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EE122B
Introduction to Biomedical Electronics

Bioelectricity, Origin and Measurement

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Spring 2015

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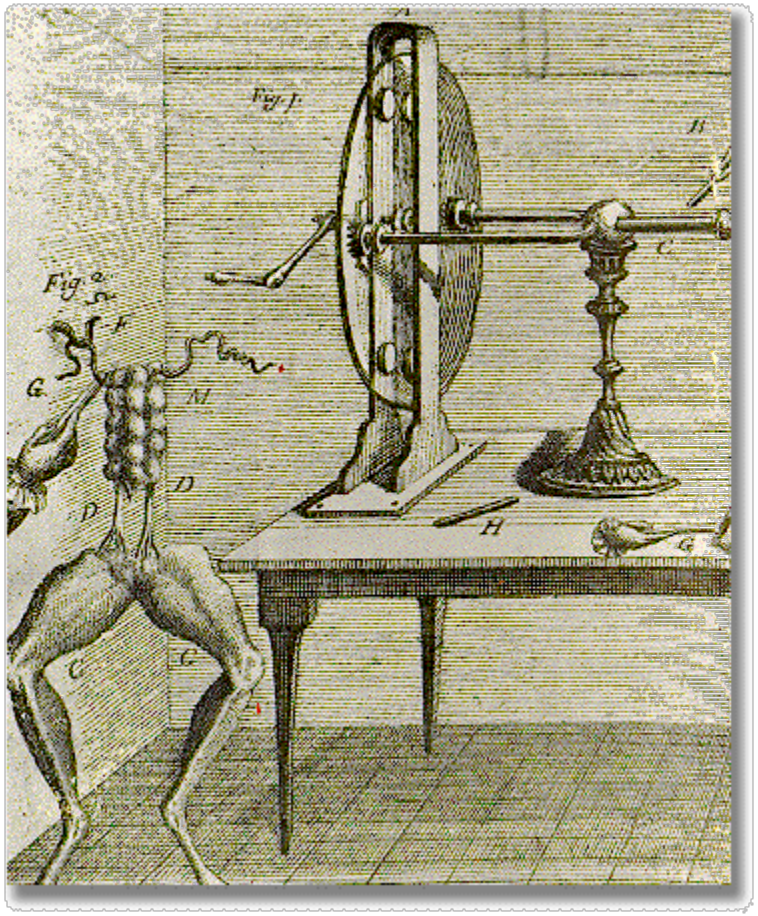
Overview

Goal: review mechanisms of bioelectric signals at the cellular level, and how to measure them.

- Origins of bioelectricity
  - Membrane biopotentials
  - Ion channels
  - Electrically-active tissue types
- Measurement of cellular bioelectricity
  - Electrode theory
  - Microelectrodes (intra- and extra-cellular)
  - Micropipette electrodes
  - Noise associated with electrode/amplification
  - Patch clamp technique
  - Other methods of biopotential measurements

Electrical Activity of Excitable Cells

How It Started…



In 1790, Luigi Galvani (1737-1798) proposes the concept of "animal electricity," supposedly different from conventional electricity, based on his observation of stimulation of frog legs.

Trying to disprove him, Alessandro Volta (1745-1827) invents the battery and shows that it is just electricity…

Why Does Bioelectricity Matter?

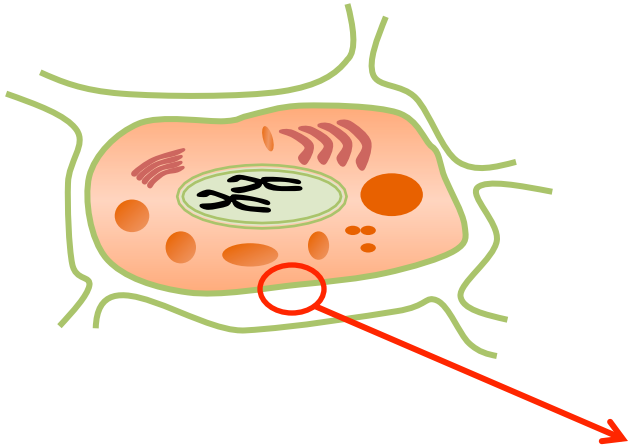
- Electrical potentials are key in the transmission of information throughout the whole body:
  - Central nervous system: memory, cognitive functions.
  - Peripheral nervous system: signaling to amuscles, feedback from sensory cells.
  - Pacing and synchronization of the heart.
- Disruption of electrical potentials causes diseases.

  Examples:
  - *Channelopathies* (disease related to ion channel malfunctions, e.g. cystic fibrosis [Cl^-], Long QT syndrome [Ca^{2+}, K^+], seizure [voltage-dependent Na^+],…). See: http://en.wikipedia.org/wiki/Channelopathy
  - *Multiple Sclerosis* (myelin "insulation" on fibers lost).

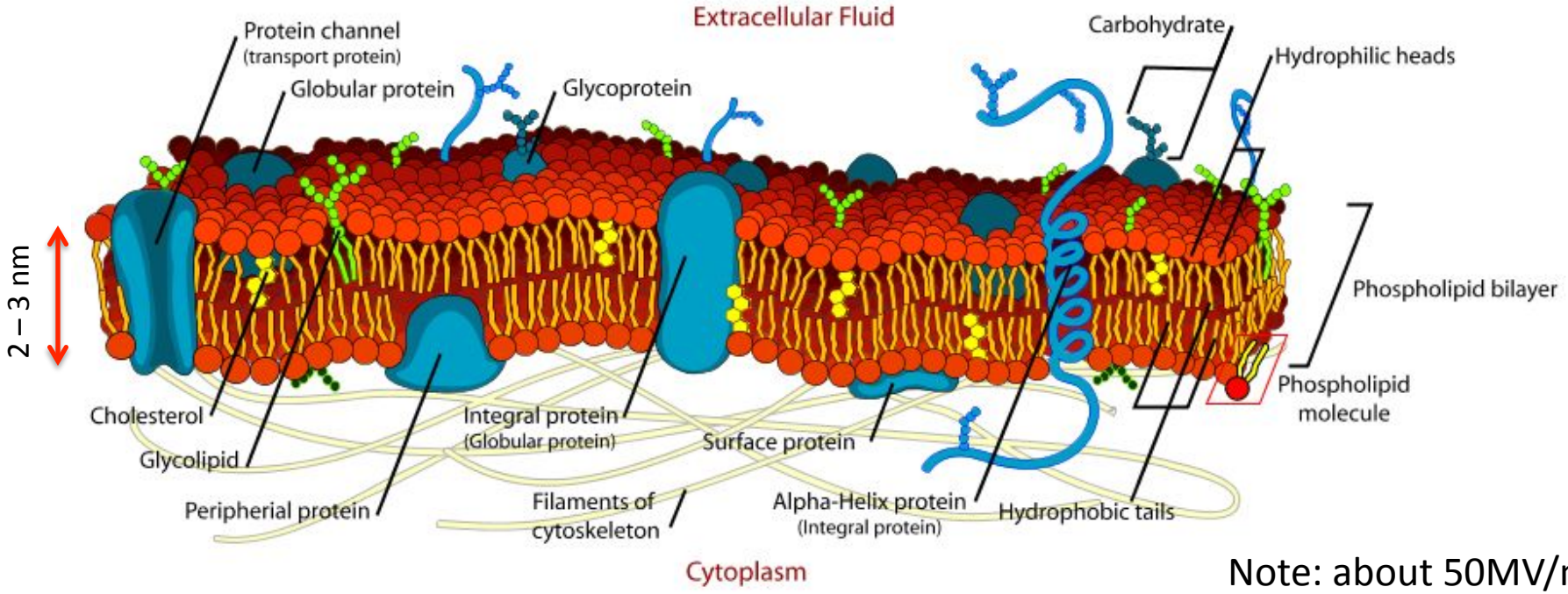
Origins of Cellular Biopotentials

- Biopotentials arise from differences in *ion concentrations* across a *semi-permeable membrane*, the cell membrane.
- Changes in *membrane permeability* give rise to transient potentials, such as action potentials.
- Rapid changes in membrane permeability is enabled by *ion channels*.

A Close Look at the Cell Membrane



- Cell membrane separates *cytoplasm* from *extracellular fluids.*
- Primary structural constituents are *phospholipids.*
- Membrane contains many other proteins serving various functions (transport, signaling, etc.).

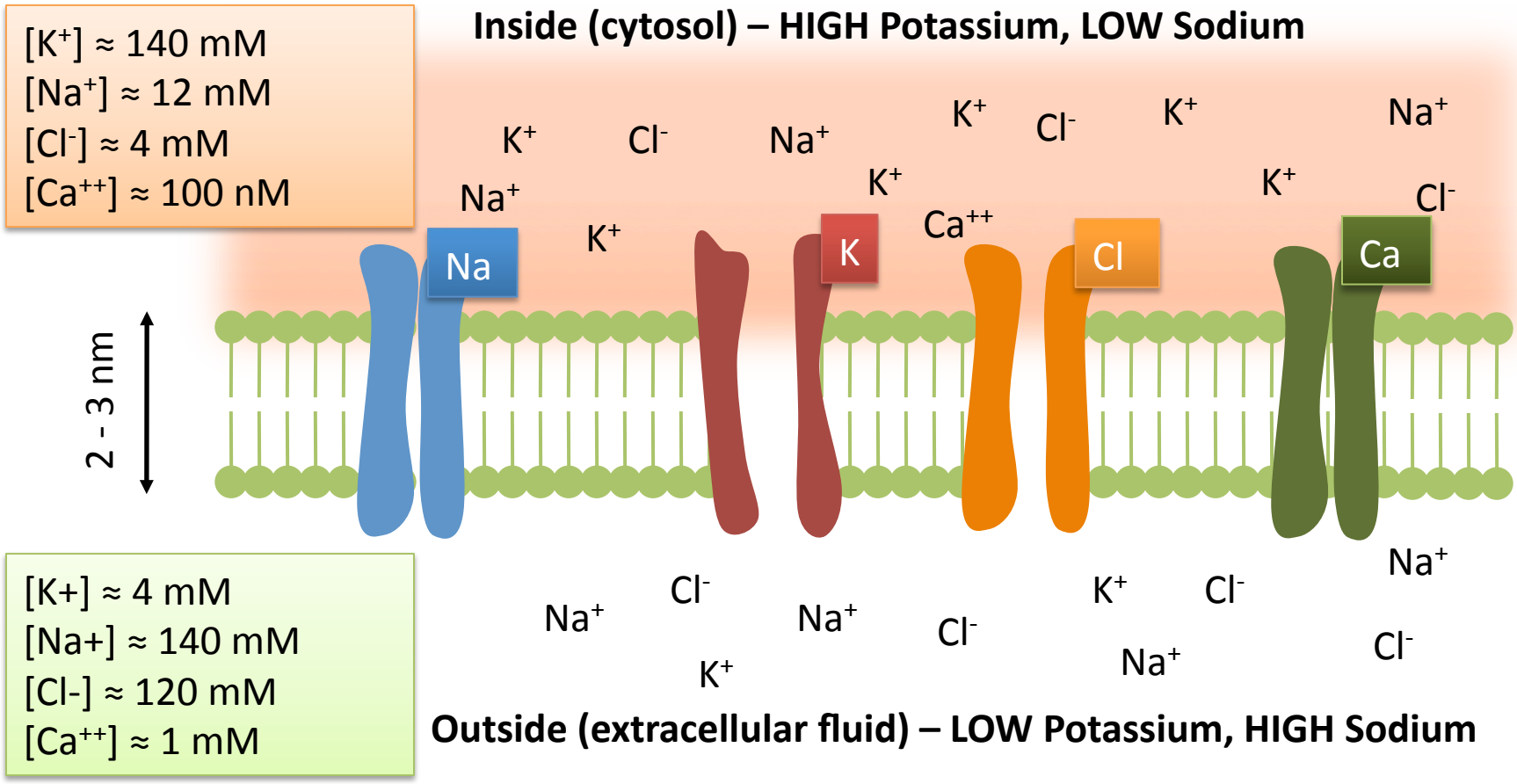


Note: about 50MV/m!

Source: www.wikipedia.org

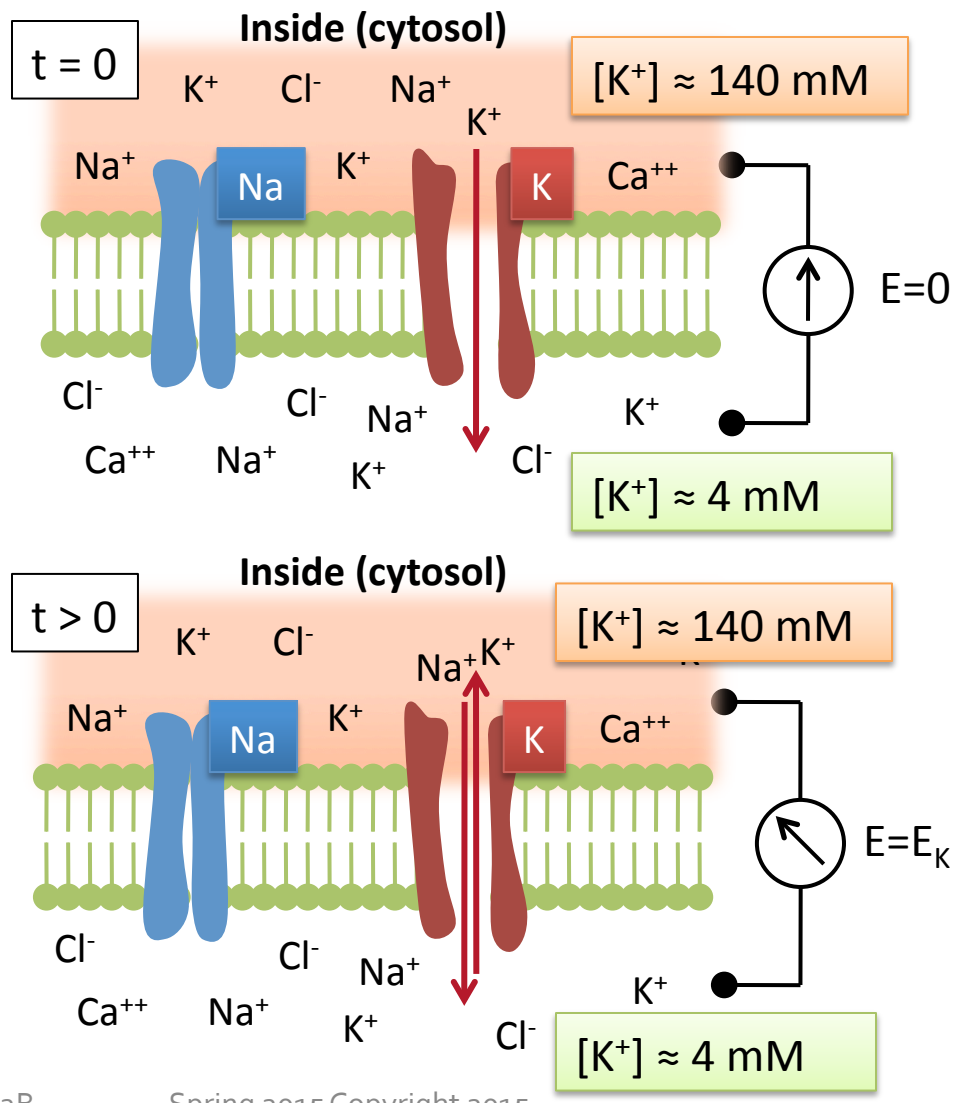
A Close Look at the Cell Membrane

- The cell membrane separates fluid with largely *different* ionic concentrations.
- *Selective permeability* through ion channels regulates currents and potentials.



Membrane Resting Potential

Assume the membrane is only permeable to potassium ions (K^+) (Na^+ channels closed):



At first, K^+ ions diffuse down their concentration gradient, leaving the inside depleted of K^+.

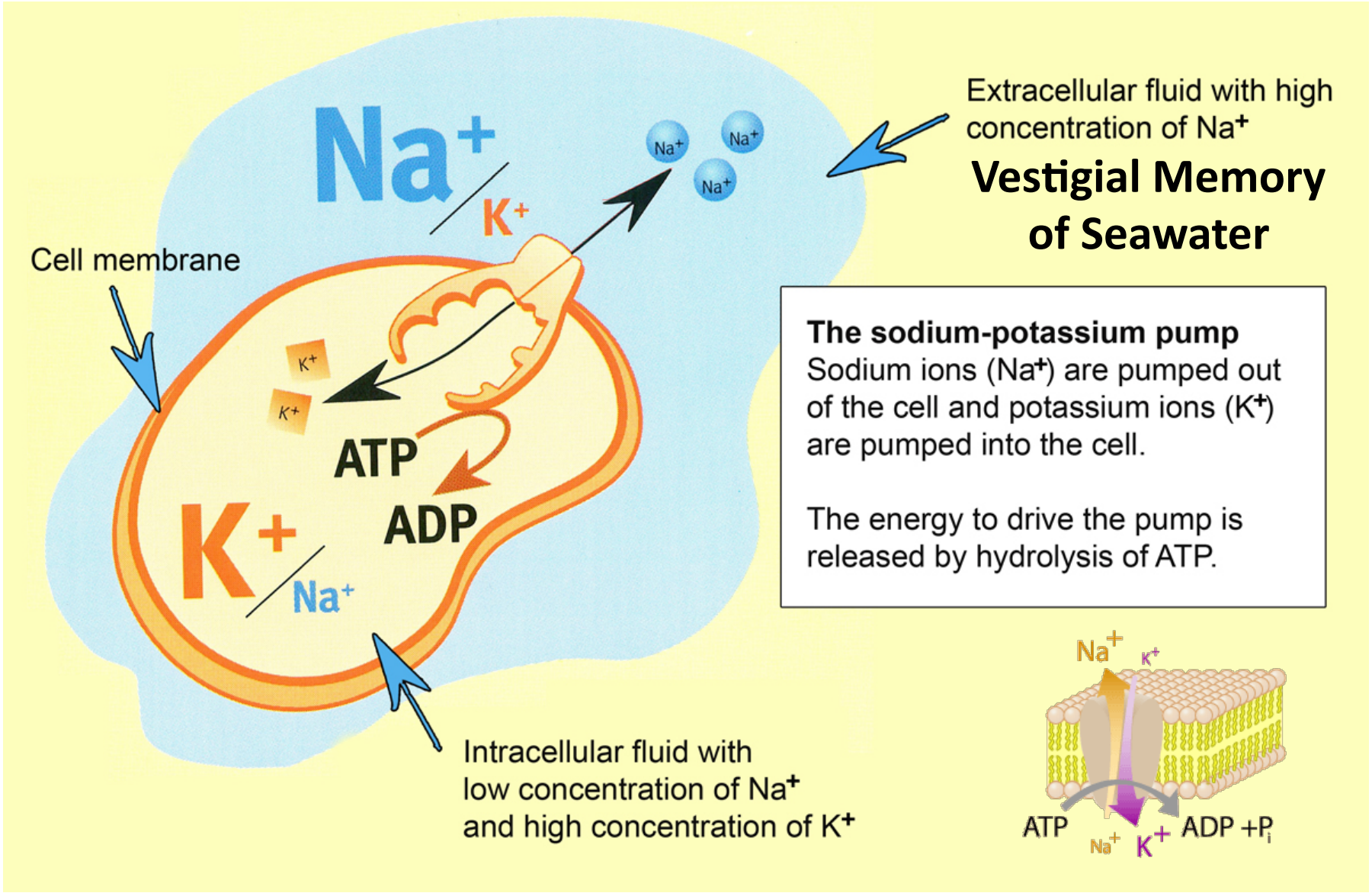
This gradually builds up a *potential difference* between the inside and outside, until the resulting electrical potential exactly counteracts the diffusion force (Donnan Equilibrium).

This potential is defined by the ***Nernst potential***:

$$E_K = \frac{RT}{nF} \ln \frac{[K]_o}{[K]_i}$$

E_K: equilibrium potential
n: valence
$[K]_I$,$[K]_o$: intra- and extracellular concentrations
R: universal gas constant
T: absolute temperature in Kelvin
F: Faraday constant

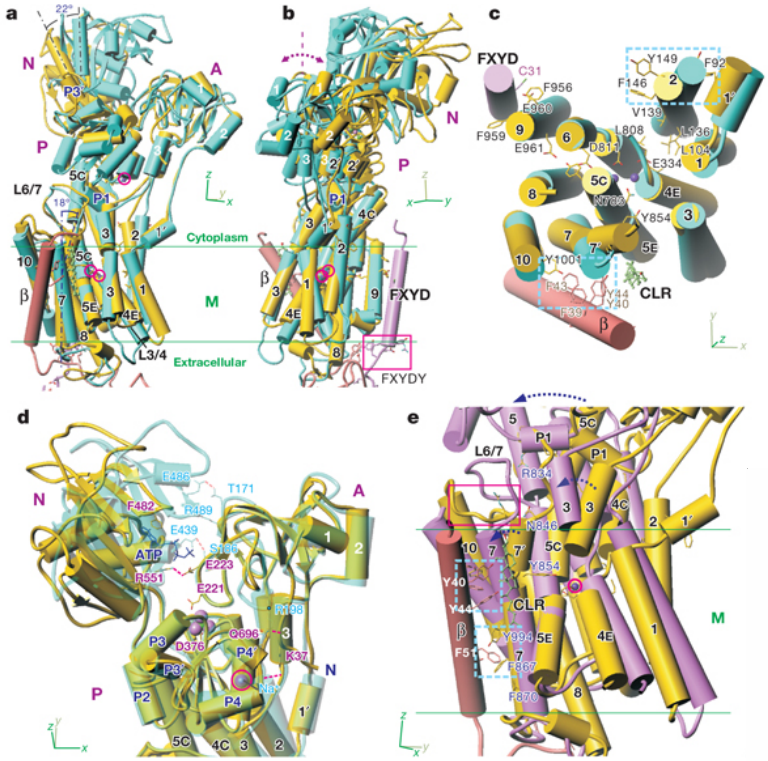
Sodium Potassium Pump: Active Transport



http://tiredoframen.wordpress.com/2012/06/13/potatoes-not-the-enemy-we-think-they-are/

http://hyperphysics.phy-astr.gsu.edu/hbase/biology/imgbio/nakpump1.gif

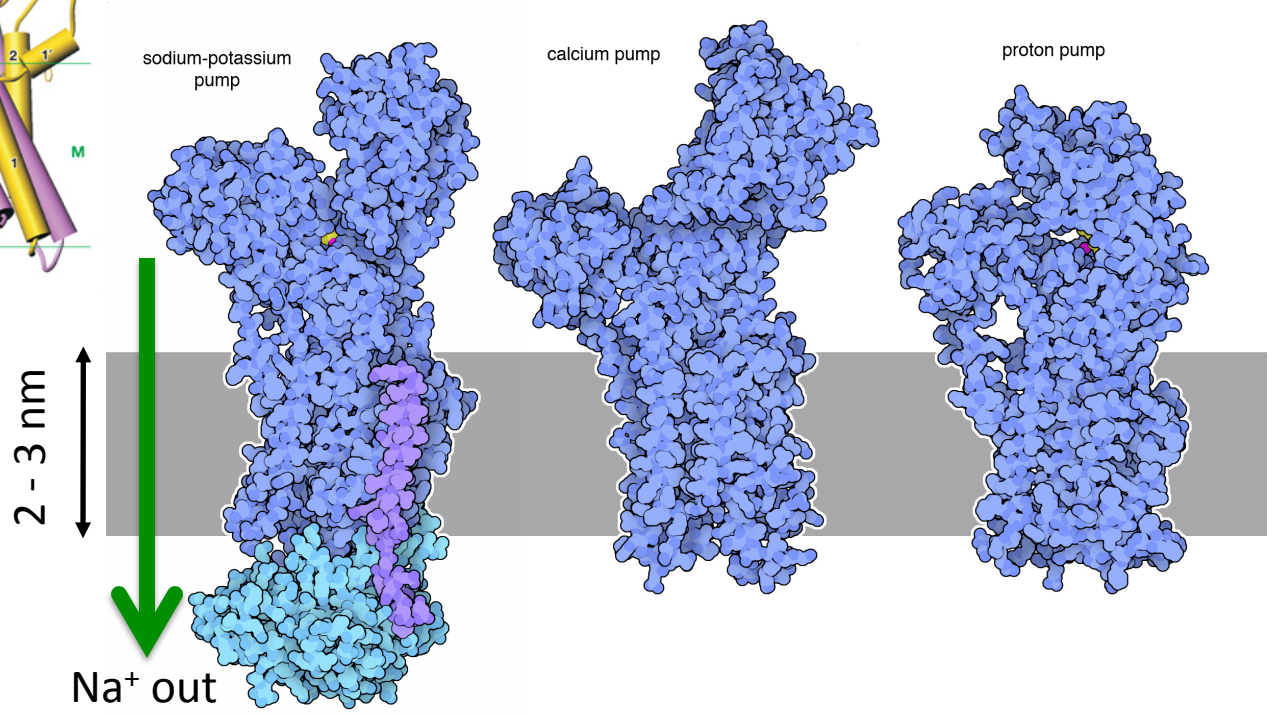
Sodium Potassium Pump: Non-Trivial!



Shinoda, et al, "Crystal Structure of the Sodium-Potassium Pump at 2.4Å Resolution," Nature, vol. 459, 21 May 2009.

Sodium is pumped out in exchange for Potassium via consumption of ATP.

Inside (cytosol) – HIGH Potassium, LOW Sodium



Outside (extracellular fluid) – LOW Potassium, HIGH Sodium

Membrane Resting Potential

Example of Nernst potentials for multiple ions in mammalian skeletal muscle (after Hille, 1992):

Ion	Extracellular concentration (mM)	Intracellular concentration (mM)	Equilibrium potential @ 37°C (mV)
Na^+	145	12	**+67**
K^+	4	155	**-98**
Ca^{++}	1.5	100 nM	**+129**
Cl^-	123	4.2	**-90**

The overall membrane potential of the cell depends on the respective influence of each ion channel type. **Depending on their permeability** (P_x), the membrane potential will vary between these potentials.

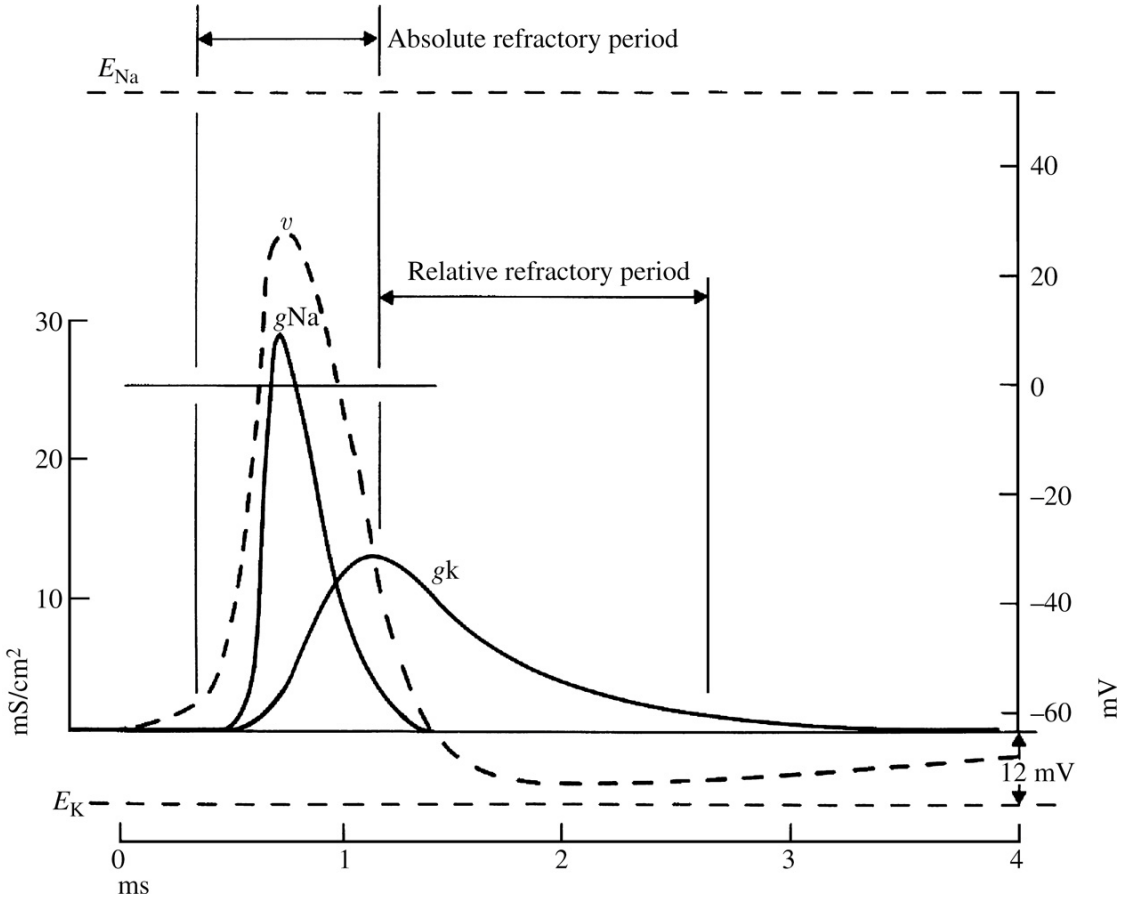
Goldman-Hodgkin-Katz Resting Potential equation:

$$E_K = \frac{RT}{F} \ln \frac{P_K[K]_o + P_{Na}[Na]_o + P_{Cl}[Cl]_i}{P_K[K]_i + P_{Na}[Na]_i + P_{Cl}[Cl]_o}$$

Tissue	Resting Potential
Smooth muscle	≈ - 50 mV
Skeletal muscle	≈ - 95 mV
Neurons, Cardiac cells	≈ - 73 – 78 mV

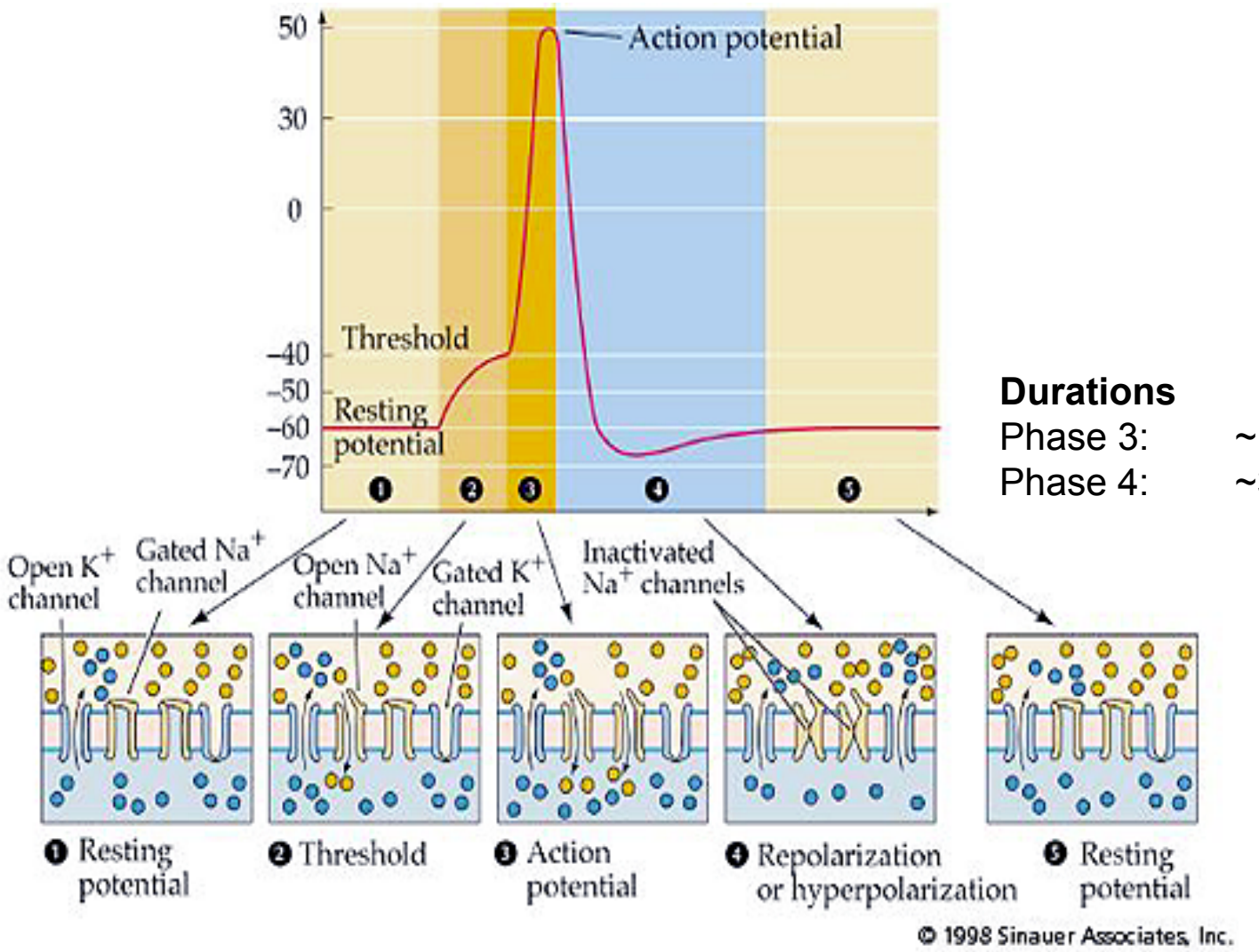
Action Potentials

Action potentials, "digital" outputs of electroactive cells, have their origin in the *voltage-* and *time-dependant conductance (permeability)* of ion channels (typically Na^+, K^+, Ca^{2+}).



Source: Medical Instrumentation, J. Webster, Wiley, 2009

Neuronal Action Potential

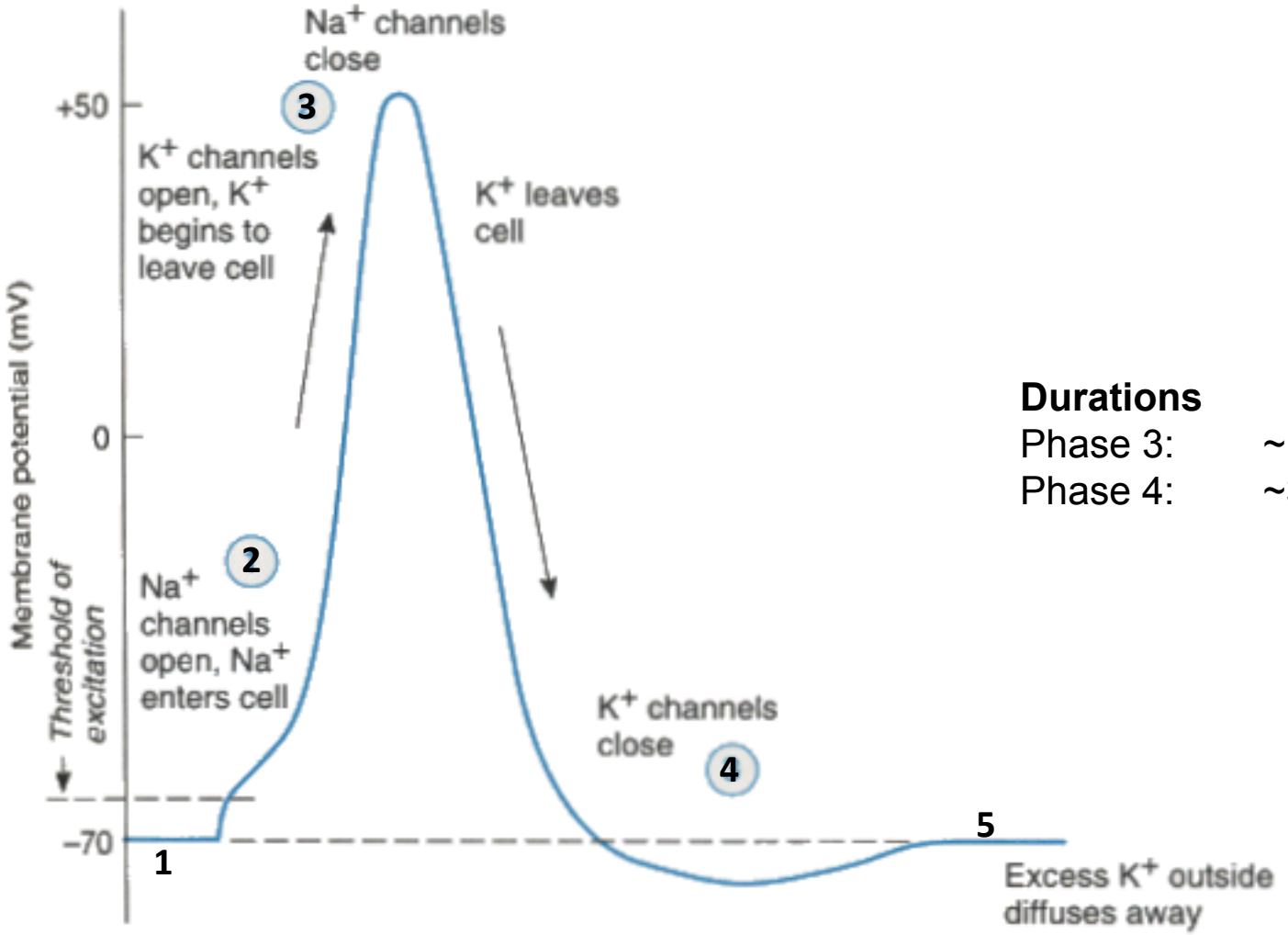


Durations

Phase 3:	~1 ms
Phase 4:	~3 ms

Source: Purves, Orians, Heller, and Sadava, "Life: The Science of Biology," Sinauer Associates/W.H. Freeman & Co., New York, 1999.

Neuronal Action Potential: Ion Flows



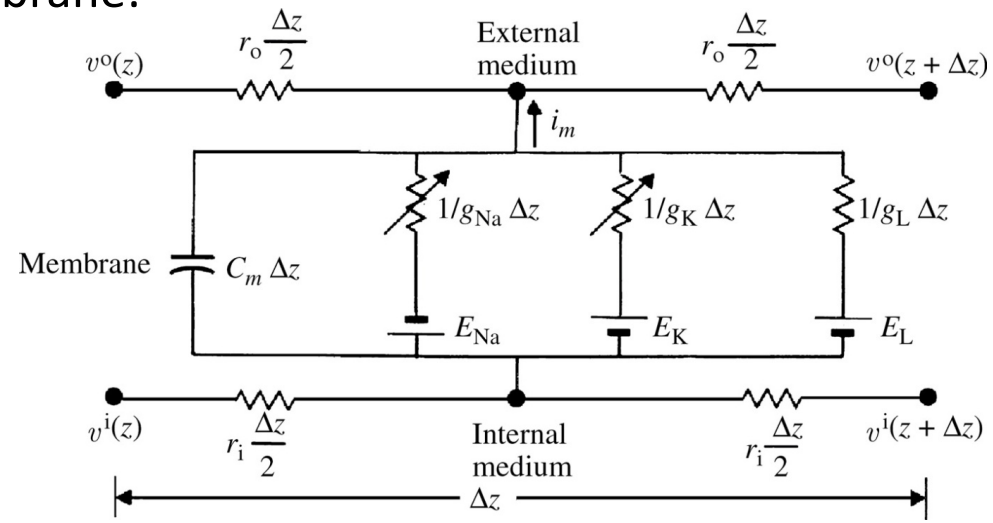
Durations

Phase 3:	~1 ms
Phase 4:	~3 ms

Source: Carlson, Niel. A. (1992). Foundations of Physiological Psychology. Needham Heights, Massachusetts: Simon & Schuster. pp. 53.

Hodgkin-Huxley Electrical Equivalent

Hodgkin and Huxley (HH) electrical equivalent of a small segment of an axon's membrane:



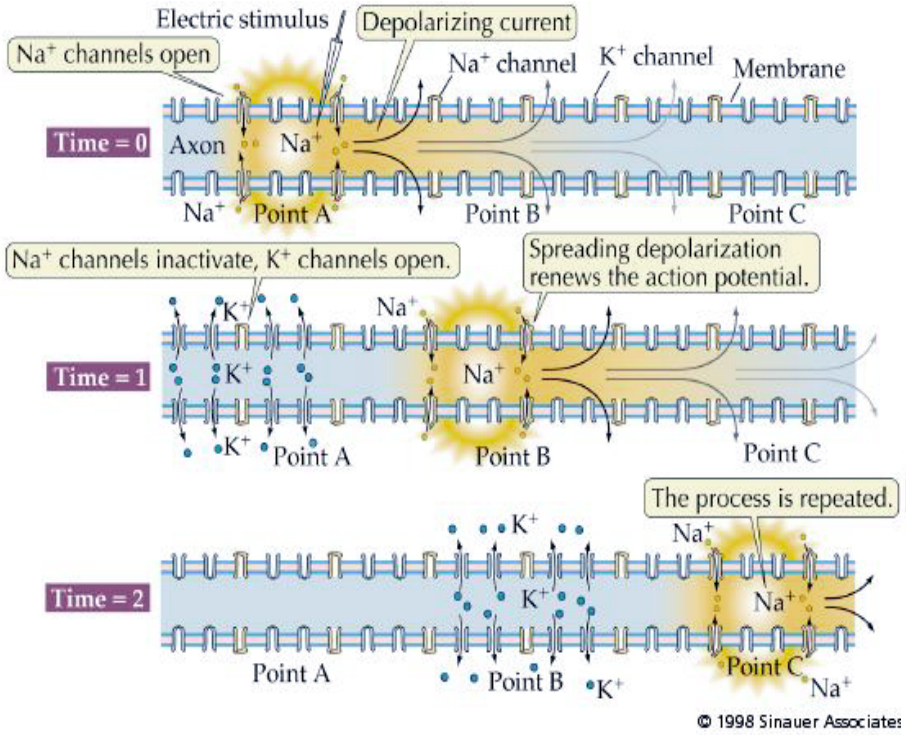
$$I_M = C_m \frac{dV}{dt} + g_K(V - E_K) + g_{Na}(V - E_{Na}) + g_L(V - E_L)$$

where, g_K and g_{Na} are time- and voltage-dependent.

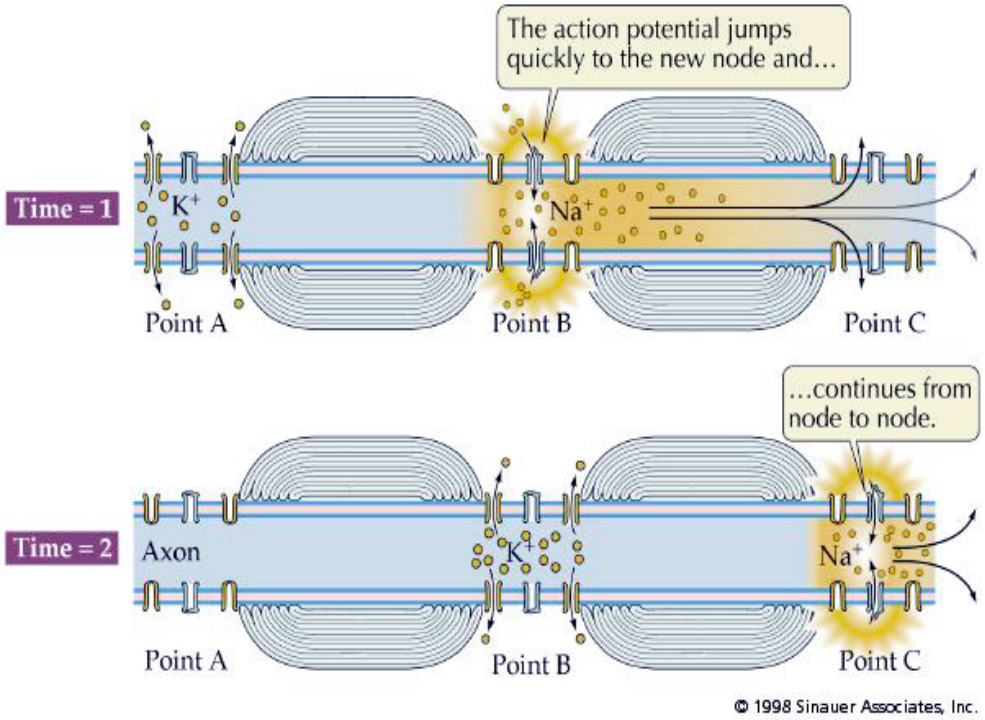
Signal can propagate along a series of these segments, and directionality comes from the fact that voltage-gated sodium channels "remember" for a while and need to "rest" (i.e., exhibit *refractoriness*).

Source: Medical Instrumentataion, J. Webster, Wiley, 2009

Nerve Action Potential Conduction



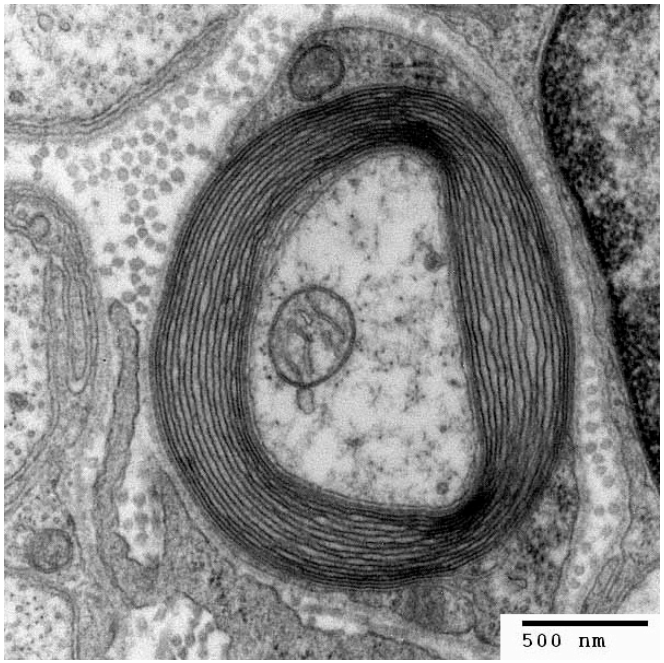
Propagation along unmyelinated axons is ***slow*** (just movement of charges across the membrane).



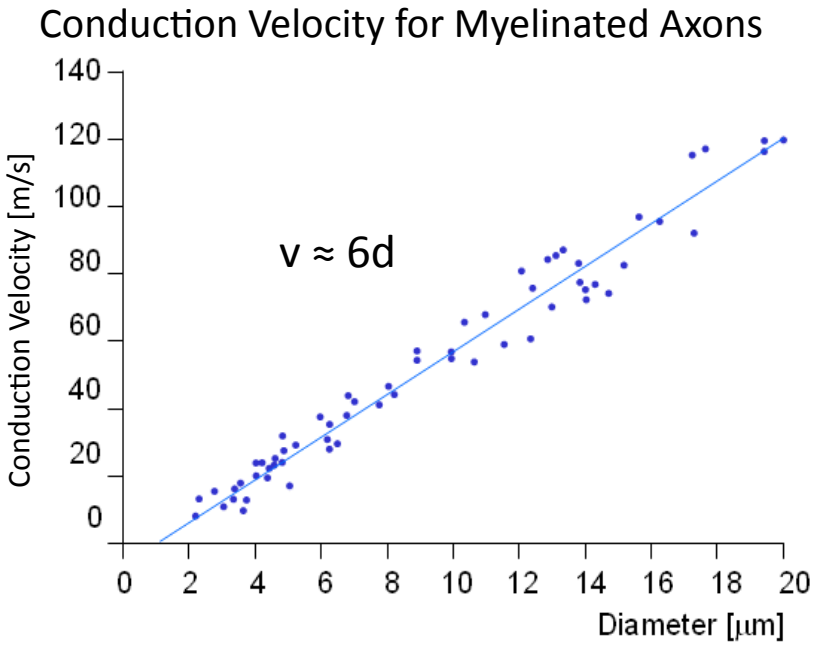
Propagation along myelinated axons is ≈ 20X ***faster*** (current forced to flow from point to point, where it is regenerated). This is *saltatory* conduction.

Courtesy Prof. H. C. Heller, Stanford University. Source: Purves, Orians, Heller, and Sadava, "Life: The Science of Biology," Sinauer Associates/W.H. Freeman & Co., New York, 1999.

Myelinated Axons



http://upload.wikimedia.org/wikipedia/en/c/c1/Myelinated_neuron.jpg



$$v = \sqrt{\frac{i_{Na\,\max}}{r_i c_m^2 V_{th}}} \quad \text{in m/s}$$

$i_{Na\,\max}$ = maximum Na current per unit length [A/m]

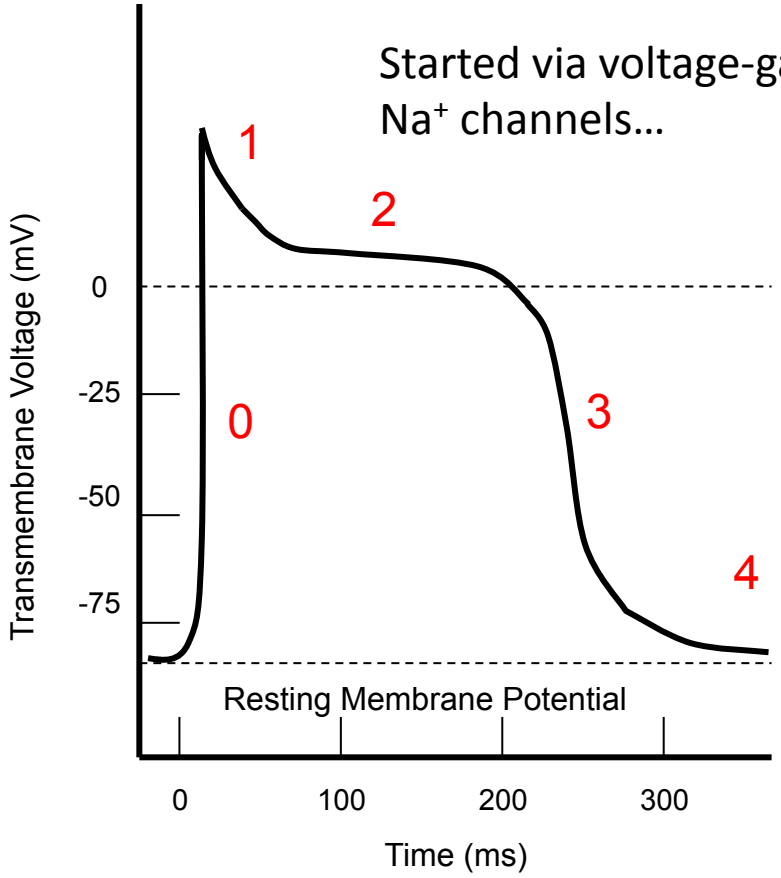
r_i = axial resistance per unit length [Ω/m]

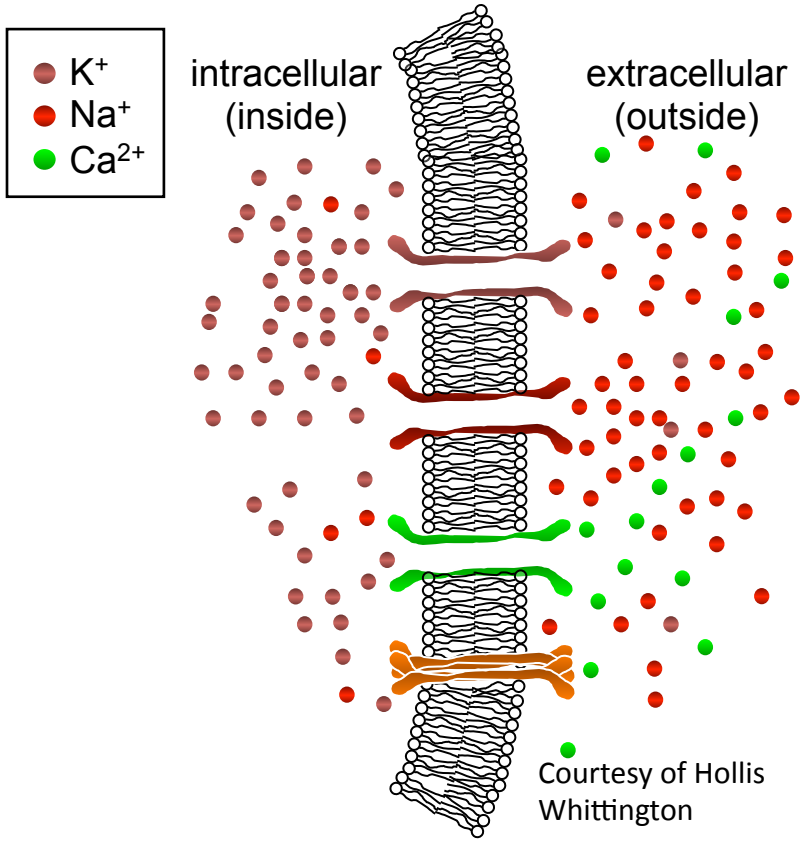
c_m = membrane capacitance per unit length [F/m]

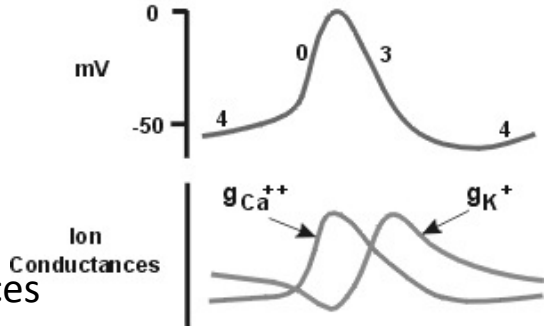
V_{th} = threshold voltage [V]

Ruch TC, Patton HD (eds.) (1982): *Physiology and Biophysics*, 20th ed., 1242 pp. W. B. Saunders, Philadelphia.

Cardiac Action Potential







0 – **Rapid depolarization** – Na+ rushes in, K^+ out
1 – **Initial repolarization** – decreased Na^+ and increased K^+ conductances
2 – **Plateau phase** – increased Ca^{2+} conductance
3 – **Repolarization** – increased K^+ and decreased Ca^{2+} conductances
4 – **Resting potential** – increased K^+ and decreased Na^+ and Ca^{2+} conductances

Other Electrogenic Cells

Beside the well-known neurons and cardiac cells, other cells are also capable of generating electrical signals:

- Muscle cells (smooth, skeletal)
- Retinal cells (cone, rods)
- Pancreatic β cells
- Osteoblasts (bone), colon cells (much slower waves)

Measurement of Membrane and Cell Potentials

Cellular Electrophysiology Techniques

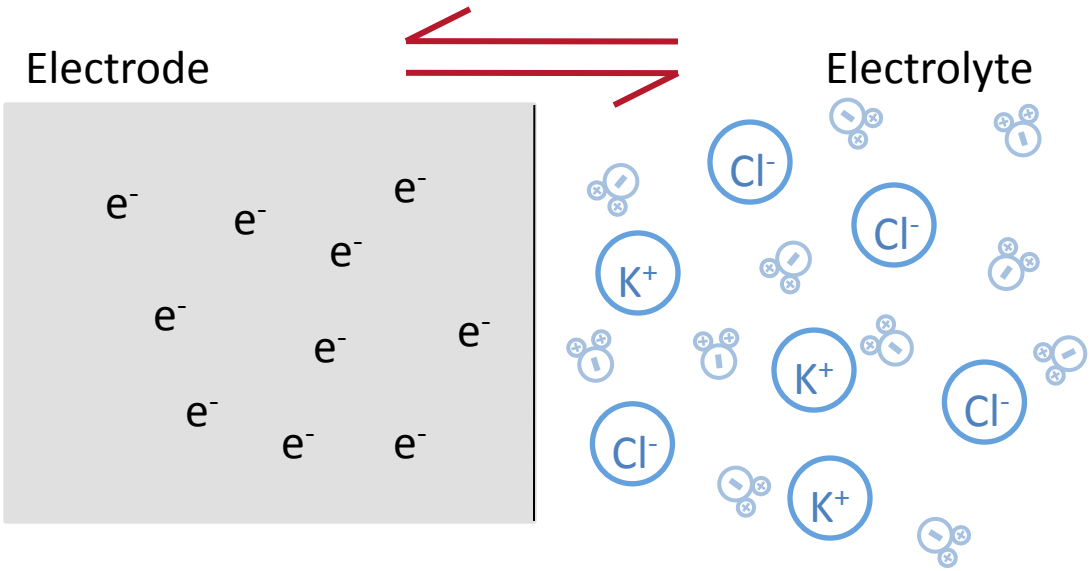
Ions and Electrons

As you have seen, cells make use of ions.

- Many types of ions used (Na^+, Cl^-, K^+, Ca^{2+},…)
- Ions are much slower than electrons (6 logs!)

Electrodes are the most common transducers to convert ionic currents into electronic currents.

The next objective is to understand how this works.



Carrier or Ion	Mobility, μ, in $cm^2/(s \cdot V)$
electron in Si	1.35×10^3
hole in Si	4.80×10^2
H^+ in H_2O	3.63×10^{-3}
OH^- in H_2O	2.05×10^{-3}
Cl^- in H_2O	7.91×10^{-4}
K+ in H_2O	7.62×10^{-4}
NH_4^+ in H_2O	7.61×10^{-4}
ClO_4^- in H_2O	7.05×10^{-4}
Na^+ in H_2O	5.19×10^{-4}
HCO_3^- in H_2O	4.61×10^{-4}
Li^+ in H_2O	4.01×10^{-4}

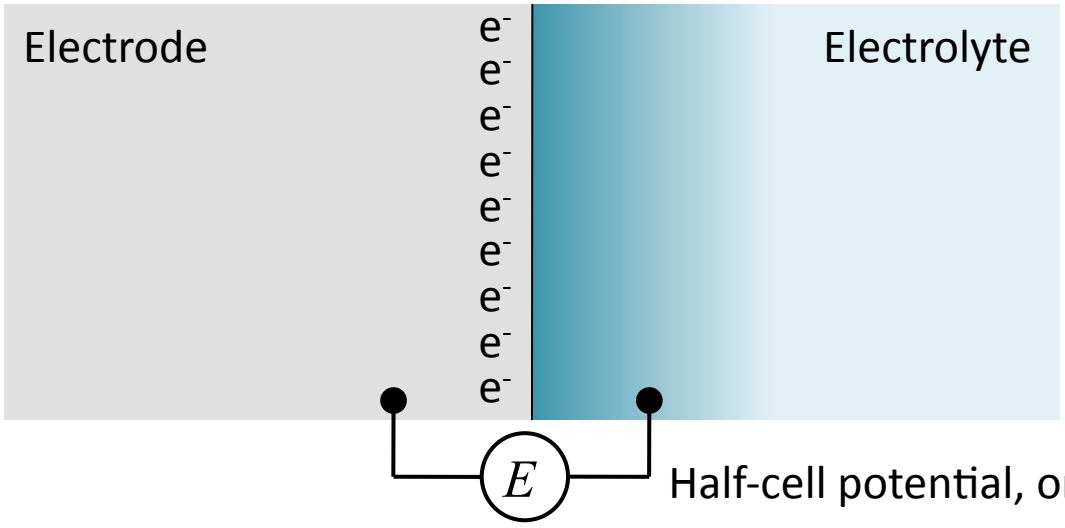
Electrode-Electrolyte Interface

When an electrode is placed into an ionic solution:

- Cations form due to oxidation of electrode ($C \leftrightarrow C^+ + e^-$), leaving electrons in the electrode.
- Neutral atoms form due to oxidation of anion ($A^- \leftrightarrow A + e^-$), giving electrons to the electrode.

Eventually a space charge builds up and electrochemically opposes the oxidation reaction. (Sound familiar? There is a Nernst potential involved.)

$$C \leftrightarrow C^+ + e^-$$
$$A^- \leftrightarrow A + e^-$$



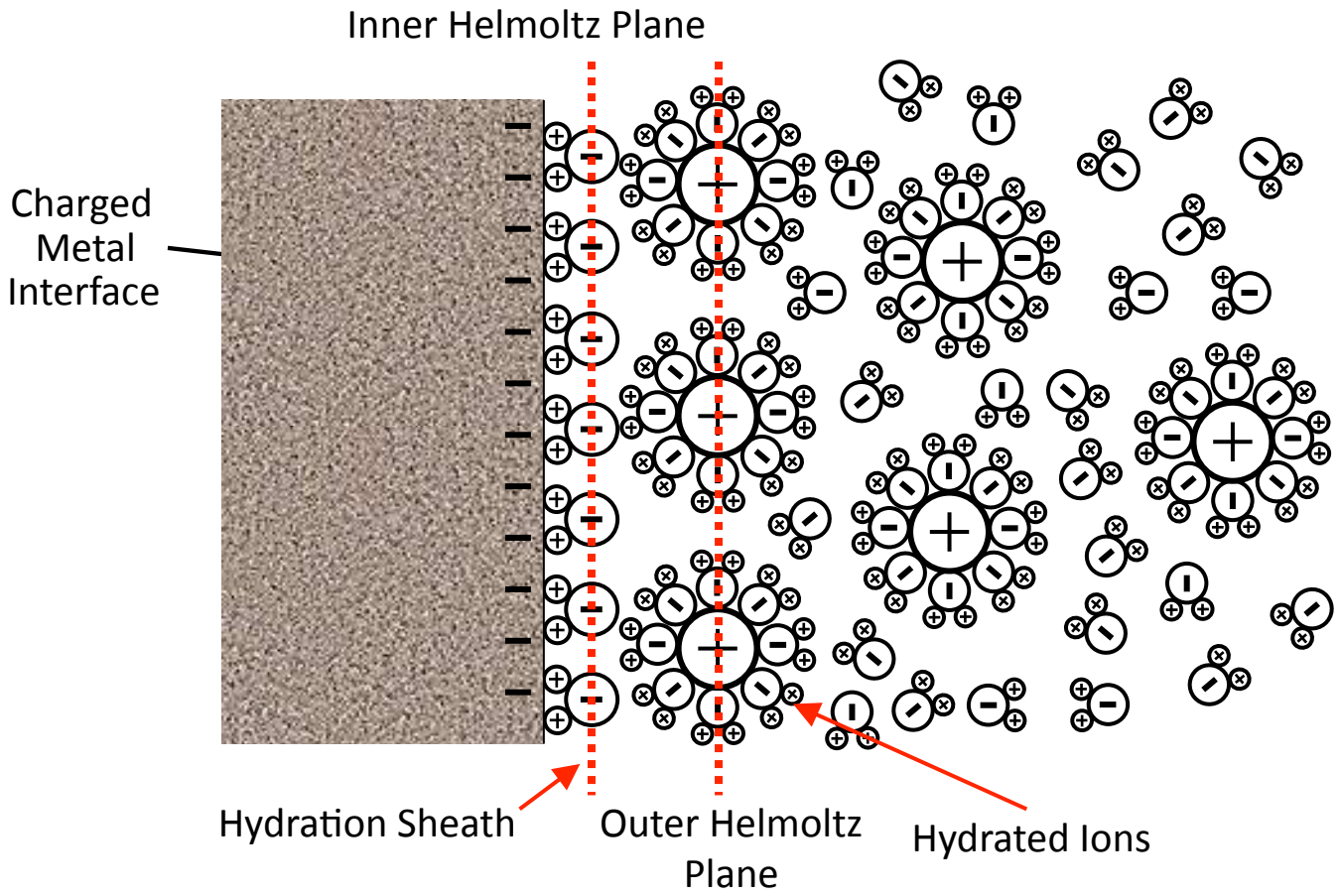
$$E = E^0 + \frac{RT}{nF} \ln Q$$

E_0: standard cell potential (25°C, 1M)
n: valence
Q: thermodynamic reaction quotient
R: universal gas constant
T: absolute temperature in Kelvin
F: Faraday constant

Half-cell potential, or electromotive force (EMF)

Electrode-Electrolyte Interface

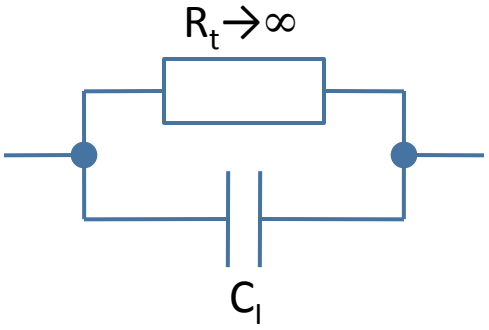
The space charge create highly-oriented layers close to the electrode, acting as a *capacitor* (10 - 20 μF/cm^2). This is the basis of the electrolytic capacitor in common use.



Electrode Polarization

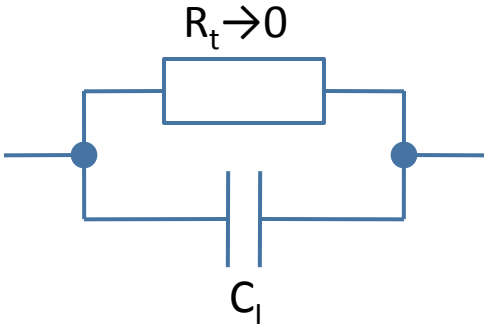
- Two main electrical transport mechanisms arise for electrodes:
  - Capacitive (displacement): AC only
  - Faradic (electrochemical): DC and large-signal AC
- Depending on which mechanism dominates, an electrode is *polarizable* (capacitive), or *non-polarizable* (Faradic):

Ideally Polarizable



Platinum, Gold, … (noble metals)

Ideally Non-Polarizable



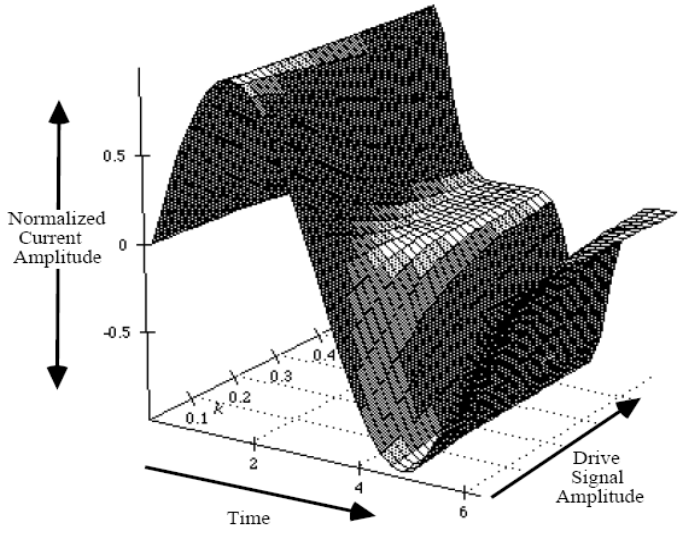
Ag/AgCl, Calomel

Electrode Materials

- Noble metals (platinum, iridium, gold)
- Organic materials (PEDOT)
- Ceramics (titanium nitride)
- Carbon nanotubes (yep, even here…)
- Silver/Silver chloride (Ag/AgCl)
  - Silver helps keeping resistance low.
  - Silver chloride participates in electrochemical reactions.
  - Fabricated by electrochemical deposition (coating) or sintering.

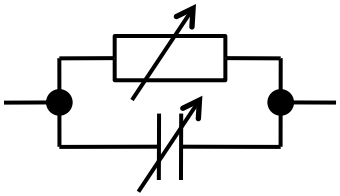
Imperfections of Electrodes

- High charge transfer resistance (Pt, Ir, Au)
  - Non-linearity at high amplitudes
  - Electrode damage
  - Irreversible reactions
  - Generation of toxic by-products



Source: G.Kovacs, Enabling Technologies for Cultured Neural Networks, 1994

- Frequency-dependence of impedance (Pt, Ir, Au)
  - Polarized electrodes have typical $1/f^{\alpha}$ impedance spectrum ($\alpha \approx 0.5$ - 1), and a constant phase.



- Electrode wear (Ag/AgCl)
  - Non-polarized electrode consumes themselves.

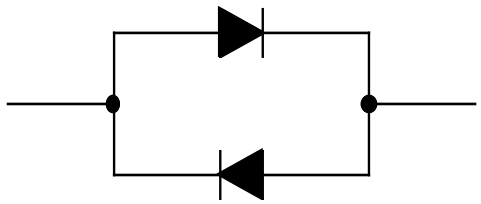
Aside: Large-Signal Electrodes

- For large-signal drive (> 50 mV or so), electrodes look like back-to-back diodes.
- It turns out that this is very similar to the solid-state case familiar to EE's.

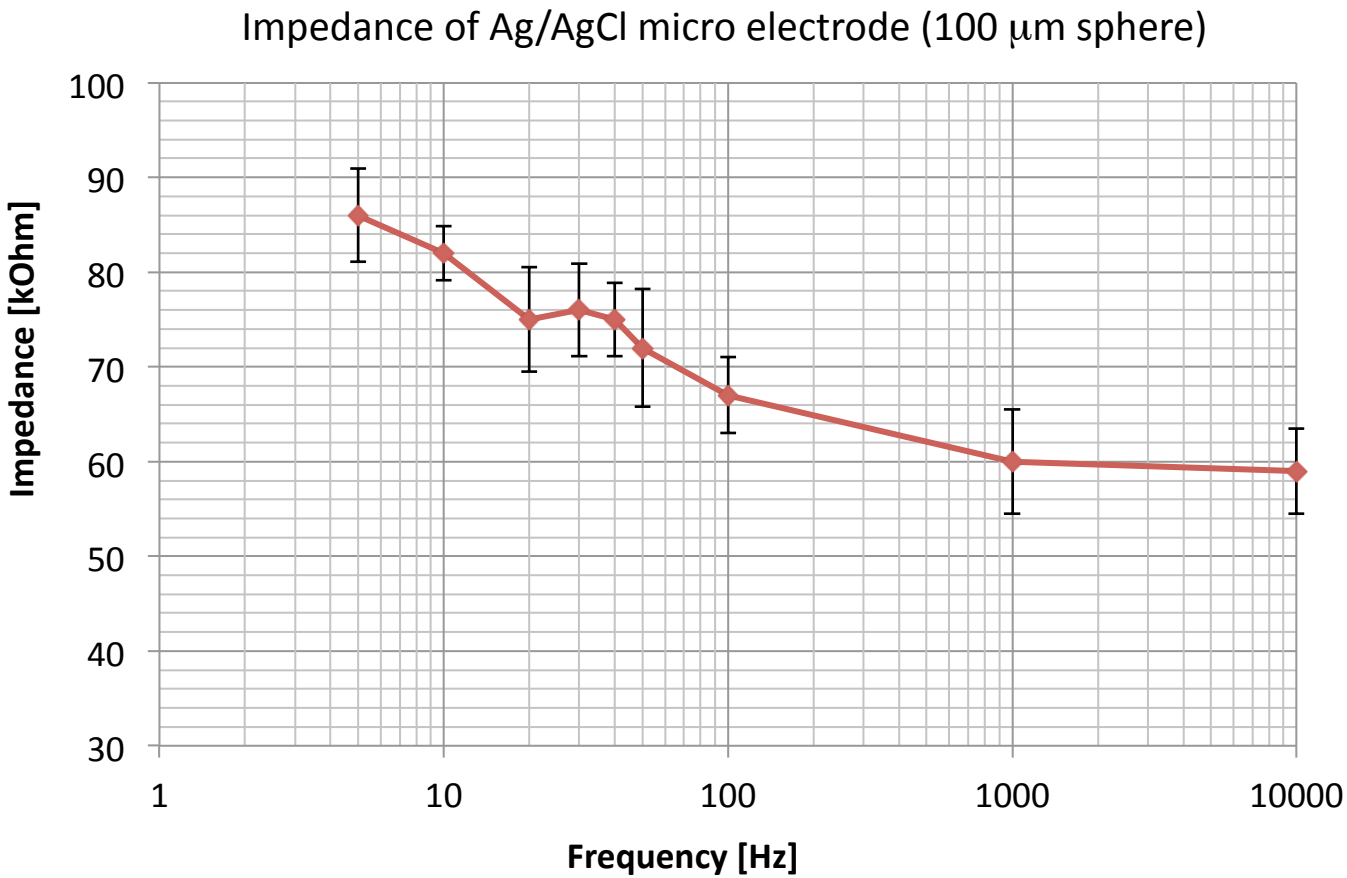
$$J = J_o\left(e^{\frac{(1-\beta)z\eta_t F}{RT}} - e^{\frac{-\beta z\eta_t F}{RT}}\right)$$

$$J = J_o\left(e^{\frac{qV}{\eta kT}} - e^{\frac{-qV}{\eta kT}}\right)$$

$$V_T = \frac{kT}{q} = \frac{RT}{F}$$



Ag/AgCl Electrode Impedance Spectrum



After: E. Larsen, J. Applied Physiology, 28(3), 1970

Pt Electrode Impedance Spectrum

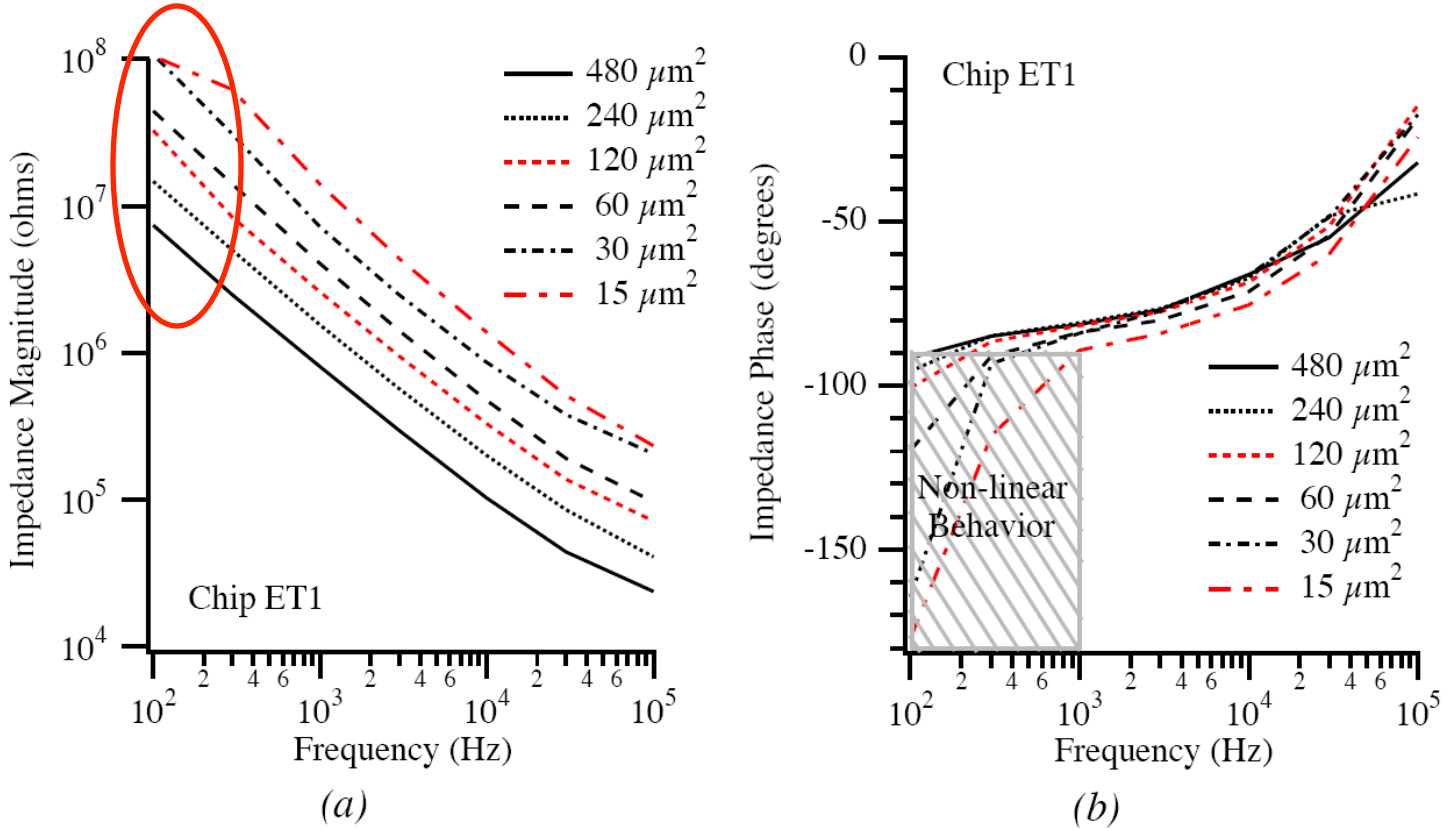
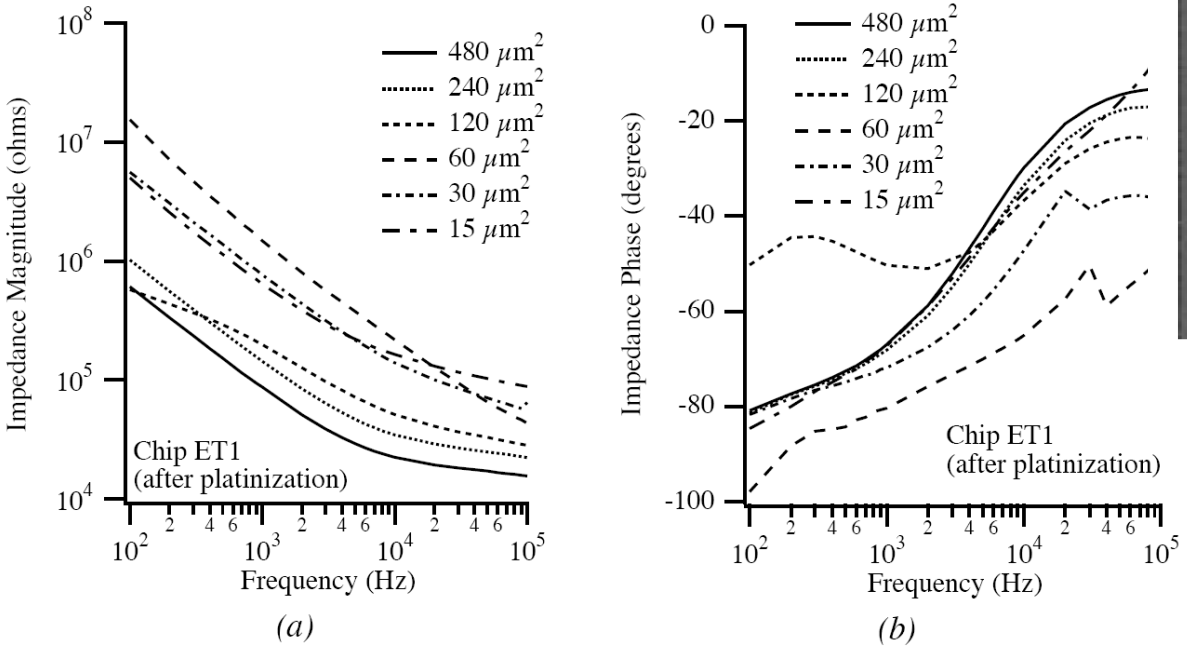
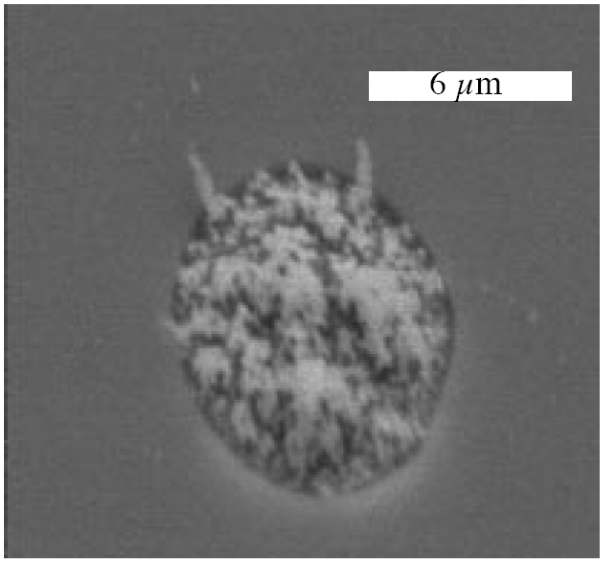


Figure 4.11: Measured impedance magnitude (a) and phase (b) for 6 different bare Pt electrodes on chip ET1. Note that around 1 kHz the phase began to drop off significantly for the smallest electrode. This was the most likely the onset of non-linearity due to excessive current density. The impedance values above 1kHz should be approximately correct for all electrode sizes.

Source: D. Borkholder, Thesis, Stanford, 1999

Reduction of Impedance





SEM View of Pt-black.

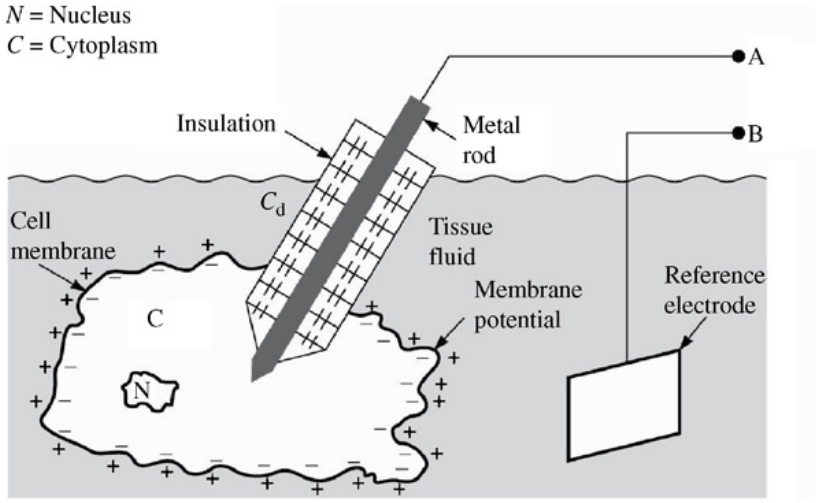
Figure 4.15: Measured impedance magnitude (a) and phase (b) for 6 different electrodes on chip ET1 following deposition of platinum black. A comparison to Figure 4.11 shows the impedance magnitude decreased by approximately one order of magnitude for this chip. The non-linear behavior at low frequencies has also been significantly diminished.

Source: D. Borkholder, Thesis, Stanford, 1999

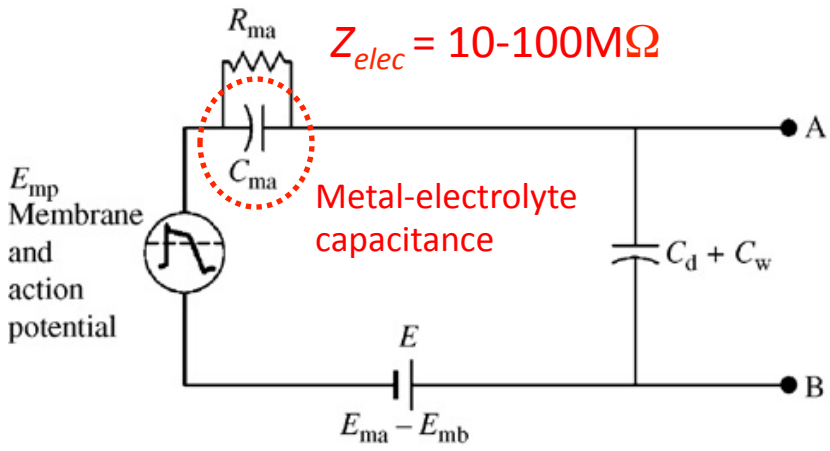
Intracellular Microelectrode Recordings

- First recordings of intracellular potentials ever made.
- Metal electrodes directly impaled into cell.
- Mostly suitable for AC recordings (metal-electrolyte impedance).

Typical Setup



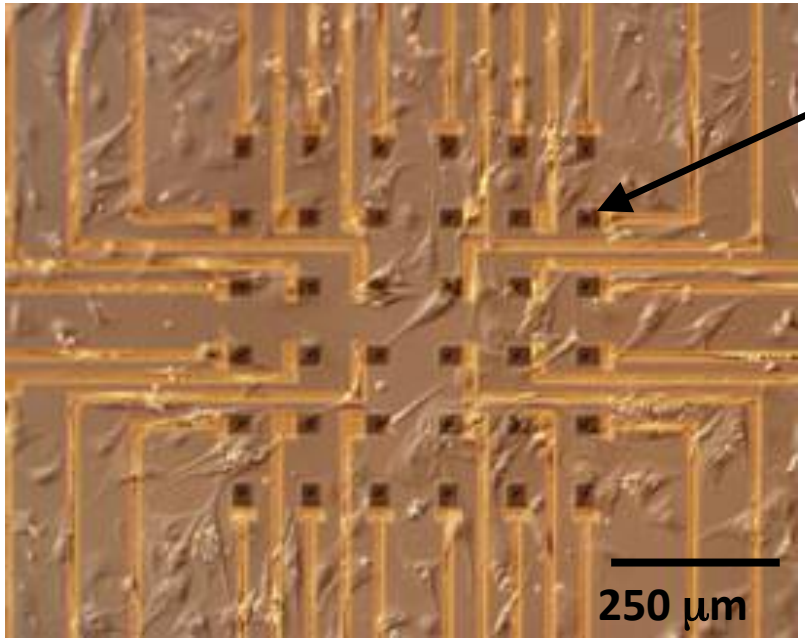
Equivalent circuit

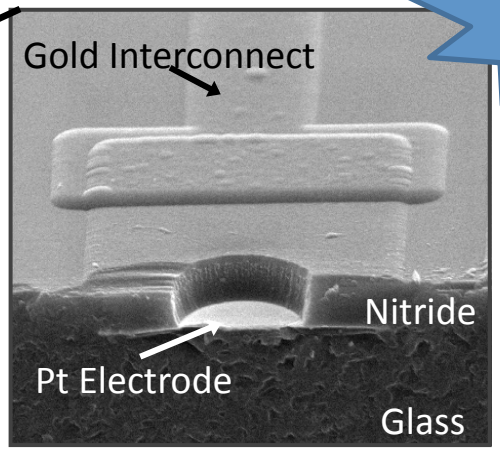


Source: Medical Instrumentation, J. Webster, Wiley, 2009

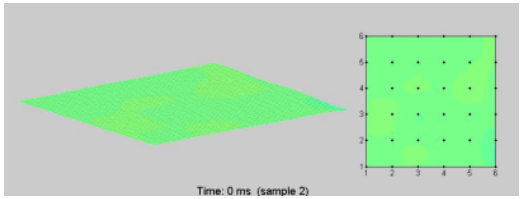
Extracellular Planar Microelectrodes

- Record extracellular potential of cells in close contact with metal electrodes.
- Arrays of planar electrodes can be built for multichannel recordings.

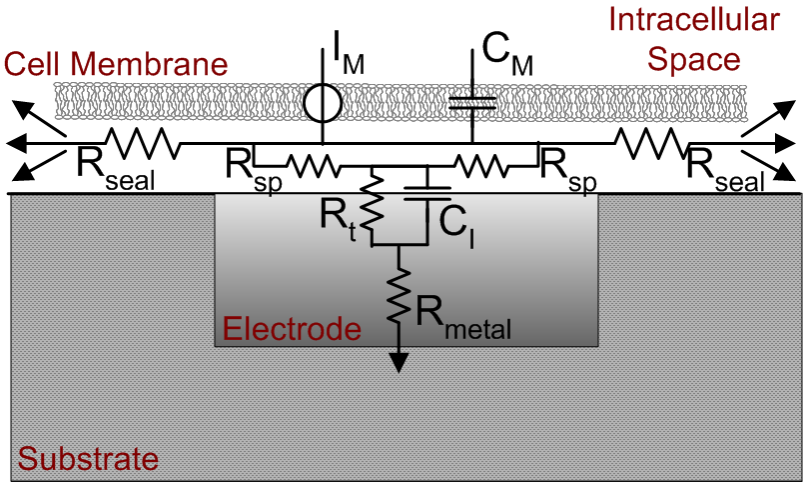




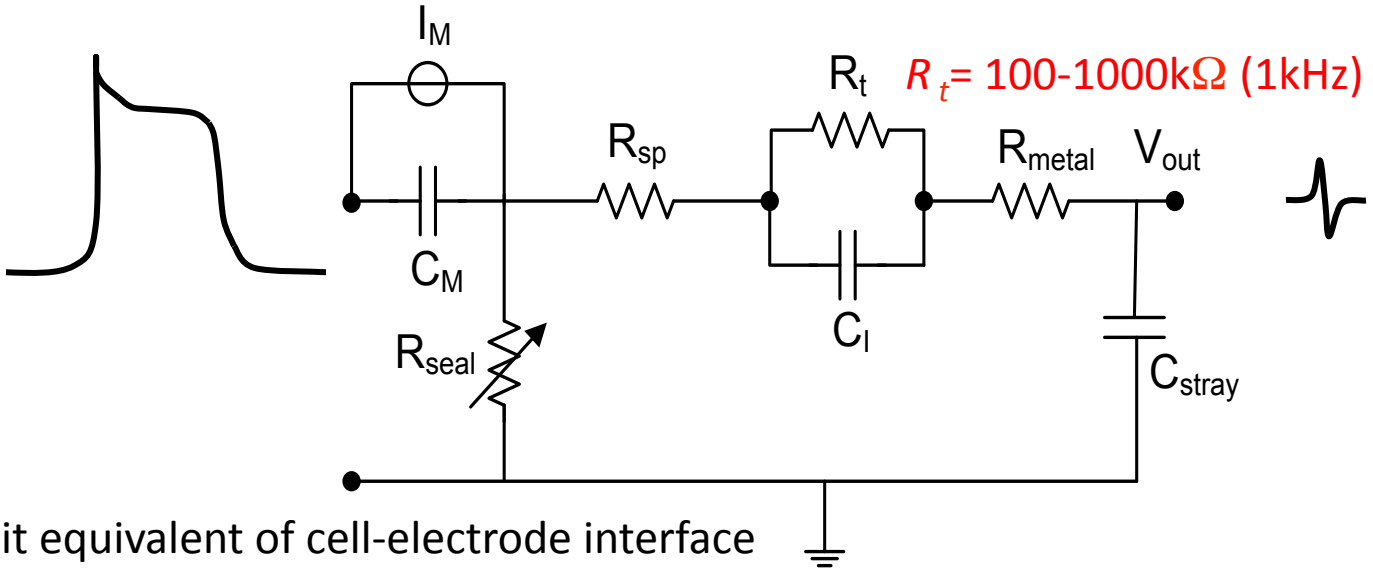
Used in Lab !



Extracellular Planar Microelectrodes



- Extracellular voltage *smaller* than intra (function of R_{seal})
  - Typically 100 - 1000 μV
- Waveform dependent on coupling
  - From AP-like to second derivative

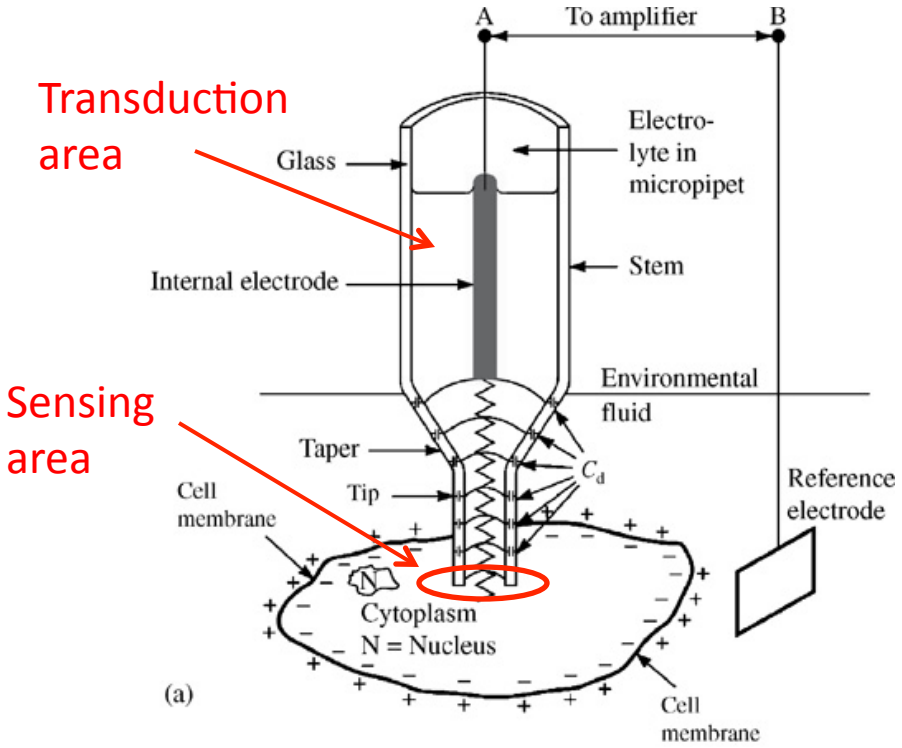


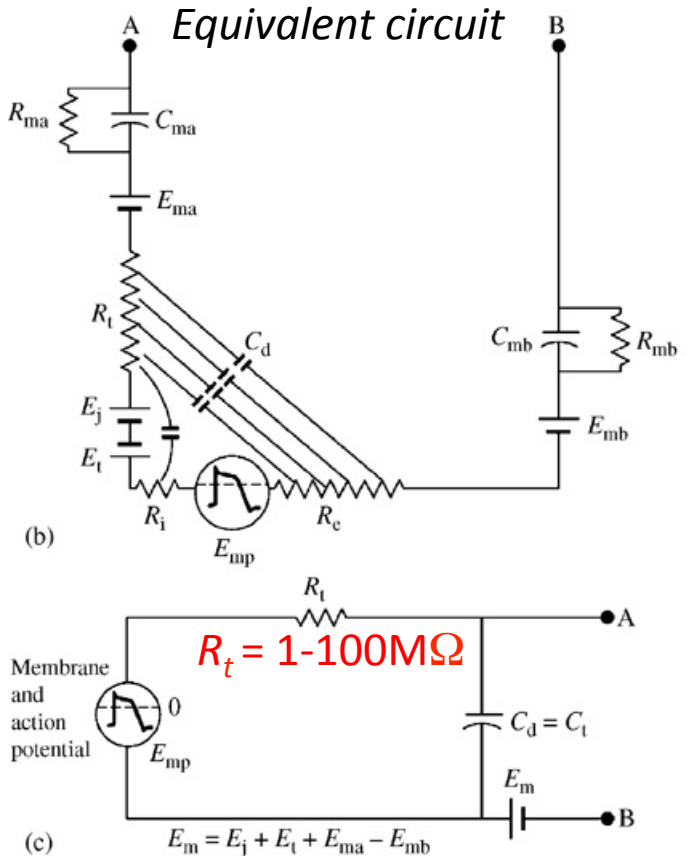
Circuit equivalent of cell-electrode interface

After: Grattarola and Martinoia, TBME 40, 1993

Micropipette Electrode Recordings

- Significant improvement upon metal microelectrodes
  - Electrolyte-filled capillary allows decoupling of signal pick-up and signal transduction.

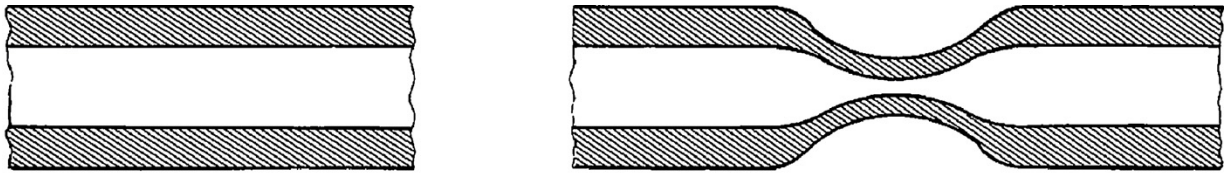




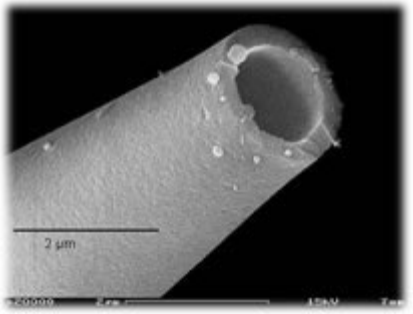
Source: Medical Instrumentation, J. Webster, Wiley, 2009

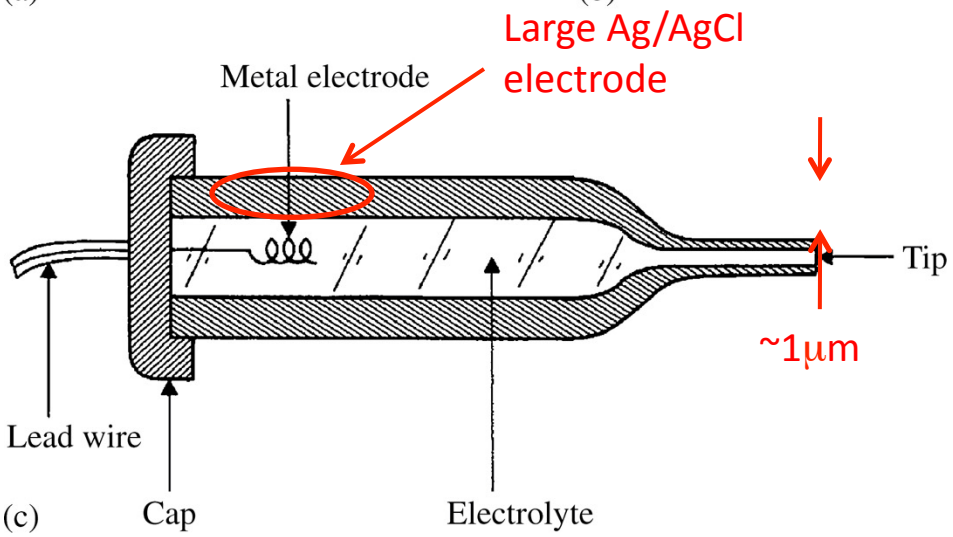
Micropipette Electrodes

Fabrication method: Pipette pulling

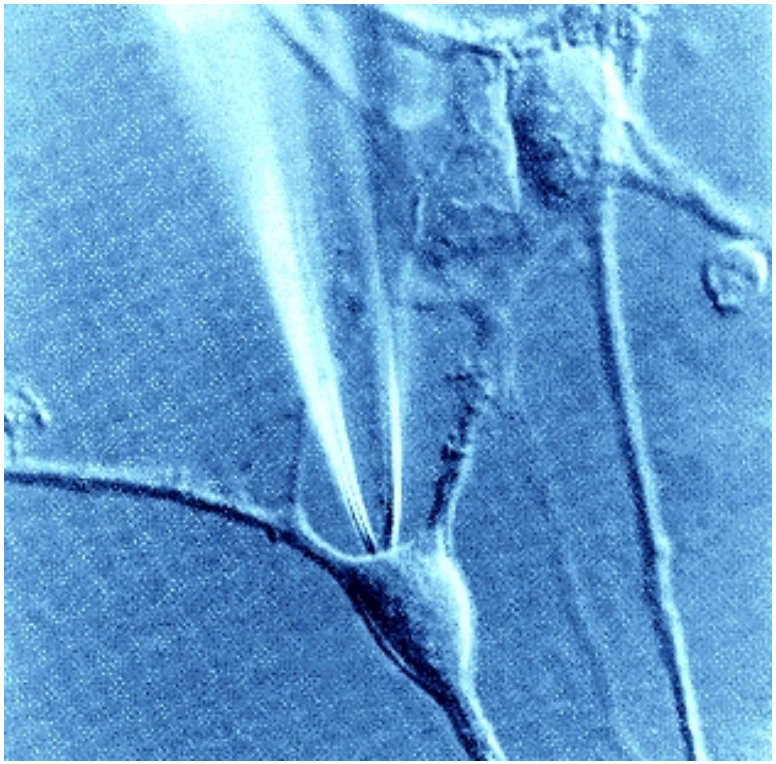


(a) (b)





(c)



Sources: Medical Instrumentation, J. Webster, Wiley, 2009, http://www.ipmc.cnrs.fr/~duprat/neurophysiology/patch.htm, Wikipedia

Challenges in Electrode Interfacing

Low-Noise Amplifiers

Refresher: Noise Sources

- Intrinsic
  - Thermal (Johnson) noise
    Due to random motion of electron across a resistor.
  - Shot noise (current noise)
    Due to quantization of current.
- "Excess noise"
  - Flicker (pink, 1/f) noise
    Due to fluctuations in device parameters.
- Extrinsic
  - Interferences

$$V_{noise,RMS} = \sqrt{4kTRB}$$

$$I_{noise,RMS} = \sqrt{2qIB}$$

$$V_{noise}, I_{noise} \propto 1/f$$

k: Boltzmann's constant
T: Temperature [K]
R: Resistance [W]
B: Bandwidth (Hz)
q: Electron charge [C]
I: Current [A]
f: frequency [Hz]

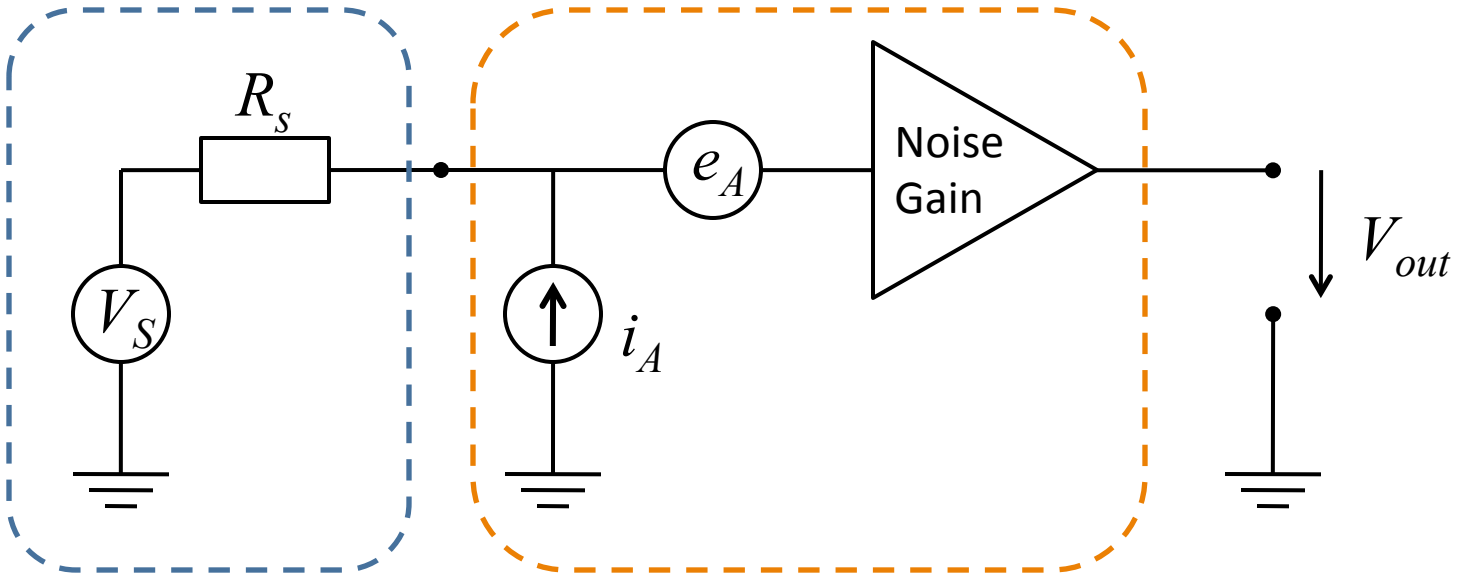
Good References: Horowitz & Hill, TI App Note SLOA 082

Noise in Amplifiers

- All reviewed electrodes are characterized by *high impedances*, and are recording signals of *low amplitudes.*
- Amplification must add minimum noise!

$$e^2 = \underbrace{4kTR_s}_{\text{Source}} + \underbrace{e_A^2 + (R_s i_A)^2}_{\text{Amplifier}} \quad \text{V}^2 / Hz$$

Note the units – the noise is a function of the bandwidth !

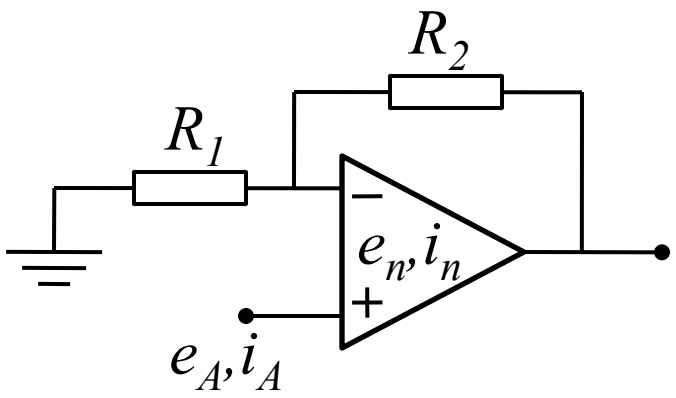


Voltage Amplifier Noise Model

Noise in Feedback Amplifiers

Amplifier noise is also affected by feedback (simplified treatment):

Non-inverting configuration:

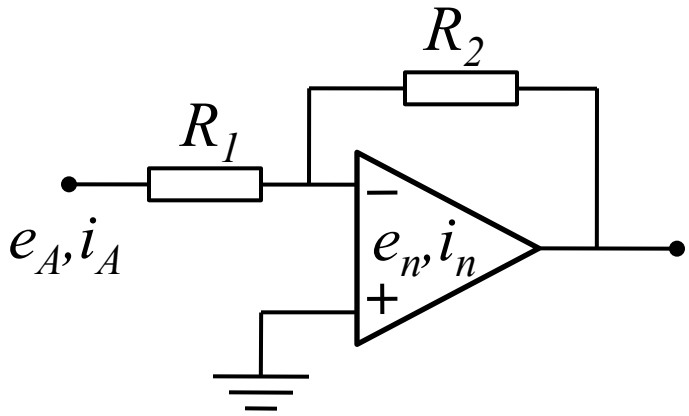


$$i_A^2 = i_n^2$$

$$e_A^2 = e_n^2 + 4kTR_{\|} + (R_{\|} i_A)^2$$

$$with\ R_{\|} = \frac{R_1 R_2}{R_1 + R_2}$$

Inverting configuration:



$$i_A^2 = i_n^2 + 4kT\frac{1}{R_2}$$

$$e_A^2 = e_n^2 + R_1^2\left(i_n^2 + 4kT\frac{1}{R_2}\right)$$

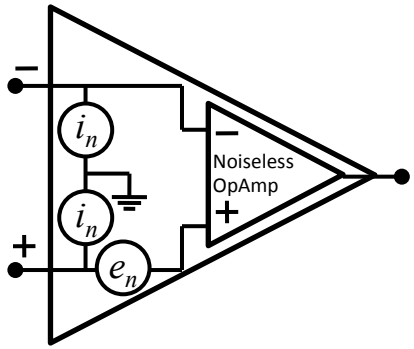
$$= e_n^2 + (R_1 i_A)^2$$

Noise in Operational Amplifiers

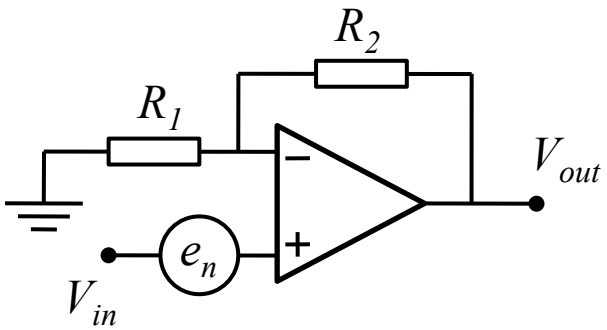
A quick aside: Closed-loop Signal Gain and Noise Gain

- **Signal gain** is gain seen by signal.
- **Noise gain** is gain seen by a voltage noise source in series with an op amp input.

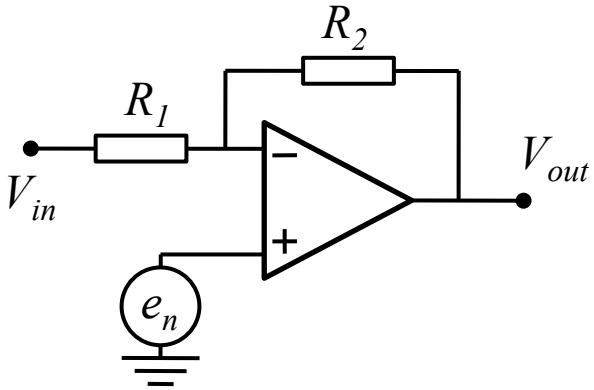
Operational Amplifier Noise Model



Non-inverting configuration



Inverting configuration



$$A_{Signal} = 1 + \frac{R_2}{R_1} \quad \longleftrightarrow \quad A_{Signal} = -\frac{R_2}{R_1}$$

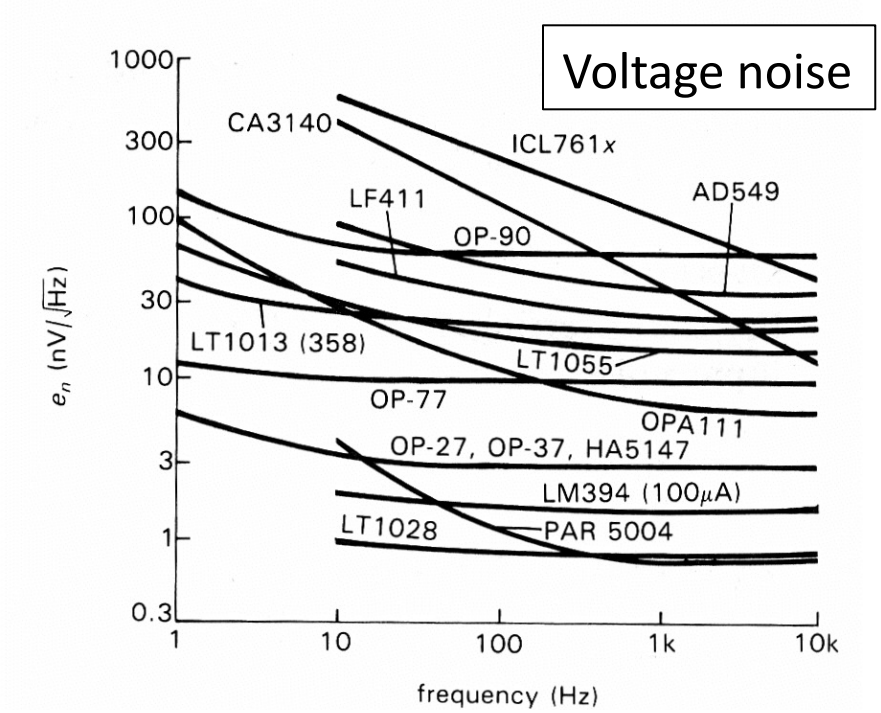
Different

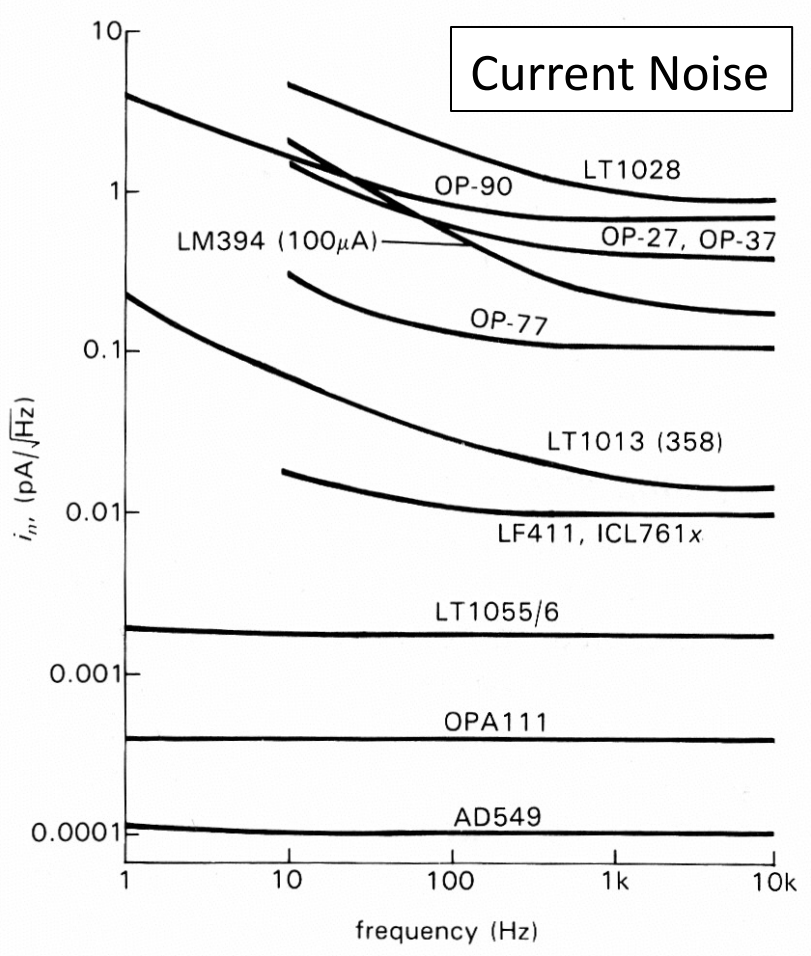
$$A_{Noise} = 1 + \frac{R_2}{R_1} \quad \longleftrightarrow \quad A_{Noise} = 1 + \frac{R_2}{R_1}$$

Same !

Which Op-Amp to Choose?

There is no free lunch. Choosing an op-amp is ALWAYS a trade-off!



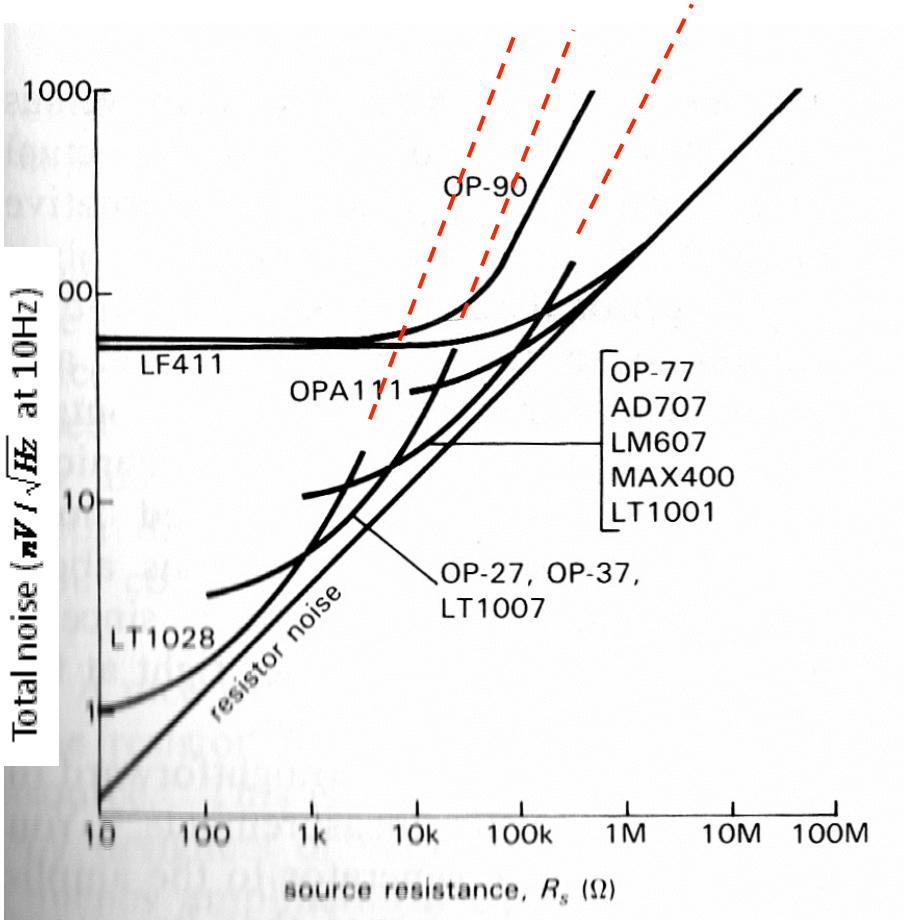


Generalities (sometimes wrong!):

- JFET/FET : Low current noise (capacitive gate)
  High voltage noise
- Bipolar: Low voltage noise
  High current noise (base current)

Source: The Art of Electronics, Horowitz & Hill, 1989

Matching Source with Op-Amp



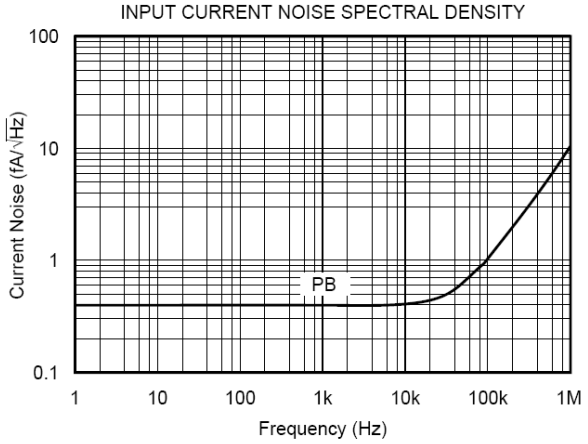
Depending on source resistance and amplifier:

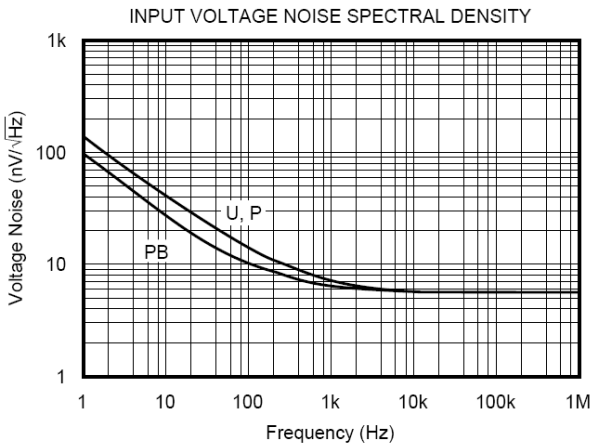
- *Voltage noise* can dominate
- *Current noise* can dominate
- *Source resistance noise* can dominate (best – means amplifier does not add noise)

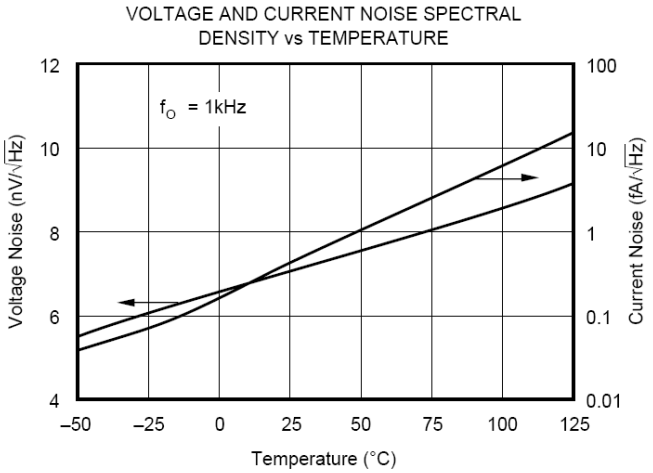
Total noise (source resistor plus amplifier, at 10Hz)

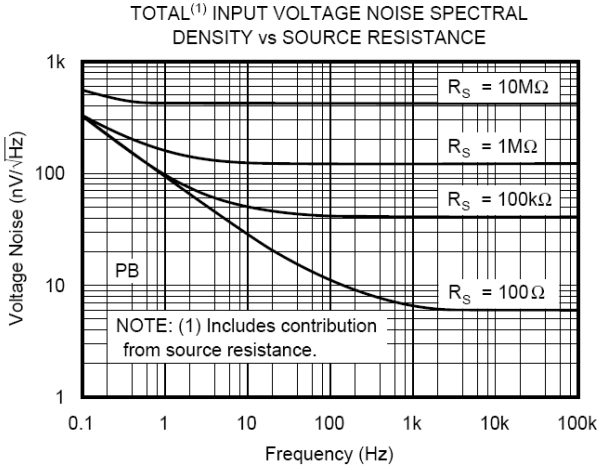
Source: The Art of Electronics, Horowitz & Hill, 1989

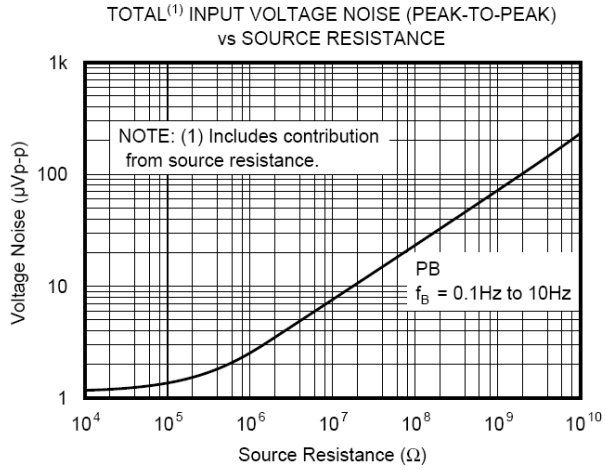
Noise in Operational Amplifiers - Example

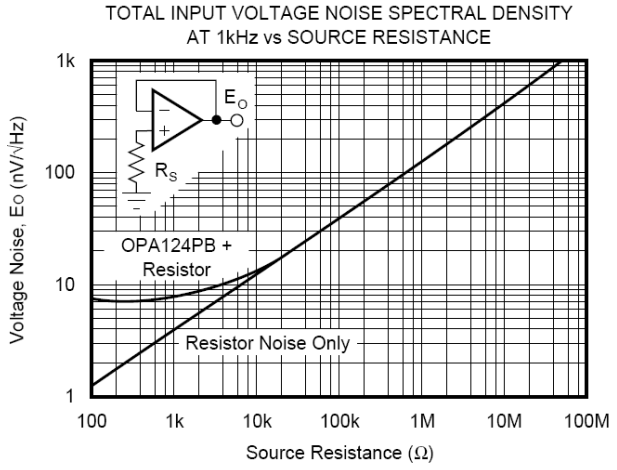












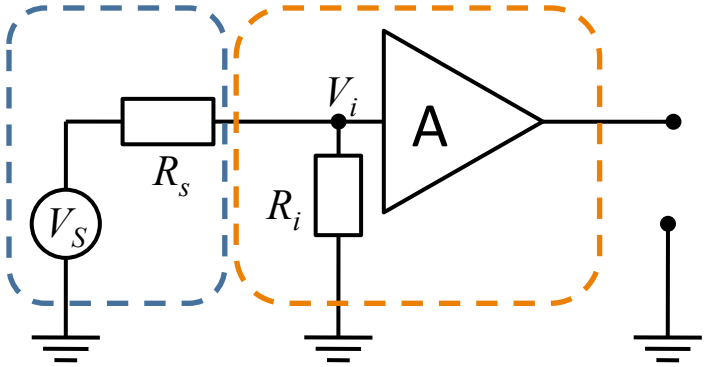
From OPA124 Low noise Precision DiFET Op-Amp Data sheet

Other Non-Idealities of Op-Amps…

…which particularly matter with large source impedances.

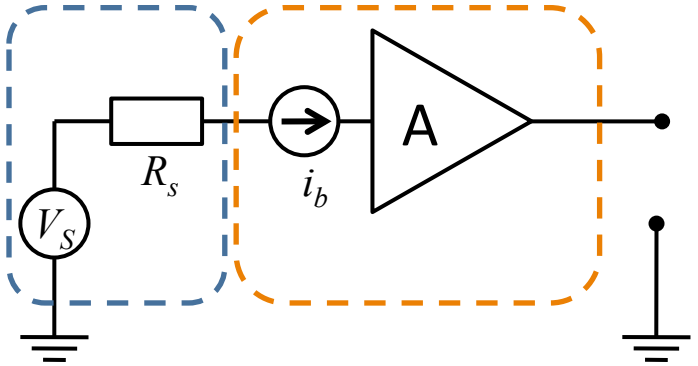
- Input impedance

  With high source impedance, low input impedance will load the source and reduce the signal.



- Input bias current

  Large input bias currents will create a DC offset across the source impedance, potentially saturating the op-amp input.



Focus on a Key Electrophysiology Technique

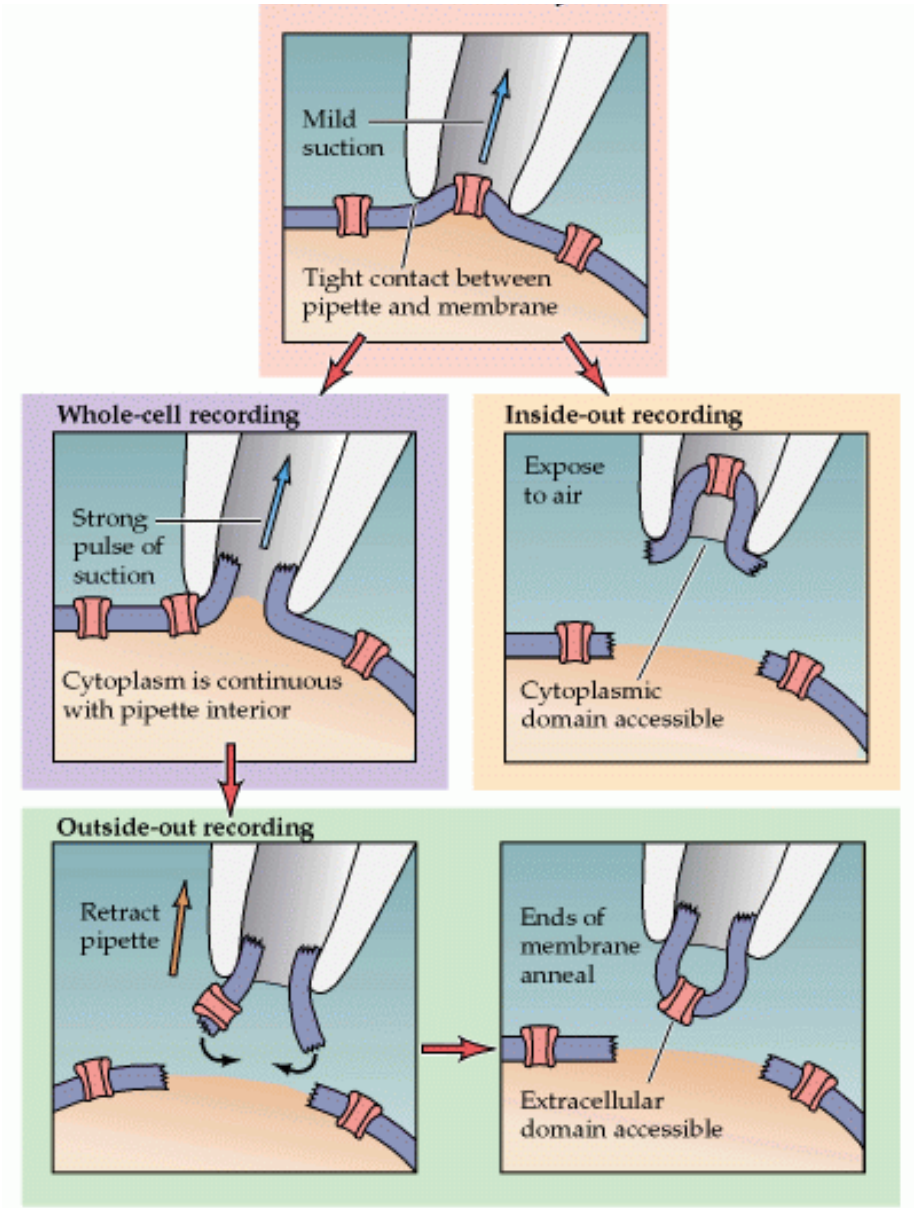
Patch-Clamping

Patch-Clamping Techniques

Essentially, current recording under voltage clamp.

Four recording modes:

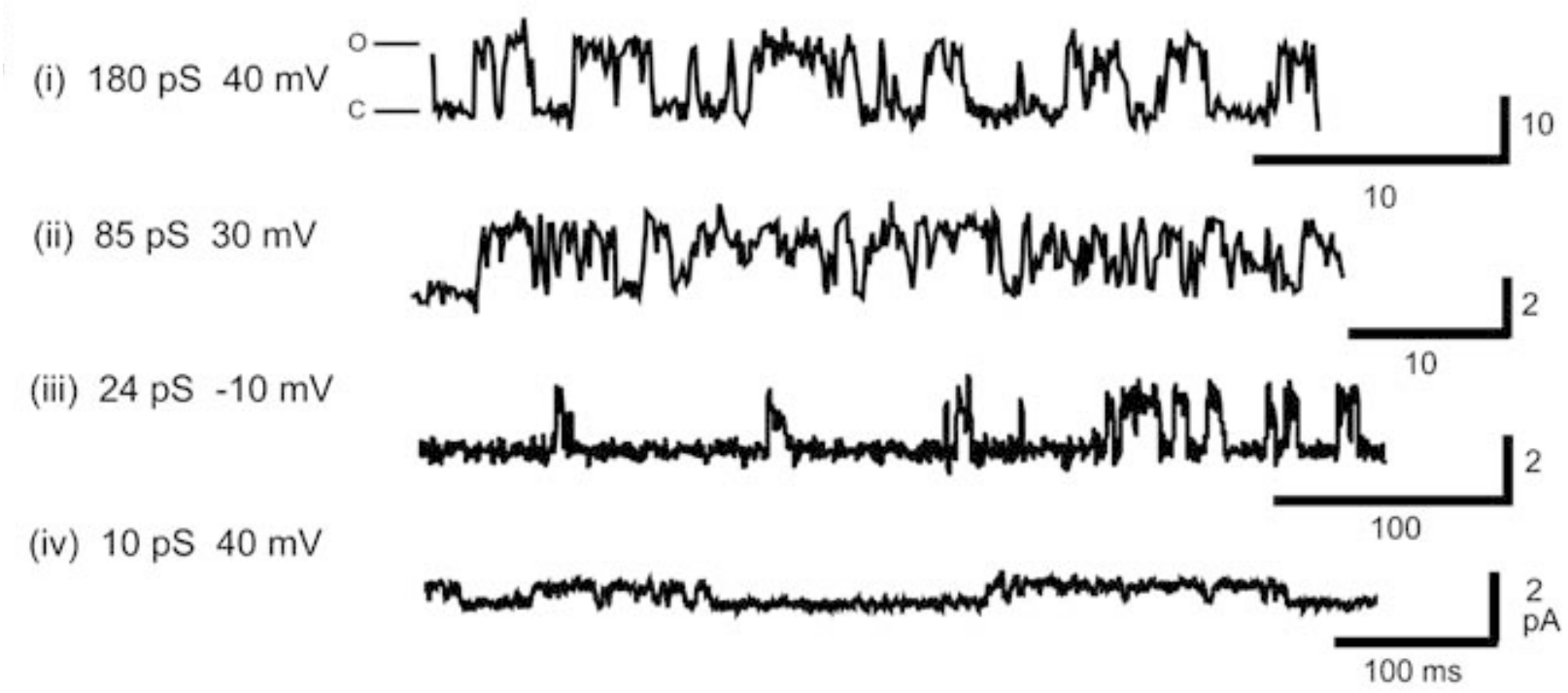
- *Cell-attached*
  (single channel recording)
- *Whole-cell*
  (access to cytosol)
- *Inside-out*
  (single channel with access to cytosol)
- *Outside-in*
  (single channel with access to extracellular fluid)



Patch Clamping Setup

Source: http://www.univ-orleans.fr/neurobiologie/ENGLISH/equipements.htm

Typical Single-Channel Recording

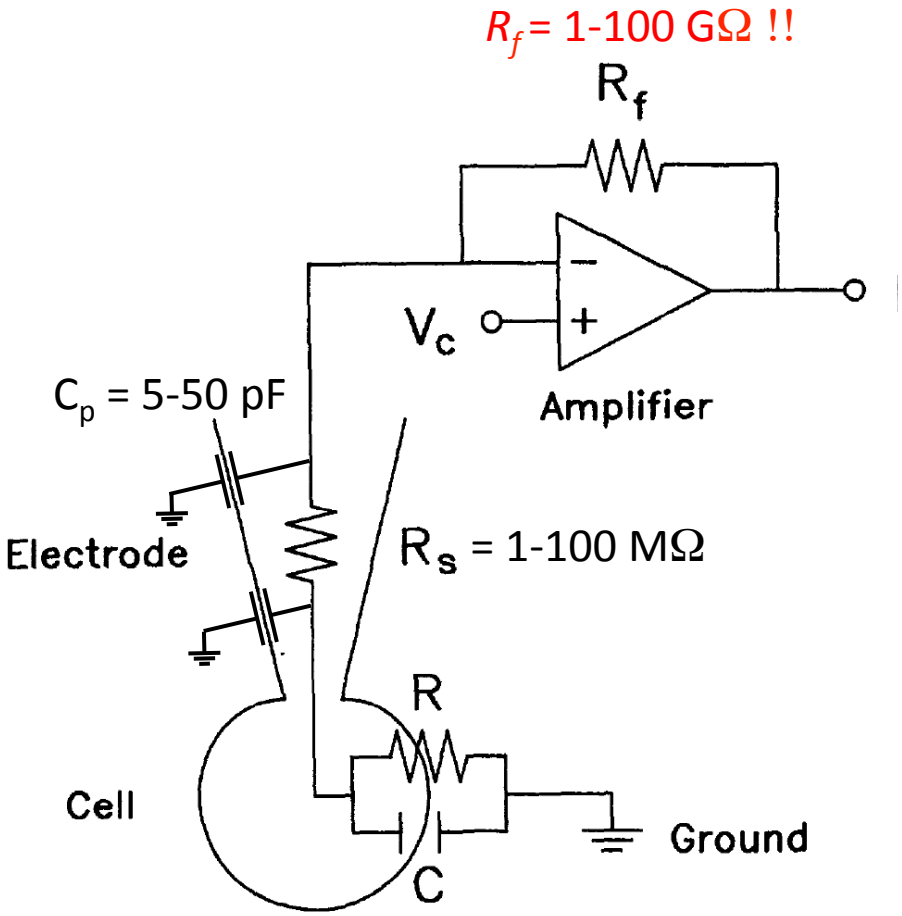


Single channel recording from electrosensory lateral line pyramidal cells (neurons), showing four classes of K^+ channels.

Source: Morales, Releasing the peri-neuronal net to patch-clamp neurons in adult CNS, Eur J Physiol, 2004

Patch-Clamp Amplifier

- Basically, a voltage-clamped, current converter.
- Challenges:
  - Noise
  - Bandwidth (capacitance compensation)
  - Offset (series resistance compensation)



Noise in Patch-Clamp Amplifiers

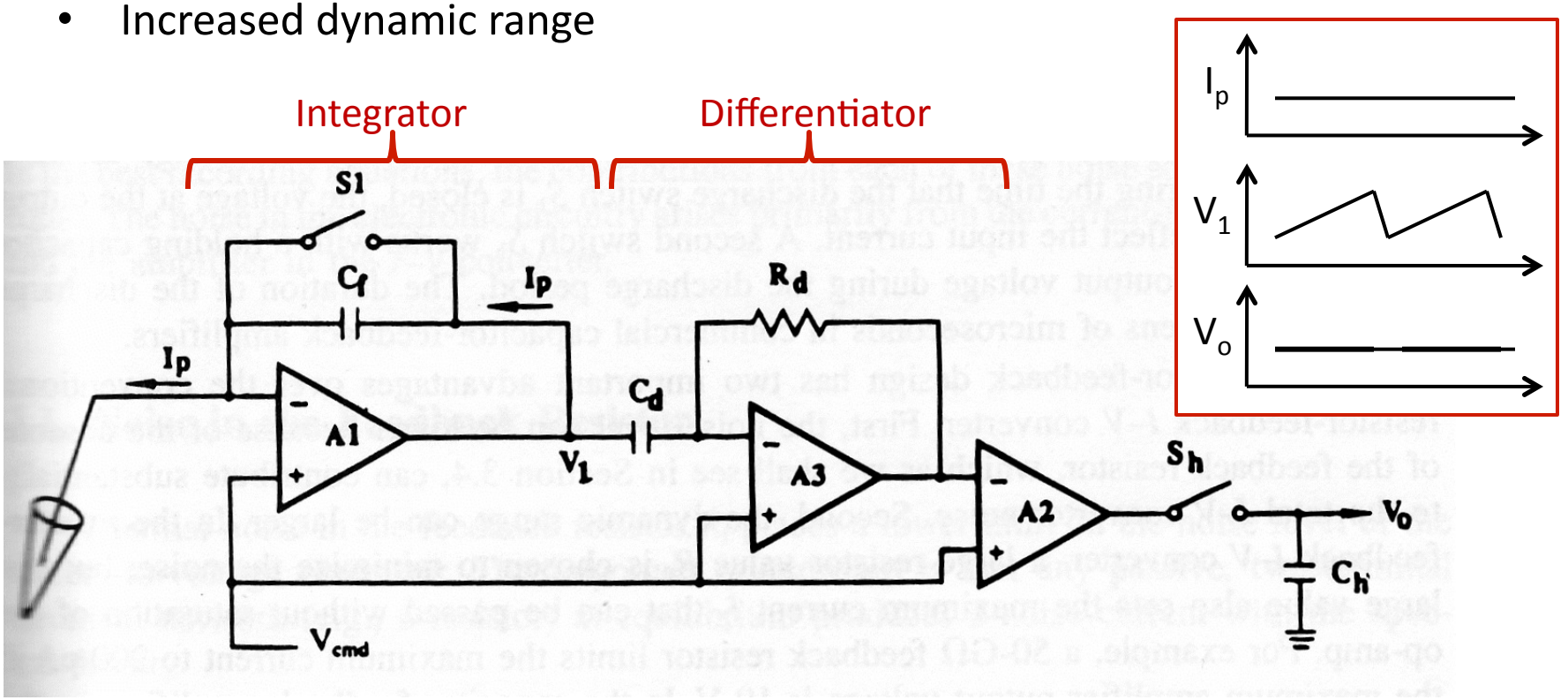
- Primary noise contributors
  - Electrode and feedback resistors
  - Capacitances, dielectric noise
  - Amplifier

- Mitigation techniques
  - First-stage close to electrode
  - Reduction of stray capacitances
    e.g., coating of electrode with polymer
  - Reduction of resistances
  - Reduction of thermal noise
    e.g., cooling

Capacitor-Feedback I-V Converter

Replace feedback resistor by a capacitor (R→∞), followed by a differentiator:

- Higher sensitivity: $V_{out} = R_d \frac{C_d}{C_f} I_p$
- Increased noise performance (no $R_{feedback}$)
- Increased dynamic range

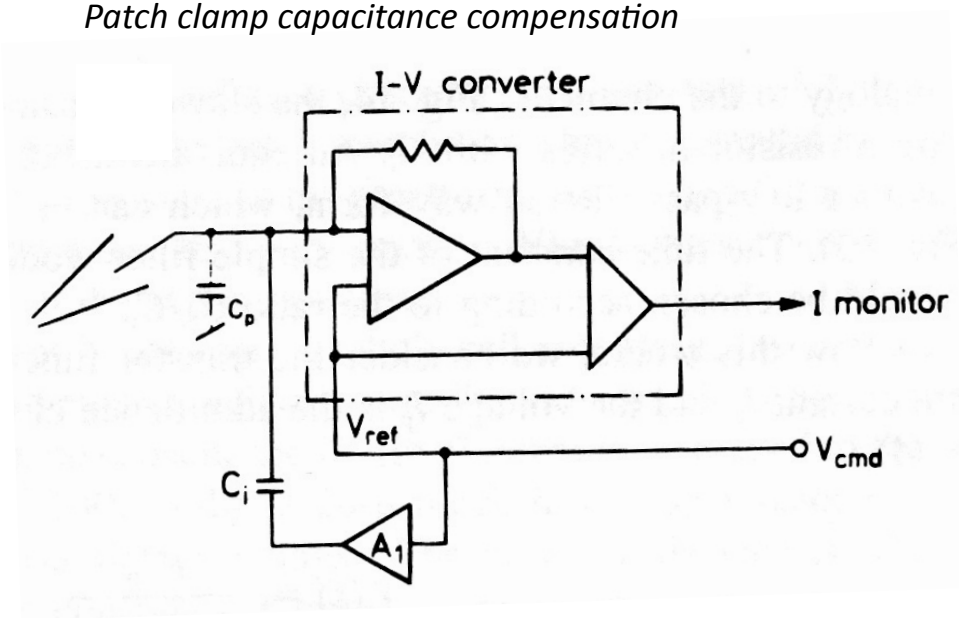


Source: Single-Channel Recording, Neher & Sackmann, 1995

Capacitance Compensation

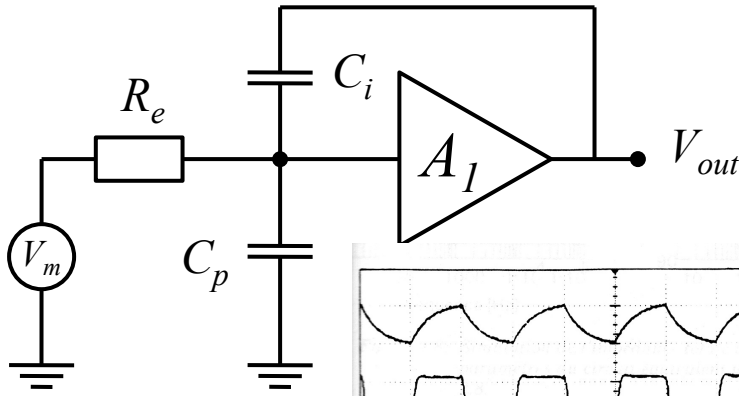
- Large current required to charge stray capacitances (or membrane in case of whole-cell).
- 'Negative capacitance' provided by separate circuit or feedback, provides alternate pathway for C_p charging.
- If $(A_1\text{-}1)C_i = C_p$, then the parasitic capacitive current is fully compensated by the injection/feedback capacitive current.

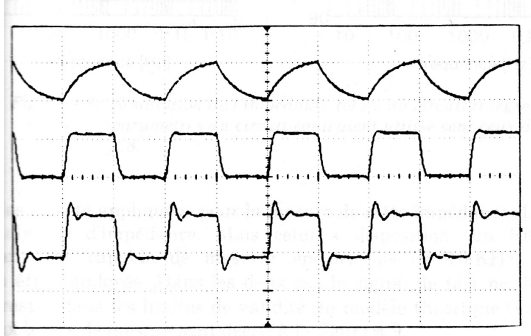
Patch clamp capacitance compensation



Source: Single-Channel Recording, Neher & Sackmann, 1995

Microelectrode (voltage amplifier) compensation





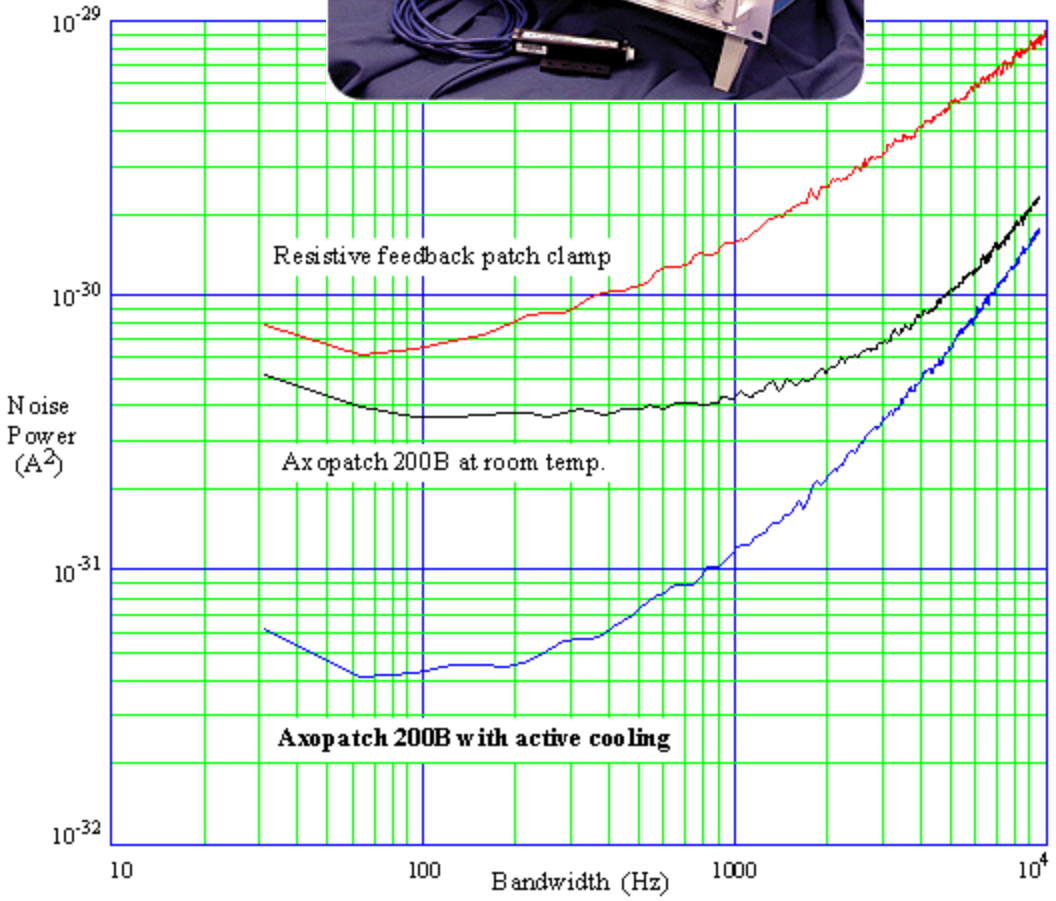
Under-, correct and over-compensation of a square input

Axon Patch-Clamp Amplifier

AxoPatch 200B (Axon CNS/Molecular Devices)

Noise < 15 fA_{RMS} for 1 kHz BW
Noise < 130 fA_{RMS} for 10 kHz BW

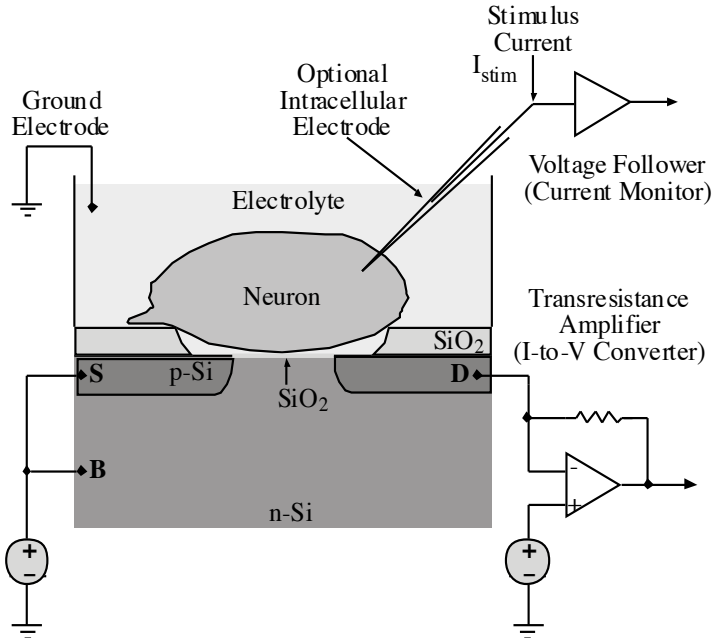
Capacitor-feedback integrating headstage, cooled (-20°C):



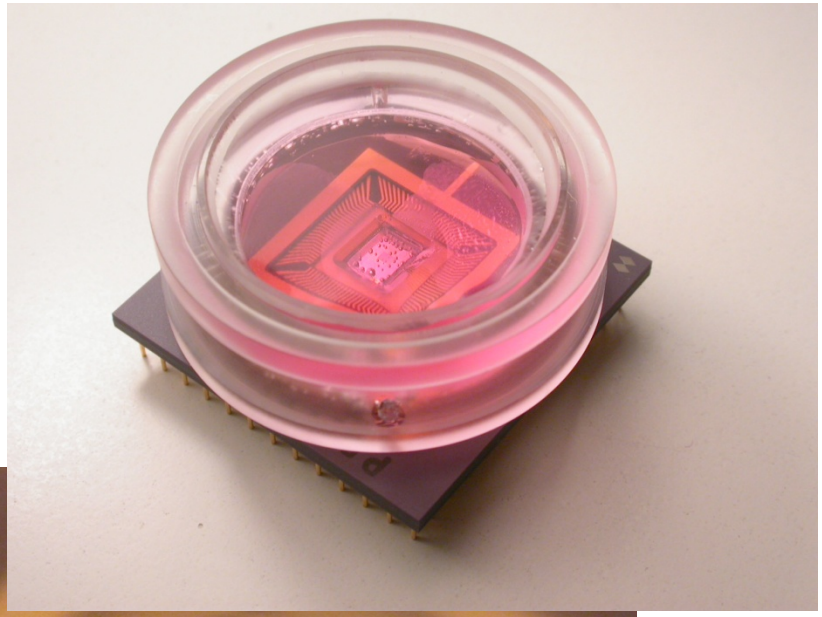
Source: www.moleculardevices.com

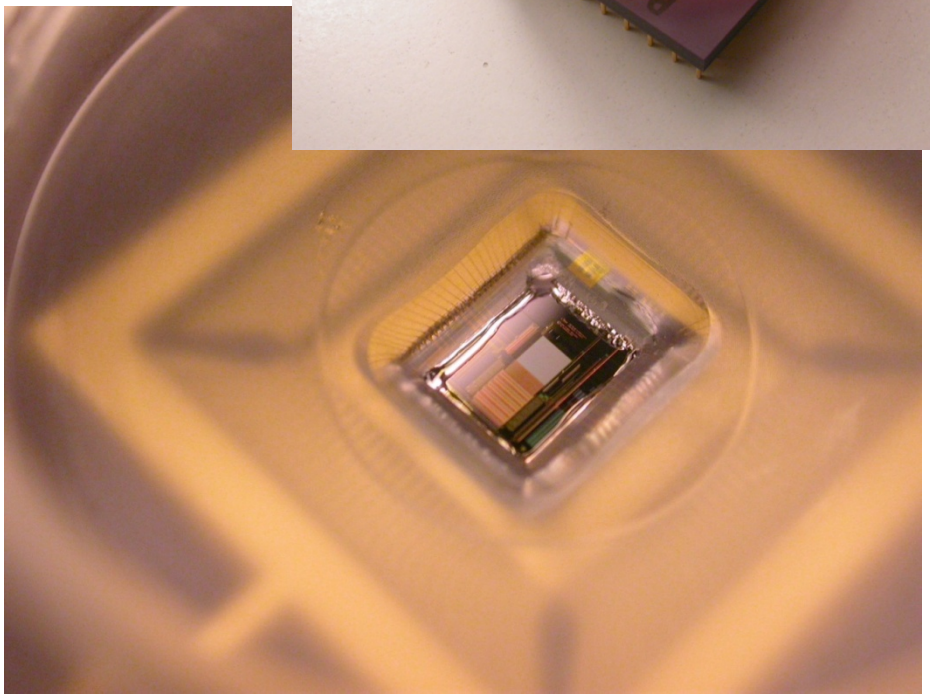
Other Electrophysiology Applications

Infineon 16,384-Electrode Array



Source: G. Kovacs, Micromachined Transducers Sourcebook, 1998

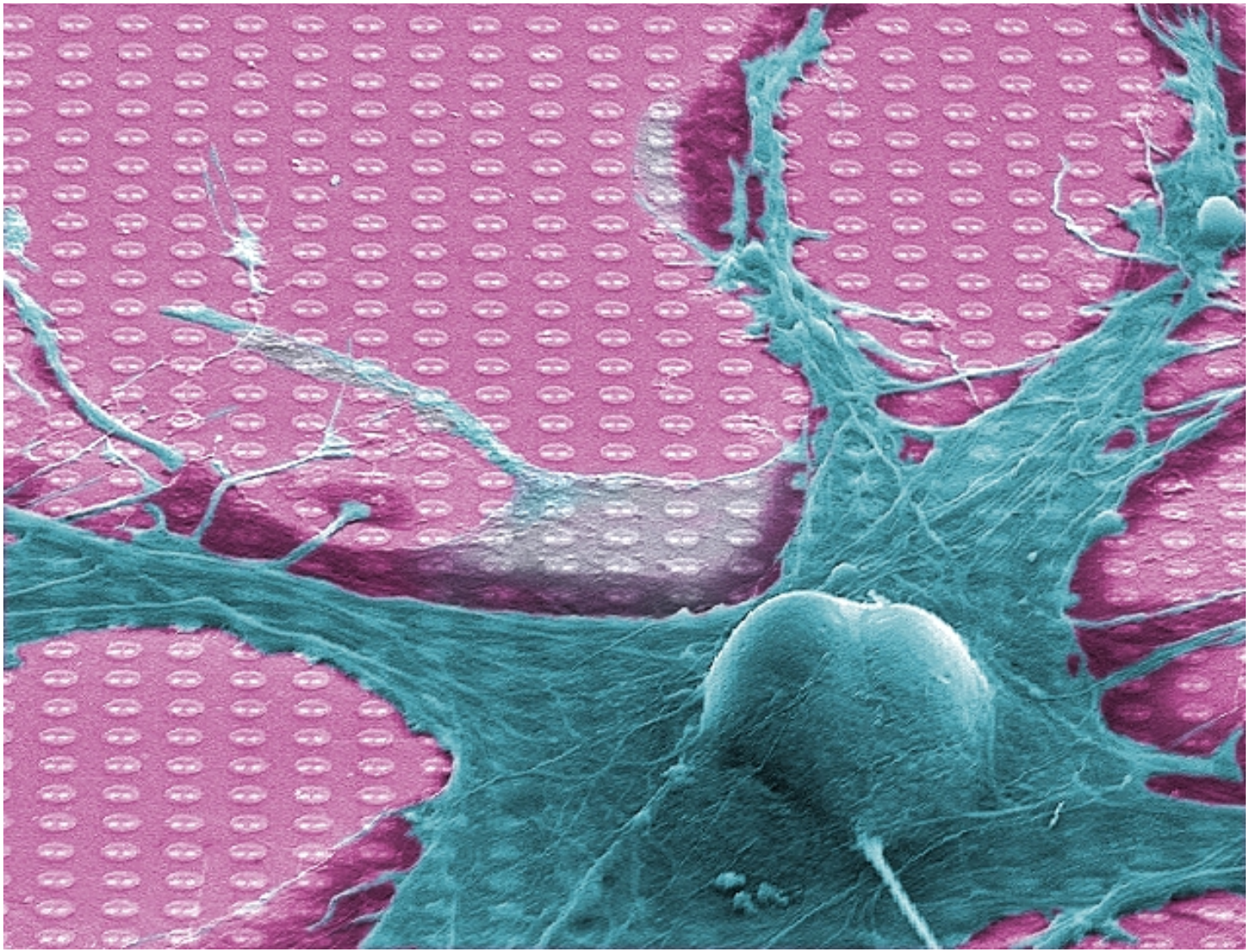




Based on Fromherz' Neuron-FET junction

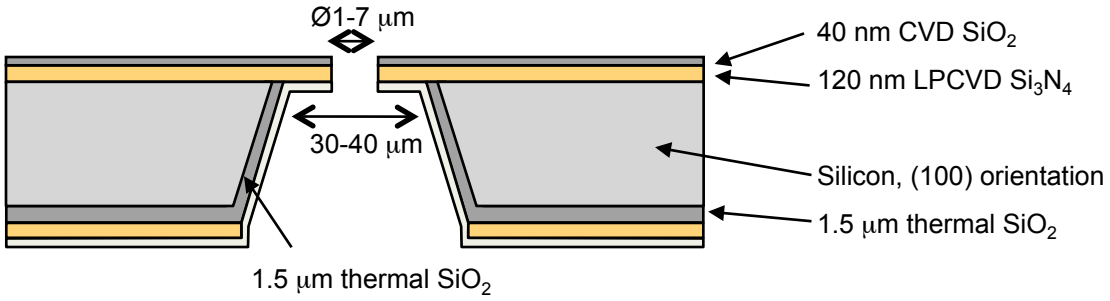
http://www.infineon.com/cms/en/corporate/press/news/releases/2003/129317.html

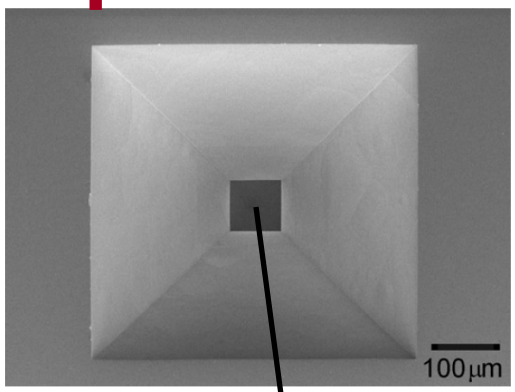
Infineon 16,384-Electrode Array

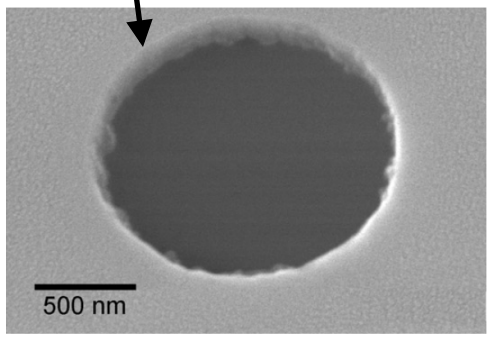


http://www.infineon.com/cms/en/corporate/press/news/releases/2003/129317.html

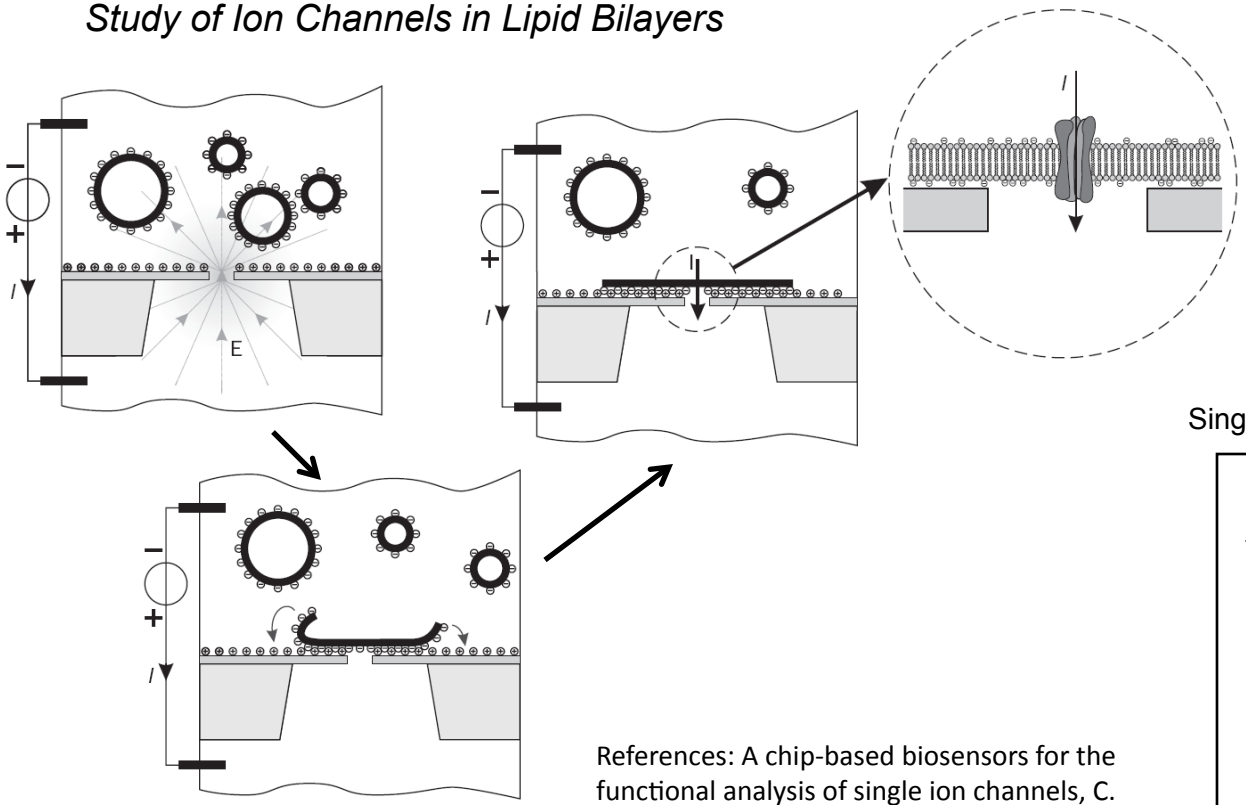
Planar Patch Clamp



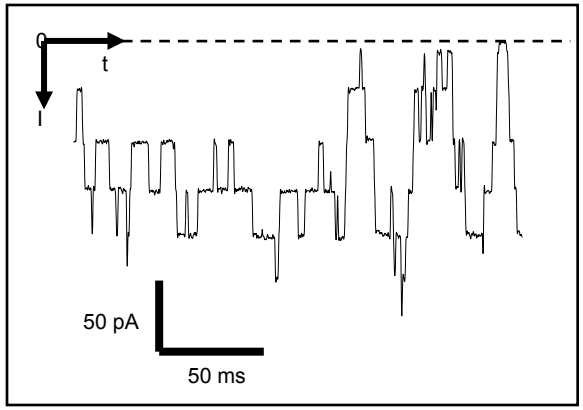




Study of Ion Channels in Lipid Bilayers



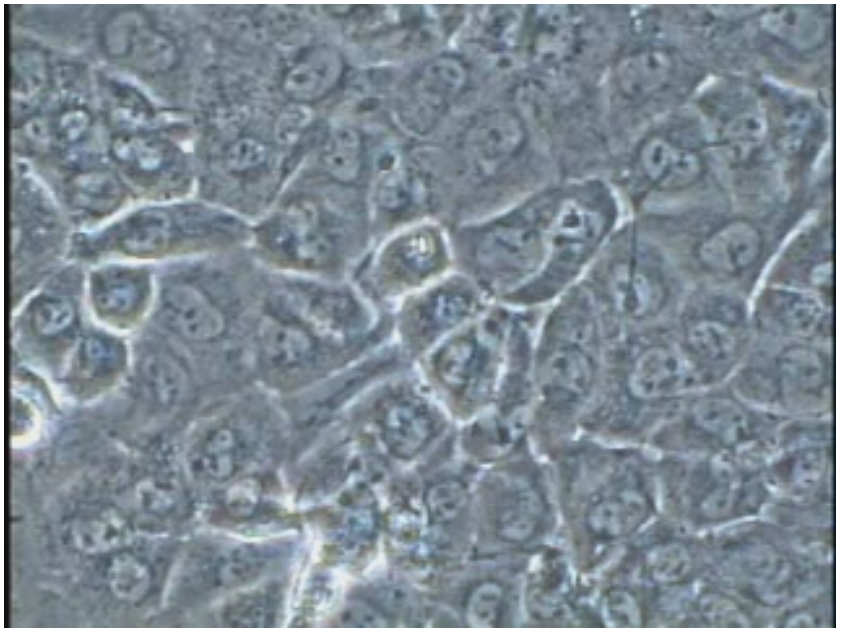
Single Alamethicin Ion Channel Recording



References: A chip-based biosensors for the functional analysis of single ion channels, C. Schmidt, et al., Angewandte Chemie, 2000

Other Means to Record Bioelectricity

- Fluorescence
  - Voltage-sensitive dyes
- Magnetic
  - SQUID (Superconducting Quantum Interference Devices)
- Electromechanical
  - Vibrating probe to measure slow-varying currents
- Acoustic
  - Acoustic signature of ions in varying magnetic fields



Lab this week

Electrophysiology - Record from live cells!