Advanced Course on High Resolution Electronic Measurements in Nano-Bio Science

Current Measurements in Bioscience

Examples of Applications

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Outline

• Introduction
  ▪ Biosensors
  ▪ Direct electrical sensing in Biology
• Current detection requirements in bio-applications:
  ▪ **Patch-clamp neural current recording**
    • Planar setup improvement
  ▪ **Exocytotic molecular detection**
    • Redox cycling
    • Stem-cells application
  ▪ **Amperometric glucose sensors**
The context

Common scientific and technological effort towards the realization of nano-bio-chemical devices
Biosensors: Two Meanings

Biosensor

- A sensor based on a biological entity
- A sensor that monitors a biological system
One of the Oldest Biosensors

The Mine Canary:
The first gas biosensor
(full-animal)
The **selectivity** (specificity) or the **sensitivity** of the biological sensor are leveraged.

**Chemical sensing applications:**
- diagnostics
- food
- environment
- homeland security
Sensing at the Interface

Use the ionic liquid as a top conformal conductive electrode

Affinity Biosensors

Probing charge transfer and induction at the interface affected by the presence of the analyte
Two Possible Approaches

1. The electrochemical current brings direct information about the biological entity (cells):
   - Ion Channels
   - Exocytosis of redox biomolecules

2. The electrochemical current is a tool to detect the binding event (molecules):
   - Glucose sensor
From Micro to Nano

The scaling of the electrode fabrication ability below the micrometer enables a direct size compatibility with cells and macromolecules.
Microelectrodes: Spatial Resolution

Sub-micrometric electrodes for electrophysiology:


Biological investigation mainly relies on optical microscopy

Advantages of direct electrical sensing:
• Label-free (save time and reagents, non intrusive)
• Quantitative
• Integration with microelectronics
• Miniaturization and portability
• Some cells generate electrical signals

Historical landmarks of electrophysiology:
• 1791 Galvani “De viribus electricitatis in motu musculari”
• 1952 Hodgkin Huxley action potential model (Nobel 1963)
• 1976 Patch-Clamp (by Neher & Sakmann, Nobel 1991)
Electrogenic Cells

Electrical signals within cells are driven by ionic gradients:

- Pumps (against diffusion, need energy)
- Ion channels (valves)
- Neurons
- Heart, muscles
Non-invasive external electrical sensing of electrogenic activity:

- Voltage
- **Current** (Patch-Clamp)
- Chemical detection of released molecules

Common trend to miniaturization, parallelization and integration
Voltage Sensing

CMOS platforms:
- Fromherz
- Hierlemann
- Martinoia
Life’s Transistors: Ion Channels

Ion-specificity

Triggering mechanisms:
- Voltage-gated
- Ligand-gated

The Patch-Clamp
Current Recording

Significant biological interest in improving the current resolution:

- Na, K channels: 1-10pA
- Ca, Cl channels: 0.1pA
- Pumps (100e^-/s): 0.016fA

**Single channel:**

- ~nA current

**Membrane rupture whole-cell:**

Axopatch 200B
Axon (Molecular Dev.)

Cooled headstage (-15°C)

Specs:
• JFET, $I_{bias} = 1\,pA$
• Gain = 1mV/pA
• BW = 140kHz
• $60fA_{rms}$ over 5kHz
• $C_f = 1pF$
• $T_{reset} = 50\mu s$
Achievable Performance

Experienced user:

- **10 kHz**
- **30 kHz**

- Single-channel event
- Good seal
- Bad seal

**Current (pA)**

- **Time (ms)**
  - 415, 420, 425, 430, 435
  - 200, 210, 220, 230, 240, 250
How to Improve the Performance

The dominating noise terms are due to the setup:

- \( C_m = \sim 0.01 \text{pF/\mu m}^2 \)
- \( R_L = \sim G\Omega \) (seal)
- \( R_S = \sim M\Omega \) (solution)

Improving the Setup

Pipette improving:
- Quartz
- Polymeric coating

Planar setup:
- Reduce stray
- Automatic
- Parallel

R. Levis & J. Rae, *Biophys. J.* 65 1993
K. Klemic et al., *Biosens. Bioelectron.* 17 2002
Whole Live Cell Odorant Sensor

N. Misawa et al., PNAS 107 (2010)
The Synapse

Chemical mediation: $5 \cdot 10^{14}$ synapses in the human brain

Exocytosis: extra-cellular release of small molecules in vesicles
Amperometric Detection of Molecules

Microelectrode:

Chemical messengers:
- Dopamine, adrenaline etc..

Current tracking:
- $5 \cdot 10^4$ molecules in a vesicle
- 10fC released in 1ms $\rightarrow \sim 10$ pA

Techniques Combination

- Patch clamp micropipet in whole cell configuration
- Carbon-fiber amperometric electrode
- Gigaohm seal
Detection of Single Exocytotic Events

Amperometric recording:

Single PC12 cells

Specs: 1pA Resolution @ <1ms

At the **limits** achievable with a standard transimpedance amplifier:

- \( R_f = 200M\Omega \)
- 0.2pF stray capacitance
- \( BW = 4kHz \)
- 1.4pA\(_{rms}\)

**Solutions:**

**Integrators**
- Switched
- \( H(s) \)
Intrinsic Amplification: Redox Cycling

Planar interdigitated electrodes

Must be reversible!

Paeschke et al. *Analytica Chimica Acta* **305** 1995

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The Bipotentiostat Configuration

\[ \begin{align*}
V_C & \rightarrow CE \\
& \rightarrow RE \\
& \rightarrow WE_1 \\
& \rightarrow WE_2 \\
& \rightarrow V_1 \\
& \rightarrow V_2
\end{align*} \]
Bipotentiostat for Redox Cycling

With catecholamines the achievable gain is $\sim 10$
High-sensitivity tracking of neurotransmitter exocytosis:

- Investigate fundamental biological mechanisms (brain)
- Addressing neurodegenerative diseases
- Early detection of stem-cell differentiation

A major biological challenge

Understand and control the differentiation triggers and paths of stem cells
Integrated Platform

Combined techniques

- Optical/electrical detection
- Microfluidics
- Intra/extra cellular recording
- Automatic
- Highly parallel

Combinatorial approach
Sub-Cellular Resolution

High-resolution spatio-temporal tracking of single exocytotic events:

A. F. Dias et al., Nanotechnology 13 2002

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The Problem of Interferences

Similar neurotransmitters (similar molecules, similar CV) may have very different biological function \(\rightarrow\) selectivity issue:

![Chemical structures and graph showing selectivity issues between nor-adrenaline and adrenaline.](image)
Solution: Fast Cyclic Voltammetry

In-vivo rat brain:

(1) Dopamine (1 μM)

400V/s $\rightarrow$ 100ms

Extension of Amperometry

If the molecule is not electroactive?

Use a specific mediator

Enzyme!
If the catalysis of the target molecule involves an electron transfer it can be detected with amperometry.

- The current is proportional to the concentration
- The process can be less efficient
- Enzymes are often immobilized on electrodes
Enzymatic Reactions for Glucose

Glucose $+ \text{O}_2 \rightarrow \text{Gluconic acid} + \text{H}_2\text{O}_2$

$\text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2\text{H}^+ + 2\text{e}^-$

Total Reaction:

Glucose $+ \text{O}_2 \xrightarrow{\text{GOD}} \text{Pt anode} \rightarrow \text{Gluconic acid} + \text{O}_2 + 2\text{H}^+ + 2\text{e}^-$

$4\text{H}^+ + \text{O}_2 \xrightarrow{\text{AgCl cathode}} 2\text{H}_2\text{O} - 4\text{e}^-$

* Glucose oxidase (GOD) is commonly used since it fairly stable & requires no cofactors or coenzymes
Commercial Glucose Sensors

Glucose Oxidase

Glucose \rightarrow \text{Gluconic acid}

Oxygen \rightarrow \text{Hydrogen Peroxide}

\[ 2H^+, 2e^- \]

ELECTRODE

\[ 5.4 \text{ mM} \]

Working electrode with immobilised enzyme (or mediator)

Medisense glucose biosensor

Contact

Conductive Carbon Track

Reference electrode (Ag/AgCl₂)
Commercial Biosensor System

Disposable electrode
Summary: Paradigm Shifting

To address the measurement challenges set by micro- and nanoscale biological systems (sup-pA and sub-ms resolution), macroscopic bench-top instruments have to be replaced by miniaturized integrated systems.

- size compatibly $\rightarrow$ sub-micrometric electrodes
- radical reduction of parasitics
- parallelization (multichannel)
- portability, low-cost
Why Integrated Electronics?

1) Extremely compact (embedded in lab-on-chip device)
2) Highly parallel (thousands of sensing channels)
3) Extremely low-cost (*only if mass production!*)
4) Good performance (close to the sensor)
5) Compatible with microcomputers and wireless systems
Towards On-Chip Sensing

Among several approaches, direct current sensing offers the but integrability with CMOS platforms
References