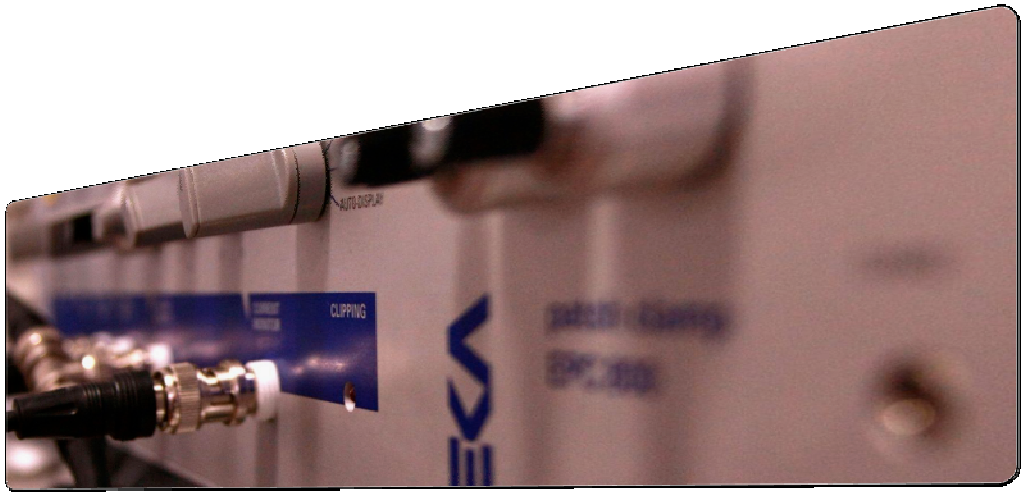

Combined Capacitance and Amperometry measurements

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Why this subject?

The selected project requires acquiring up to 5 channels:

- The current and voltage traces of two amplifiers
- The cell capacitance trace, computed online.

The acquisition templates must deal with the requirements of the experimental goal and the complex conditions for the capacitance computation.

These requirements allow to focus on some features in PATCHMASTER which help in handling such complex experiments, particularly:

- Trace Assignments,
- The Protocol Editor,
- Key Assignments.

Additionally, this project demonstrate the usefulness of the EPC10/Double when measuring multiple signals from multiple amplifiers.

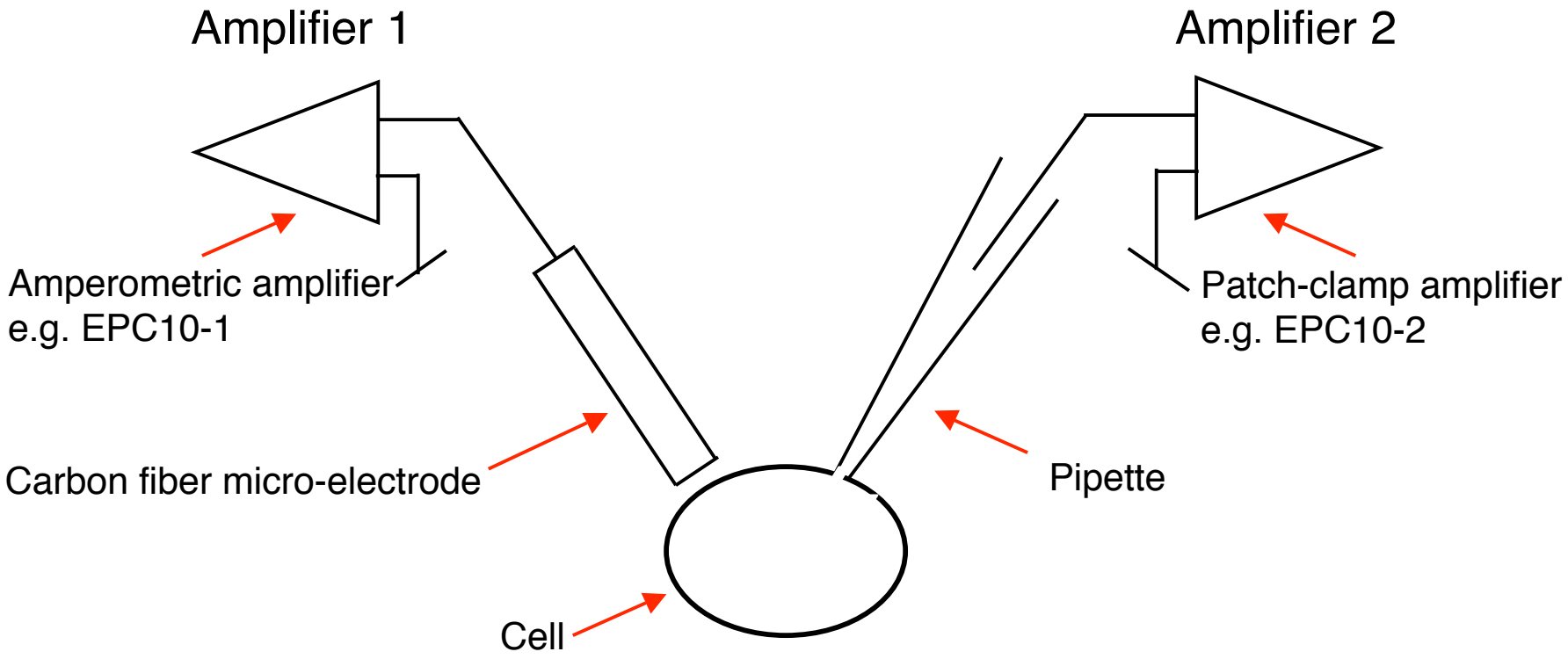
Goals

I will show how to perform combined measurements of amperometric currents and cell capacitance traces using PATCHMASTER.

I will guide you through:

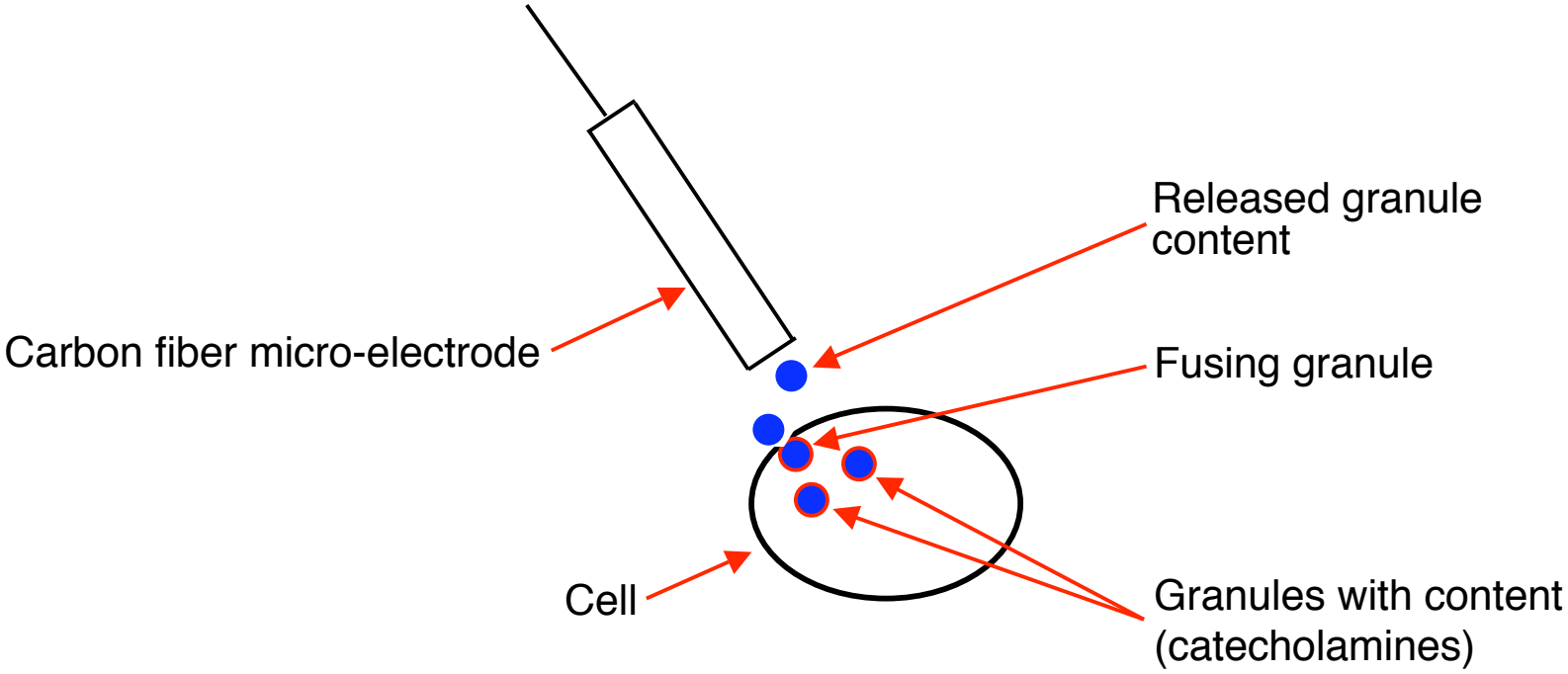
- Configuring PATCHMASTER for this task
- Defining the trace assignments
- Defining the required stimuli (PGF templates)
- Preparing event protocols to harness the complexity of these measurements

Experimental Setup

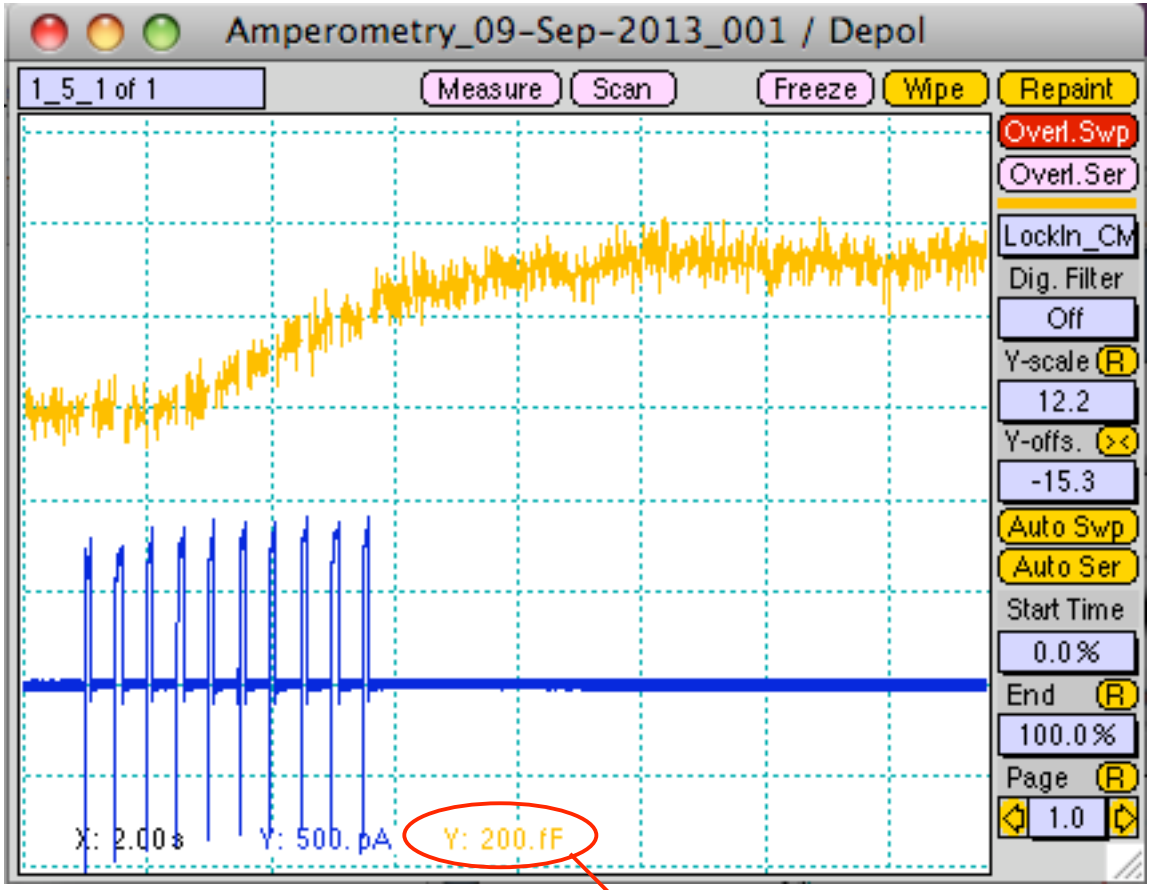


Schematic of exocytosis

Granule fusion and granule content release:
- Fusion leads to increase in membrane capacitance (CM).
- Released granule content gets oxidized, when it reaches the electrode.



Example recording



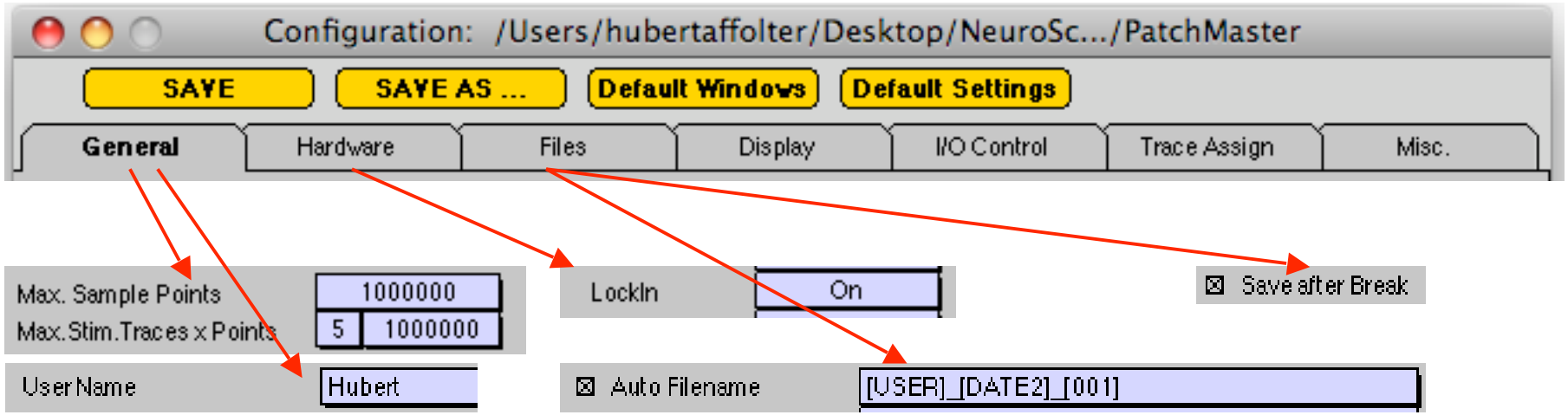
Cell capacitance →

Cell current →

200 fF => very high resolution!

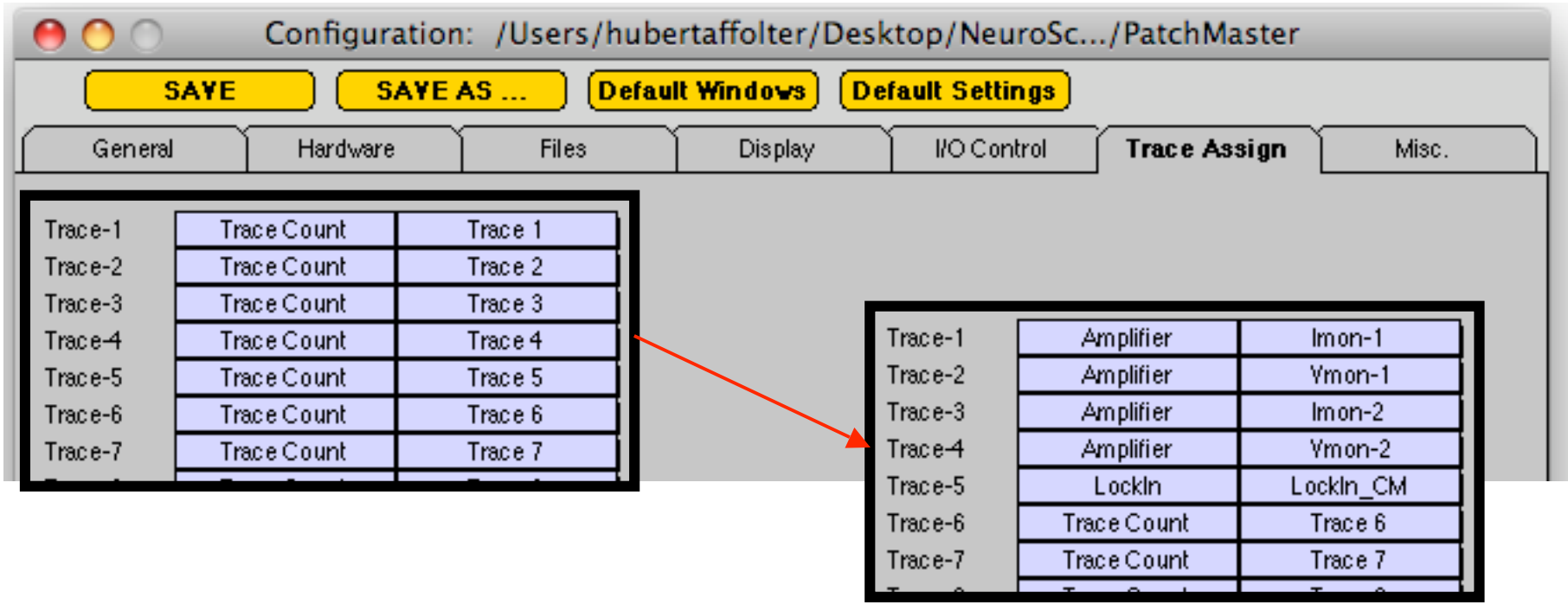
Data are from recordings acquired during the course:
Microelectrode Techniques For Cell Physiology
30th Workshop 4-18 September 2013, Plymouth, UK

Configuration Settings



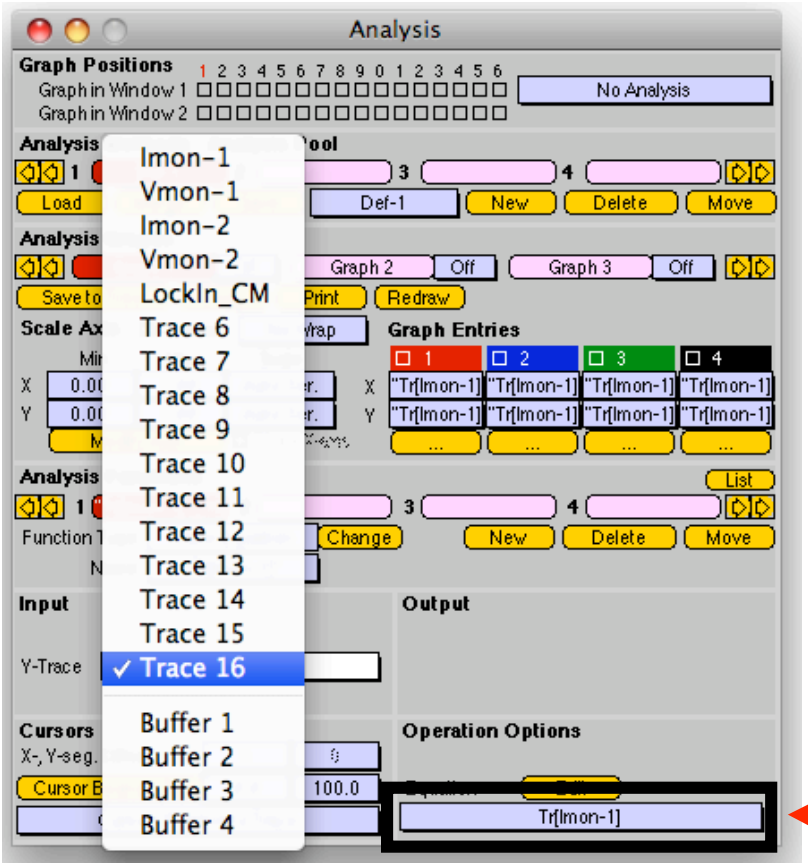
- Increase Max Sample Points: Maximal number of samples in acquired trace
- Increase Max Stim Points: Maximal number of samples in stimulus / channel
- Activate LockIn support and computation
- Activate storing partially acquired traces
- Automatic filename generation
- User name

Configuration Settings: Trace Assignments

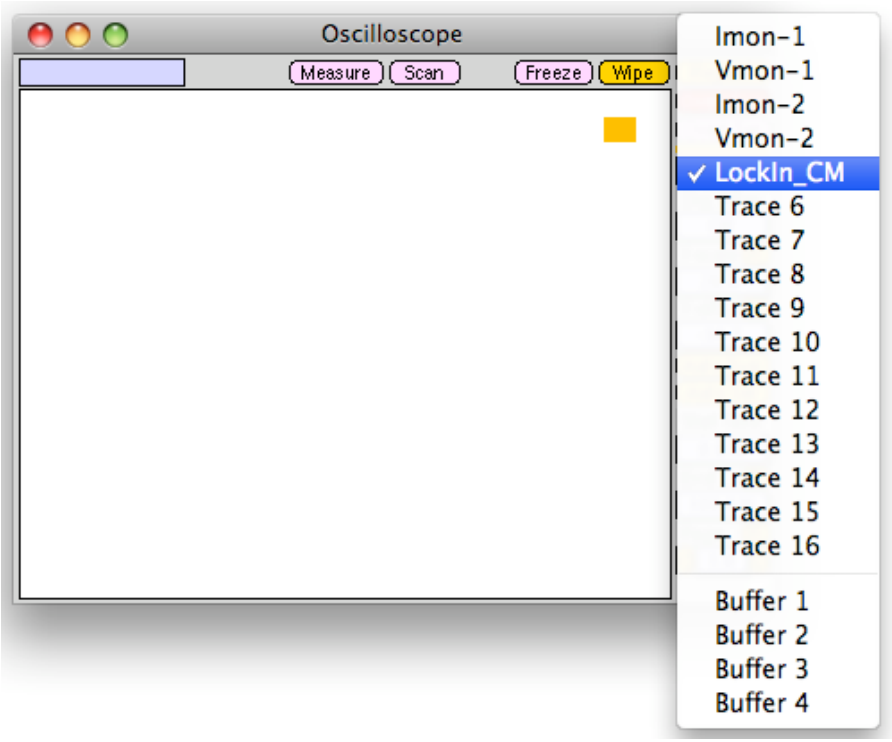


Assign inputs from selected sources to fixed traces, i.e., PATCHMASTER will assign traces (in lists, equations, etc.) to a named, fixed position.
Here, assignments are made for both amplifier inputs plus the cell capacitance trace.

Resulting selection lists when using trace assignments



Equations !



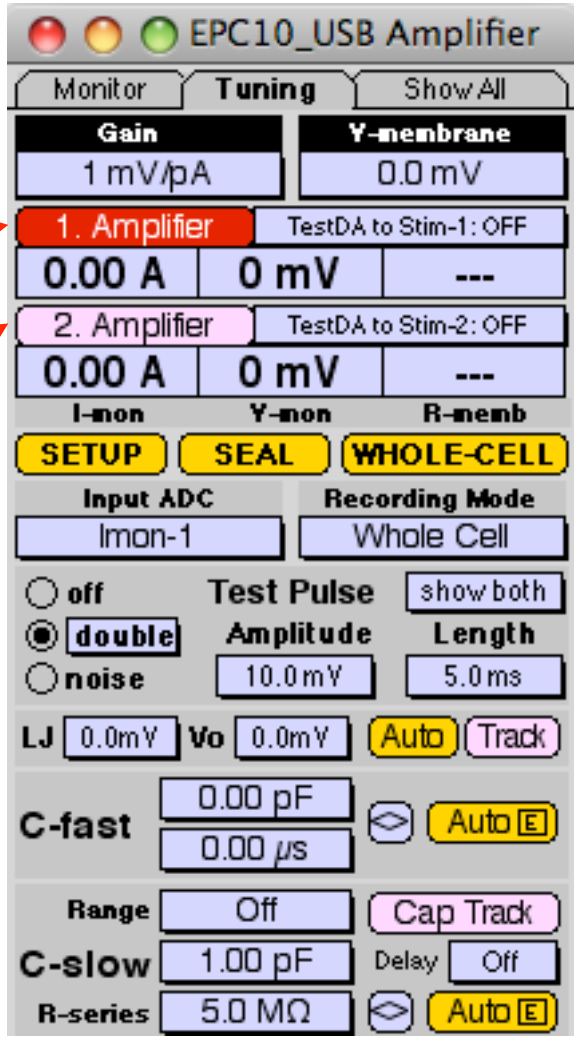
Amplifier requirement

2 amplifier boards:

Amplifier for amperometry:
- Applying the controlling voltage
- Measuring current of amperometry electrode

Amplifier for patch-clamping:
-Applying clamping voltage and current
- Measuring cell voltage and current
- Measuring capacitance: derived from cell current

- Here, an EPC10/USB Double is used.



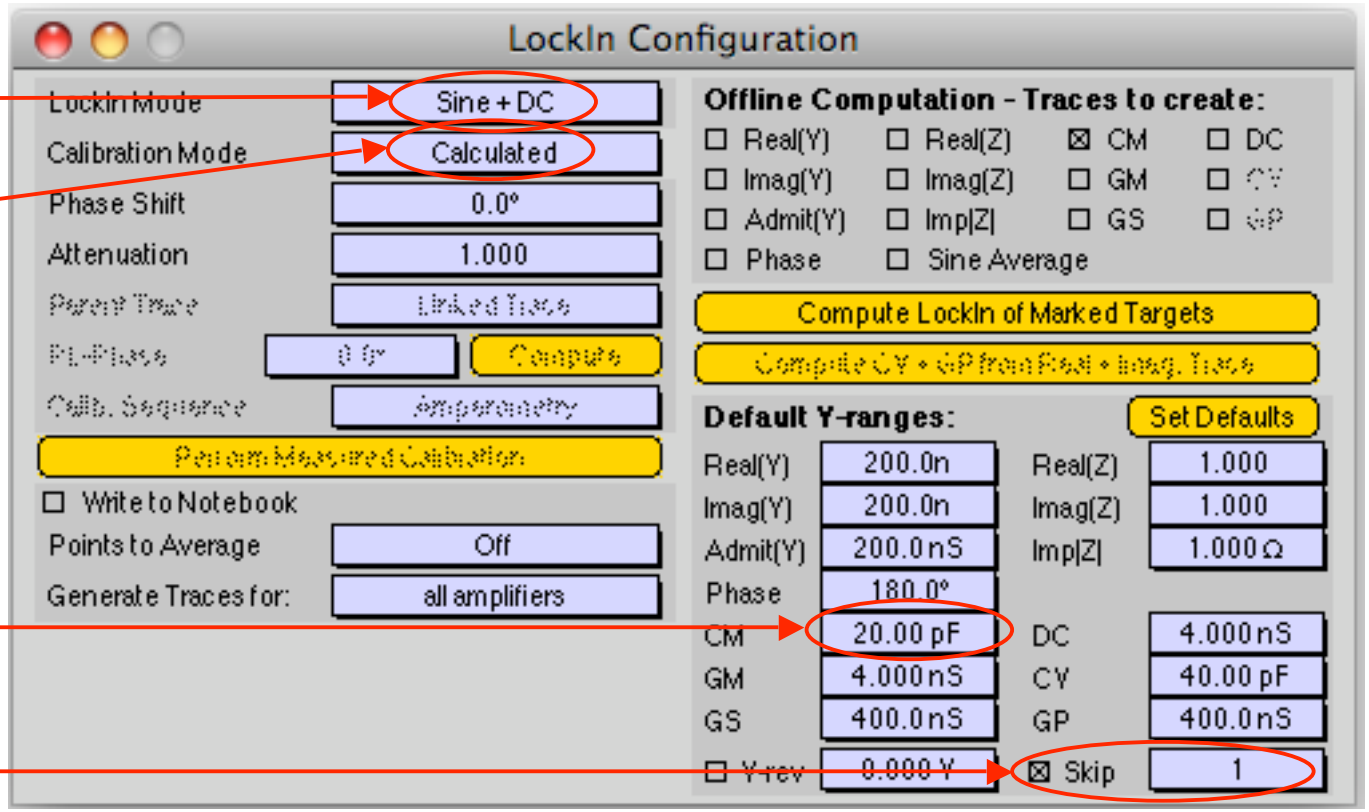
Defining basic LockIn parameters

LockIn computation mode

Calibration mode

Expected cell size

Cycles to skip

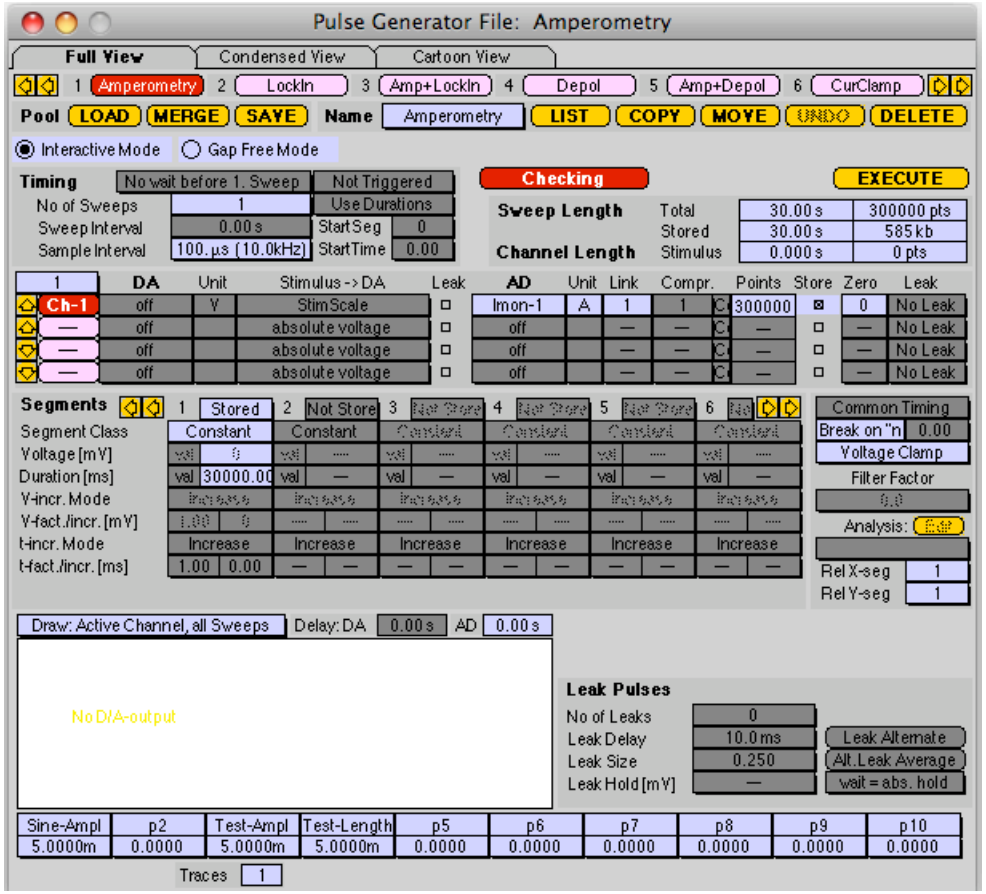


Amperometric measurements

Amperometric measurements were performed by applying a constant voltage of 650mV on the carbon fiber micro-electrode.

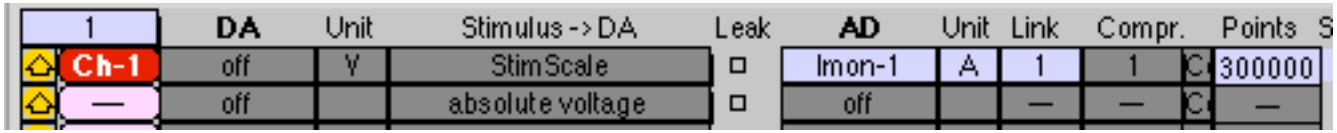
Prepared Stimuli

7 stimulation templates

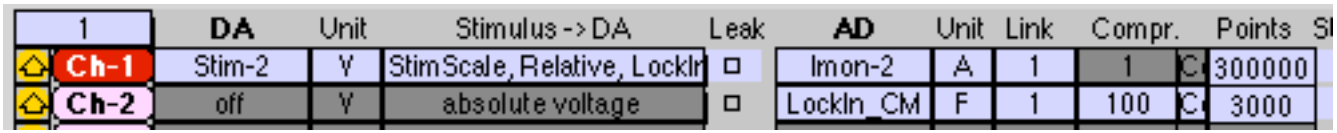


The 7 stimulation templates

1. “Amperometry”
- Used to monitor the current of the amperometric electrode.
- 1 segment: acquire and display 30s overview.
- No stimulation: Amperometry and cell potentials remain unchanged from the amplifier holding potentials.
- Acquire just one trace: The amperometric current



2. “LockIn”
- Used to monitor the cell capacitance.
- 1 segment: acquire and display 30s overview.
- Stimulation: Apply the required sinewaves relatively to the amplifier holding potential.
- Acquire 2 traces:
-- The cell current.
-- The cell membrane capacitance derived from the cell current.



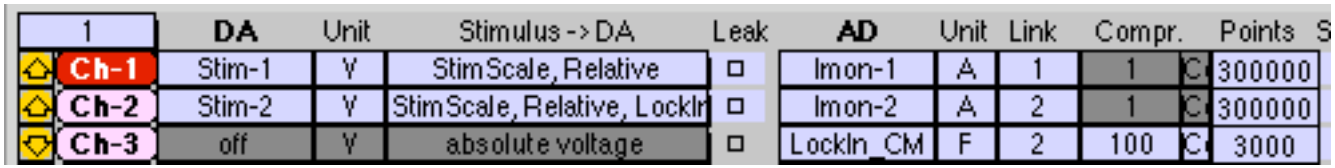
1	DA	Unit	Stimulus -> DA	Leak	AD	Unit	Link	Compr.	Points
Ch-1	Stim-2	V	StimScale, Relative, LockIn	□	Imon-2	A	1	1	300000
Ch-2	off	V	absolute voltage	□	LockIn_CM	F	1	100	3000

3. “Amp+LockIn”
- Used to monitor:
 -- Current of the amperometry electrode
 -- Cell current
 -- Cell membrane capacitance derived from cell current

- 1 segment: acquire and display 30s overview.

- Stimulate 2 outputs:
-- Apply voltage relatively to the amplifier amperometric potential.
-- Apply the required sinewaves relatively to the amplifier holding potential.

- Acquire 3 traces:
-- The amperometric current.
-- The cell current.
-- The cell membrane capacitance derived from the cell current.



1	DA	Unit	Stimulus -> DA	Leak	AD	Unit	Link	Compr.		Points	S
Ch-1	Stim-1	V	StimScale, Relative	□	Imon-1	A	1	1	C	300000	
Ch-2	Stim-2	V	StimScale, Relative, LockIn	□	Imon-2	A	2	1	C	300000	
Ch-3	off	V	absolute voltage	□	LockIn_CM	F	2	100	C	3000	

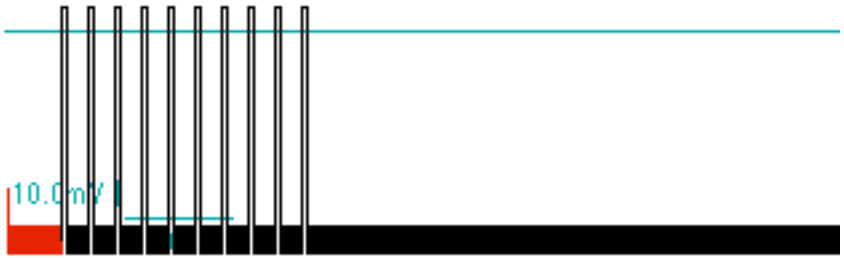
4. “Depol”

- Depolarizes the cell 10 times and monitors the evoked cell current response and the incremental change in cell capacitance.

- 22 segments

- Stimulate on one Stim-Dac:
-- Apply the 10 depolarizing voltage pulses.
-- Apply the required sinewaves. Note that no sinewave is applied while the cell is stimulated!

- Acquire 2 AD-traces:
-- The amperometric current.
-- The cell current.



1	DA	Unit	Stimulus -> DA	Leak	AD	Unit	Link	Compr.	Points
Ch-1	Stim-2	V	StimScale, LockIn	□	Imon-2	A	1	1	156100
Ch-2	off	V	absolute voltage	□	LockIn_CM	F	1	100	1561

5. “Amp+Depol”
- Same as “Depol”. Additionally, acquire the current of the amperometric electrode as well.

1	DA	Unit	Stimulus -> DA	Leak	AD	Unit	Link	Compr.		Points	S
Ch-1	Stim-2	V	StimScale, LockIn	□	Imon-2	A	1	1	C	91000	
Ch-2	off	V	absolute voltage	□	LockIn_CM	F	1	100	C	910	
Ch-3	off	V	absolute voltage	□	Imon-1	A	1	1	C	91000	
—	off		absolute voltage	□	off		—	—	C	—	

6. “CurClamp”
- Used to monitor the settling of the cell potential after switching to current clamp.
- 1 segment: acquire and display 30s overview.
- No stimulation: leave holding potentials/current of the amplifiers unaffected.
- Acquire 1 trace: The cell voltage.

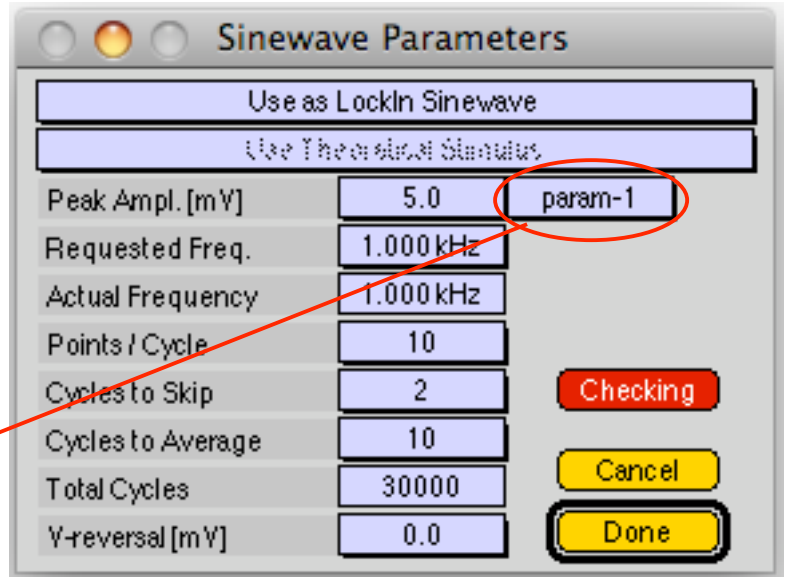
7. “cc+stim”

- Find the extend of current injection required to evoke an action potential in current clamp mode.
- 3 segments
- Stimulate on one Stim-Dac:
-- Start at the given current injection amplitude (here 5pA),
-- Increment the current injection by 2pA after each sweep,
-- Repeat for a total of 10 sweeps.
- Acquire 2 AD-traces:
-- The cell voltage.
-- The cell current.

1	DA	Unit	Stimulus -> DA	Leak	AD	Unit	Link	Compr.	Points
Ch-1	Stim-2	A	StimScale	□	Vmon-2	V	1	1	2200
Ch-2	off	V	absolute voltage	□	Imon-1	A	1	1	2200

The parameters for the LockIn stimulation

The parameters are defined in the “Sinewave” window of each PGF-stimulus. In this collection, all sinewave amplitudes were defined via one of the PGF-parameters visible at the bottom of the PGF-window. This allows to consistently change the sinewave amplitudes of all stimuli from one central place.

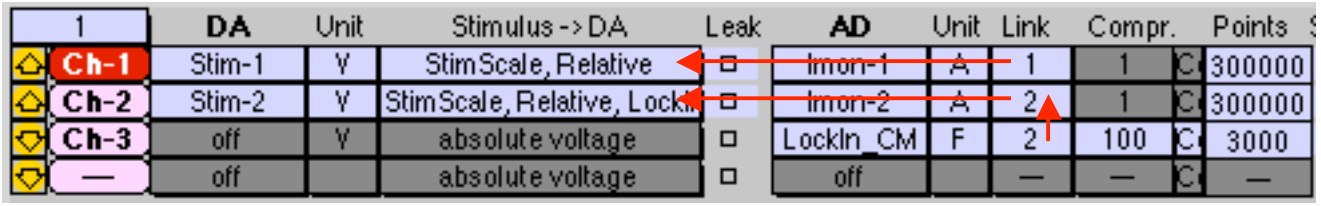


Sine-Ampl	p2	Test-Ampl	Test-Length	p5	p6	p7	p8	p9	p10
5.0000m	0.0000	5.0000m	5.0000m	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Configuring a PGF-Template for the LockIn stimulation and computation

The cell current AD-channels are linked to their respective stimulus DA-channels:
- Imon-1 originates from Stim-1 => AD-Link = 1
- Imon-2 originates from Stim-2 => AD-Link = 2

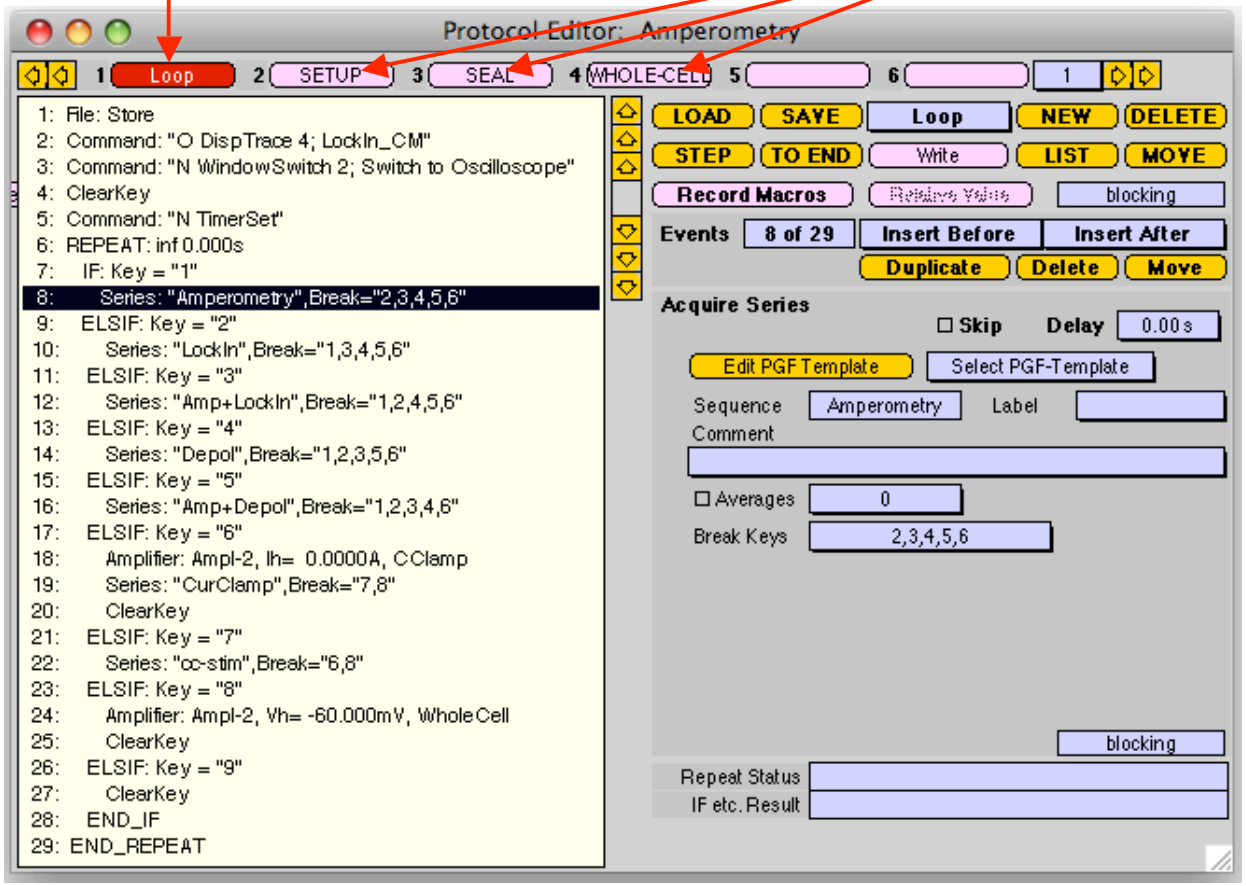
The capacitance AD-channel is linked to its parent cell current AD channel:
-LockIn_CM originates from Imon-2 => AD-Link = 2
That linked to AD-channel points to the stimulus with the sinewave stimulation.



Prepared Event Protocols

1 “main” experimental protocol loop

3 amplifier protocols



The main experimental protocol:
1. Set the initial conditions, then
2. Loop till experiment is terminated.

The single protocol events:

- File Store -> Activate storing; create new file, if none is opened for writing.
- Command "O DispTrace 4; LockIn_CM" -> Make the LockIn_CM trace the selected trace in the oscilloscope.

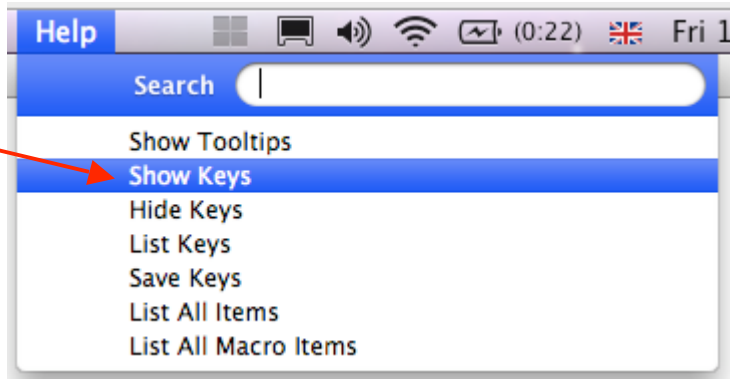
3. Command "N WindowSwitch 2; Switch to Oscilloscope"
4. ClearKey -> Clear the key buffer.
5. Command "N TimerSet" -> Reset the timer.
6. REPEAT -> Start the main repeat loop = Repeat until stopped.
7. IF Key = "1"
8. Series "Amperometry" -> Acquire the amperometric current in vc-mode.
9. ELSIF Key = "2"
10. Series "LockIn" -> Acquire the cell capacitance.
11. ELSIF Key = "3"
12. Series "Amp+LockIn" -> Acquire amperometry and cell capacitance.

```
13.    ELSIF Key = "4"
14.      Series ”Depol”          -> Depolarize the cell, acquire cell current and capacitance.
15.    ELSIF Key = "5”
16.      Series "Amp+Depol”  -> Depolarize cell, acquire amperometry, current, and CM.
17.    ELSIF Key = "6"
18.      Amplifier Ampl-2, Ih=  0.0000A, Cclamp        -> Set current clamp mode.
19.      Series ”CurClamp”    -> Let the cell establish its resting potential.
20.      ClearKey                -> No active key = Suspend acquiring
21.    ELSIF Key = "7"
22.      Series ”cc-stim”        -> Find the injection current which evokes an action potential.
23.    ELSIF Key = “8”
24.      Amplifier Ampl-2, Vh= -60.000mV, WholeCell -> Set voltage clamp mode.
25.      ClearKey                -> No active key = Suspend acquiring
26.    ELSIF Key = "9"
27.      ClearKey                -> No active key = Suspend acquiring
28.    END_IF
29.  END_REPEAT
```

Additional keys controlling program flow

Keys: “b” for break and “s” for stop

Items in the Help menu can be used to show or hide “key” assignments.
Key assignments are stored in and retrieved from the “PatchMaster.key" file.



Notes

Multiple “Break keys” during acquisition: Available only in version 2x80.
“Break key” during acquisition in version 2.73 and earlier: “N” for “Next”.

Centering LockIn_CM trace: Use the “<>” function in the Oscilloscope window.
Its default key assignment is key “numeric*”.

Centering is very important because:
- The cell capacitance (example on slide 6) is about 13pF.
- Expected changes are in the range of 0.1pF, i.e. less than 1%.
- To see such a small change, the trace must be displayed heavily amplified.
- This most often causes the signal ending off-screen.
- Centering brings the signal back into vision.