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# 1. Capacitance Measurements using LockIn Extension

## 1.1 Introduction

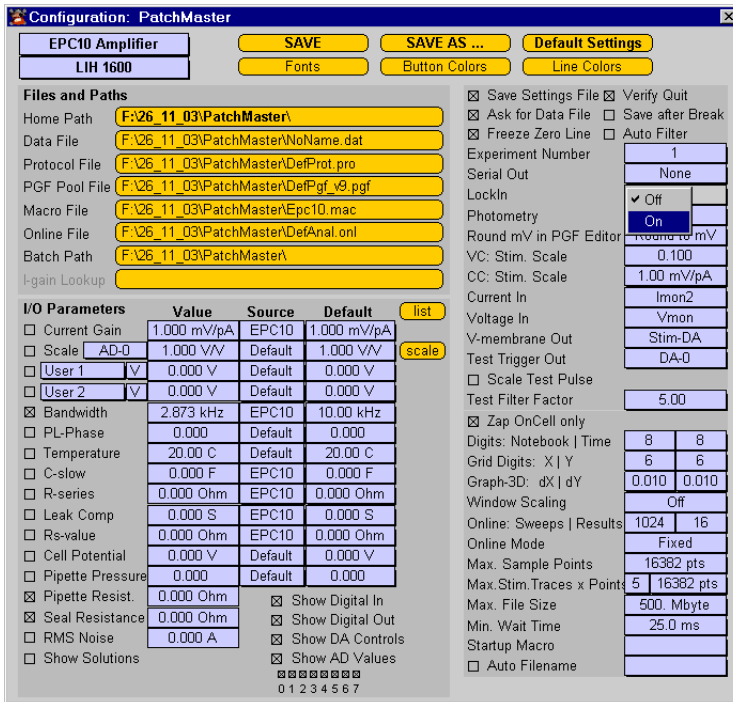
Membrane capacitance ( $C_m$ ) measurements have been used extensively to study exocytosis and endocytosis in individual cells. Currently, the most popular techniques for measuring changes in  $C_m$  utilize a sinusoidal voltage stimulus and process the resulting sinusoidal current using a phase-sensitive detector or "lock-in amplifier" implemented either in hardware or software. A software based phase sensitive detector is only a small part of the LockIn extension of the PATCHMASTER software. LOCKIN together with an EPC 10 or EPC 9 comprises a unique "virtual" instrument with unprecedented capabilities for performing cellular admittance measurements without the need of external filters. Admittance can be measured even when the current monitor signal is profoundly altered with the use of capacitance and/or series resistance compensation. Thus, estimates of the actual values of equivalent circuit parameters (rather than just relative changes) can be generated from bona fide admittance measurements.

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## 1.2 Tutorial: Using the LockIn Extension

Here we make our first capacitance measurement using a model circuit and look at how LOCKIN works together with capacitance compensation. We used an EPC 10 Single for this tutorial, but most settings are identical for EPC 10 Double / Triple amplifiers and EPC 9 Single, Double and Triple amplifiers. We will explicitly mention the particular amplifiers, where it is required.

### 1.2.1 Activating the LockIn extension

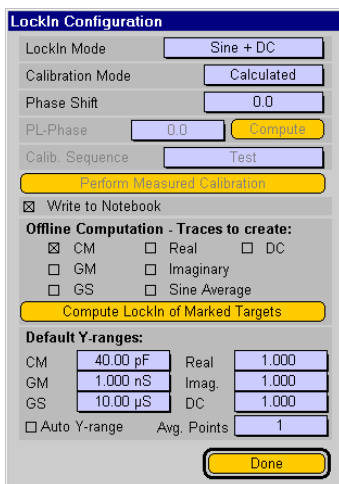


By default, the LockIn extension of PATCHMASTER is inactive. To activate the extension, we have to open the configuration dialog and switch on

LockIn in the right section of the dialog. This step only has to be done the first time one uses LockIn. The setting is stored in the PATCHMASTER configuration file "Patchmaster.set".

### 1.2.2 LockIn configuration


Immediately after switching on the LockIn extension, the LockIn configuration dialog comes up. Please set the LockIn Mode to "Sine + DC". This is the recommended mode, if you are using an EPC 10 or EPC 9 amplifier. If you want LockIn calculations to be written to the Notebook, Write to Notebook should be checked. At the moment, no other settings need to be made. We will come back to the LockIn Configuration dialog later.



*Tip:* The Default Y-ranges section can be used to set reasonable display ranges according to the expected values.

### 1.2.3 Creating a PGF sequence

The next step is to prepare a PGF sequence, that can be used for  $C_m$  measurements. Please open the Pulse Generator dialog ('F8' key). We

could either modify an existing sequence or create a new one from the scratch. Let us create a new sequence this time. Please click on the yellow arrow button  at the right upper corner of the dialog to get to the last sequence in the opened PGF pool. A click on an empty button will create a new sequence and you are prompted to enter a name for the new sequence. I would suggest to use the name "LockIn".


PATCHMASTER automatically creates a new and very simple protocol: it just consists of one "Constant" segment with a duration of 10 ms. 10 ms is quite short, therefore we will increase the duration to 100 ms. Furthermore, we have to change the **Segment Class** to "Sine", since at least one sine wave segment is required for the LockIn. Of course, one can also use other segment classes (Constant, Ramp, Square) in the same sequence, for example to define a depolarizing voltage step at the beginning of the sweep. But LockIn data can only be calculated from "Sine" segments.

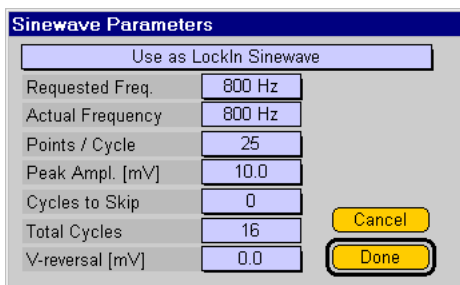
The **Sample Interval** is set to 50  $\mu\text{s}$ . This corresponds to a sampling rate of 20 kHz. Please keep that sampling rate in mind. We will need that information later on.



Segments			<input checked="" type="checkbox"/> Store 1	<input type="checkbox"/> Store 2	<input type="checkbox"/> Store 3	<input type="checkbox"/> Store 4	<input type="checkbox"/> Store 5	<input type="checkbox"/> Store 6		
Segment Class			Sine	Constant						
Voltage [mV]	hold	V-mem	val	---	val	---	val	---	val	---
Duration [ms]	val	100.00	val	---	val	---	val	---	val	---
V-incr. Mode			Increase	Increase	Increase	Increase	Increase	Increase		
V-fact./incr. [mV]	1.00	0	---	---	---	---	---	---	---	---
t-incr. Mode			Increase	Increase	Increase	Increase	Increase	Increase		
t-fact./incr. [ms]	1.00	0.00	---	---	---	---	---	---	---	---

For the calculation of the LockIn parameters, we need to know the whole cell conductance. The conductance can only be measured, if the holding potential is not "0". Therefore, you should not forget to enter a holding potential for the "Sine" segment. One can either enter a fixed value, for example "-70 mV" or set **Voltage** to "hold". If **Voltage** is set to "hold", the holding potential from the amplifier dialog is used.

If a "Sine" segment exists in the PGF sequence an additional button appears in the dialog: . This button can be used to modify the parameters (frequency, amplitude, ...) of the sine wave segments in a protocol.



In this dialog, you can decide, if the "Sine" segments in the sequence should be used for LockIn measurements (**Use as LockIn Sinewave**) or just as a simple sine wave stimulus (**Use as Simple Sinewave**). Of course, we want to use it as LockIn sine wave.

Now, we increase the **Requested Frequency**. A frequency of 800 Hz is quite good for most approaches. All segment durations must be integral multiples of the sine wave period. Therefore the **Actual Frequency** might be slightly different from the **Requested Frequency**. An appropriate value is calculated by the software and cannot be changed.

Please note, that the **Sample interval** in the PGF sequence is adopted, upon changing the setting for **Requested Frequency** or **Points/Cycle**. It is intuitive that for higher frequencies higher sampling rates are required. If you want to reduce the sampling rate, you can decrease the number of **Points/Cycle**. We will do that and set **Points/Cycle** to "25".

**Important note:** *The number of **Points/Cycle** should never be below 10, otherwise the accuracy of the  $C_m$  measurement is dramatically reduced.*

The setting of 10 mV for the sine wave amplitude is fine and a **V-reversal** of 0 mV is also correct for our model circuit. The **V-reversal** setting is actually not very critical if  $G_m$  is low. A value of zero is often used in the common situation where  $G_m$  is low and the actual reversal potential is unknown. Now, we can go back to the PGF sequence.

**Important note:** *Since we decided to use the "Sine" segment*

in the sequence for LockIn measurements, the entry "Stimulus → DA" in the PGF sequence has automatically been changed from "StimScale" to "StimScale, LockIn" upon closing the *Sinewave Parameters* dialog. This "LockIn" flag is important. Without that flag, no  $C_m$  data can be calculated.

The DA output is already set to Stim DA. The Stim DA is the stimulus output of the amplifier. The "LockIn" flag in the "Stimulus → DA" is already set, so there is nothing left to do in that section. It would be possible to use additional DA outputs (e.g. for triggers), but for the moment, one DA is sufficient.

1	DA	Unit	Stimulus -> DA	Leak
Ch-1	Stim-DA	V	StimScale, LockIn	<input type="checkbox"/>
Ch-2	off	V	StimScale	<input type="checkbox"/>
---	off		absolute voltage	<input type="checkbox"/>
---	off		absolute voltage	<input type="checkbox"/>

The AD input is set to "Imon2", the current input of your amplifier. This is undoubtedly a good idea, however, we need an additional trace to store the  $C_m$  value. A click in an empty AD field opens a long list of available inputs. What we need is a "LockIn\_CM" trace. You will see, that the compression factor is automatically set to "25" (the value, we have set for *Points/Cycle* in the *Sinewave Parameters* dialog). That means, 25 data points are in one cycle and one  $C_m$  value is calculated from each cycle, therefore you will get 80  $C_m$  data points in that 100 ms sweep.

AD	Y	Link	Compr.	Points	Store	Zero	Leak
Imon-1	A	1	1	2000	<input checked="" type="checkbox"/>	1	No Leak
LockIn CM	F	1	25	80	<input checked="" type="checkbox"/>	0	No Leak
off		---	---	---	<input type="checkbox"/>	---	No Leak
off		---	---	---	<input type="checkbox"/>	---	No Leak

Please note, that *Link* is set to "1". This tells PATCHMASTER to use the first trace (the current trace) for calculating the LockIn information. This point is not crucial as long as you have only one current trace and one LockIn trace, however, it is important if you should have more than one current trace (e.g. if you are using a Double or Triple amplifier).

*Store* is active for both traces. It would also be possible to store only the LockIn information and to discard the current data. However, in most

cases, it is reasonable to store both traces.

### 1.2.4 Amplifier Settings

Now its time for the experiment. Switch the model circuit into the 10 M $\Omega$ m setting to simulate a 10 M $\Omega$ -pipette that is open to the bath solution. Hit the space bar on your keyboard to activate the **Amplifier** window.

You can correct pipette offset potentials by adjusting the  $V_0$  value or you can alternatively click on the **Auto  $V_0$**  button to let PATCHMASTER do this correction automatically for you. The same is done by calling the macro **SET-UP**.

Now simulate a pipette sealed to the membrane by switching the model circuit into the middle position. Make an automatic fast capacitance cancellation by clicking on the **Auto CFast** or the **On-Cell** macro button.

To break into the cell, set the switch of the model circuit to its bottom or 0.5 G $\Omega$ m position. Make an automatic slow capacitance cancellation by clicking on the **Auto CSlow** or **WHOLE-CELL** macro button. With the **V-membrane** control change the pipette holding potential to -70 mV. Now we are ready to run the pulse protocol we defined before.

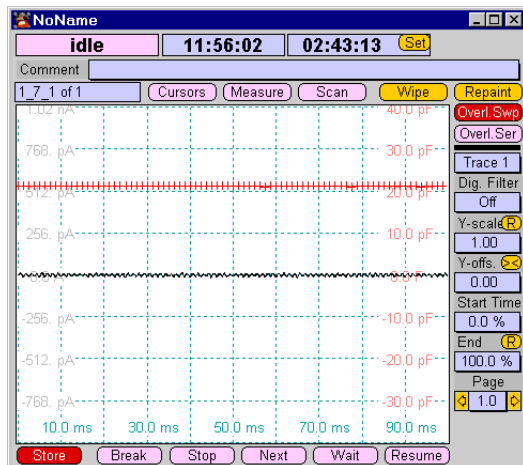
### 1.2.5 Running the PGF sequence

In PATCHMASTER, PGF sequences can be run from the protocol editor. However, it is not necessary to define a new protocol - just click on the **LockIn** button to start the sequence.



The results are displayed in the oscilloscope screen: as defined in the PGF sequence, you can see the current trace and a second trace with the LockIn data.

*Tip: Change the display labeling to Grid + Values in order*



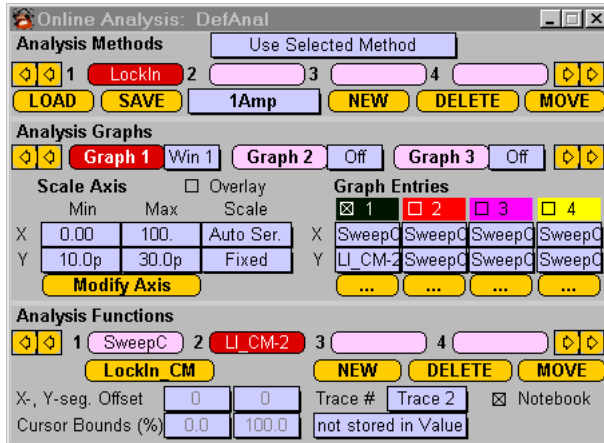
to display the axis scaling.

By default, all data points are connected by lines. If you want to see the individual data points, you can modify the Trace properties in the Display menu. The MC-10 model circuit in the 0.5 GOhm position has a capacitance of 20-22 pF. The calculated " $C_m$ " values are well within this range.

## 1.2.6 Online Analysis

In the oscilloscope screen, the LockIn information is displayed with a high time resolution. Even fast membrane capacitance changes can be observed in that way.

However, in some experiments, the expected  $C_m$  changes are in the range of several seconds or minutes. In that case, we should use the Online Analysis to monitor the changes in membrane capacitance. For that purpose, it is necessary to modify the LockIn PGF sequence: we will increase the No of Sweeps from 1 to 100. That is all we have to do for the moment.



The handling of the **Online Analysis** is quite similar to that of the pulse generator. Instead of acquisition sequences in the PGF, various analysis methods can be defined in the Online Analysis dialog. The pool of analysis methods is shown at the top of the dialog. Please click on an empty button to create a new analysis method. You can either copy the settings from an existing method or create a new method. Again, we will start from the scratch. I assume, you are already familiar with the Online Analysis settings? So, I don't need to go into details.

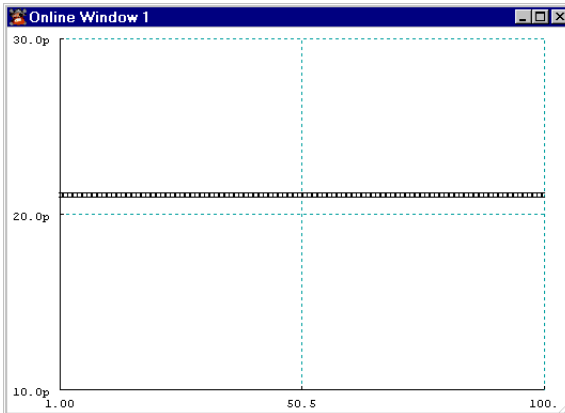
By default, all graphs are switched off. Therefore, we need to choose one of the two Online Analysis windows (Win 1 or Win 2) for **Graph 1**. Furthermore, we have to activate **Graph Entry No 1** to display the online analysis results.

Finally, we have to decide, what parameters (**Analysis Functions**) should be analyzed.

We chose "Sweep Count" for the X-axis and "LockIn Cm" for the Y-axis. Please take care, that **Trace #** for the "LockIn Cm" parameter is set to "Trace 2". Remember: the Lockin trace in our PGF sequence is the second trace.

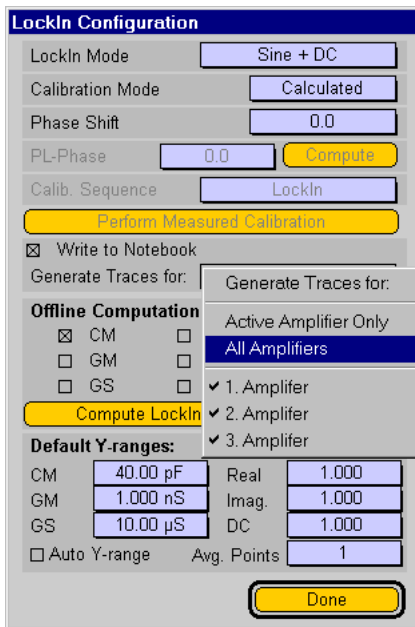
If we now run the "LockIn" PGF sequence, one LockIn data point per sweep is plotted in the Online Analysis window. This single point is the mean value from all  $C_m$  values within a sweep.

Function Type					
<input type="radio"/> Sweep Count	<input type="radio"/> Y-Analysis	<input type="radio"/> Trace Param.	<input type="radio"/> Math	<input type="radio"/> Trace	<input type="radio"/> Spectra
<input type="radio"/> Online Index	<input type="radio"/> Extremum	<input type="radio"/> C-slow	<input type="radio"/> Constant	<input type="radio"/> Trace	<input type="radio"/> Frequency
<input type="radio"/> Time	<input type="radio"/> Maximum	<input type="radio"/> R-series	<input type="radio"/> a + b	<input type="radio"/> Q = Integral	<input type="radio"/> Distribution
<input type="radio"/> Timer Time	<input type="radio"/> Minimum	<input type="radio"/> Rs-value	<input type="radio"/> a - b	<input type="radio"/> 1 / ( trace )	<input type="radio"/> Histogram
<input type="radio"/> Real Time	<input type="radio"/> Mean	<input type="radio"/> Leak Comp.	<input type="radio"/> a * b	<input type="radio"/> 1 / ( Q )	<input type="radio"/> Histogram Amp
<input type="radio"/> X-Segment	<input type="radio"/> Integral	<input type="radio"/> M-conductance	<input type="radio"/> a / b	<input type="radio"/> ln ( trace )	<input type="radio"/> Histogram Bins
<input type="radio"/> Amplitude	<input type="radio"/> Variance	<input type="radio"/> Cell Potential	<input type="radio"/> a in b	<input type="radio"/> ln ( Q )	
<input type="radio"/> Duration	<input type="radio"/> Slope	<input type="radio"/> Seal Resistance	<input type="radio"/> abs	<input type="radio"/> log ( trace )	
<input type="radio"/> Peak Amplitude	<input type="radio"/> Time to Peak	<b>Sweep Param.</b>	<input type="radio"/> log	<input type="radio"/> log ( Q )	
<input type="radio"/> Segment Time	<input type="radio"/> Anodic Q	<input type="radio"/> User 1	<input type="radio"/> sqrt	<input type="radio"/> Trace Time	
<input type="radio"/> Scan Rate	<input type="radio"/> Cathodic Q	<input type="radio"/> User 2	<input type="radio"/> 1/a		
	<b>Lock-In</b>	<input type="radio"/> Temperature	<input type="radio"/> 1/log		
	<input checked="" type="radio"/> LockIn_CM	<input type="radio"/> Pip. Pressure	<input type="radio"/> 1/sqrt		
	<input type="radio"/> LockIn_GM	<input type="radio"/> Int. Solution			
	<input type="radio"/> LockIn_GS	<input type="radio"/> Ext. Solution			
		<input type="radio"/> Digital-In			
		Name	LI_CM	<input type="button" value="Done"/>	<input type="button" value="Cancel"/>



**Tip:** If you know the expected range of membrane capacitance values, you should use a *Fixed* scale for the Y axis. 10 to 30 pF is a good range for the MC-10 model circuit.

## 1.3 Cm measurements with Double or Triple amplifiers



If a Double or Triple amplifier is used, the LockIn configuration dialog looks slightly different. An additional control allows selecting the amplifiers, which can be used with the LockIn extension. By default, all available amplifiers are selected and you should keep that setting as long as you do not have a good reason to change it.

For LockIn measurements with multiple amplifiers, we have to add additional traces to our PGF sequence. For stimulation of the second amplifier, we add the DA output channel `Stim-2`, which is the stimulus output of the second amplifier board. Furthermore, we have to set the LockIn flag for that DA channel.

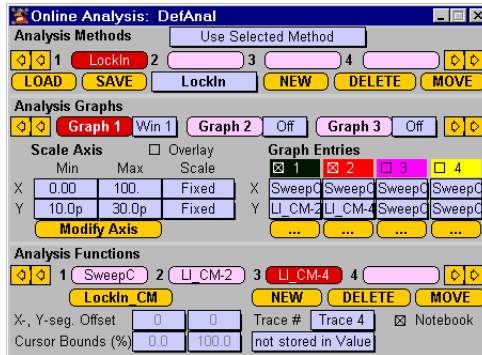
Channel 3 is set to `Imon-2`, the current input of the second amplifier board. Finally, channel 4 is set to `LockIn Cm`. Let's have a look on the Link

1	DA	Unit	Stimulus -> DA	Leak	AD	Y	Link	Compr.	Points	Store	Zero	Leak	
Ch-1	Stim-1	√	StimScale, LockIn	<input type="checkbox"/>	Imon-1	A	1	1	C	2000	<input type="checkbox"/>	0	No Leak
Ch-2	Stim-2	√	StimScale, LockIn	<input type="checkbox"/>	LockIn CM	F	1	25	C	80	<input type="checkbox"/>	0	No Leak
Ch-3	off	√	StimScale	<input type="checkbox"/>	Imon-2	A	2	1	C	2000	<input type="checkbox"/>	0	No Leak
Ch-4	off	√	StimScale	<input type="checkbox"/>	LockIn CM	F	3	25	C	80	<input type="checkbox"/>	0	No Leak

column:

1. Ch-1: AD channels are always linked to a DA output channel. Therefore Imon-1 is linked to the corresponding Stim-1 DA output.
2. Ch-2: LockIn channels are "virtual" traces. The data of a virtual trace does not depend on a DA output, but is calculated from another "AD" channel. This LockIn Cm channels uses Ch-1 as data input, therefore the Link is set to "1".
3. Ch-3: This current channel is linked to Stim-2, the stimulus DA output of the second amplifier.
4. Ch-4: Ch-4 is a "virtual" channel and therefore is linked to "its" current channel.

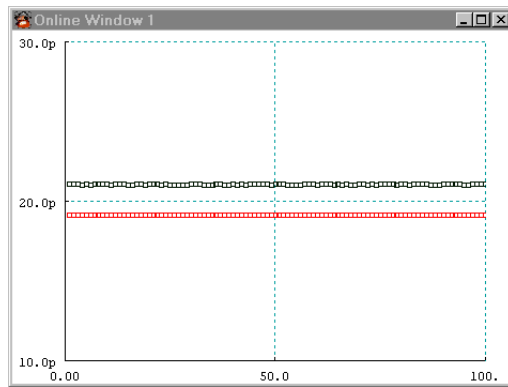
Of course, we want to monitor the measures  $C_m$  values in the Online Analysis. Therefore, we have to modify the Online Analysis settings. However, the required changes are quite moderate.



The  $C_m$  trace from the second amplifier is intended to be displayed in the same window. Therefore, we activate an additional Graph Entry for

Graph 1. The X-axis for that entry can also be set to Sweep Count, but for the Y-axis, we need a new Analysis Function. So, click on an empty entry in the Analysis Functions section and select LockIn\_Cm. It is now important to set the Trace # to "4", since the  $C_m$  trace from the second amplifier is Ch-4 in the PGF template.

Before starting the PGF sequence, you should make all required settings for both amplifiers as described in the section "Amplifier Settings" on page 7. Afterwards, you can run the protocol.



We used two model circuits with slightly different "membrane capacitances". One is slightly below, the other slightly above 20 pF. Because we knew about those values, we could set the scaling range in the Online Analysis dialog to the fixed values "10 p" and "30 p".

**Note:** If you want to enter fixed values for the X or Y scale, you should take care to enter "10 p" and not just "10", otherwise the display range will be set to 10 - 30 F, instead of 10 - 30 pF.