NEU503 Lecture

Instrumentation

for Electrophysiological Studies of Excitable Tissues

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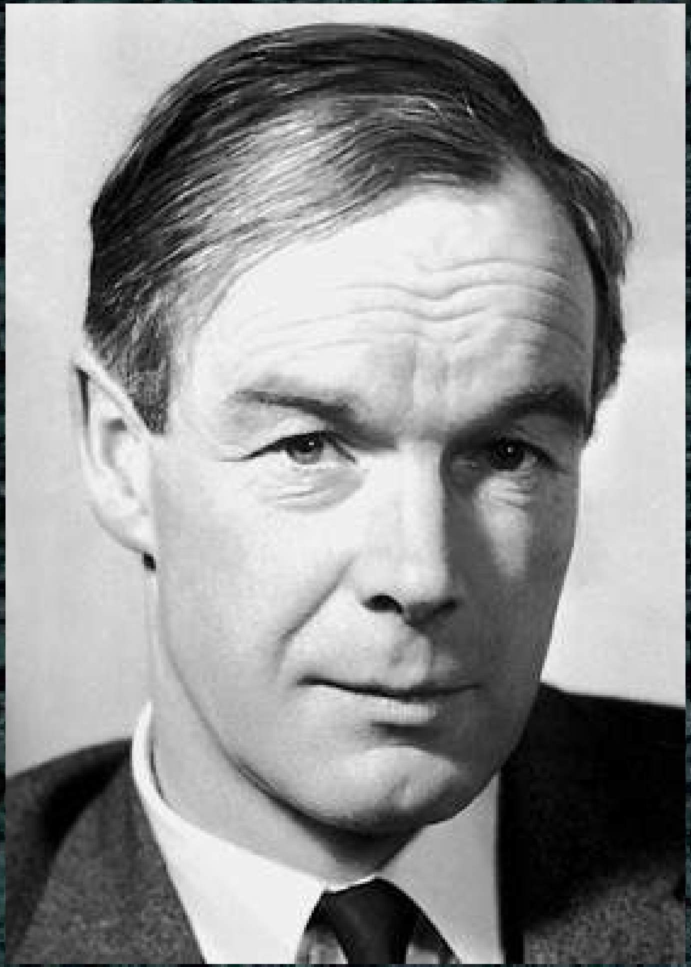
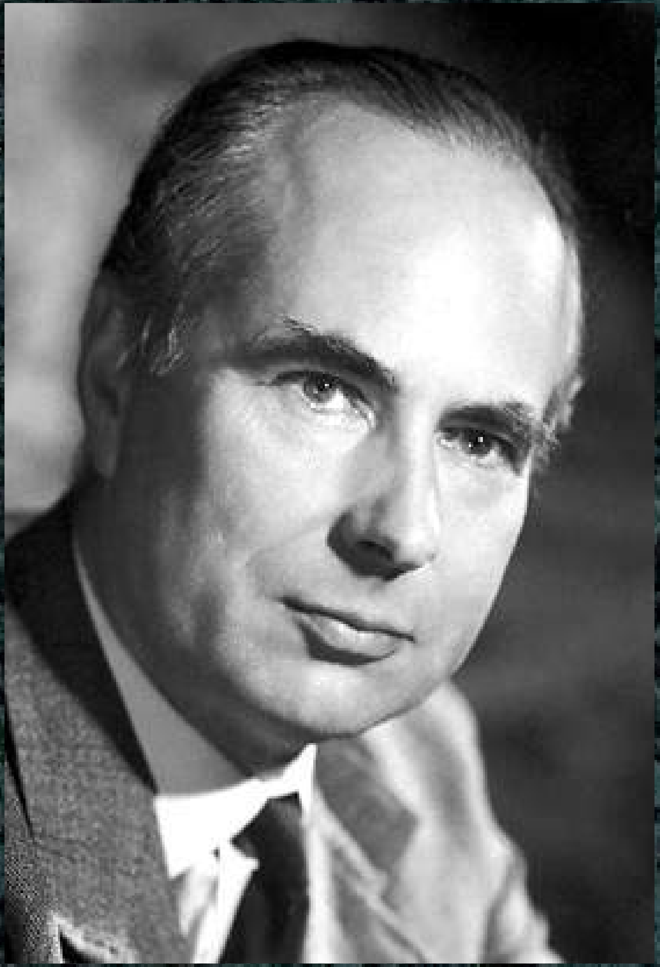
University of Rhode Island



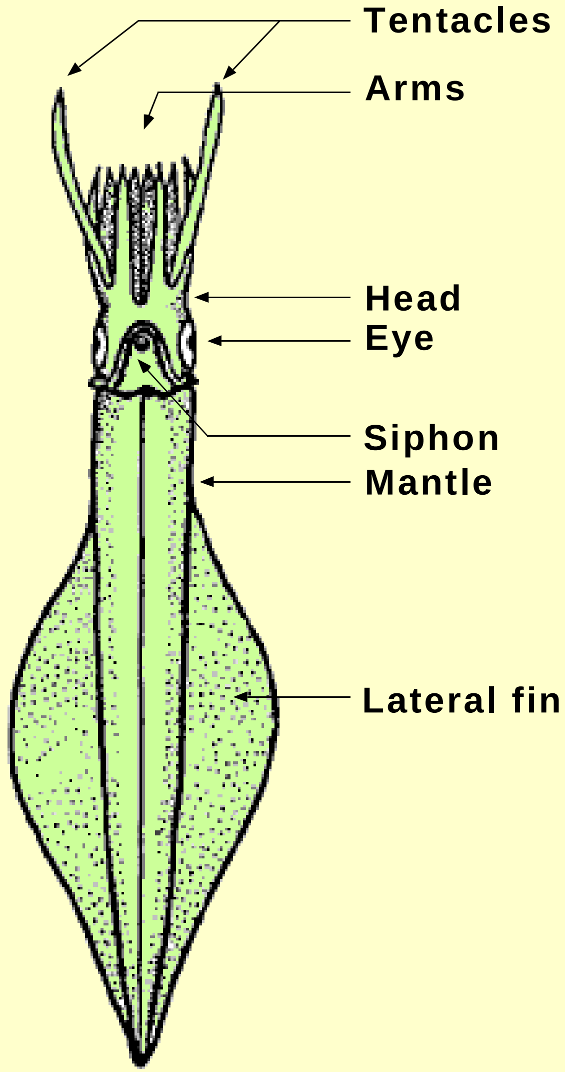


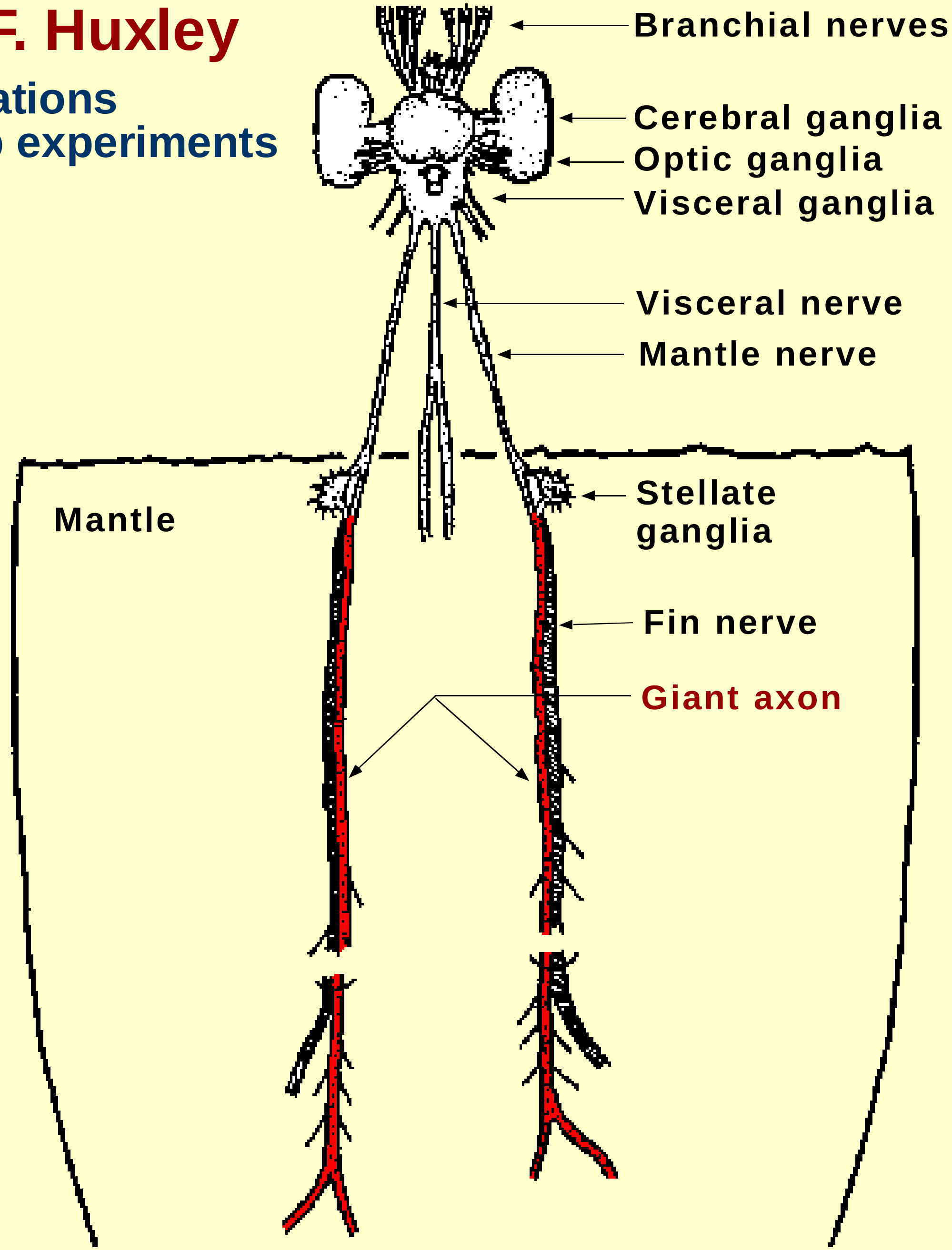
Allen L. Hodgkin
1914-1998

Andrew Huxley
1917-2012

A. L. Hodgkin and A. F. Huxley

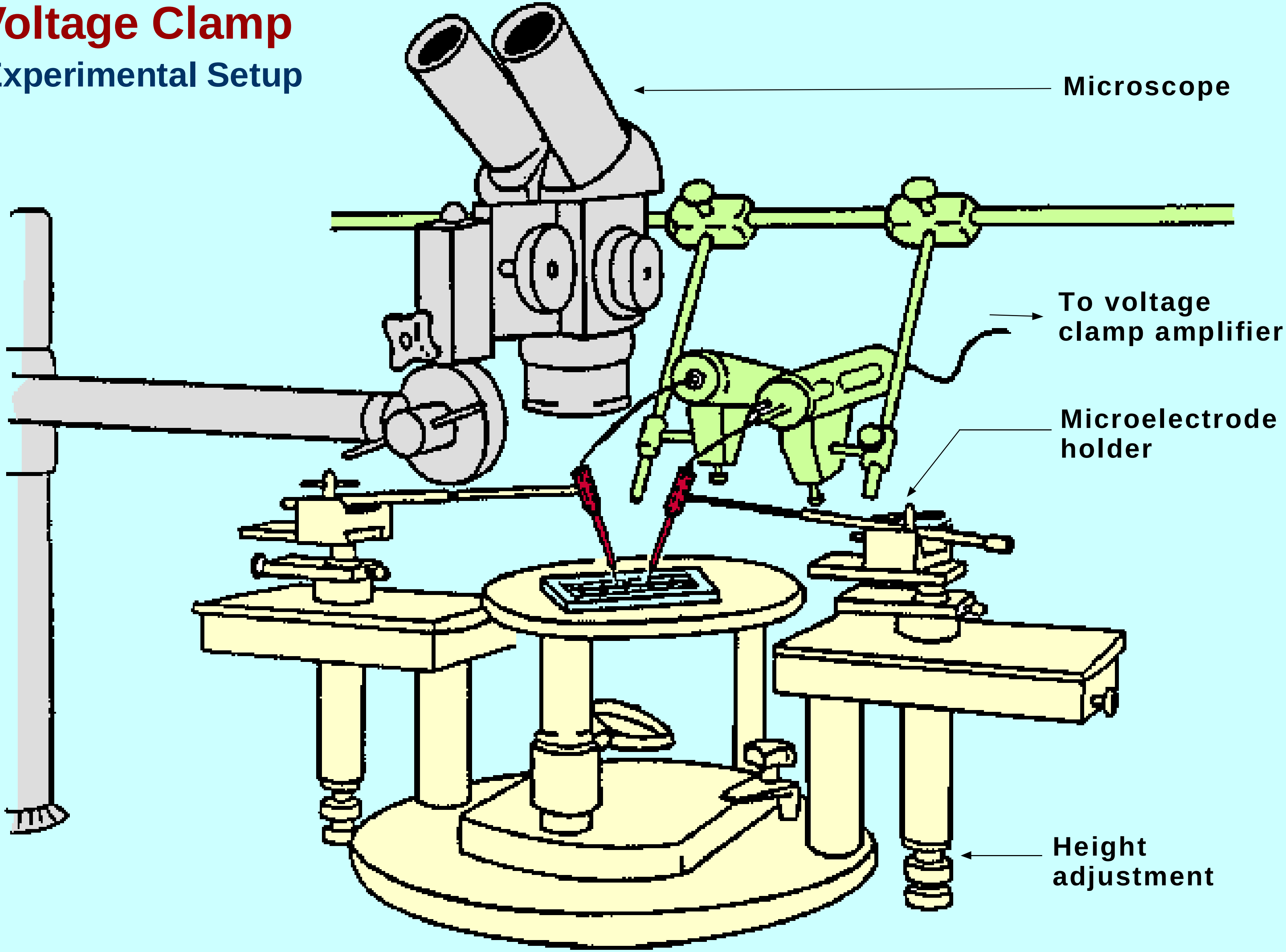
Neuronal current-voltage relations determined by voltage clamp experiments in the squid giant axon, 1952





Voltage Clamp

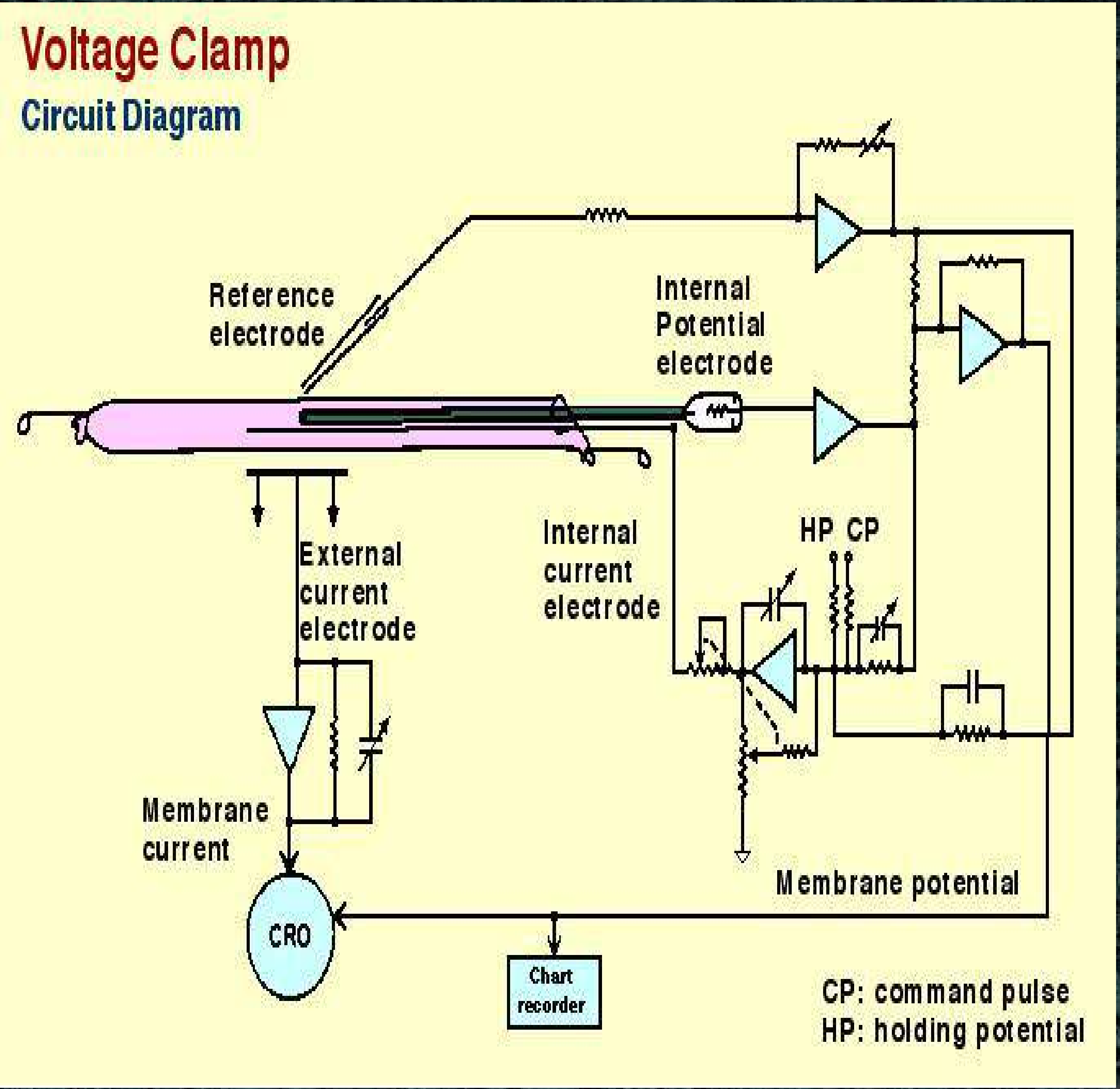
Experimental Setup



Neuroscience

Voltage Clamp

- Invented by G. Marmont & K. Cole (independently) in 1949.
- Used by A. L. Hodgkin & A. F. Huxley in 1952 to identify the ionic currents responsible for the action potentials in the squid giant axon (Nobel Prize 1963).



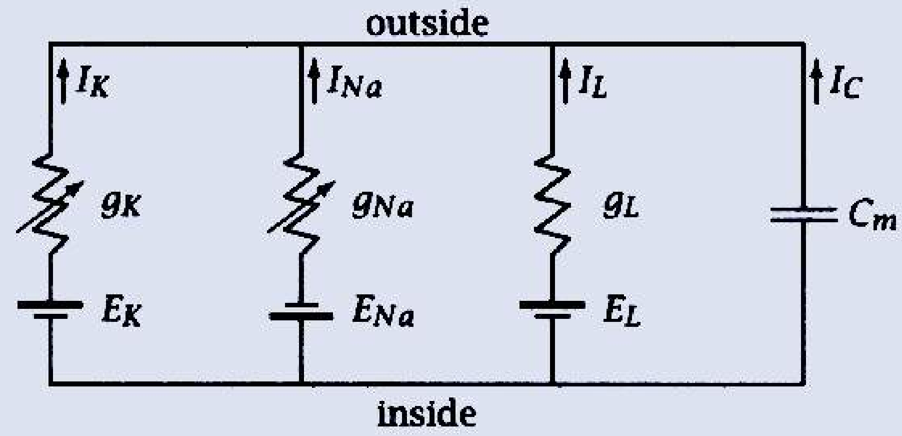
Hodgkin-Huxley Equations

$$C_m \frac{dV}{dt} = -g_L\left(V - V_L\right) - \bar{g}_{Na} m^3 h\left(V - V_{Na}\right) - \bar{g}_K n^4\left(V - V_K\right)$$

$$\frac{dm}{dt} = \alpha_m\left(V\right)\left(1 - m\right) - \beta_m\left(V\right)m$$

$$\frac{dh}{dt} = \alpha_h\left(V\right)\left(1 - h\right) - \beta_h\left(V\right)h$$

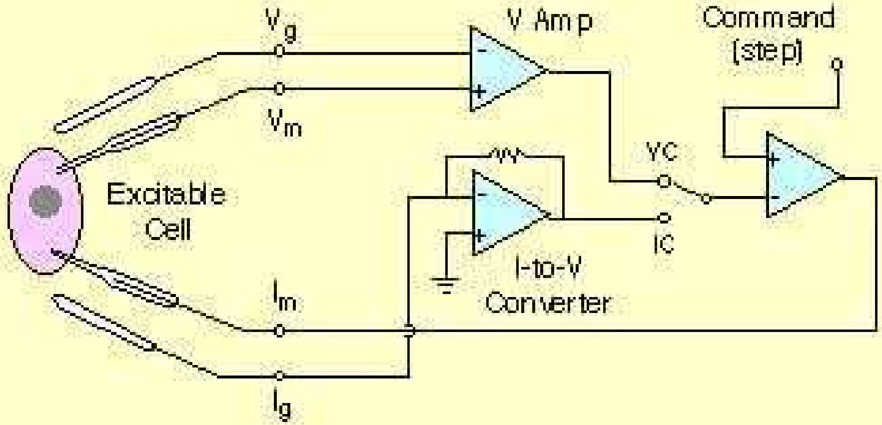
$$\frac{dn}{dt} = \alpha_n\left(V\right)\left(1 - n\right) - \beta_n\left(V\right)n$$



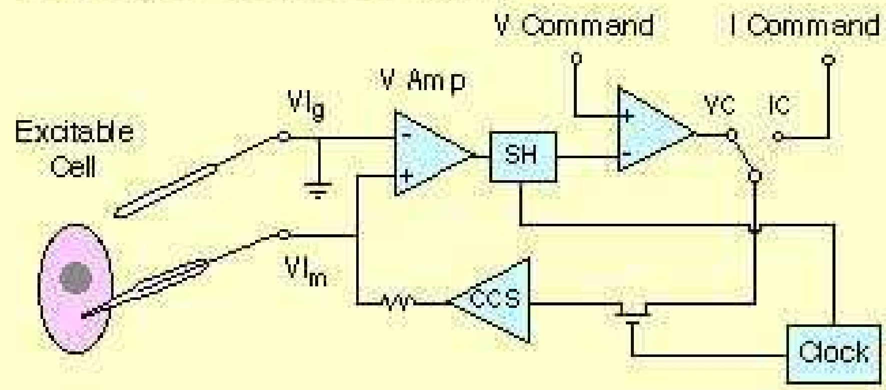
a set of 4th-order nonlinear time-varying differential equations

Variations of Voltage Clamp

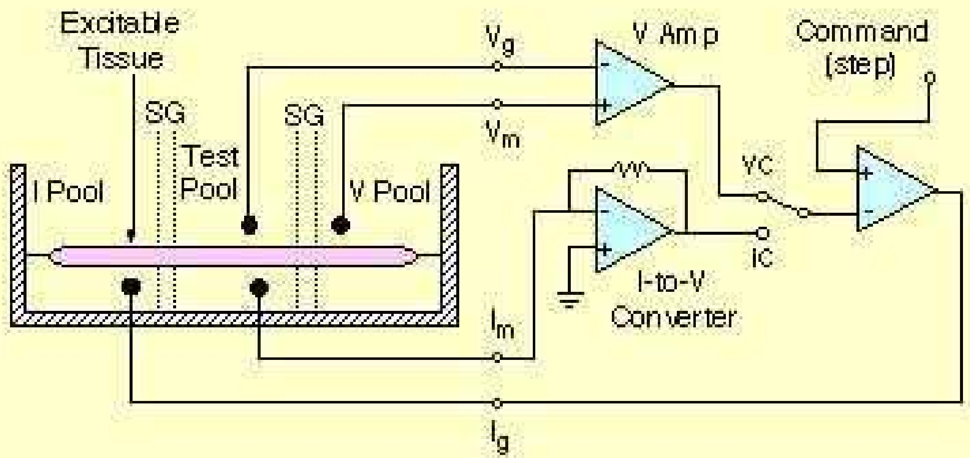
A. Two-Electrode Clamp



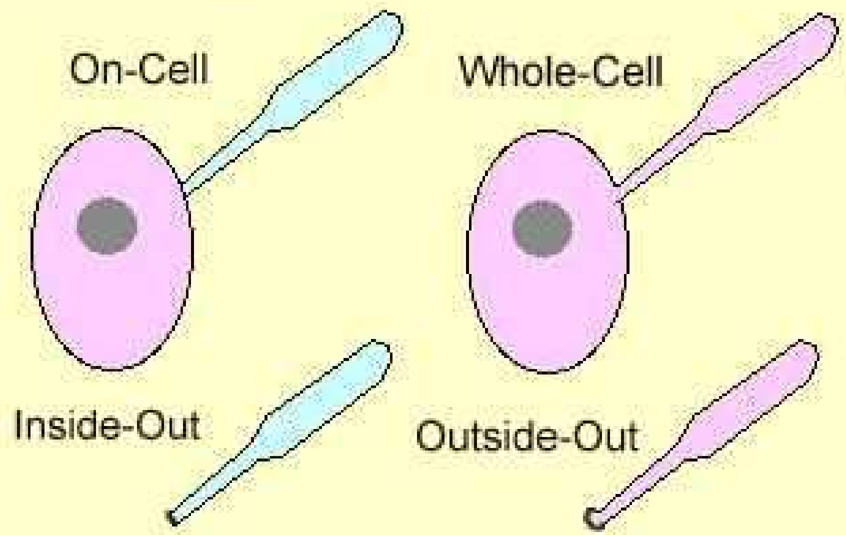
B. Single-Electrode Clamp



C. Double-Sucrose-Gap Clamp

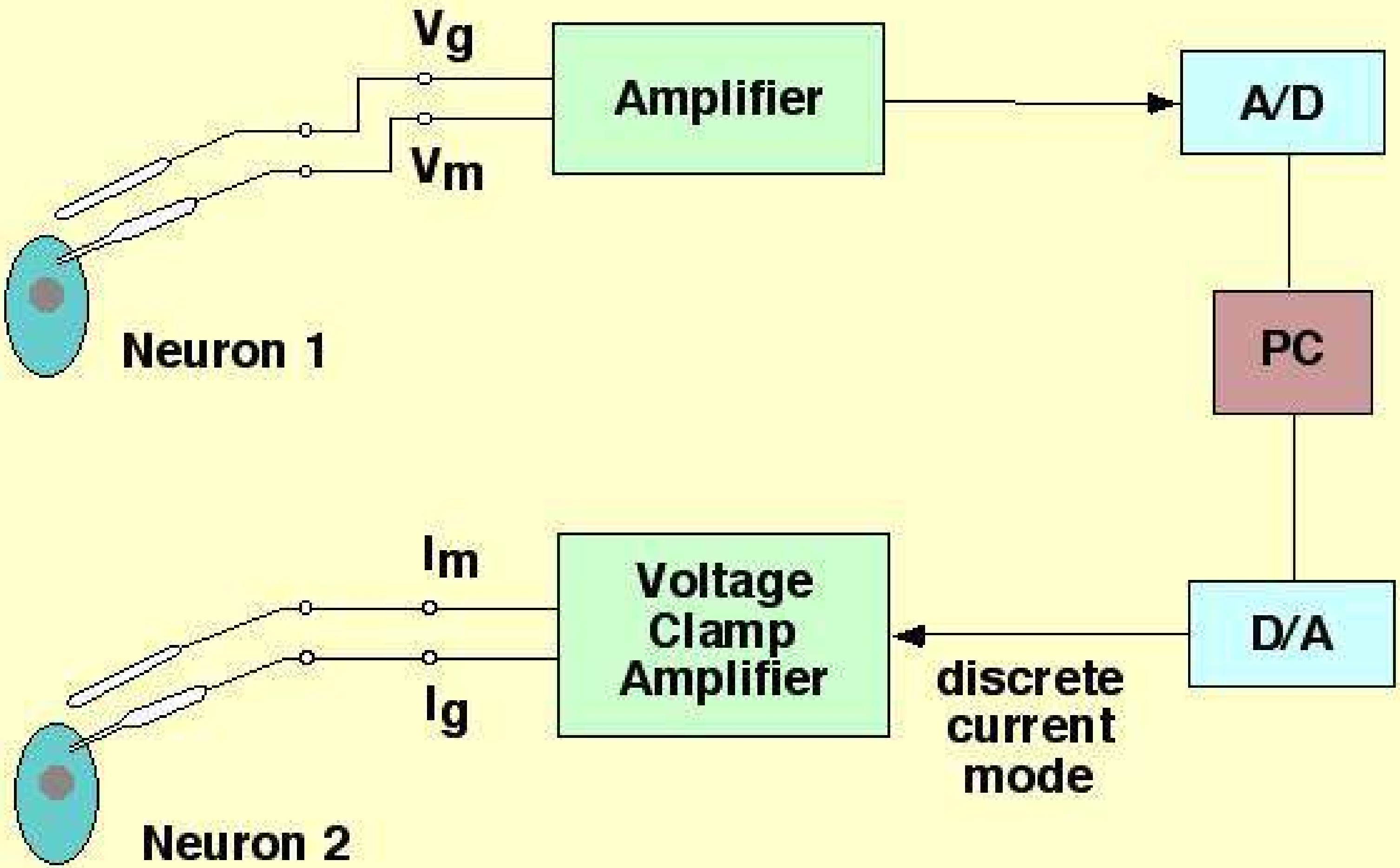


D. Patch Clamp

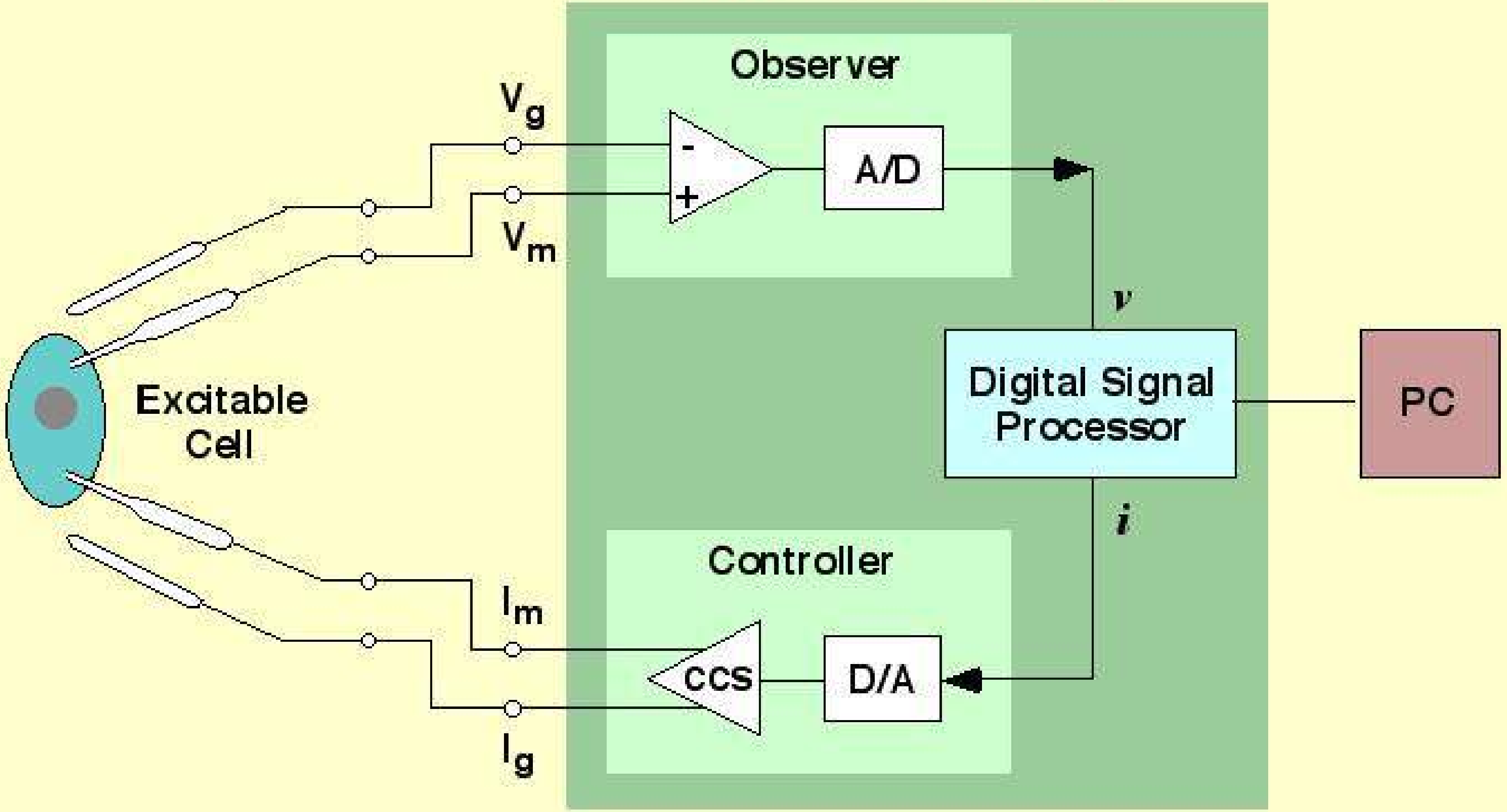


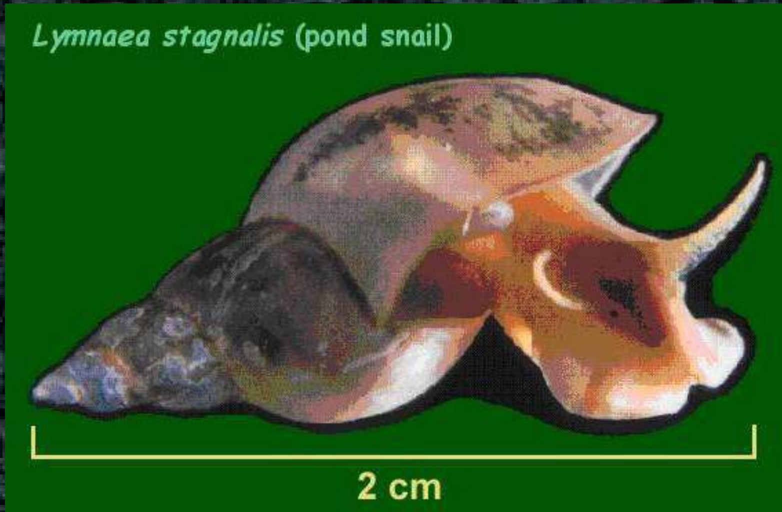
Dynamic Clamp - an Artificial Synapse

Robinson 1993
Sharp et al. 1993



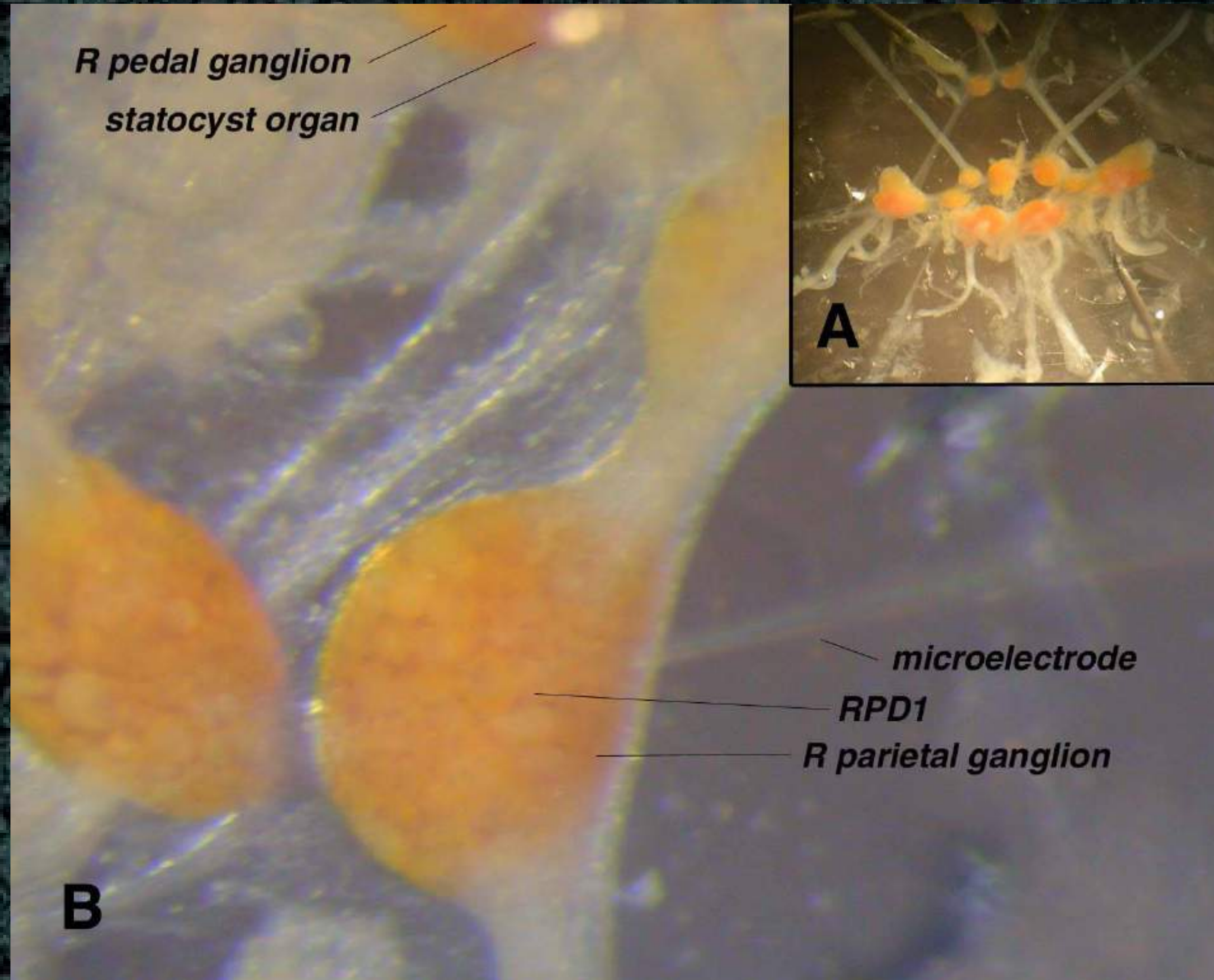
Universal Clamp

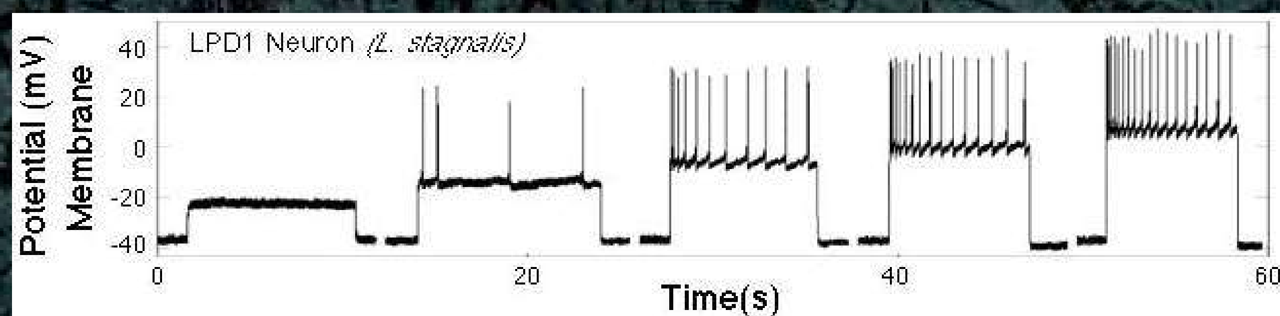




Electrophysiology

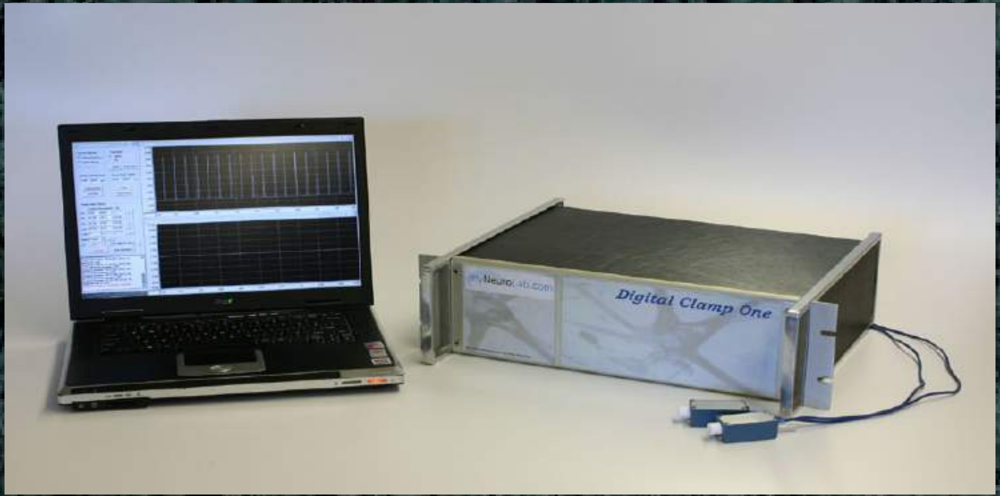
Microelectrode experiments with *Lymnaea stagnalis* (pond snail)

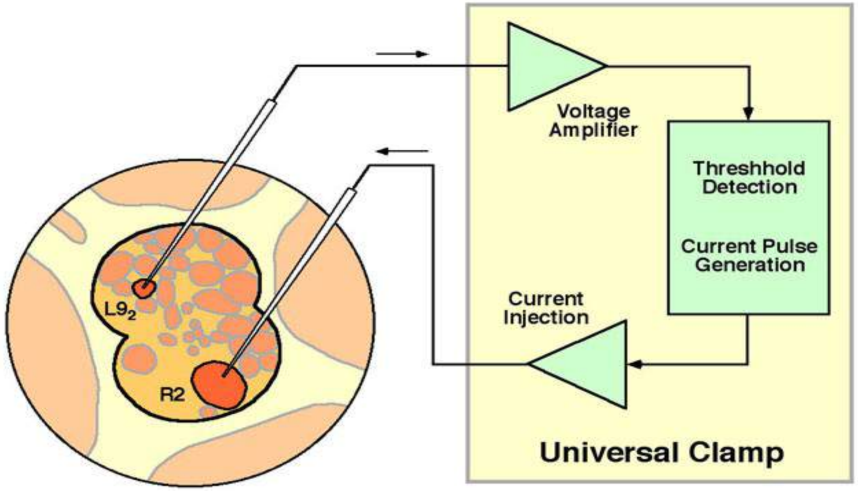


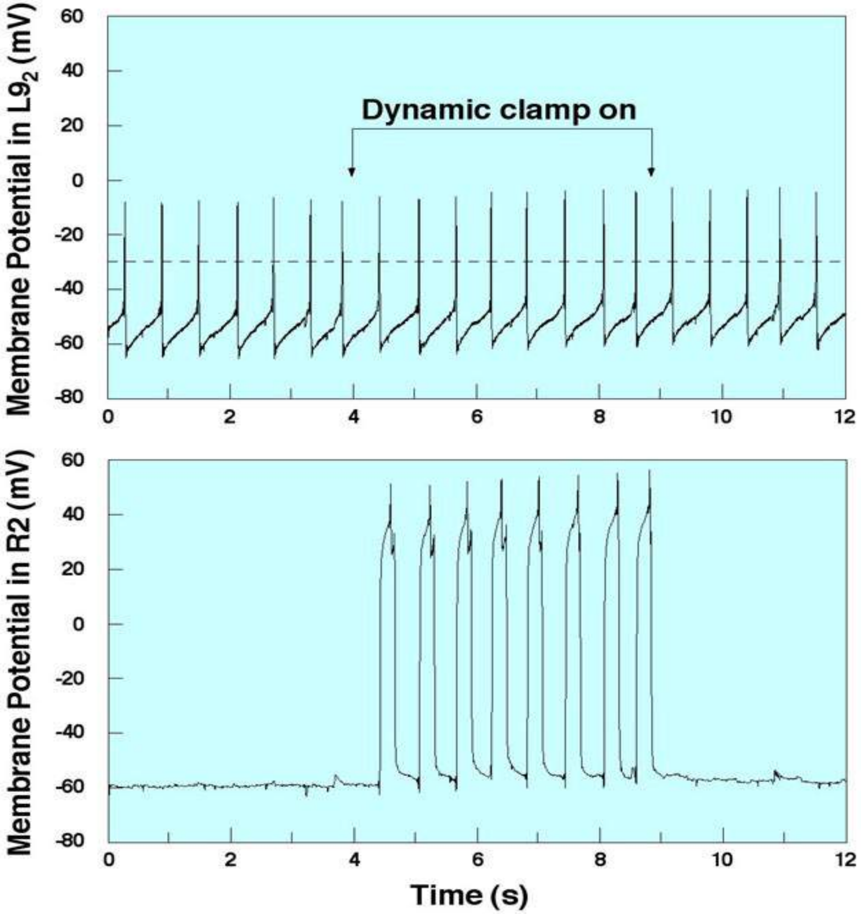


Universal Clamp

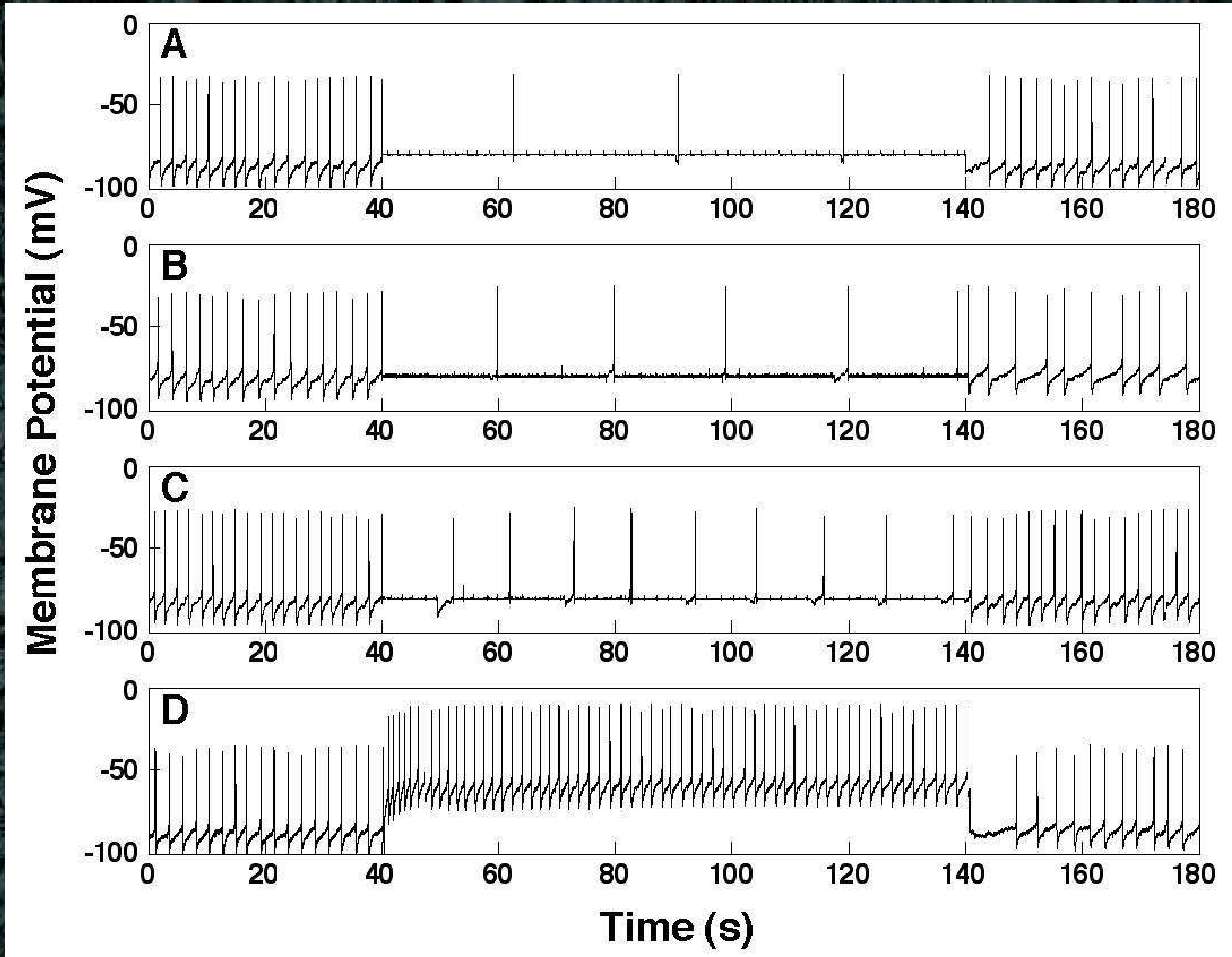
A novel instrument for controlling neuronal signals via feedback control provided by a digital signal processor chip (US and international patents 2009), funded by the National Institutes of Health



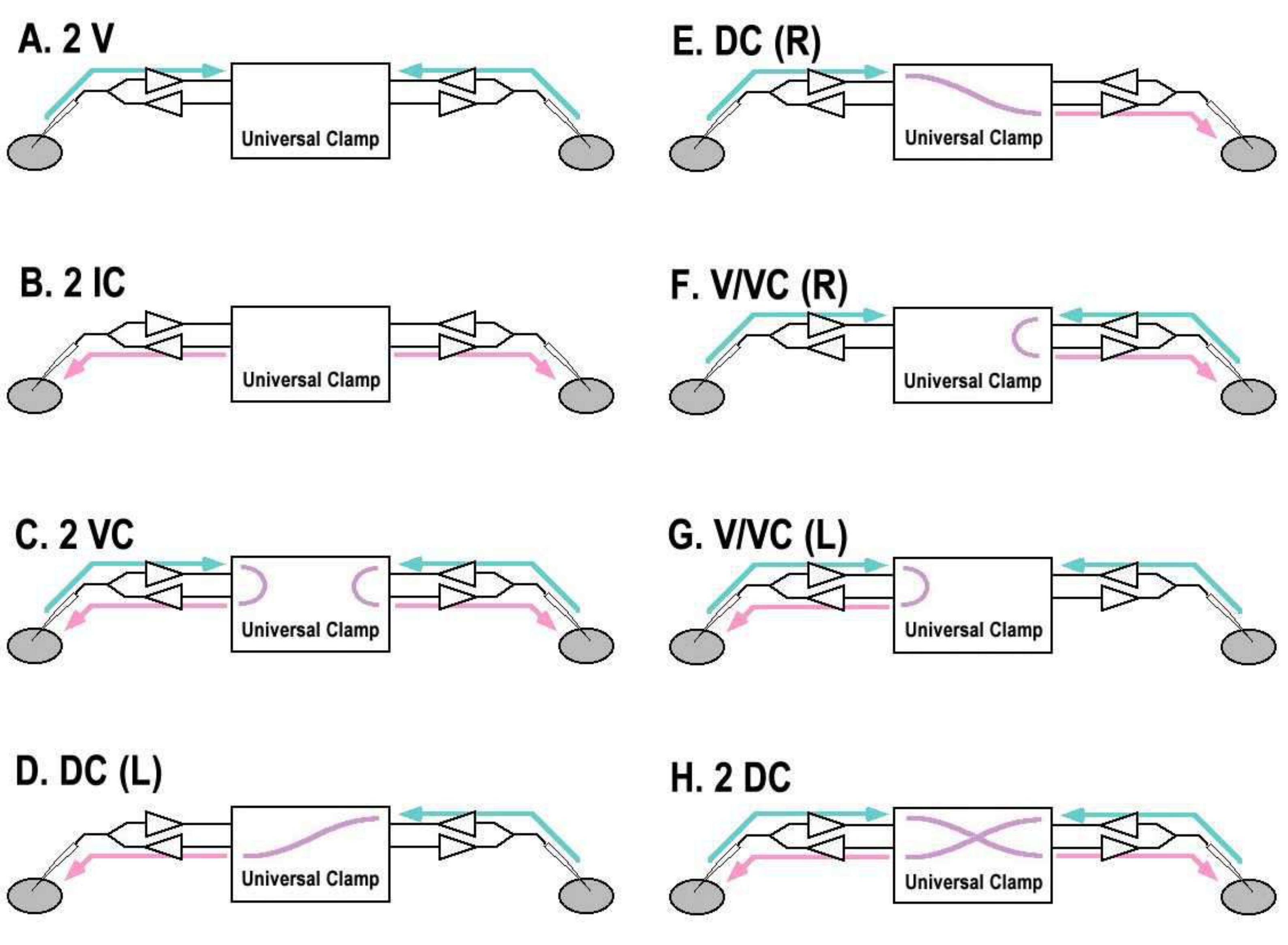




Intermittent Voltage Clamp



Possible Connectivity for a Universal Clamp with 2 Headstages



V:
voltage measurement

VC:
voltage clamp

IC:
current clamp

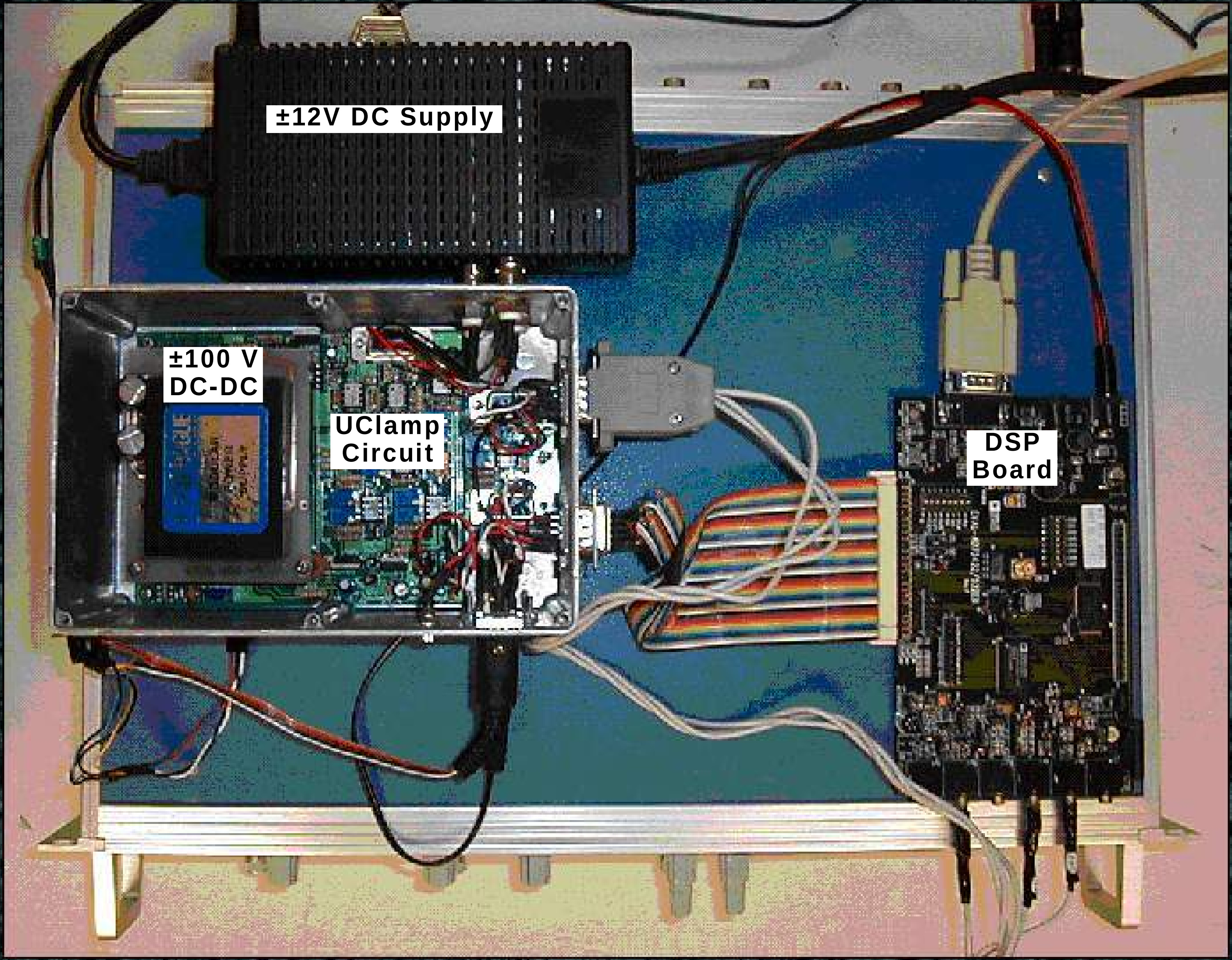
DC:
dynamic clamp

V/VC:
voltage controlled VC

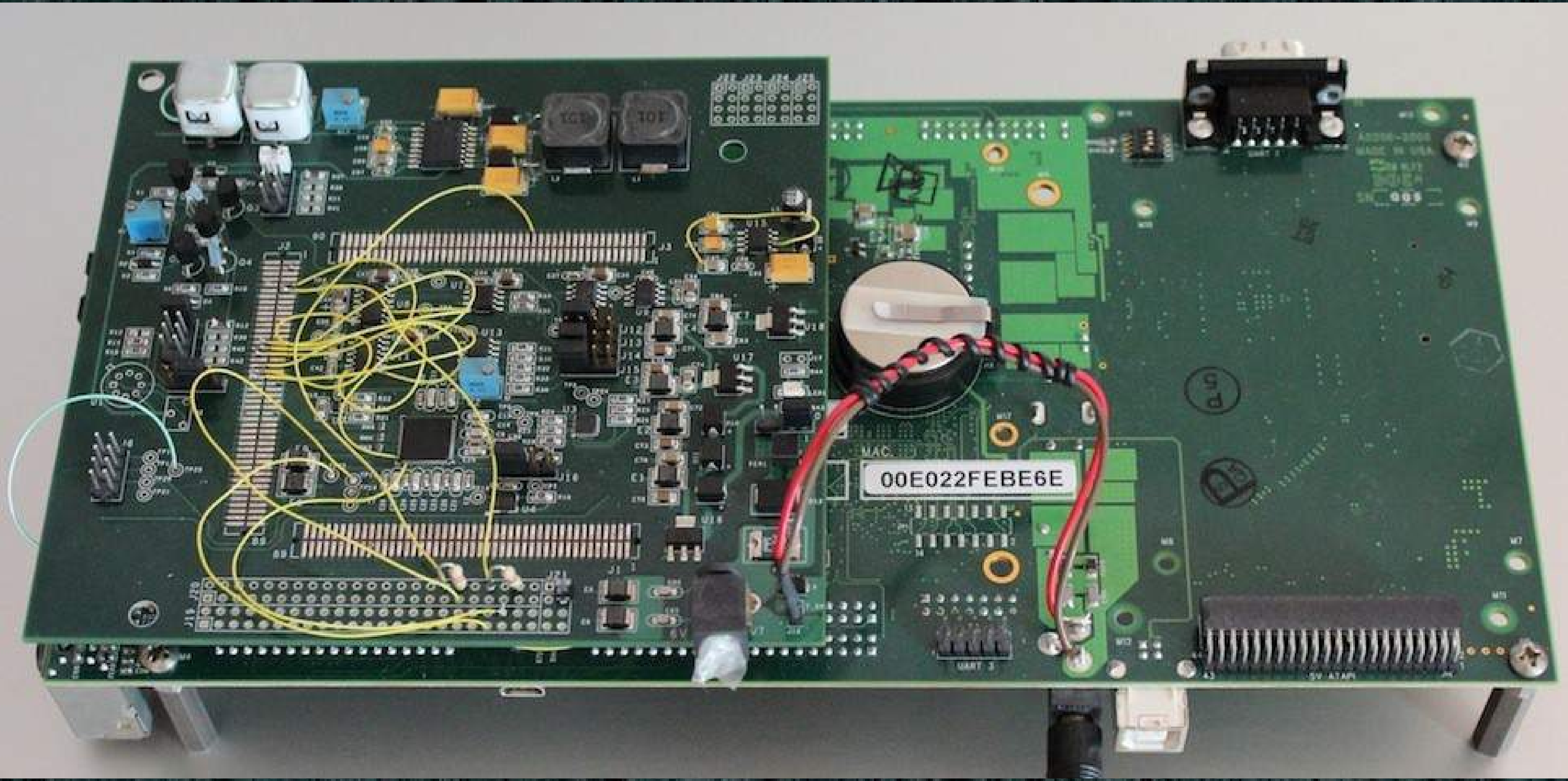
R: right

L: left

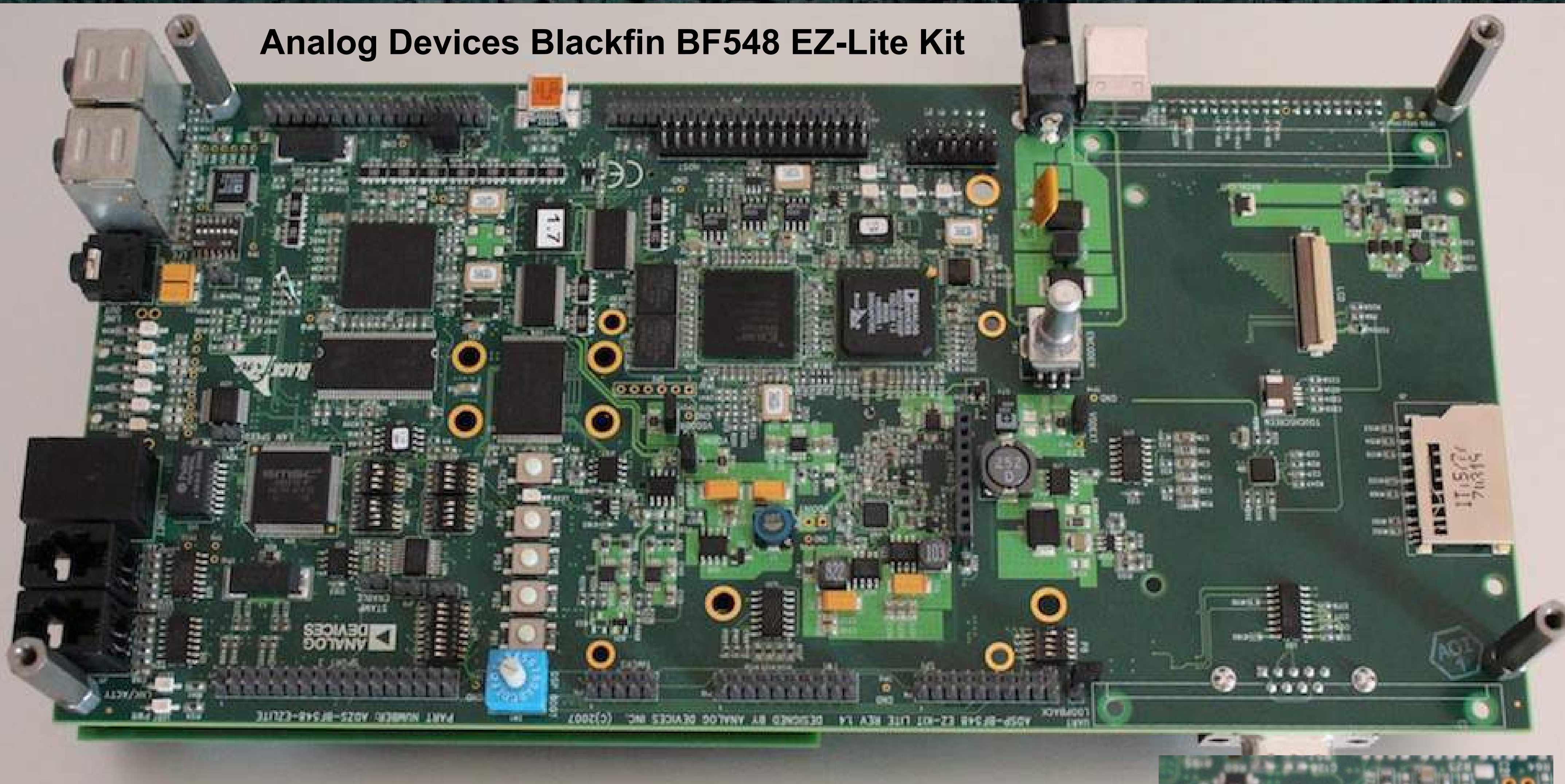
Universal Clamp – α prototype



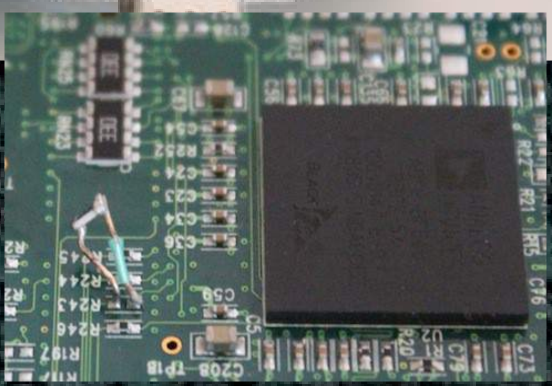
Universal Clamp – β prototype



Top view

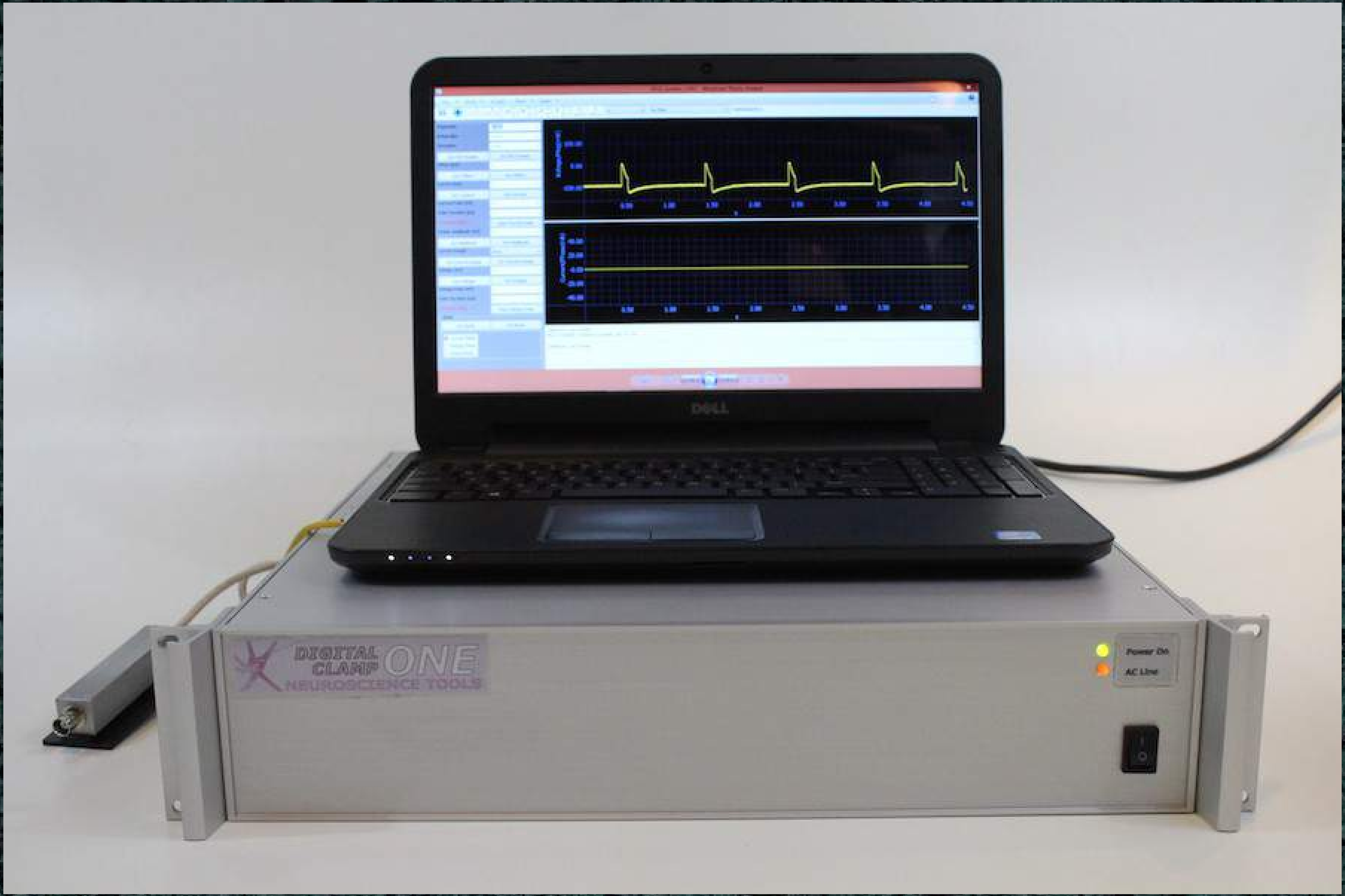


Bottom view

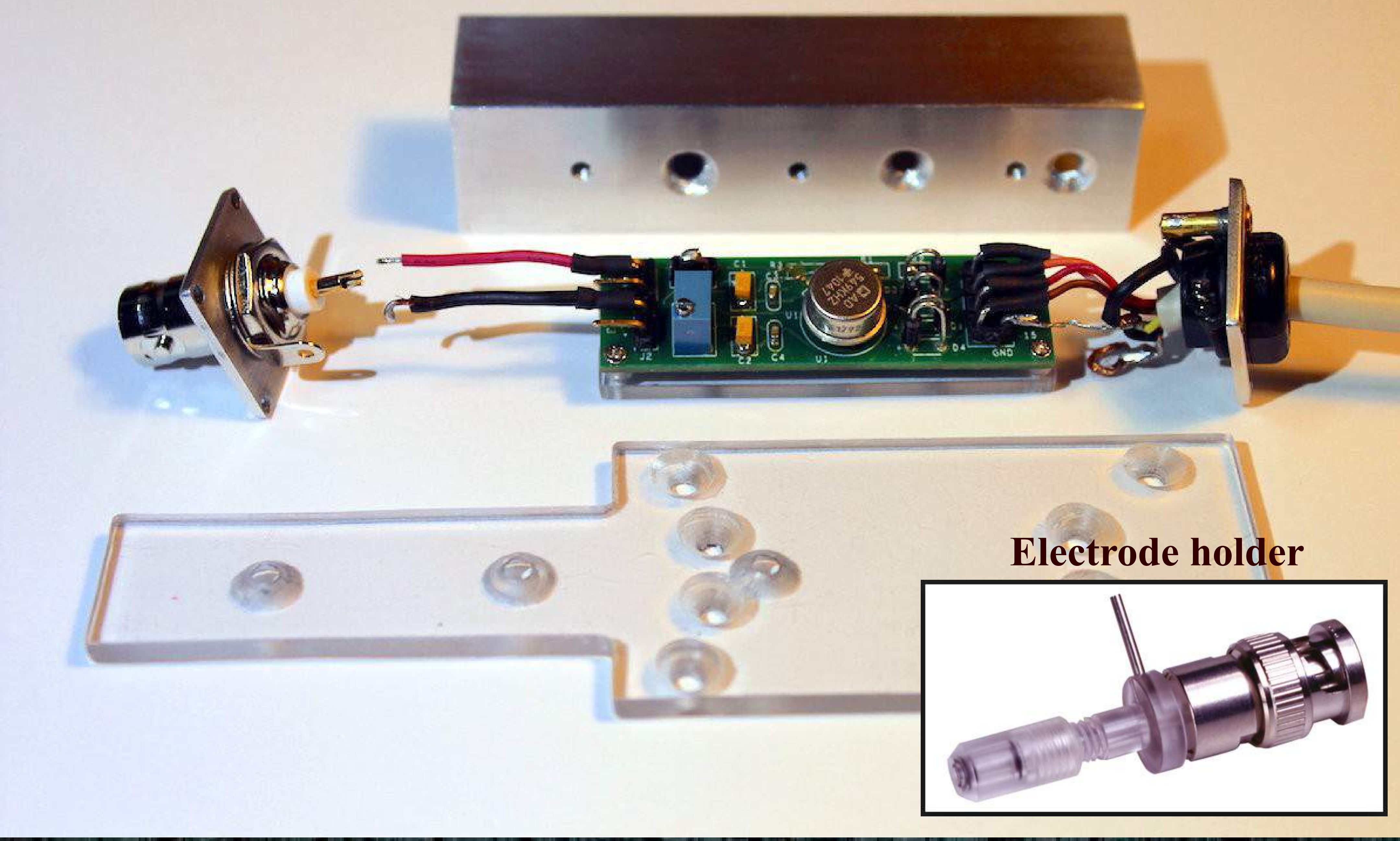


Universal Clamp – β prototype

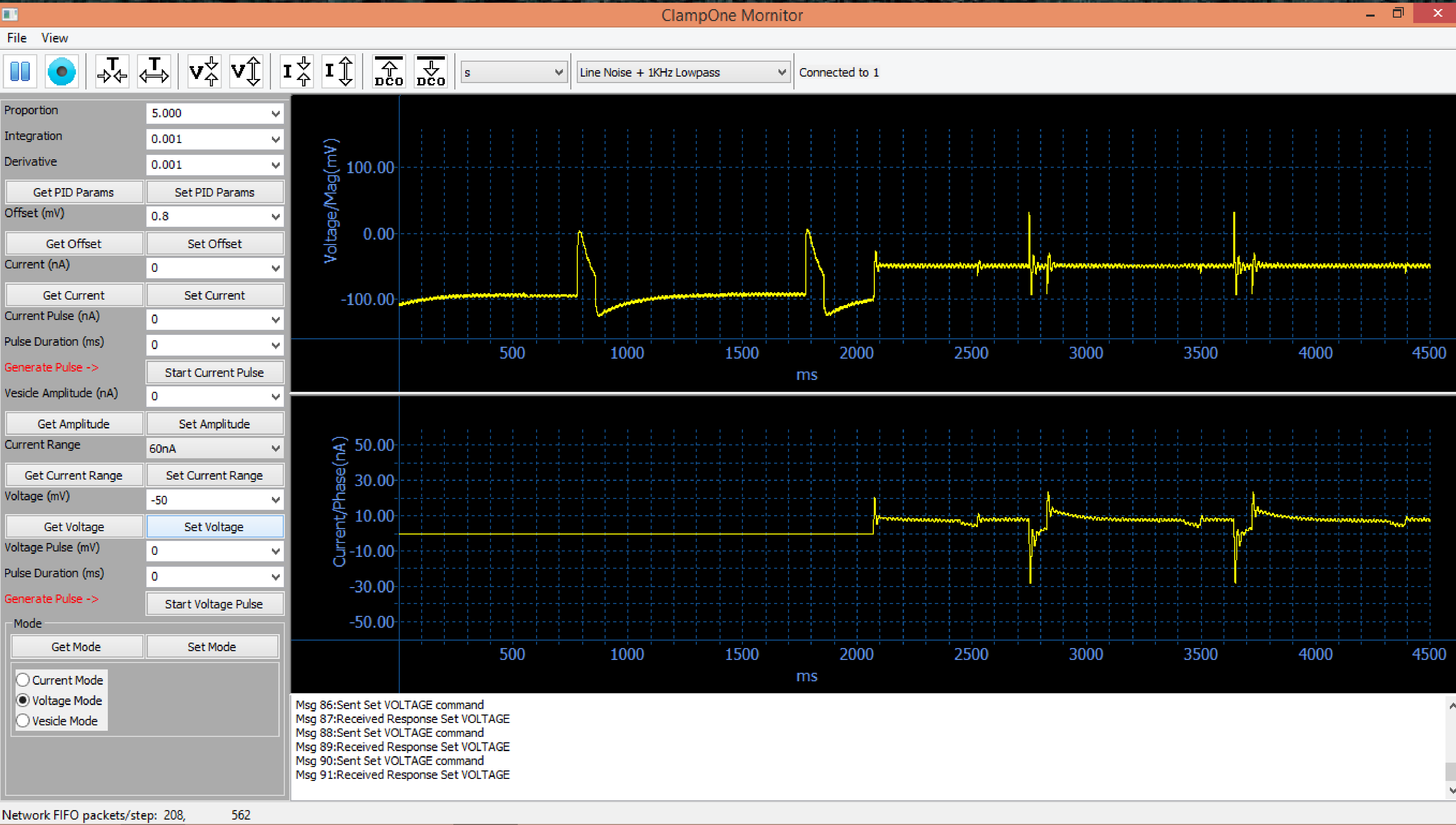
Universal Clamp – β prototype



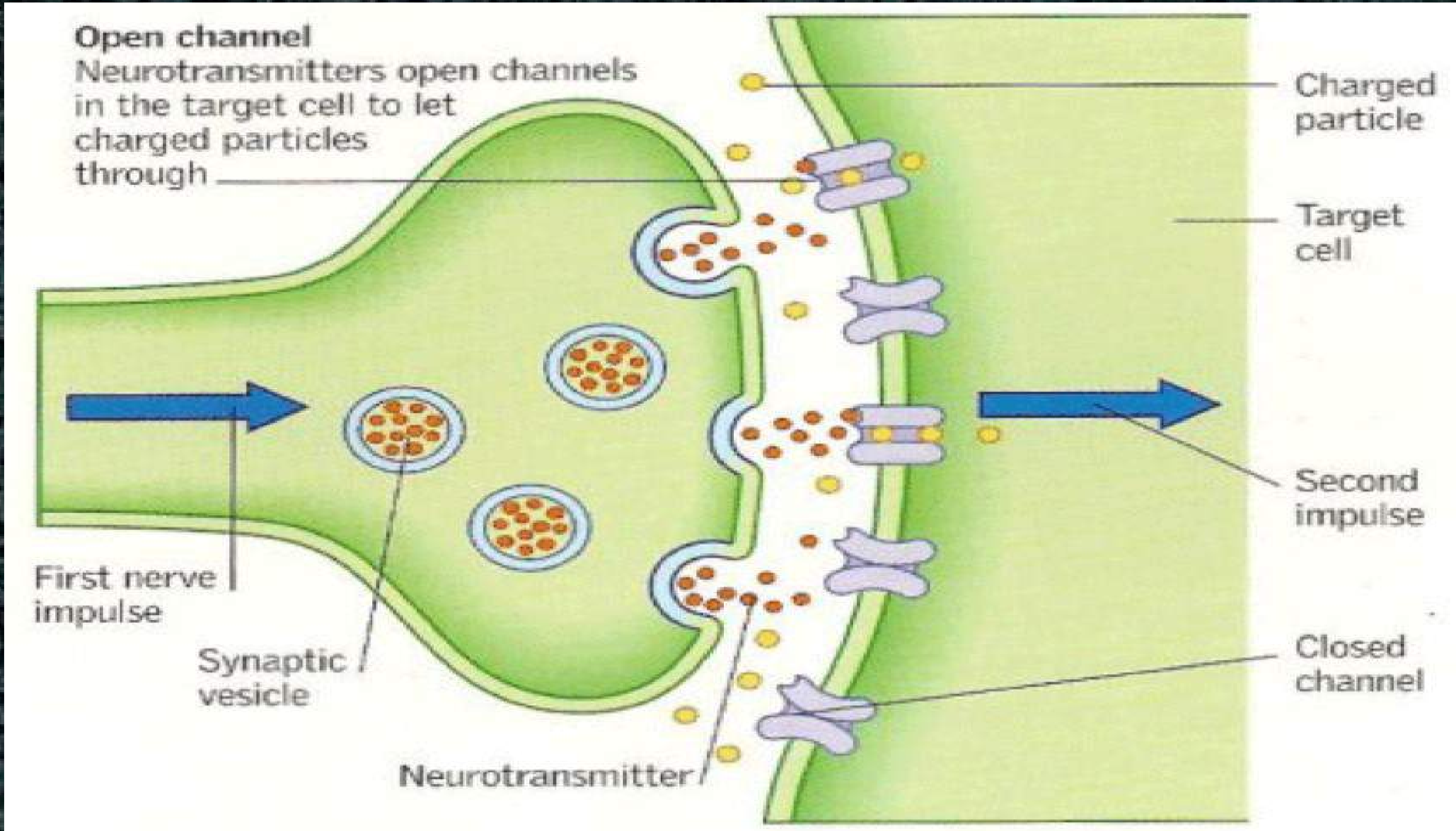
Universal Clamp – Headstage Assembly



Universal Clamp – Instrumental Panel



Neurotransmitters are released from synaptic vesicles of the presynaptic neuron and bind to receptors on the postsynaptic neuron, thereby triggering an impulse through the second neuron.



Courtesy of David Darling/Encyclopedia of Science

Can we monitor exocytosis/endocytosis activities?

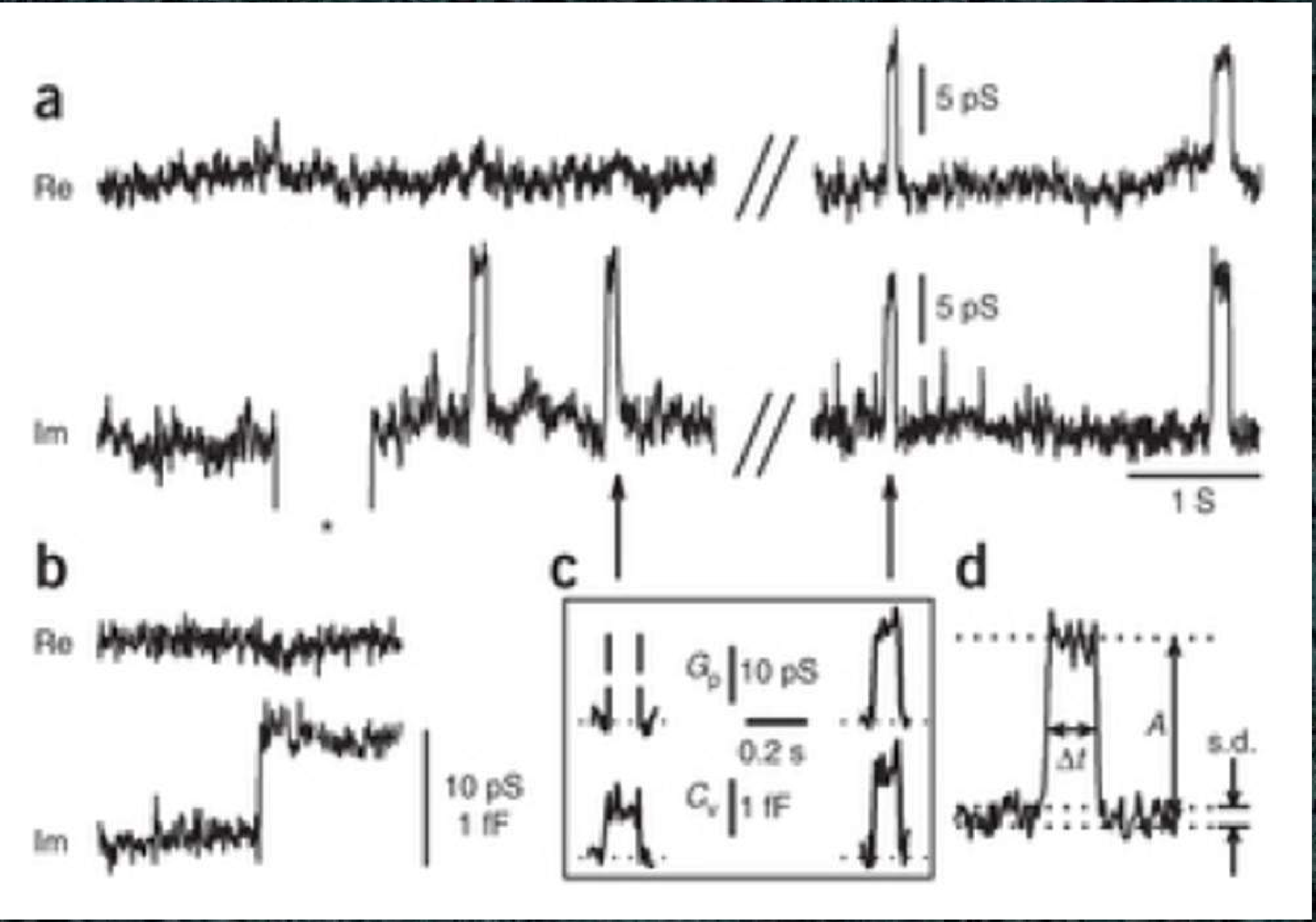
Specific cell membrane capacitance
= 0.9 μF/cm^2 (fairly constant)

Whole cell capacitance
= 3–12 pF (pico: p = 10^{-12})

Capacitance signal from a single vesicle
= 1–15 fF (femto: f = 10^{-15})

A vesicle event changes the cell capacitance by ~1/1000. A delicate and fast instrument can detect the resulting phase shift in a sinusoidal wave.

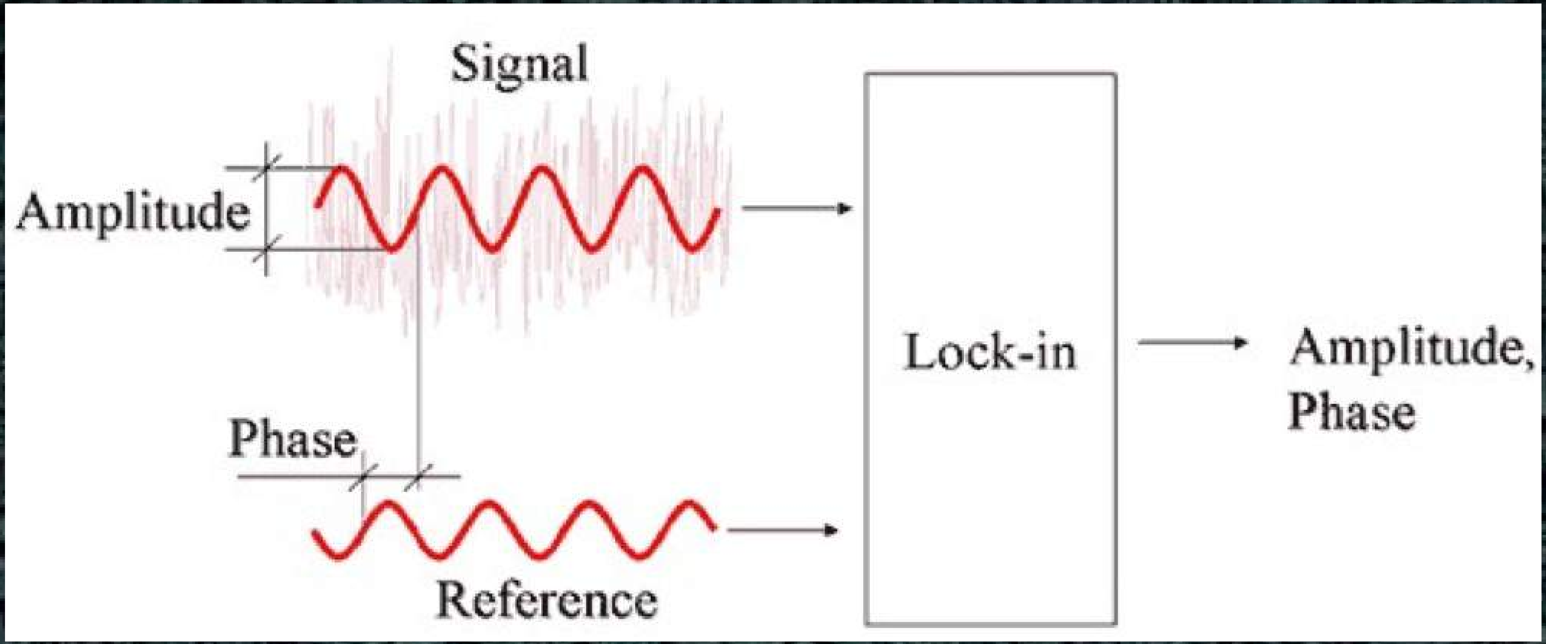
High-resolution membrane capacitance measurements for the study of exocytosis and endocytosis. Rituper et al., Nature Protocols 8:169–83, 2013



Exam Question for NEU 503

Describe the four different configurations of the patch clamp in terms of the instrumentation technique and the experimental preparation.

Lock-in Amplifier



Proceedings of the 26th Annual International Conference of the IEEE EMBS
San Francisco, CA, USA • September 1-5, 2004

Optimization of Multi-Frequency Techniques used for Cell Membrane Capacitance Estimation

S.F. Lempka, D.W. Barnett
Department of Biomedical Engineering, Saint Louis University, St. Louis, MO, USA

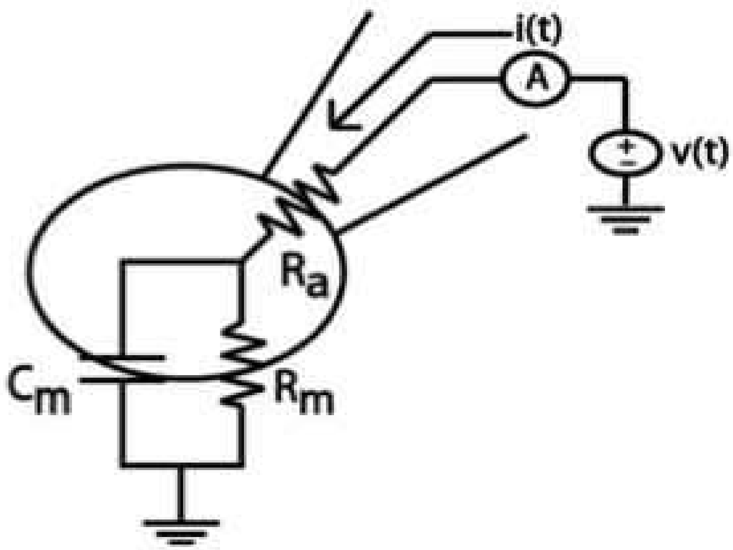


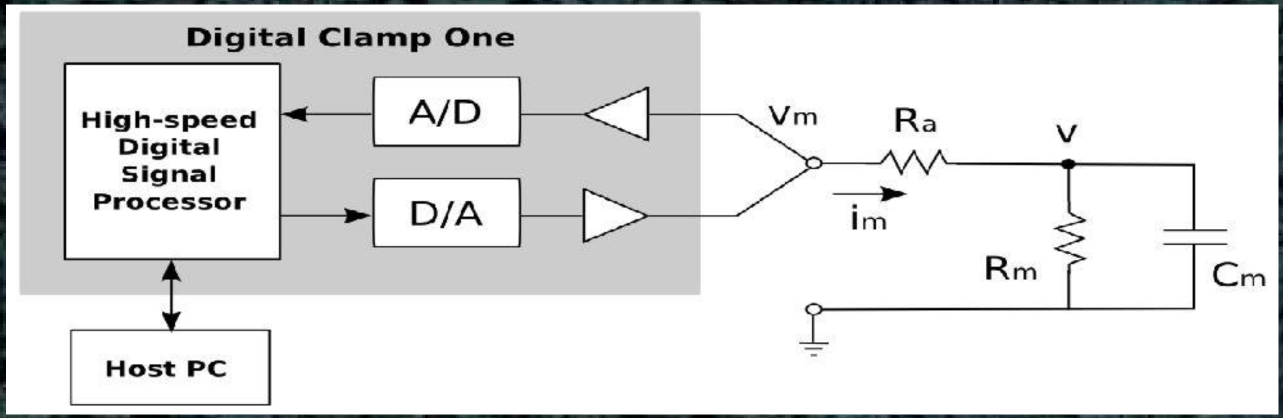
Figure 1: Three-element circuit model of a patch-clamped cell.

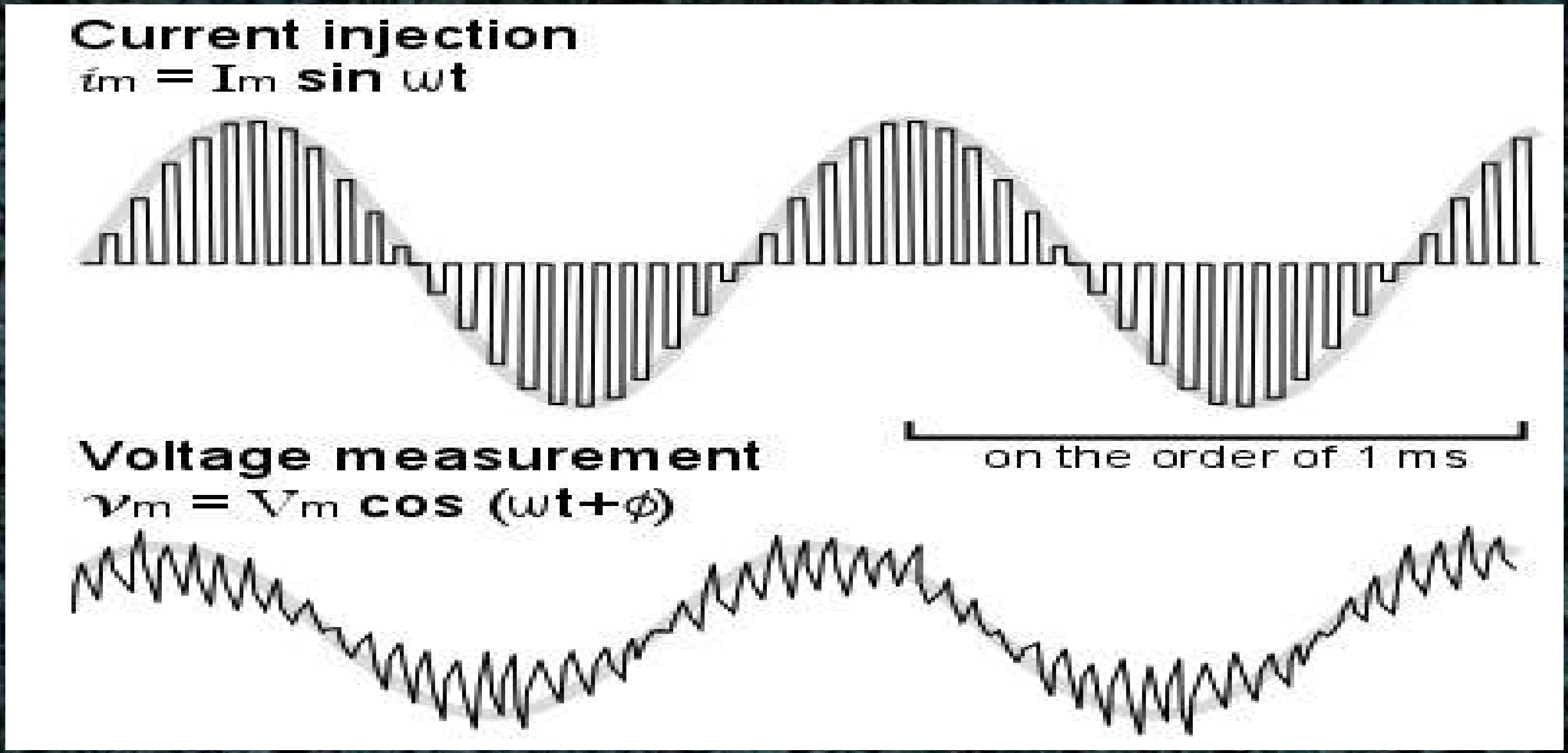
Capacitance estimation techniques are generally digital-based and involve phase detection of a single-frequency sinusoidal stimulus with a lock-in amplifier. The phase-shifted current flowing in result to a sinusoidal voltage superimposed on the DC holding potential is decomposed into real (in-phase) and imaginary (quadrature) components through the use of a phase detector. When scaled by the magnitude of the signal, the real and imaginary components represent $A(\omega)$ and $B(\omega)$ of the cell admittance function ($Y(\omega)$) evaluated at the frequency ω. $Y(\omega)$ is equal to the following,

$$Y(\omega) = A(\omega) + jB(\omega) = \frac{1+\omega^2 R_m R_p C_m^2}{R_T(1+\omega^2 R_p^2 C_m^2)} + j\frac{\omega R_m^2 C_m}{R_T^2(1+\omega^2 R_p^2 C_m^2)}, \quad (1)$$

where $R_T = R_a + R_m$ and $R_p = R_a * R_m / R_T$.

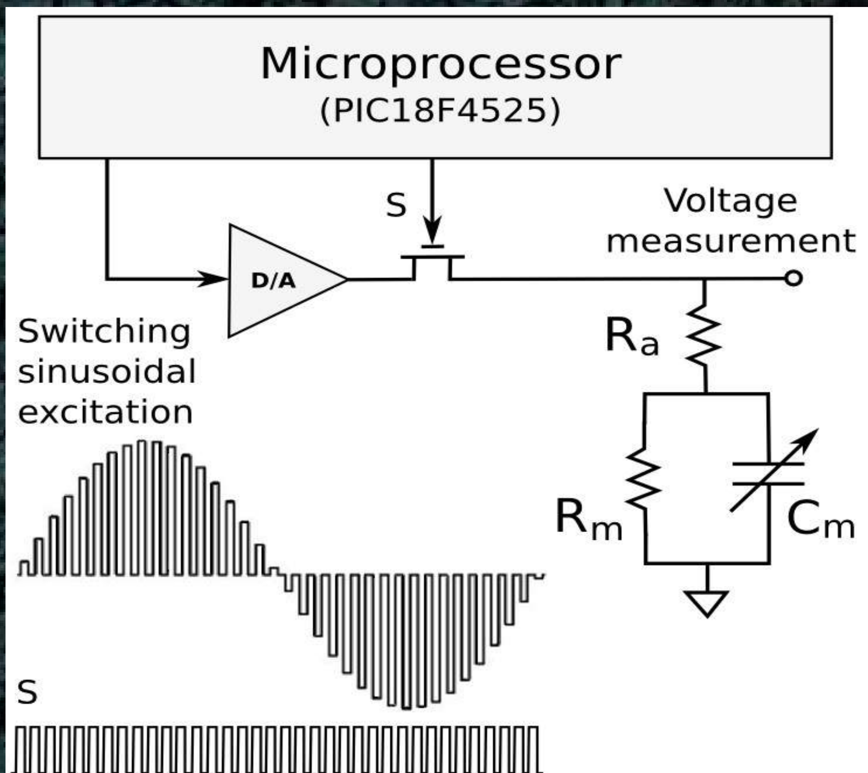
Cellular Capacitance Measurement with the Universal Clamp

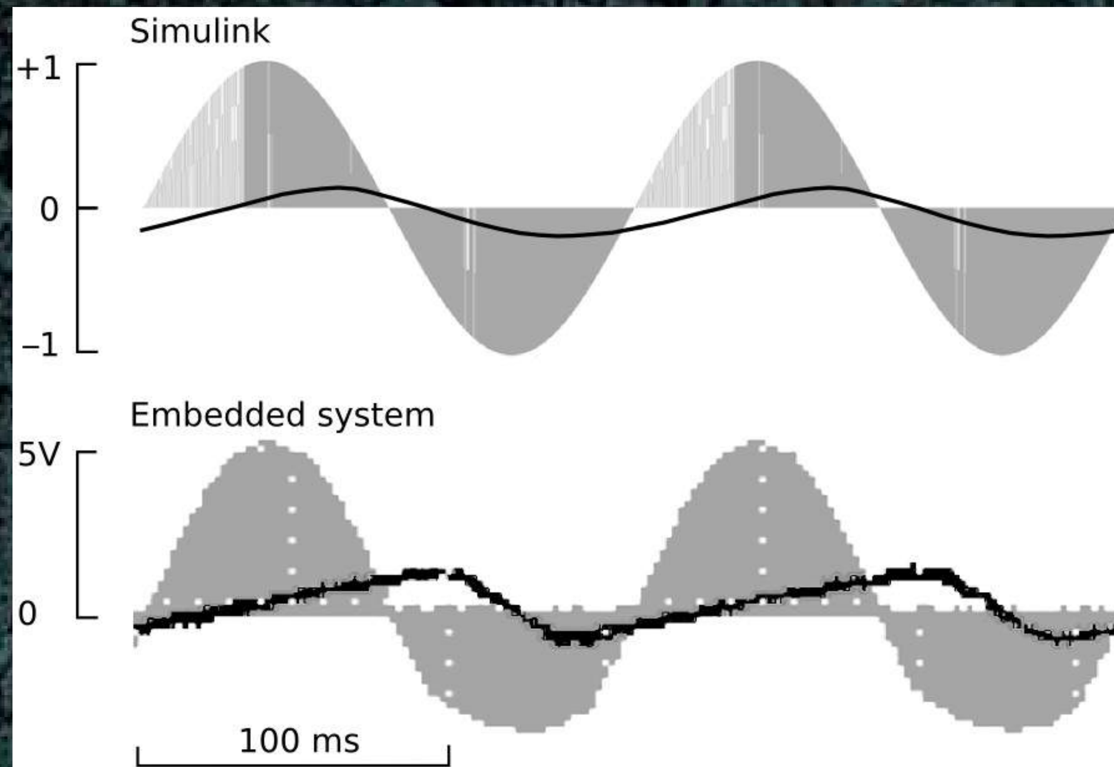


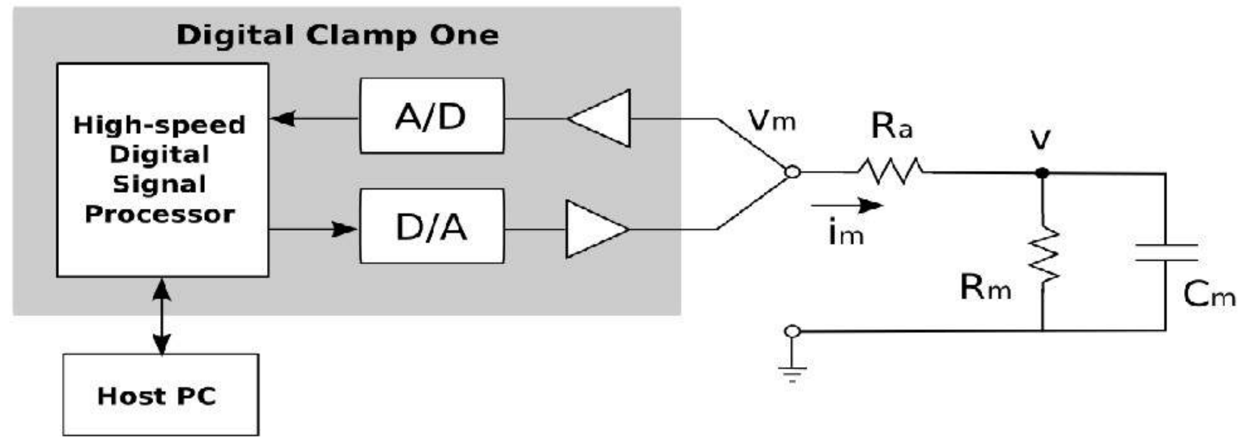


Instrumentation for cell capacitance measurements: switching sinusoidal excitations for studying cell membrane transport.

40th Northeast Bioengineering Conference, Boston, MA, April 25-27, 2014.







First, the electrode resistance R_a *is measured.*

Kirchhoff's current law results in:

$$i_m = C_m v' + \frac{v}{R_m} = \frac{v_m - v}{R_a}, \quad \textit{where} \quad v' = \frac{dv}{dt}$$

$$v_m' - R_a i_m' = \left(\frac{1}{C_m} + \frac{R_a}{R_m C_m}\right) i_m - \frac{1}{R_m C_m} v_m$$

$$v_m' - R_a i_m' = \left(\frac{1}{C_m} + \frac{R_a}{R_m C_m}\right) i_m - \frac{1}{R_m C_m} v_m$$

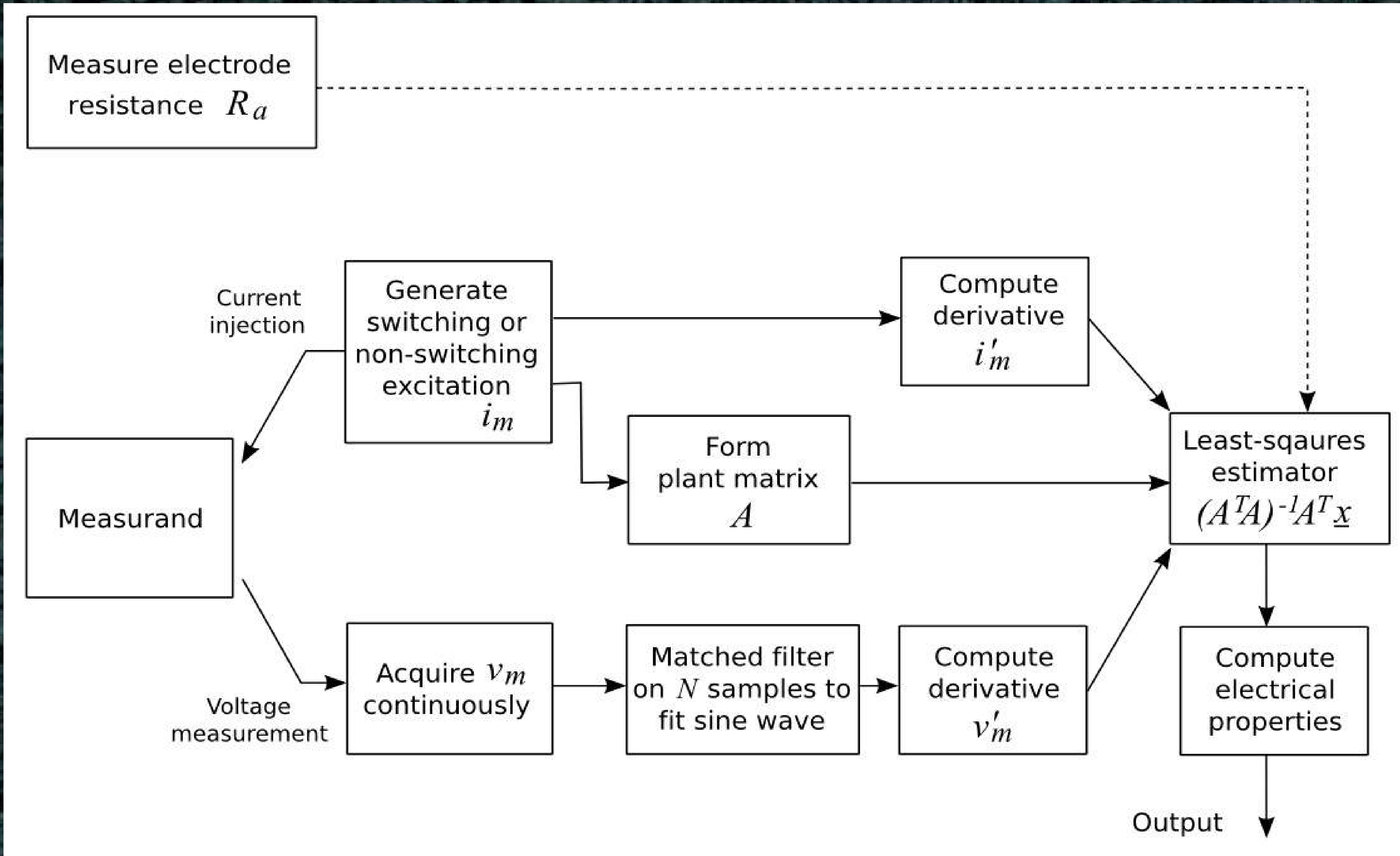
Take N sample points over a full time period $2\pi/\omega$.

$$\begin{bmatrix} x_1 \\ x_2 \\ \cdots \\ x_N \end{bmatrix} = \begin{bmatrix} i_{m1} & v_{m1} \\ i_{m2} & v_{m2} \\ \cdots & \cdots \\ i_{mN} & v_{mN} \end{bmatrix} \begin{bmatrix} \frac{1}{C_m} + \frac{R_a}{R_m C_m} \\ -\frac{1}{R_m C_m} \end{bmatrix}$$ or $$\underline{x} = A \underline{\theta}$$

Form a least-squares estimator: $\underline{\hat{\theta}} = (A^T A)^{-1} A^T \underline{x}$

where $\underline{\hat{\theta}} = \begin{bmatrix} \theta_1 \\ \theta_2 \end{bmatrix}$ and $\begin{bmatrix} R_m \\ C_m \end{bmatrix} = \begin{bmatrix} -\frac{\theta_1}{\theta_2} - R_a \\ \frac{1}{\theta_1 + R_a \theta_2} \end{bmatrix}$

Signal flow diagram for continuous monitoring of cell capacitance



Summary

1. *Time-domain formulation for a linear least-squares estimator has the advantage of providing fast, accurate, and continuous measurements of the electrical properties of a cell.*

2. *The concept of the lock-in amplifier – improving signal-to-noise ratio with sinusoidal modulations – was used to simplify the formulation and integrated into the Universal Clamp based a high-speed signal processor.*

3. *For future work, very small phase shifts related to vesicle activities remain to be a challenge and may require a phase-lock-loop type of algorithm for accurate measurements.*

Acknowledgements

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In Memory of
Prof. Robert B. Hill

1930-2013