

# Real-time monitoring of membrane capacitance in *Xenopus* oocytes with PULSE and X-CHART

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Membrane Capacitance ( $C_m$ ) correlates closely with membrane surface area and is therefore exploited widely to follow plasma membrane dynamics. For instance,  $C_m$  measurements in secretory cells allow the resolution of exocytotic events on the level of single vesicles and a millisecond time scale. Herein, information about  $C_m$  is computed from the current response to a sine-wave voltage stimulus (i.e., „impedance“ or „admittance analysis“, a „frequency-domain“ approach).

In large cells such as the widely used *Xenopus* oocytes that are mostly studied using the two-electrode voltage clamp (TEVC) technique, this approach does not work as nicely, due to fundamental physical givens. Several approaches to  $C_m$  measurements via TEVC have been described that employ other stimuli such as ramps, saw-tooth or steps. These „time-domain“ methods lacked the appeal and power of its frequency-domain counterpart, being relatively cumbersome, slow, and coarse. On the other hand, the time-domain approaches were still far from their theoretical limits, suggesting considerable room for improvement.

We therefore tried to make  $C_m$  measurement via TEVC simpler and better. First, we modified the stimulus and algorithm used to compute  $C_m$ , the so-called „paired ramps“ approach (Fig. 1). Second, we used PULSE and X-CHART to implement the paired ramps. The combination of these two software packages permits to do two things simultaneously that seem to be mutually exclusive: On the one hand, run fast stimuli and extract two crucial parameters from the current trace with a high repetition rate, on the other hand, simultaneously translate these parameters into plain  $C_m$  values, collect and display them online over hours. Technical details of our method, the results of extensive performance testing, and background information can be found in a recent article in the Biophysical Journal (see below).

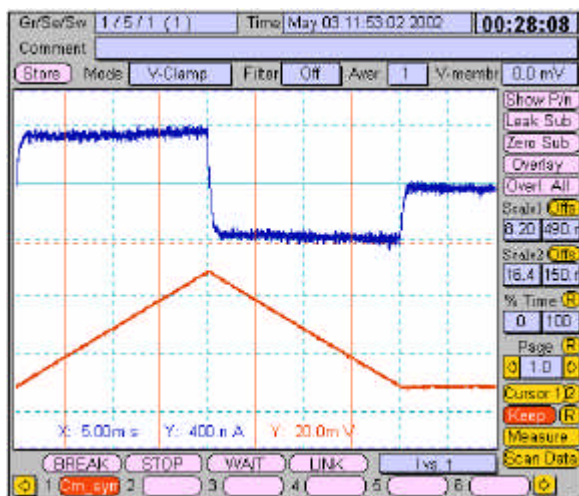


Figure 1:

$C_m$  measurement using paired ramps (screenshot oscilloscope window). In the two-electrode voltage-clamp mode, a specific stimulus („paired voltage ramps“, brown trace) is applied to a *Xenopus* oocyte. From the observed current response (blue trace), the „online analysis“ function of PULSE determines two current integrals (time windows indicated by red vertical lines). These integrals correspond to capacitive charges  $Q_A$  and  $Q_B$  that allow one, together with the known height  $V$  of the ramp stimulus, to calculate membrane capacitance according to  $C_m = (Q_A - Q_B) / 2V$ .

Implementing  $C_m$  measurements for TEVC boils down to installing a few short files with the proper instructions for PULSE and X-CHART; no extra hardware and no alteration of the recording arrangement are required (Fig. 2). At the push of a menu button,  $C_m$  can be measured with high time resolution, precision and accuracy. In practice,  $C_m$  changes of 0.5 nF are resolved routinely at several Hertz. Both values can be improved further for either speed or precision. The test stimuli per se are not a challenge to voltage clamp amplifiers. As always with TEVC, however, large currents may compromise clamp fidelity and thus also  $C_m$  measurements. Possible countermeasures include maximizing proportional gain, use of amplifiers that afford integral feedback, and series resistance compensation.

