "The Pulse Generator File" and "protocol Conf\_1").

To get an overview on the phase setting, start protocol "ScanPhase". From a starting phase of 150° the phase is increased in 5° steps. At every phase two LockIn measurements are made, toggling the C-fast compensation by 50 fF inbetween. The resulting DeltaCm values are displayed in the upper graph and the DeltaGm values in the lower graph. The correct phase is reached if DeltaCm goes through its maximum and DeltaGm through its minimum. When the measurement passed the correct phase setting, terminate the protocol with the F12 key. The phase at minimum of DeltaGM is then automatically entered as LockIn Phase in the LockIn configuration.

Fine tune the LockIn Phase with the protocol "AdjustPhase". Start the protocol. As starting phase angle the last entry in the LockIn configuration is prompted. Press OK. The DeltaGM values are displayed in two graphs. The upper graph provides an overview and the lower graph displays the DeltaGM value at higher resolution. The second graph is wiped every 20 measurements. Use the keys "1" and "2" to increment and decrement the phase by 10°,

respectively. Use the keys "3" and "4" to increment and decrement the phase by 0.5°, respectively. Again, the optimal phase is found, when the DeltaGM has reached a minimum. Terminate the phase adjustment by pressing the "F12" key.

You might find that the first measurement gives an outlying result that leads to a disadvantageous scaling of the graph. In such a case stop the protocol by pressing "F12" and restart it.

*Amplitude Calibration:*

At the given phase angle we now calibrate the amplitude of our Cm measurements. As calibration reference we use the C-Fast compensation of the EPC 10. Start the protocol "GetAttenuation". Starting from its auto compensation value the C-fast compensation will be reduced by 10 fF at a time. Then the Cm is measured with the LockIn and plotted against the difference in C-Fast compensation. This cycle is repeated 5 times and the slope is calculated by linear regression. The slope is the scaling factor for the amplitude (ratio of measured / expected capacitance change) and is set as "Attenuation" in the LockIn configuration.

**NOTE:** *In case of a negative slope, please reduce the LockIn phase angle by 180° (LockIn configuration) and repeat the amplitude calibration.*

**Typical Phase and Attenuation Settings**

The listed values were measured with a 1.5 pF capacitor connected to the headstage of an EPC 10 revision J. The capacitance measured with the C-fast compensation was: 2.37 pF (1.5 pF + 0.87 pF input capacitance of EPC 10, Tau = 91.5 nsec).

	<i>Rec. Mode: OnCell, Gain: 50 mV/pA, Filter 1: 30 kHz Bessel, Current recorded from Imon-1 (Filter 1 output), Sampling Frequency 200 kHz</i>			
	<i>Stim Filter: 2 μs</i>		<i>Stim Filter: 20 μs</i>	
<b>Sine Freq.</b>	<b>Phase</b>	<b>Attenuation</b>	<b>Phase</b>	<b>Attenuation</b>
20 kHz	266.0 °	0.944	164.5 °	0.356
25 kHz	241.5 °	0.855	129.0 °	0.240
40 kHz	174.0 °	0.597	40.5 °	0.078

## **Practical Guide**

Open “OnCell\_Cm.pgf” and define the amplitude and frequency of your sine wave in the “Sine Wave Settings” dialogue of the sequences “Rec20kHz”, and “SineTest” (we recommend 25 kHz for on-cell measurements of exo- and endocytic capacitance changes; 40 kHz, if the fusion pore is the main object of the study). Choose the maximally possible number of points per cycle (i.e. 8 for 25 kHz). For the optimal stimulus frequency for on-cell measurements of non-linear capacitance (charge movement in channels and transporters please refer to the original literature (Fernandez, 1982; Gale, 1997; Oliver, 1999).

Minimise electromagnetic pick-up of the setup (grounding and shielding) and optimise setup for short, thick-walled borosilicate or quartz pipettes, used at a steep angle (low immersion depth).

Pull sturdy pipettes, coat with sylgard (2-5 times) up to 50-75  $\mu\text{m}$  from the tip (closer it would affect sealing)

## **Calibration**

In your desired configuration (closed pipette, pipette above bath, on-cell configuration) adjust the amplifier settings (you might use the protocol *conf\_1* to set all the crucial parameters). Perform the *Phase Calibration* and *Amplitude Calibration* described above.

## **Measurement**

Obtain a high resistance ( $>10$  GOhm) on-cell configuration with very little suction. Choose the recording settings (amplifier and pgf). If you wish, you could now confirm or fine-tune the phase setting, calibrate attenuation. Finally acquire a data sweep.

## **Post-recording correction of the phase settings**

If channel flickers occur at a sufficient rate, use them for recalculation from current. Else you might want to apply slight suction manually or with a triggered air pump to elicit small suction pulses. Those result in an increase of patch capacitance.

Use lock-in configuration dialogue of Patchmaster for recalculation of apparent capacitance and conductance with a different phase until conductance changes are not any longer reflected in the capacitance trace (channel flicker) or the suction-evoked capacitance changes are not reflected in the conductance trace.

Alternatively use the IGOR calculation macro for  $\text{Re}$ ,  $\text{Im}/\omega$ ,  $G_p$  and  $C_v$