Un biochip per elettrostimolazione cellulare

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Outline

- Introduction & background
- A biochip for genetic manipulation of single-cells
  - The single cell electroporation biochip
  - Electrical models of the biochip
  - Monitoring cell-electrode adhesion
- Other related activities
  - ISFET-based DNA sensors
  - EGFET-based DNA sensors
- Conclusions

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Introduction & background

- *Biotechnology means any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use.*

  (definition by the UN Convention on Biological Diversity)

- Biotechnology is nothing really new
  - First farmers selected crops (peas, barley, wheat) in Fertile Crescent in 8000 B.C.
  - Insulin produced from genetically modified bacteria *Escherichia Coli* since 1978 (Genentech)

Introduction & background

- More and more Biotech drugs are blockbusters
  - An example: Epogen (Erythropoietin) introduced by AMGEN in 1989
  - World sales of EPO: 10B$ in 2004
- Is gene therapy behind the corner?
  - Control disease-causing genes on a person-to-person basis
- For a genetic-related disease, need to understand
  - Has gene XXX a role?
  - What if it is suppressed?
  - What if over-expressed?
  - …and so on…

Introduction & background

- Our aim: developing innovative microelectronics tools for molecular biology
- A field under growing interest
  - Microfuidics
  - DNA identification (DNA microarrays)
  - Quantitative assays (ELISA, RT-PCR, Q-PCR, …)
  - Single cell manipulation
  - Recording of electrical activity of living cells
  - Proteomics (protein microarrays)
  - Implanted devices (not just pacemakers...)
  - …

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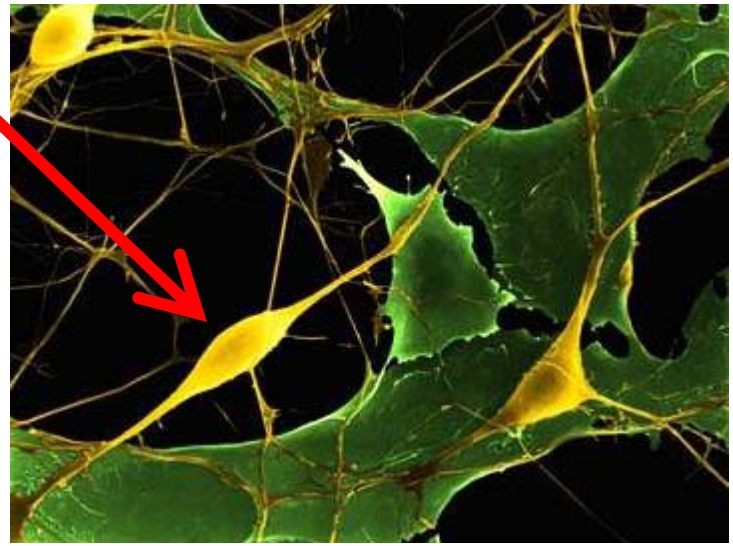
The single cell electroporation biochip

- A theoretical experiment…
  - You are a biologist studying the function of a gene implied in Alzheimer disease
  - Your models are rat neurons growing *in vitro*
  - The gene must be activated only in this neuron

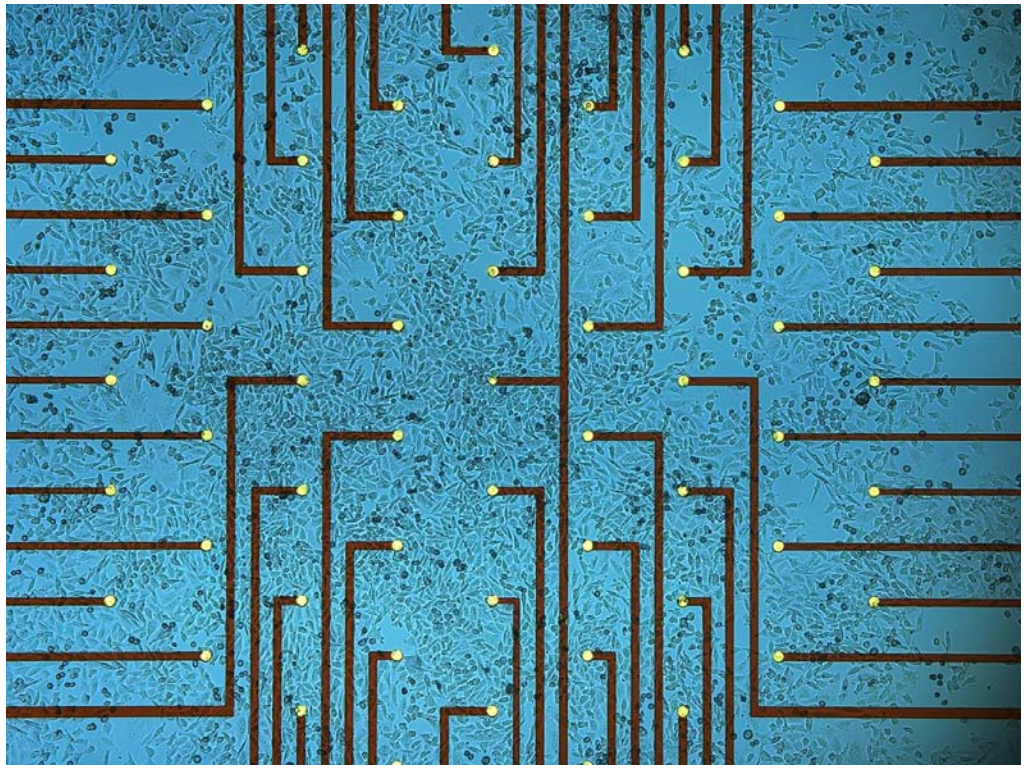


The single cell electroporation biochip

- Several methods are available to force the expression of a given gene in cultured cells
  - Chemical
  - Viral
  - Mechanical
- All of these work in a population of cells
  - Results averaged over the population
  - Large spread of statistical data
  - Unable to modify a single cell in a culture

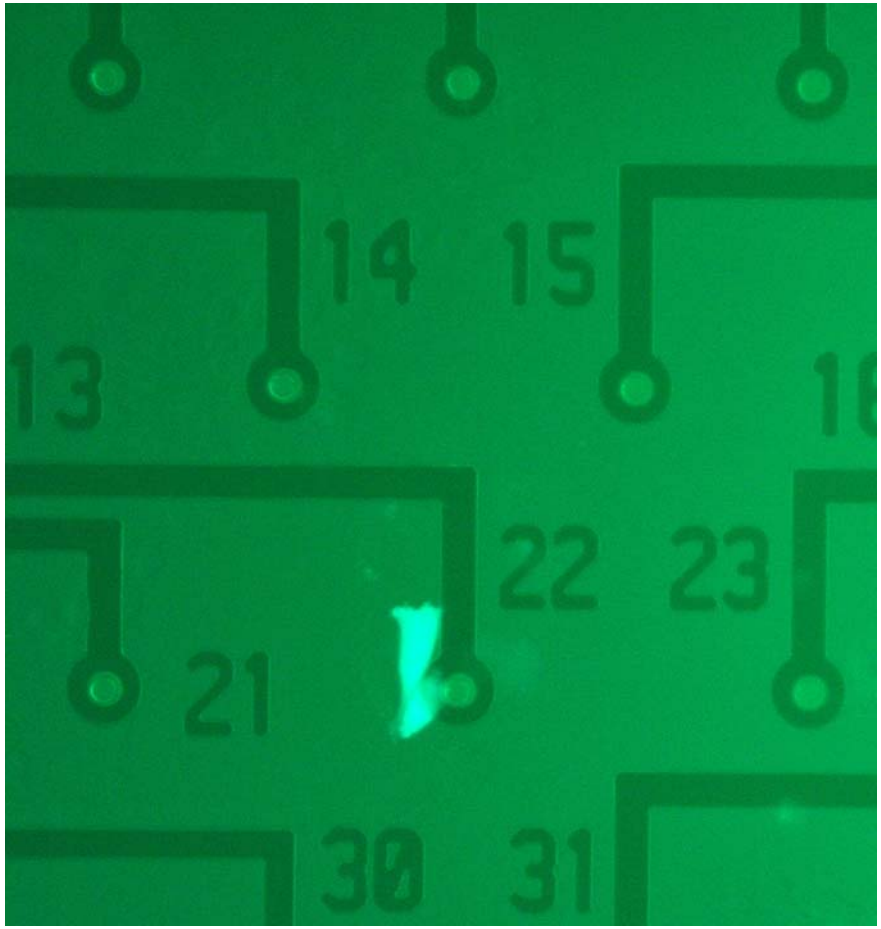
The single cell electroporation biochip

- Realize an array of microelectrodes on a silicon biochip
- Cultivate cells on chip surface
- Apply a voltage to one electrode
  - The voltage is transferred to the cell growing above the electrode
  - Temporary pores open in the cell membrane (**electroporation**)
  - Molecules in solution enter the cell cytoplasm
  - The membrane reseals



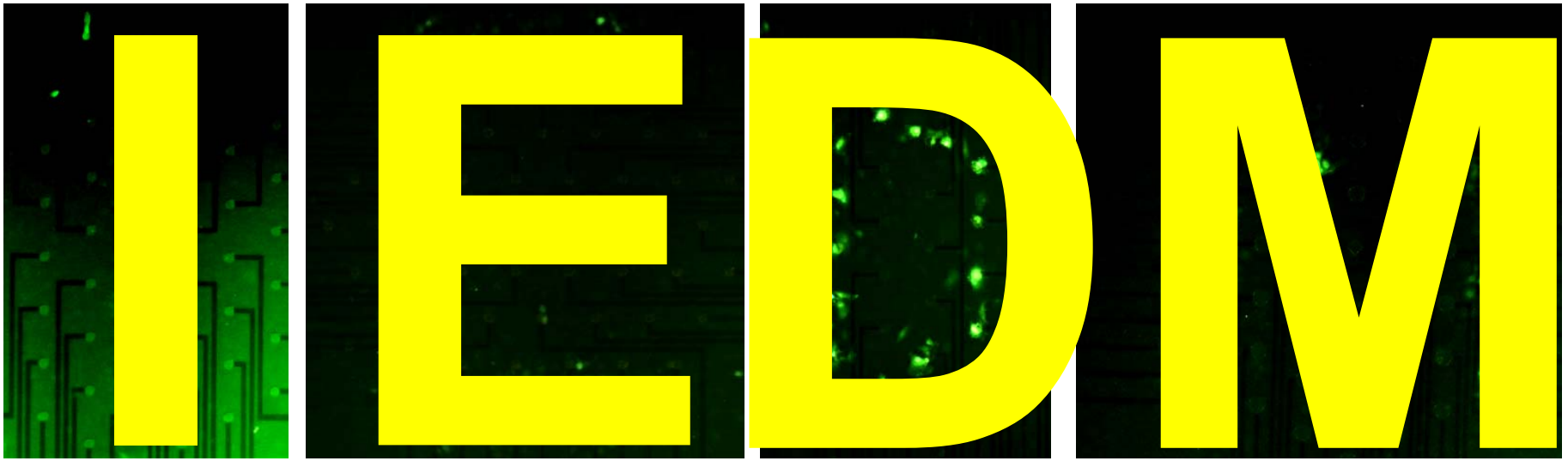
The single cell electroporation biochip

- A GFP (Green Fluorescent Protein)-encoding plasmid is inserted into a single CHO cell (el. 22)
- After 24h, the selected cell divided: both daughter cells express the GFP



The single cell electroporation biochip

- Any pattern can be realized in the same way



L. Bandiera, et al, IEDM, 2006

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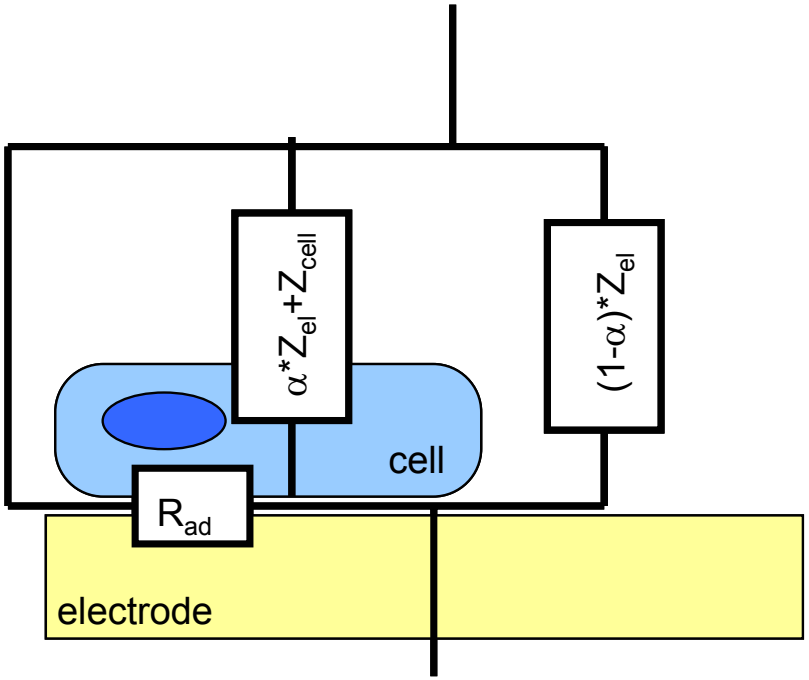
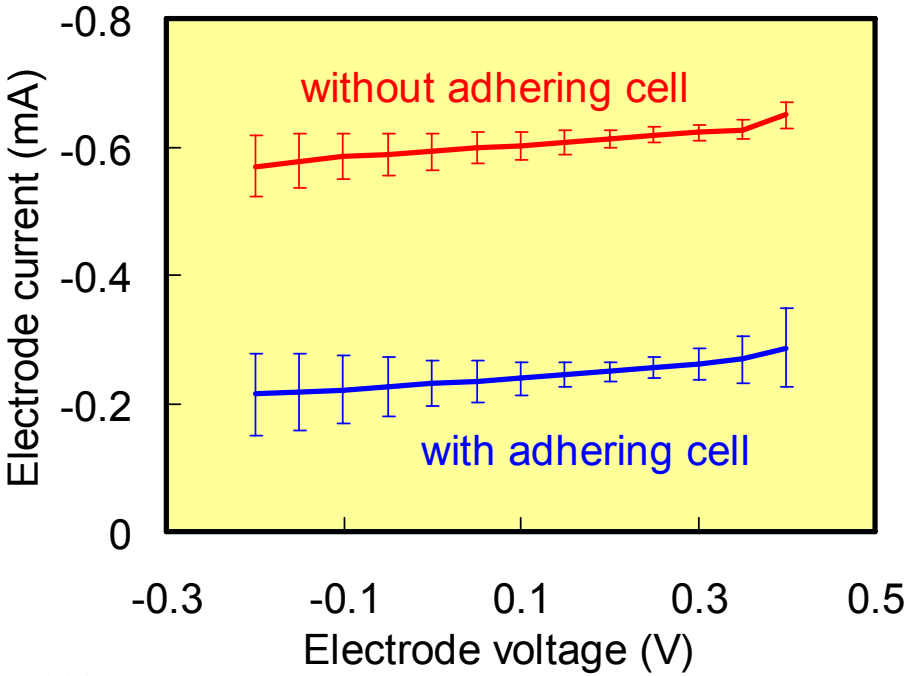
Electrical model of the biochip

- An electrical model is needed in order to
  - Design effective and non invasive electroporation stimuli
  - Allow reliable operation of the device



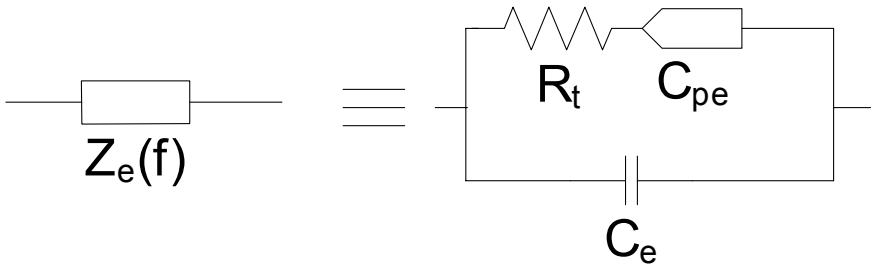


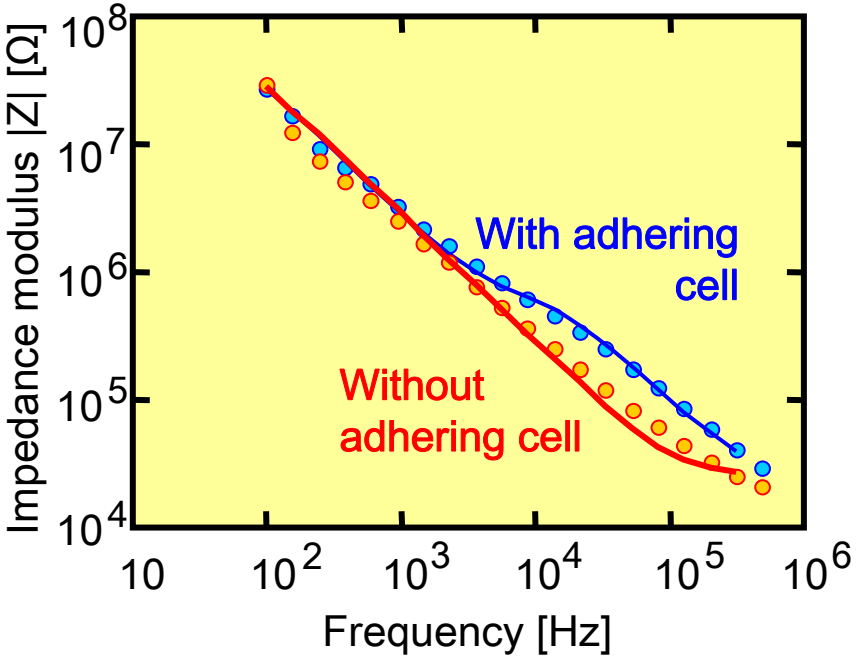
L. Bandiera, et al, IEDM, 2006. G. Cellere, et al., ECS Trans. 2007.

Electrical model of the biochip

- Electrode impedance
  - Charge transfer resistance R_t
  - Double layer capacitance C_e
  - Constant phase element C_{pe}

  (electrochemical system nonlinearities)





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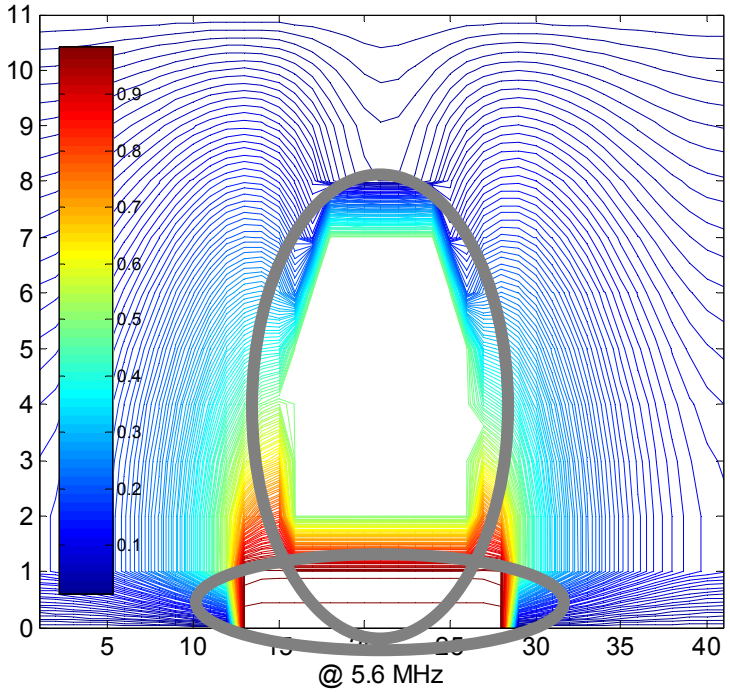
Electrical model of the biochip

- To study for example what happens to the potential on the cell membrane when it is not exactly above the electrode we need a distributed model
- Spatially Distributed Transfer Network approach
  - The electrode - electrolyte - cell system is divided in a grid
  - Each grid branch is described by an impedance whose value is derived from the concentrated model
- Similar to finite element methods (FEM) but no linearization of equations is needed (not at this stage)

Electrical model of the biochip

- How is distributed the voltage above the electrode?



Electrode

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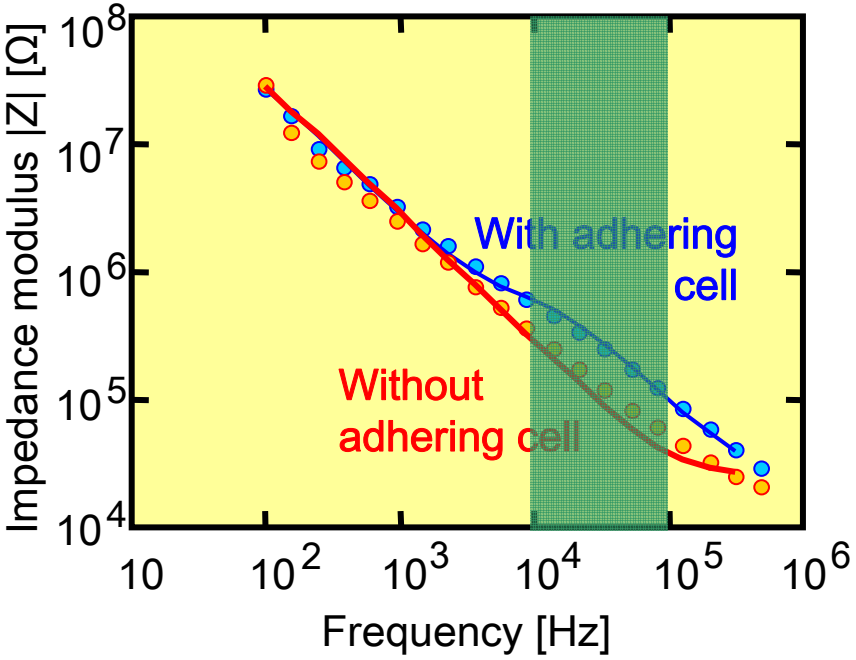
Monitoring cell-electrode adhesion

- Some questions
  - Is it possible to detect the presence of a cell by using electrical measurements only?
  - Is it possible to quantitatively evaluate the quality of cell adhesion?
- Use EIS (Electrochemical Spectroscopy Impedance) measurements over a limited frequency rage



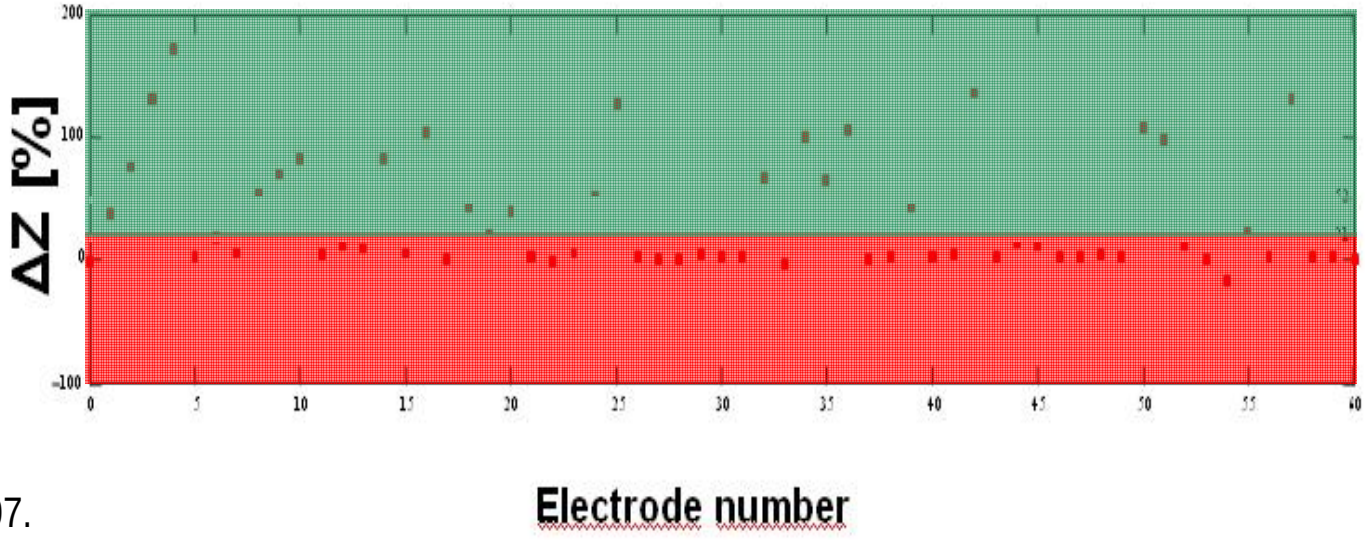
Cellere, et al., ECS Trans. 2007.

Monitoring cell-electrode adhesion

- Impedance @66kHz for different electrodes of the same chip
- $$\Delta Z = \frac{Z_{withcell} - Z_{withoutcell}}{Z_{withoutcell}}$$
- If ΔZ>20%, we can assume that a cell is adhering to the electrode

Cell above the electrode

No cell above the electrode



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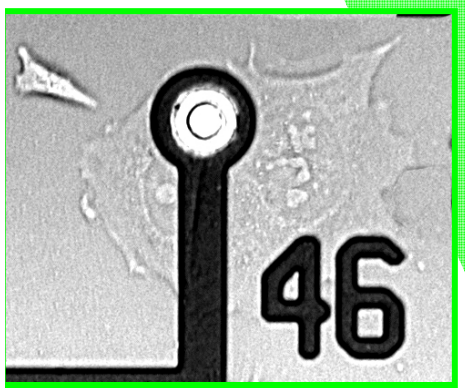
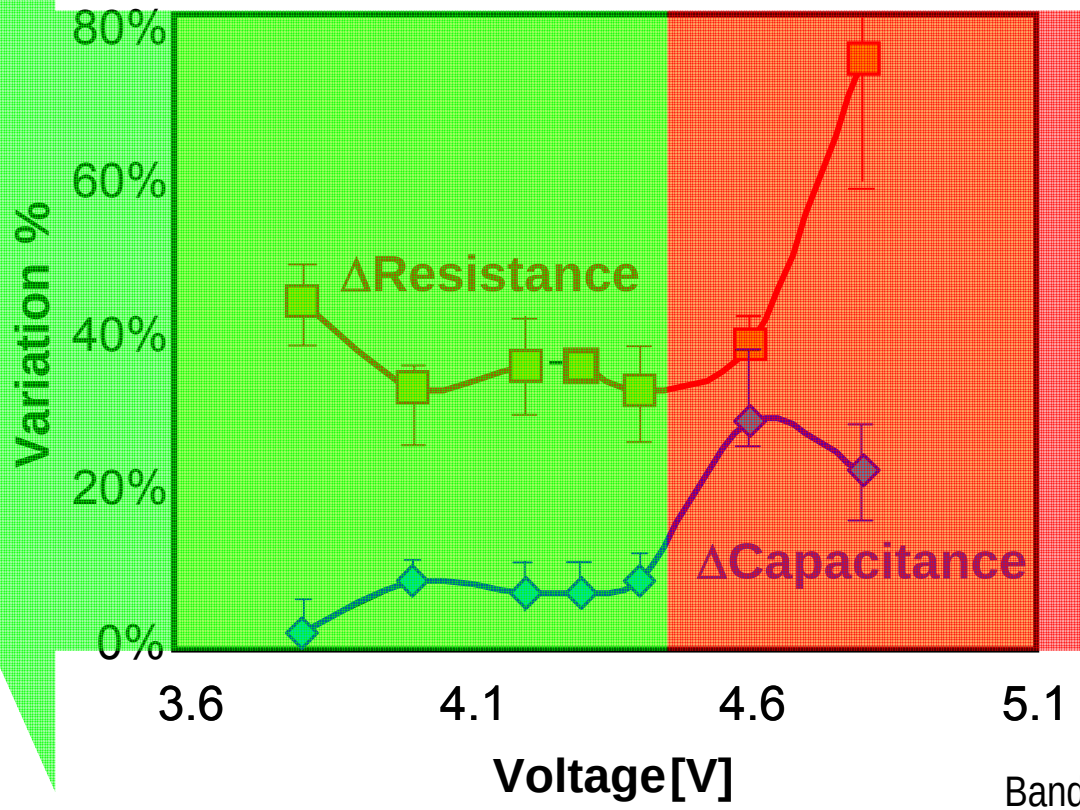
Monitoring cell-electrode adhesion

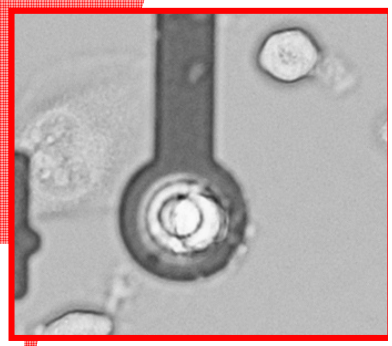
- Changing the stimulation voltage changes the stimulus effect on the membrane

No change in morphology
High cell viability





Morphology change
Low cell viability



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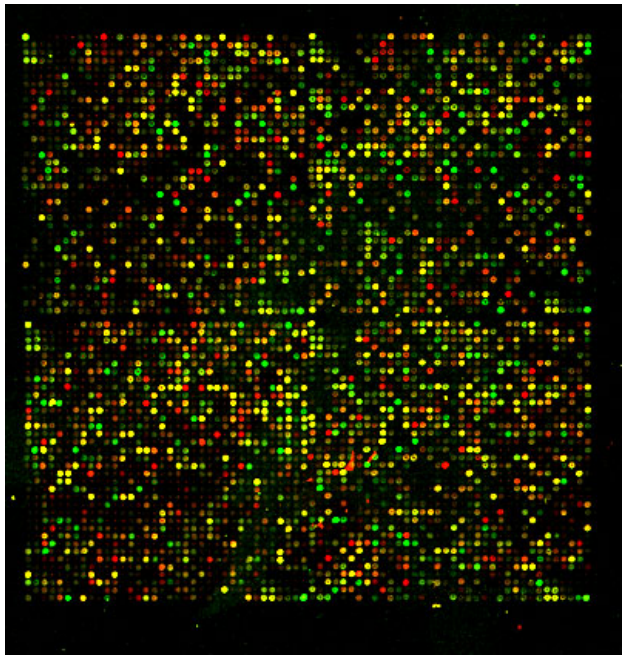
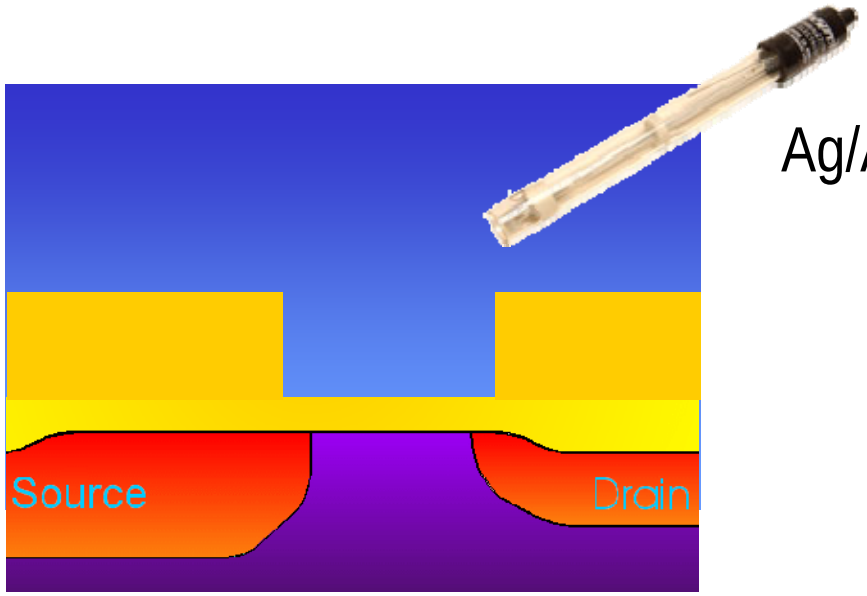
Other related activities

- Fluorescence-based DNA microarrays are used to understand the expression of many genes at once
- Drawbacks of microarrays:
  - Not reusable
  - No information on hybridization kinetics
  - Low signal-to-noise ratio
  - Expensive
- DNA molecule carries an intrinsic negative charge
  - Can we develop an all-electrical system to quantify gene expression?



ISFET-based DNA sensor

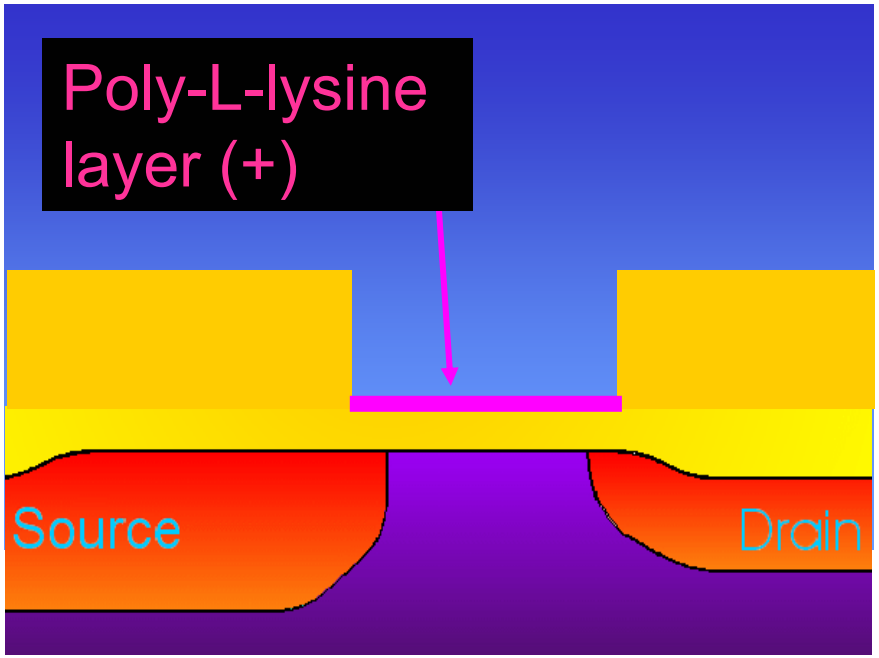
- First implementation: ISFET
  - Ion-Sensitive Field-Effect-Transistor
  - Basically, a MOSFET without the metal gate
  - Gate oxide (SiO_2-Si_3N_4 stack) is exposed to solution

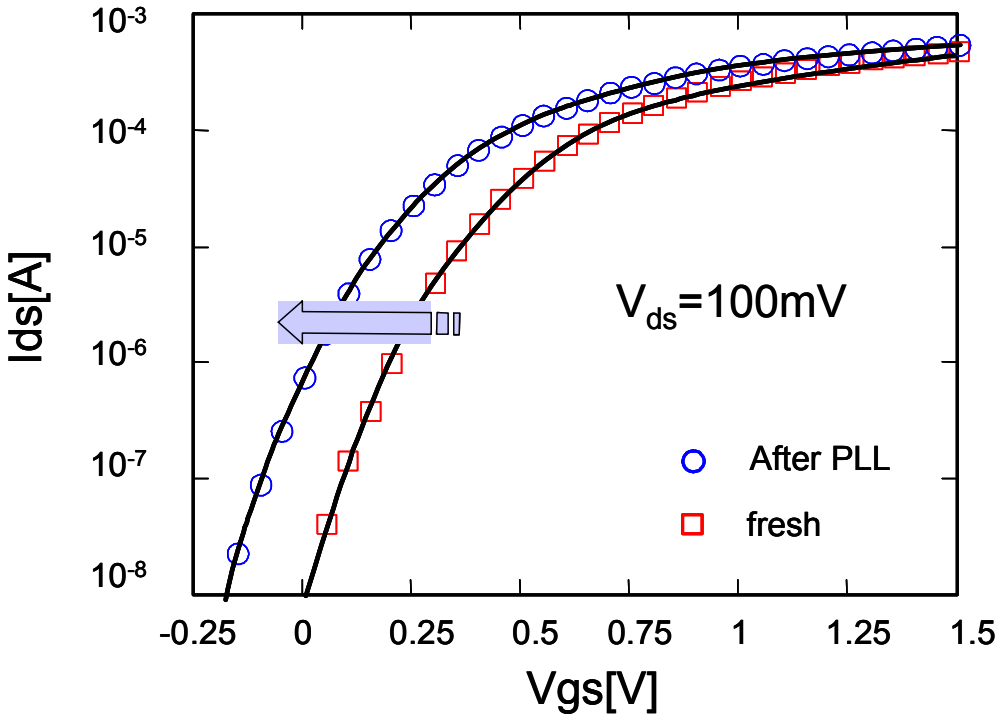


Ag/AgCl reference electrode

ISFET-based DNA sensor

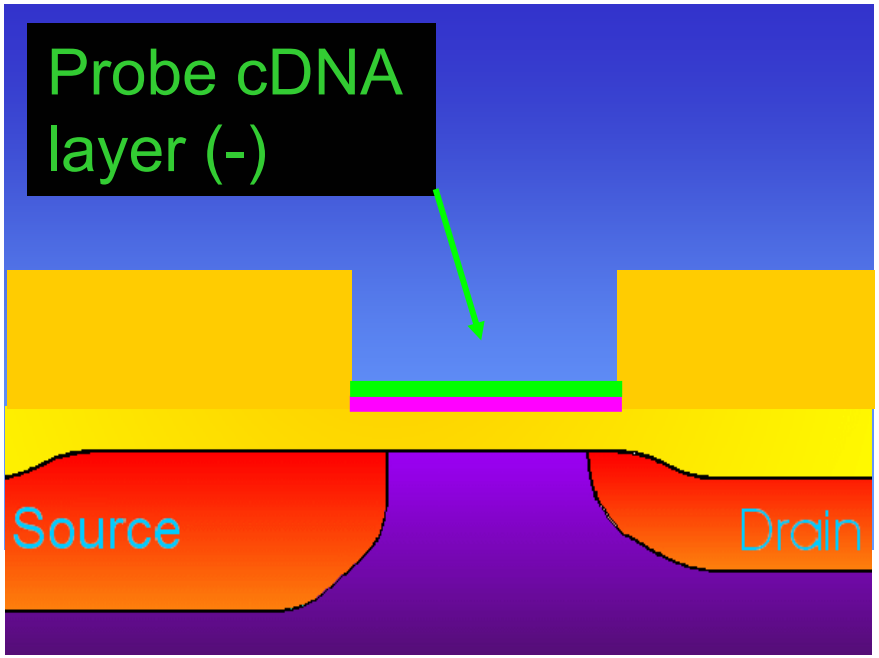
- Step 1: depose (positively charged) poly-L-lysine (PLL) on gate oxide
- $Q_{PLL}=(1.5\pm0.65)x10^{-4}C/m^2$

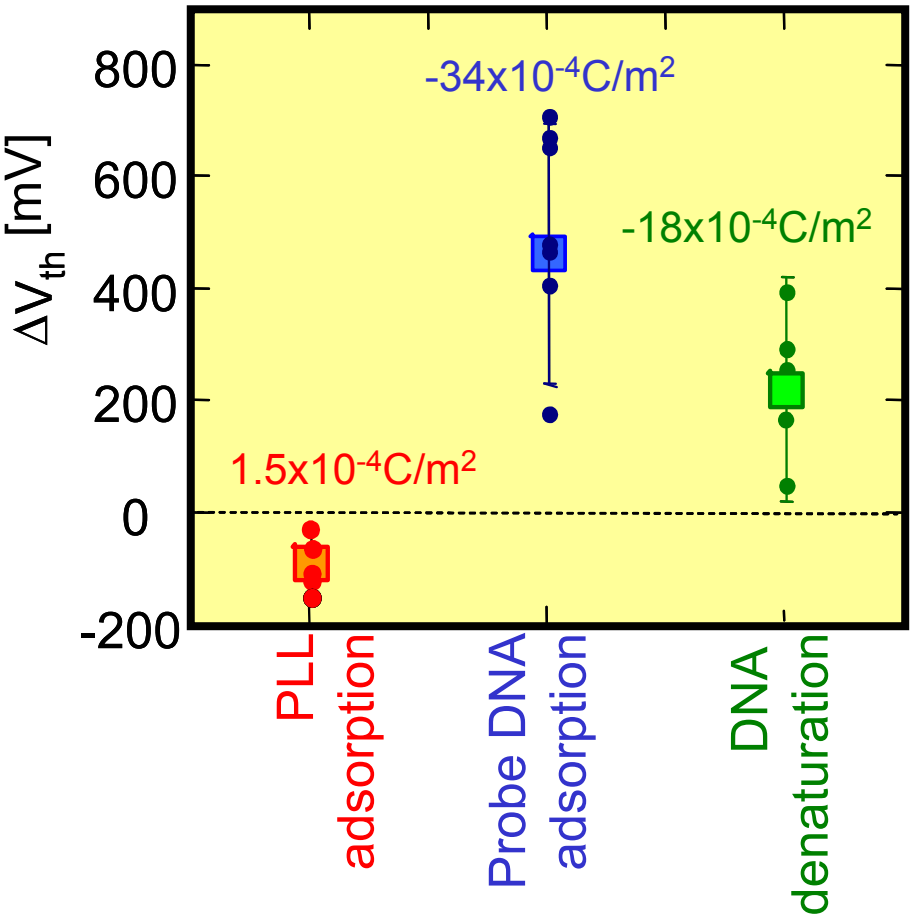




ISFET-based DNA sensor

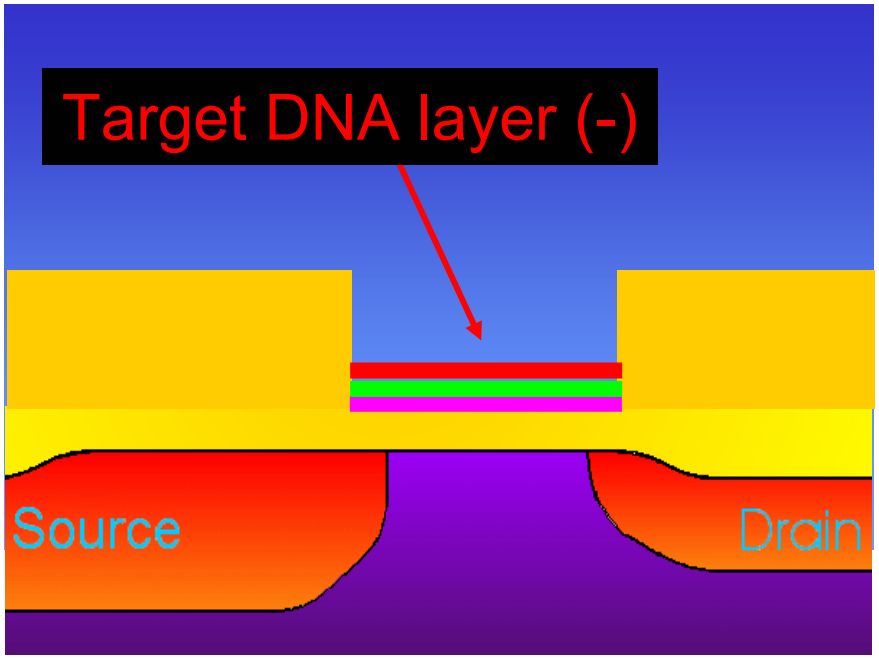
- STEP 2: depose probe (known) DNA
- DNA carries a negative charge →
  - V_{TH} shifts rightward

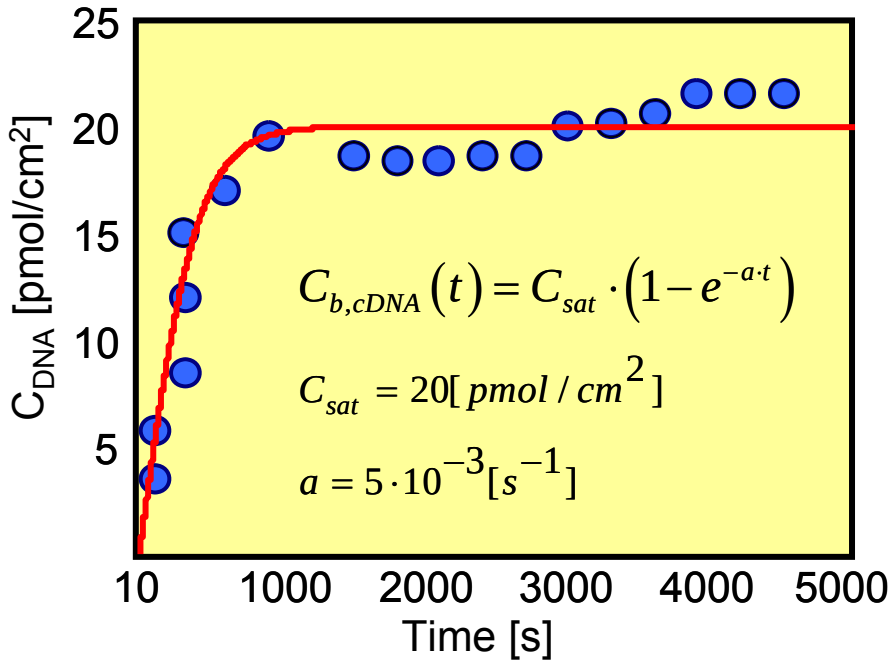




ISFET-based DNA sensor

- Step 3: depose target (unknown sequence) DNA
  - Matching → V_{TH} shift
  - Non matching → nothing happens





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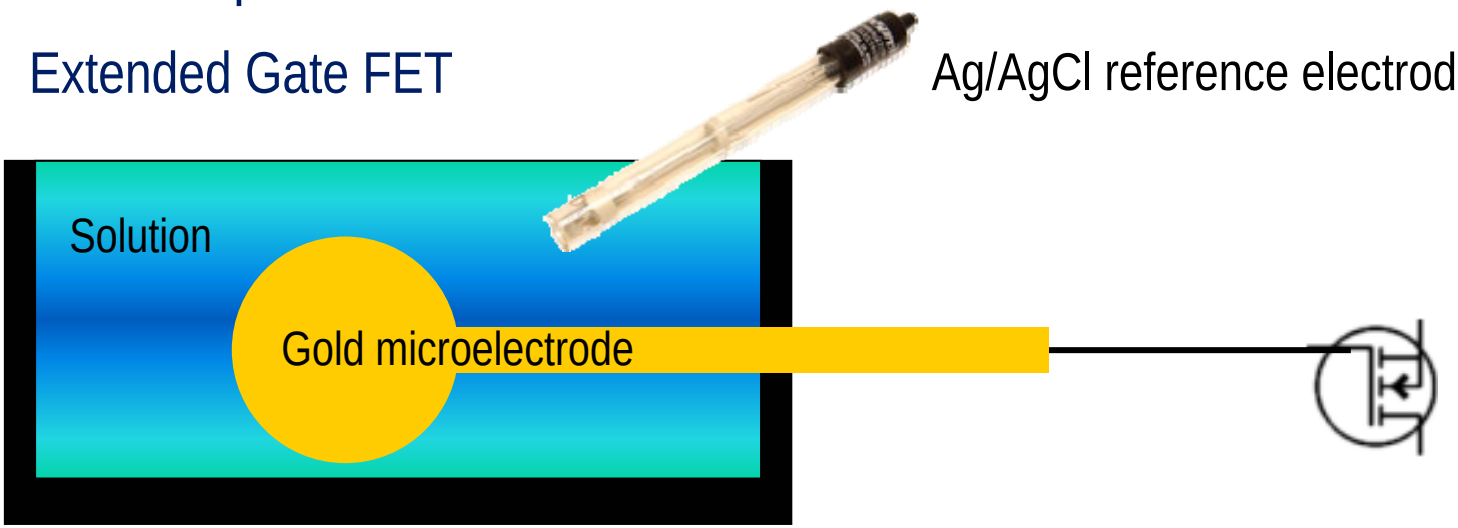
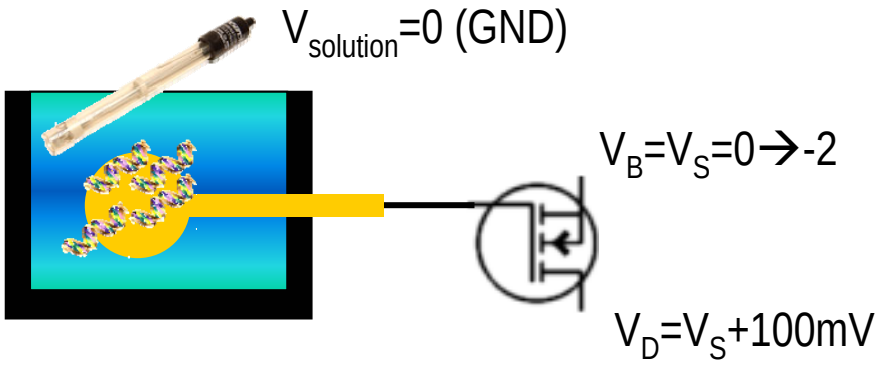
EGFET-based DNA sensor

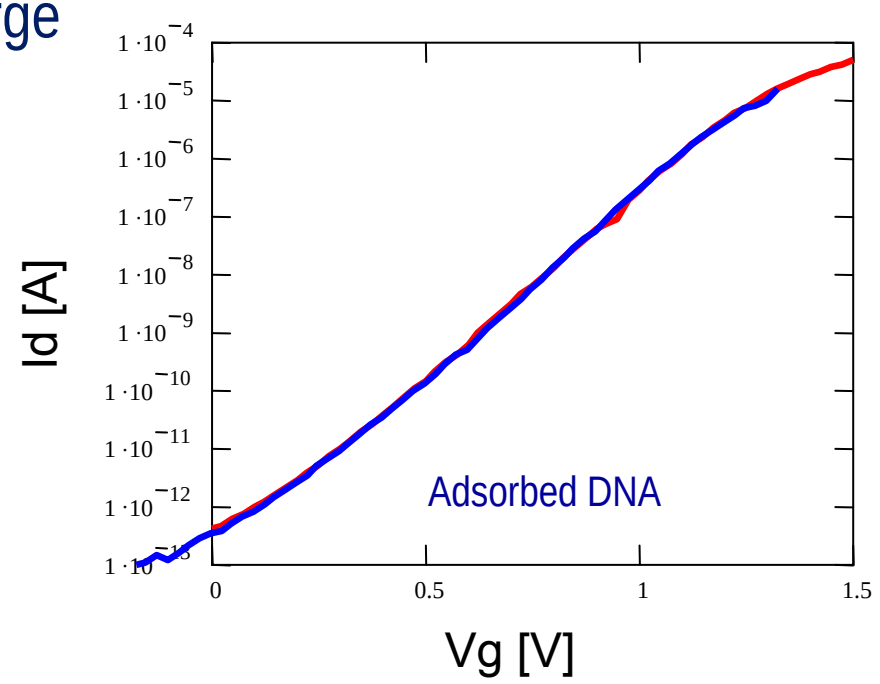
- ISFET are
  - Very sensitive to solution and to process conditions
  - Expensive devices (~CMOS process flow in small batches)
- move to simpler devices: EGFET
  - Extended Gate FET



EGFET-based DNA sensor

- Thiol-modified DNA adsorption onto gold microelectrode
- V_{TH} increases due to DNA negative charge





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Conclusions

- Innovative tools for biological applications
  - Electrical stimulation if living (cultured) cells
  - Electrical detection of DNA sequence
- Our research interest
  - HW/SW design
  - Electrical modeling
  - Reliability
- Work in close collaboration with biology and nanoscience
  - A stimulating and challenging interdisciplinary environment!
  - CIVEN, Fisiology Dept., CRIBI, FBK, VIMM, Biosilab, Columbia Univ.