Biochip Platforms:current milestones and challenges ahead

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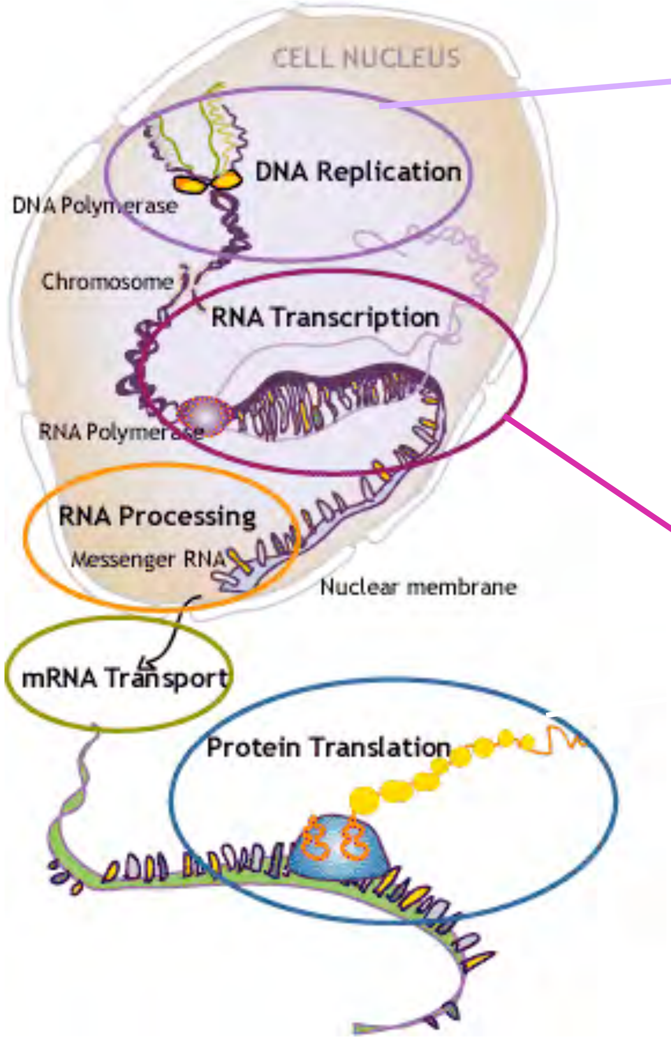
Biochip: characteristics

- **Microfabricated** (silicon) substrate
- **Active**: integrates sensing and sample handling, controlled delivery functions
- **Biological sensing elements:** analyte detection based on bio-molecular reactions
- **Biocompatible**: is not impacted by and does not disrupt biochemical processes

Applications

- Diagnosis (target: protein biomarkers….) → **reliability**
- Expression analysis (target: protein or mRNA) → **throughput**
- Gene-based tests (identification, predisposition to diseases, to drugs) → **selectivity**
- Drug delivery (in vivo) → **bio-compatibility**

Analytes



Gene-Tests (DNA-based tests)

reveal

the sequence of the DNA molecule to

identify

- mutations which could lead to diseases
- sequences that indicate specific answers to drugs

Expression Analysis

reveal

the sequence of the RNA molecule

or the presence of **protein** (biomarkers)

to identify

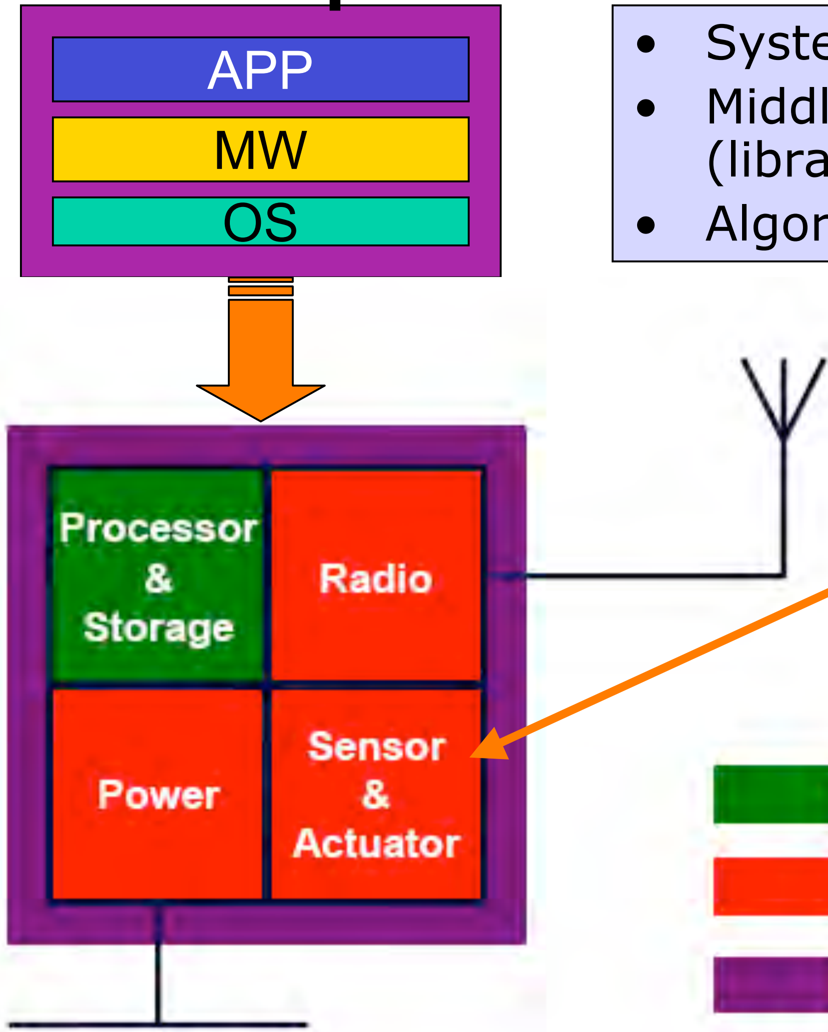
specific conditions of the cell

Why a Biochip platform?

- Increase productivity through reuse
  - Components
  - Interfaces
- Simplify the design process
  - Helps multi-disciplinary integration
  - Separation of concerns (much more relevant for heterogeneous technologies)
- Facilitate the creation of derivatives
  - Conquer low volume markets with low investment
  - Fast design space exploration
- Configurability (post-fabrication) is a requirement
  - In-field tuning (in a lab, point-of-care)

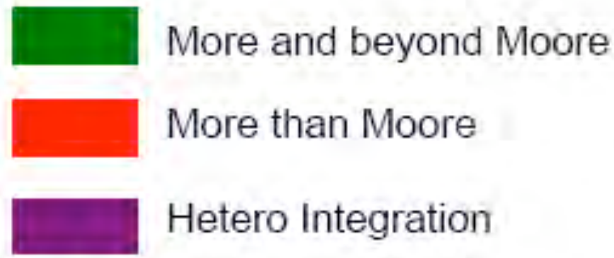
Biochip Platforms: Components

- signal processing circuits
- communication module
- power supply unit



- System SW
- Middleware (libraries, APIs)
- Algorithms

- bio-molecular receptors
- substrate-linker
- Transducer
- Sample manipulation



Bio-molecular components

Forms of Biological receptors

Form	Advantages	Disadvantages
Purified	sensitive, no side reactions	expensive, unstable
Whole cell	stability, possibility of multi-step reactions	side reactions
Tissue slice	minimal preparation, natural environment	slow diffusion, side reactions

Biological Receptors

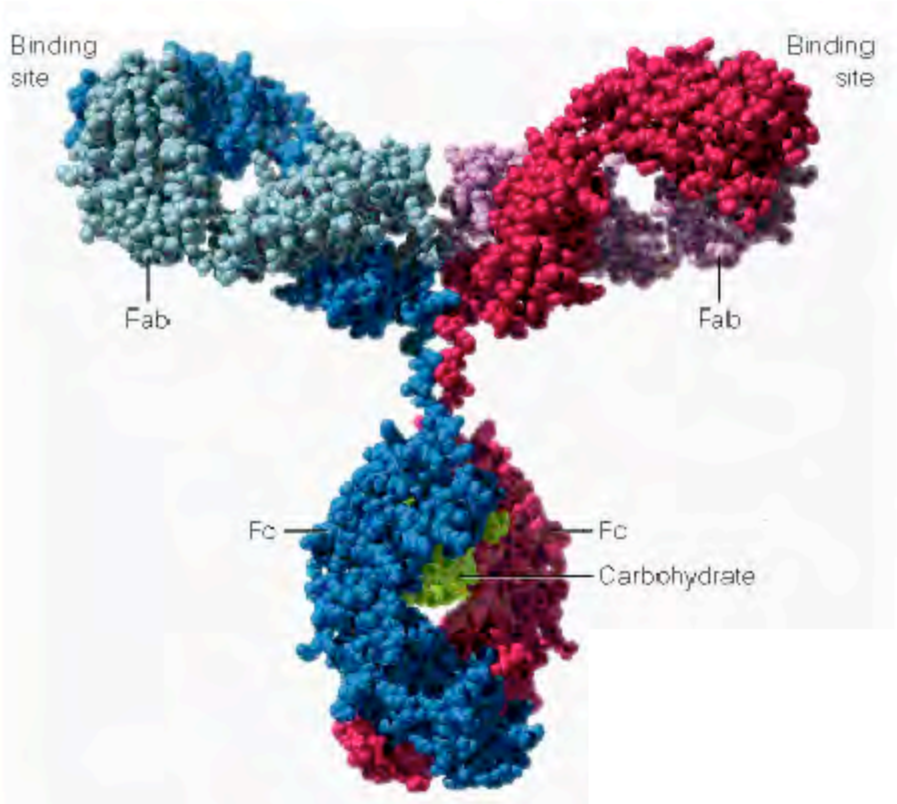
Antibodies/Antigen

- Highly-selective interaction, very tight binding, ultrasensitive

- Antibodies can be raised almost again any antigen.

- Disadvantage: No intrinsic amplification

- Labelling: Radio-isotopes, Enzymes, Fluorescet probes, chemiluminescent probes, metal tags



Biological Receptors

Nucleic Acids

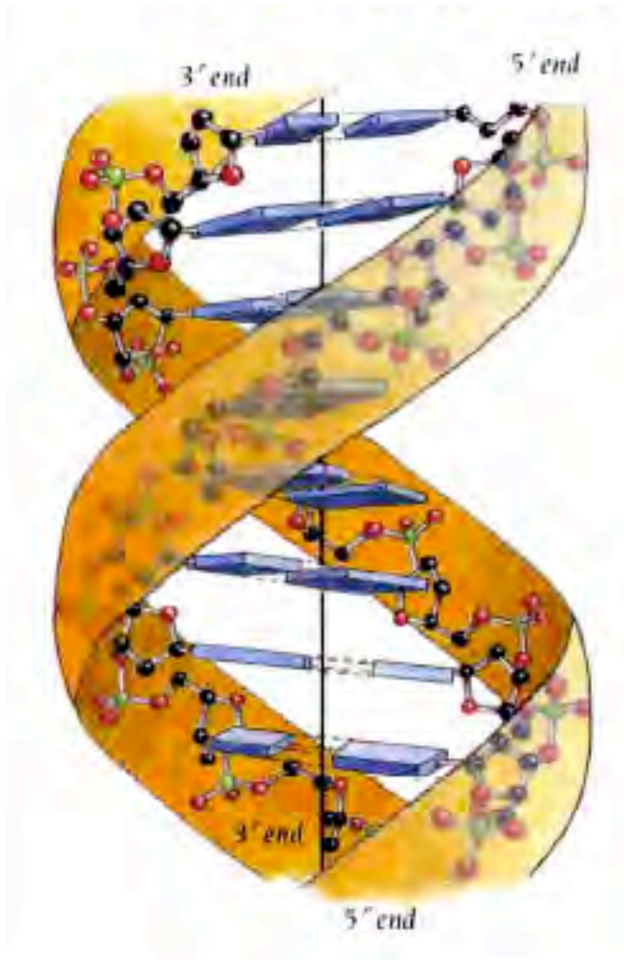
- DNA and RNA dignostic sensors in chip format; used to detect genetic disorders and expression levels of protein in parallel

- Receptors can be synthesised in the lab

- Hybridization can be controlled conveniently by temperature, ionic strength and pH

- Hybridization can be analysed by UV-spectroscopy

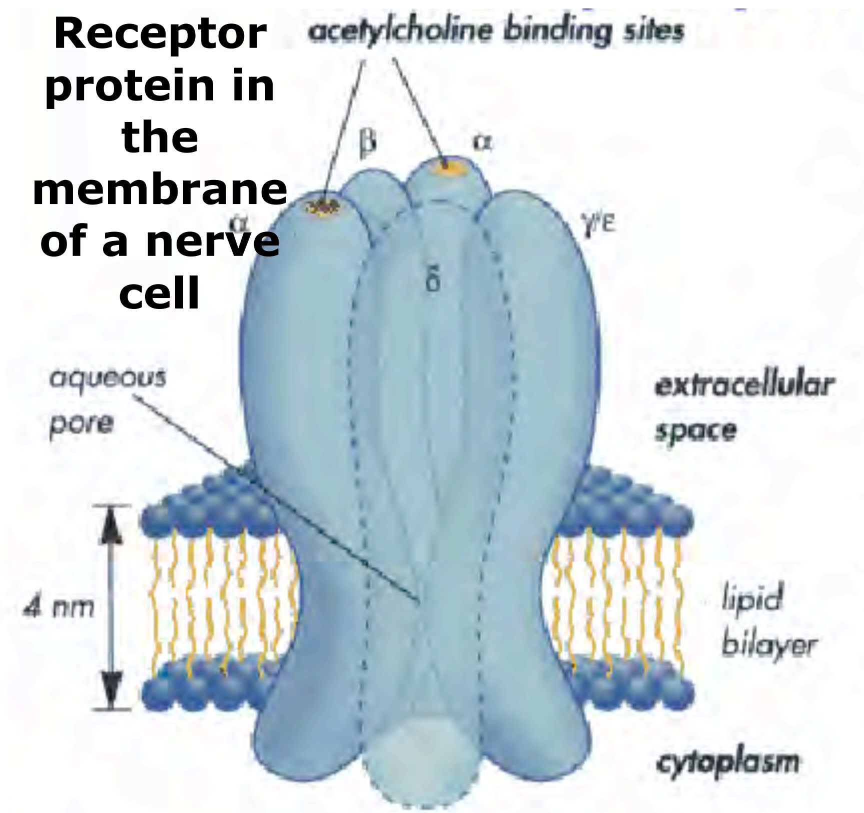
- Disadvantage: No intrinsic amplification -- Labelling: Radio-isotopes, Enzymes, Fluorescet probes, chemiluminescent probes, metal tags



Biological Receptors

Receptor proteins

- Many receptor proteins are on the surface of cells and embedded into membranes
- Applications mainly expected in detection of neuro-transmitters, hormones, neuro-active drugs, anaesthetics
- Highly selective but often difficult to isolate.
- Interfacing cell biology with microelectronics: nerve cells on chips



Transducer components

Detection principle based on changes of	Electronic/Silicon-based sensing element
Mass or Surface Stress	Bio-modified Cantilevers/SAW devices
Refractive Index	Bio-modified Surface of porous silicon
Electrochemical Activity	Bio-modified Elelctrodes
Interface Electrical parameters	Bio-modified Elelctrodes
Charge	Field-effect devices
Absorbance	Photodetectors
Light emission	Photodetectors

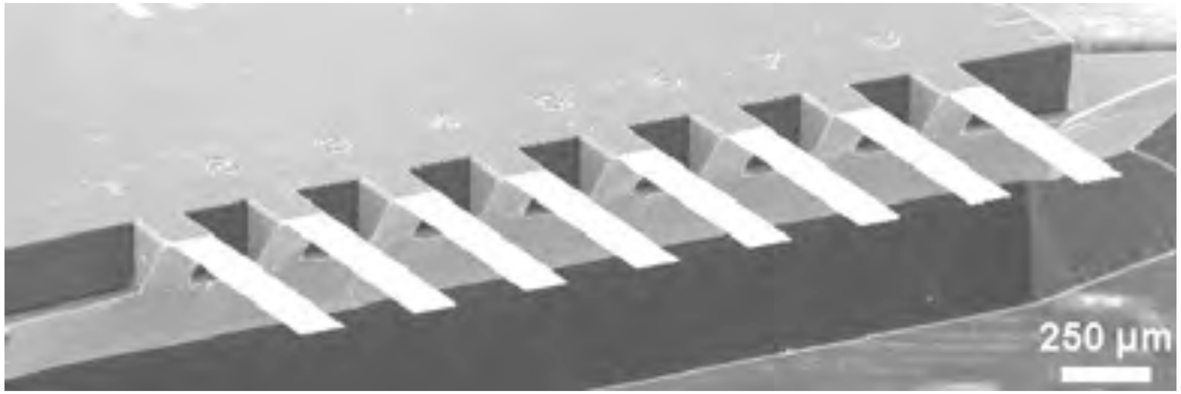
Detection principle based on changes of
Mass or Surface Stress

Electronic/Silicon-based sensing element
Bio-modified Cantilevers

- **Surface stress – Mechanical Displacement (Bending)**
- IBM *Fritz et al. Science 288, 316 (2000)*
- Deflection of about 10 nm for a 16-mer oligonucleotide target.

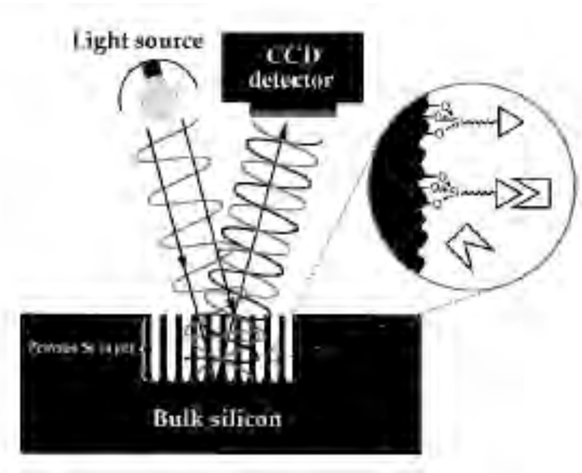
eight identical silicon cantilevers
250 *μ*m pitch

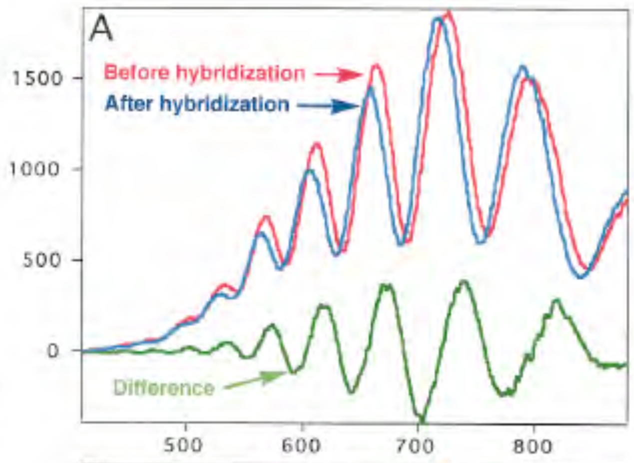
length of 500 *μ*m
width of 100 *μ*m
thickness of 0.5–1 *μ*m



Detection principle based on changes of

Refractive Index





Electronic/Silicon-based sensing element

Bio-modified Surface of porous silicon

- The silicon oxide surface of the porous layer can be modified
- Reflection of white light at the top and bottom of the PSi layer results in an interference pattern (Fabry-Perot fringes). The reflectometric interference spectrum is sensitive to the refractive index of the PSi matrix.
- Interactions of the molecular species with their recognition partners immobilized on the surface induce a change in the refractive index of the semiconductor, giving rise to wavelength shifts in the fringe pattern that can be easily detected and quantified.

Lin, Science, vol. 278, pp-840-842 (1997)

Detection principle based on changes of

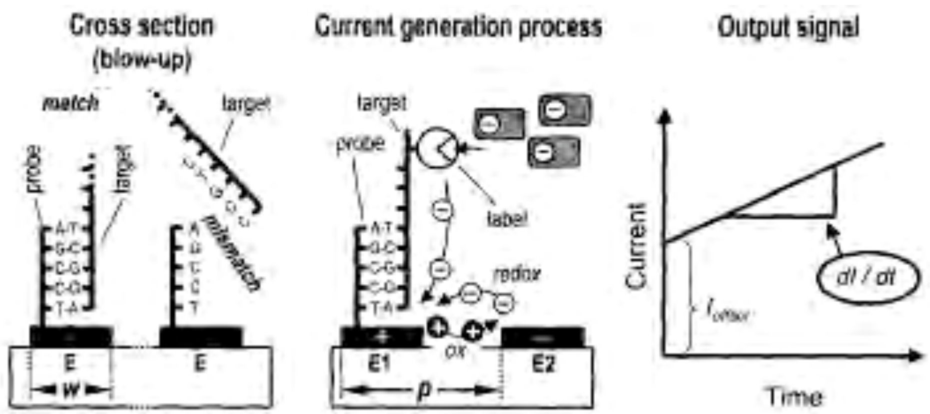
Electrochemical Activity

Electronic/Silicon-based sensing element

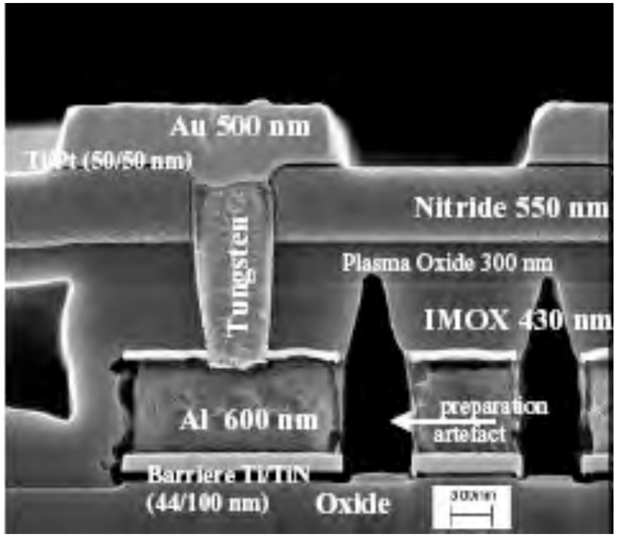
Bio-modified Elelctrodes

Sensor array based on 0.5 µm, 5V standard CMOS

Measurement Circuits on-chip



Enzymatic and redox-cycling processes



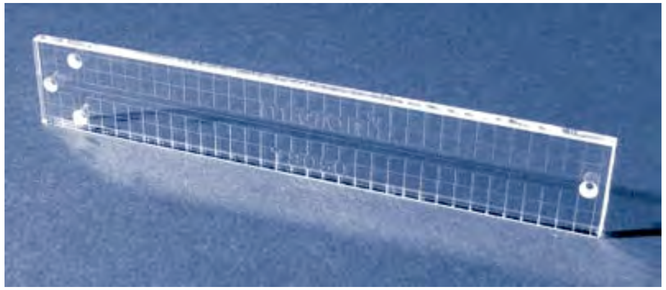
Additional process steps to expose and contact gold sensor electrodes

Infineon technologies

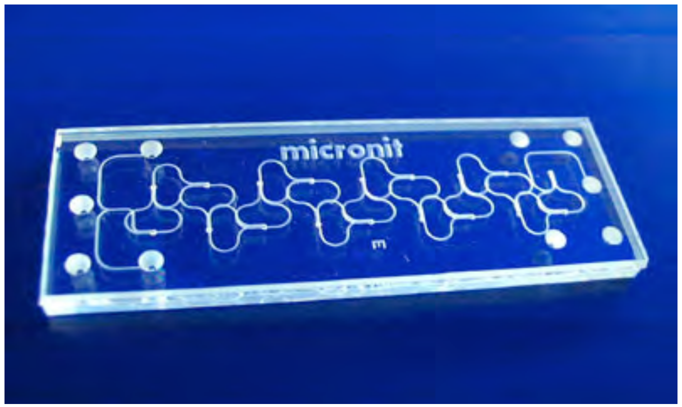
Microfluidic components

- Common microfluidic functional blocks

Micro-wells



Micro-channels



Micro-needles

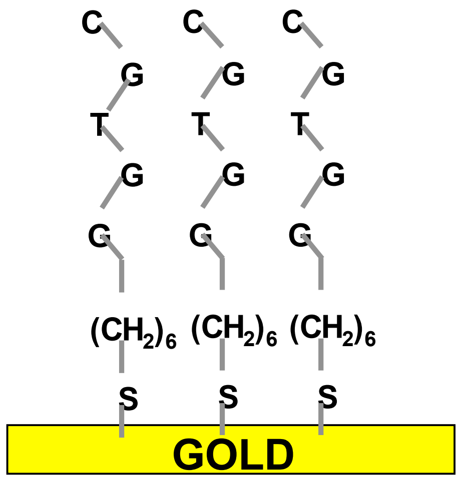
Micro-pipette

Configurability

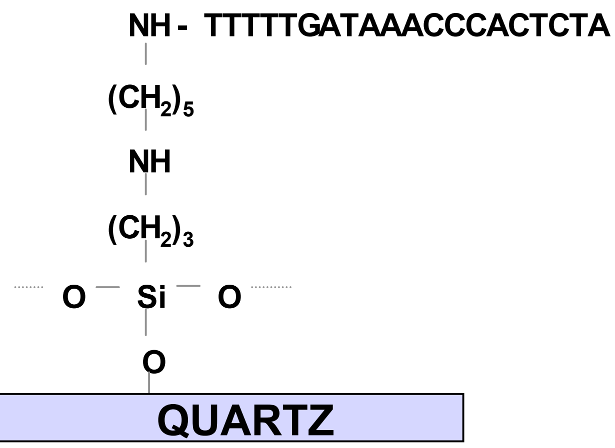
- Digital+Analog configurable electronics (well known)
- Bio-configurability:
  - different substrate-linker layers (between transducer and probes)
  - different receptors
  - different chemical signal amplification strategies
- Analogy with mask programmable devices

Substrate-linker layers

- Gold+thiols



- Oxides+silane-based monolayers



Micrel Lab Research Activities on Biochips

More Moore

Signal processing

Communication module

Power supply unit

More than Moore

Bio-molecular receptors

Substrate-linker

Transducers

Sample manipulation

Application of molecular recognition in course of investigation

- detection of protein biomarkers for cancer diagnosis and monitoring (*Xeptagen, Parco Tecnologico Vega, Venice, Italy*)
- detection of RNA sequences for the diagnosis of viral infections (expression analysis) (*Istituto Spallanzani, Roma, Italy*)
- Label-free high-throughput expression analysis (Comparative analysis of biochip devices with respect to standard fluorescence scanner-based microarray tests) (*CRIBI, Padova, Italy*)

Mass produced, Reliable, Low-cost, Portable

Detection Principle based on changes of
Conductive Substrate/solution interface **impedance**

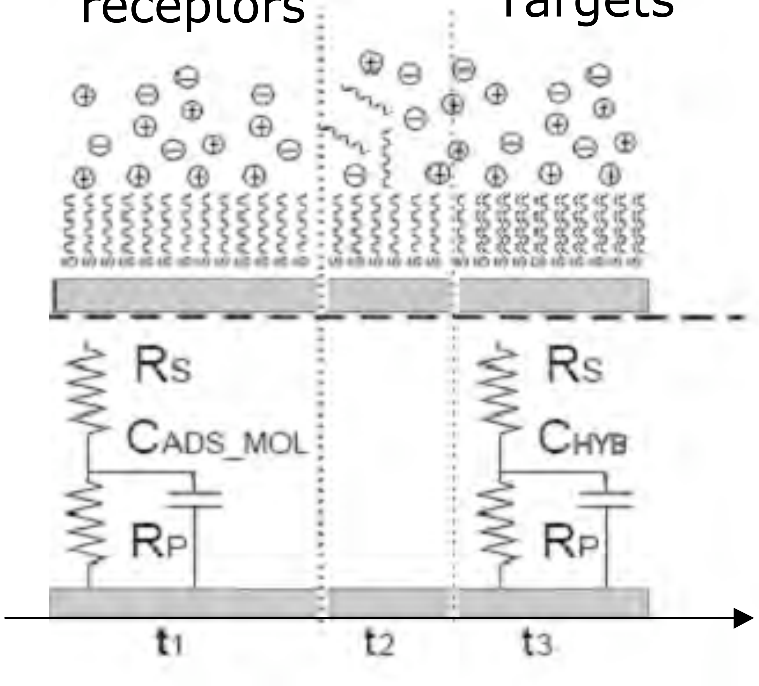
Substrate-linker layer
Ultra-clean **gold layer** modified with thiol/alkanthiol linkers

Transducer
Capacitance measurement circuit

Detection Principle based on changes of Conductive Substrate /solution interface impedance

Bio-Sensing receptors

Detected Targets

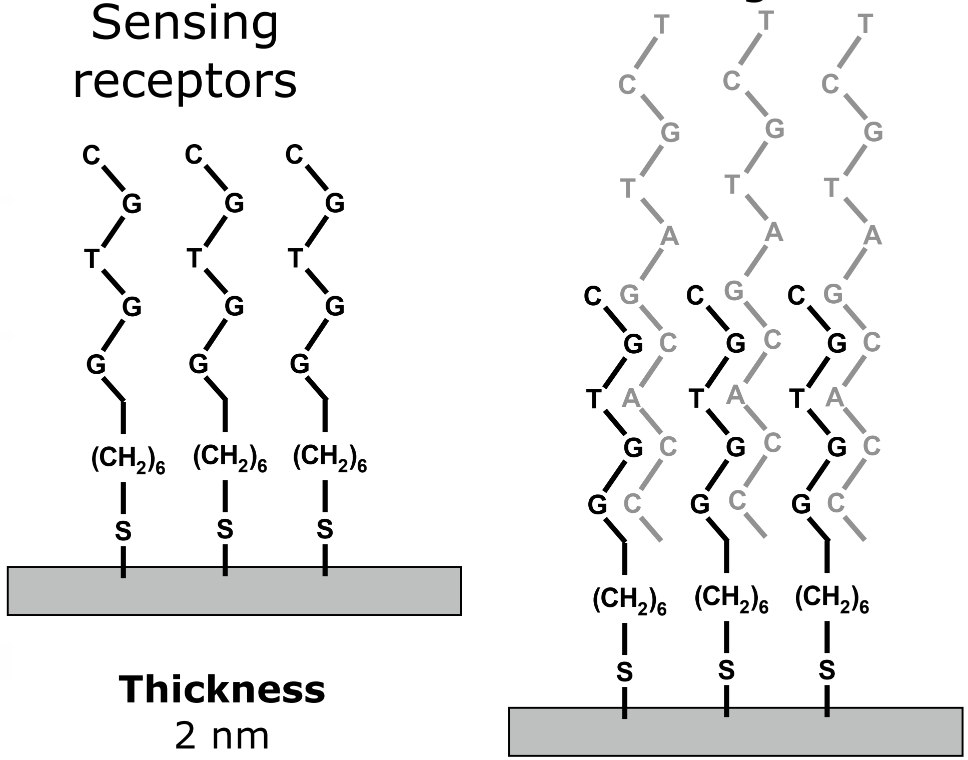


$C_{ADS\_MOL} > C_{HYB}$

(-30 %)

Bio-Sensing receptors

Detected Targets



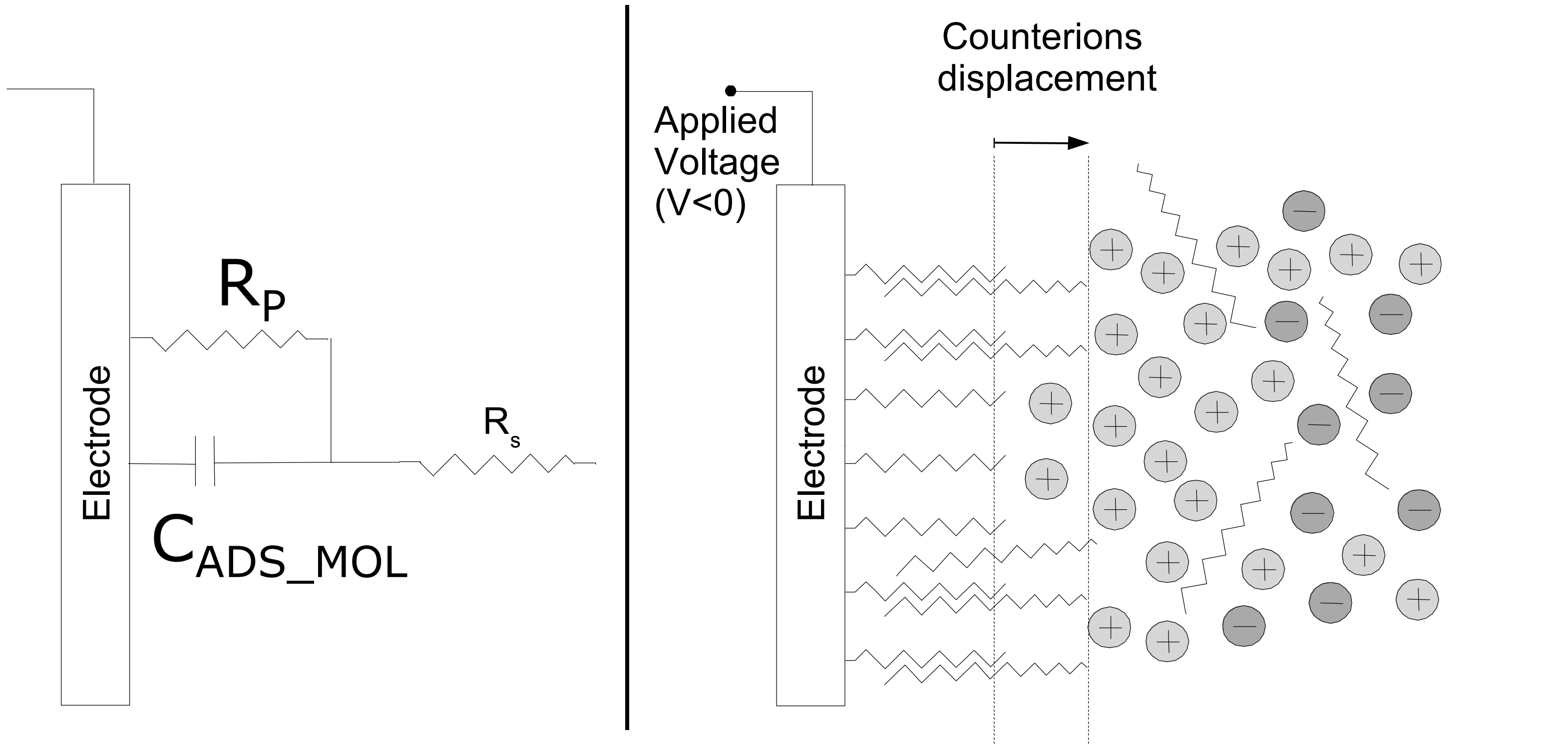
Thickness
2 nm

Permittivity
1.9

Thickness
5.5 nm
Permittivity
2.5

Meas:[Peterlinz et al., 1997, Yang et al., 1997 Kelley et al., 1998]

Conductive Substrate /solution interface impedance

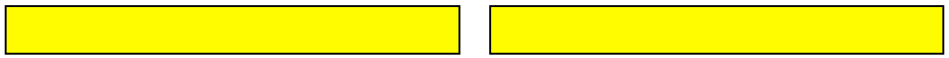


Substrate-linker layer

Ultra-clean gold layer modified with thiol/alkanthiol linkers

Cleaning step

Plasma etching or chemical pirana etching



<u>Protocol:</u>

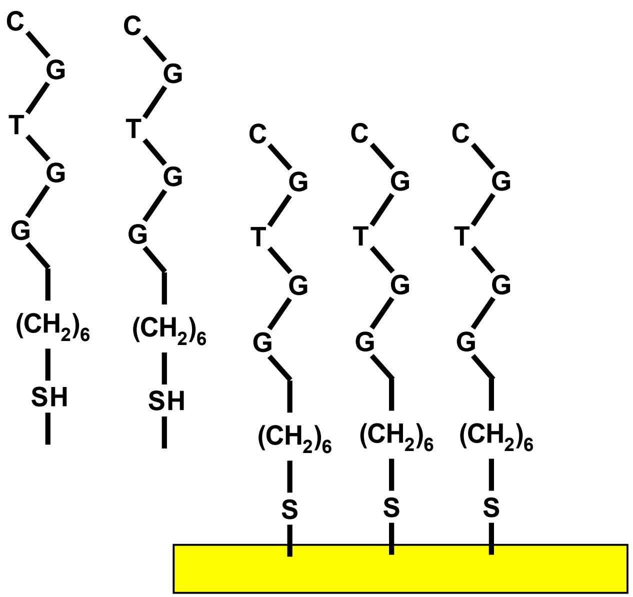
plasma oxigen

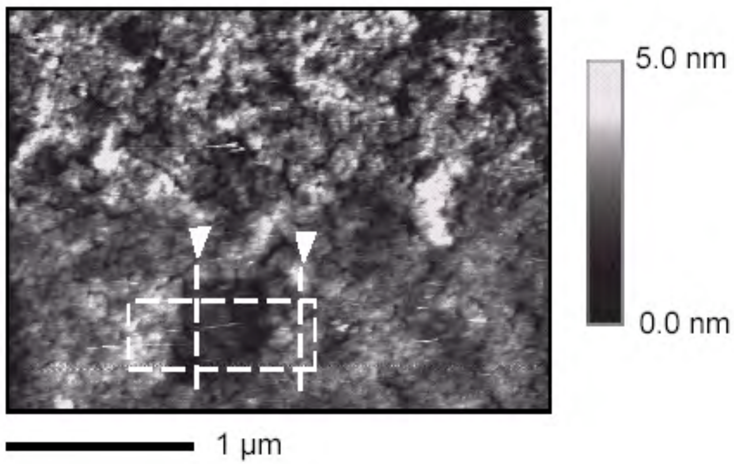
20 min at 200 W

Bio-modification step

<u>Protocol:</u>

3 μM probes concentration in Na_2HPO_4 (1 M) 18 hours

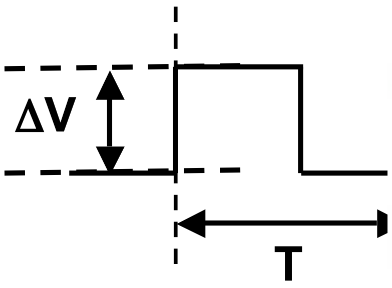


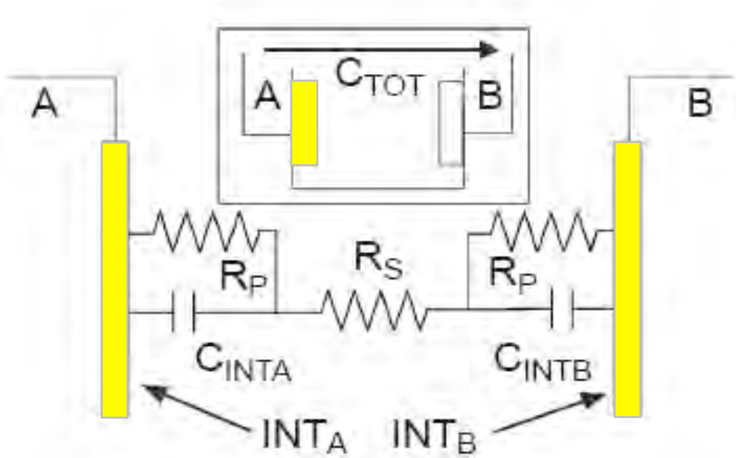


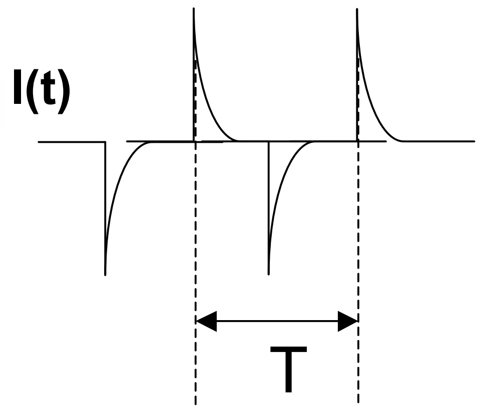
AFM-Nanoshaving Technique

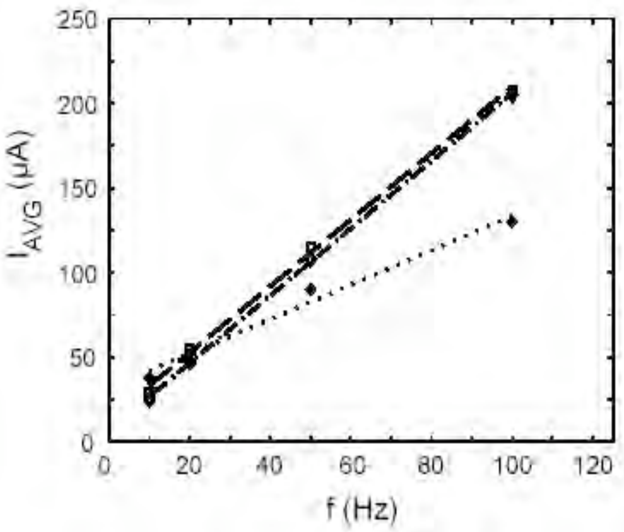
Transducer

Integrable capacitance measurement circuit

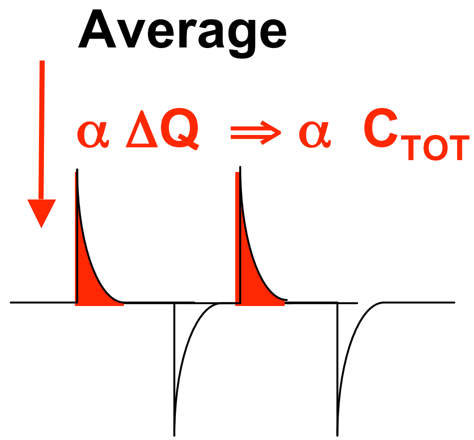




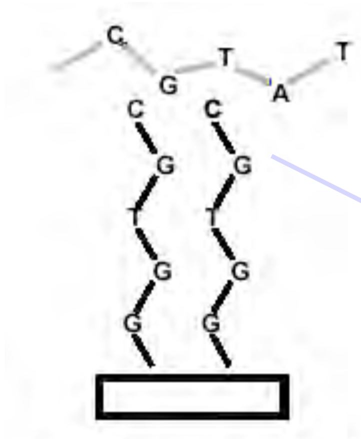




$$I_{avg} = I_{DC} + C_{TOT} \Delta V f$$

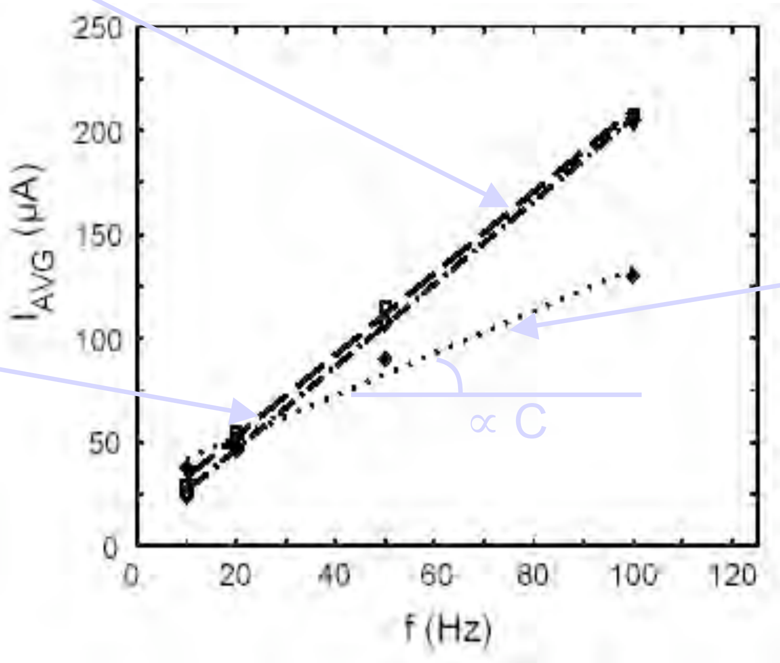


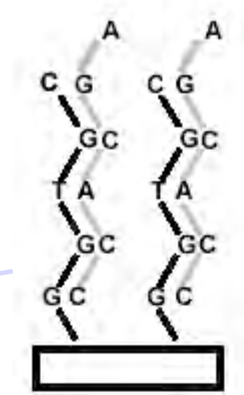
Experimental Results I



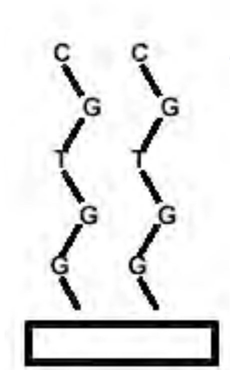
Non Complementary

Total capacitance variation: 52%





Complementary

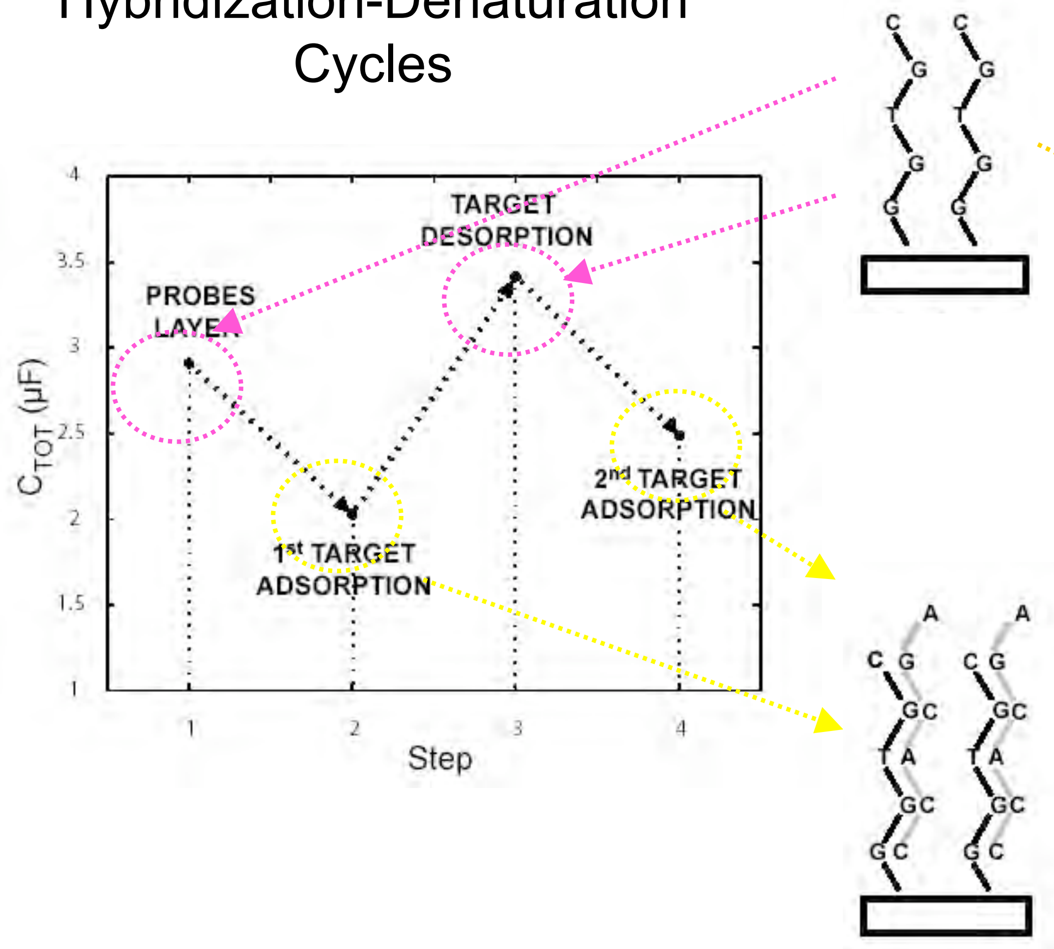


Probes

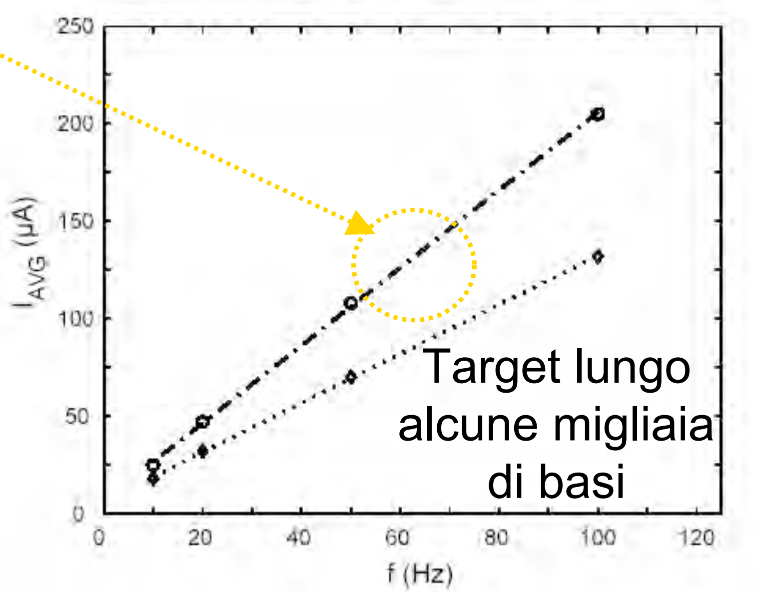
C. Guiducci, C. Stagni, G. Zuccheri, A. Bogliolo, L. Benini, B. Samorì, B. Riccò,” Biosensors and Bioelectronics, vol. 19, pp. 781–787, 2004.

Experimental Results II

Hybridization-Denaturation
Cycles



On-field target

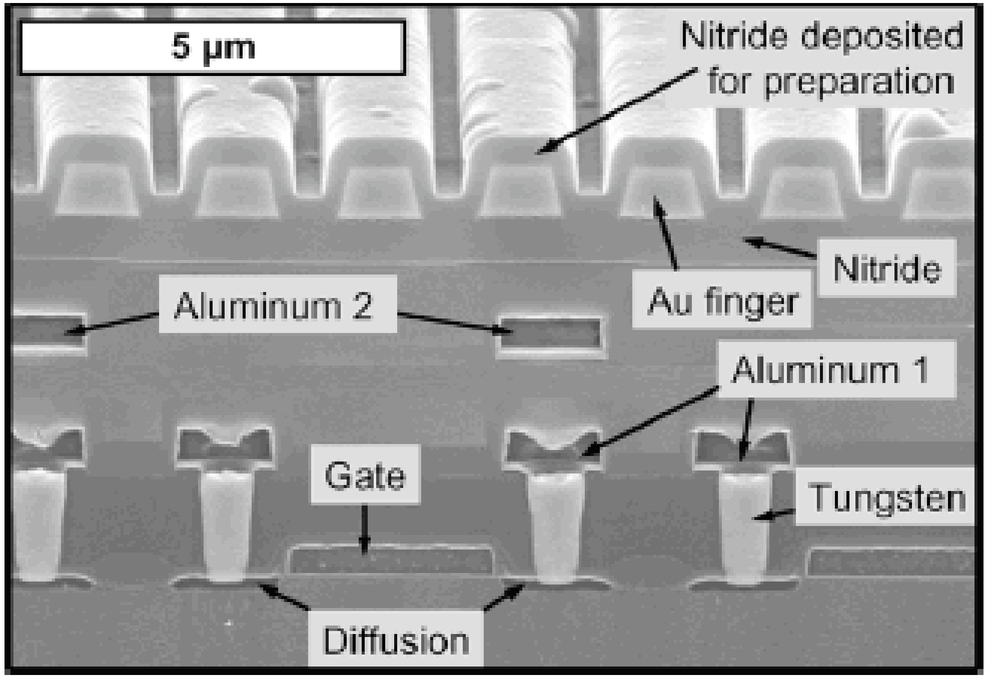


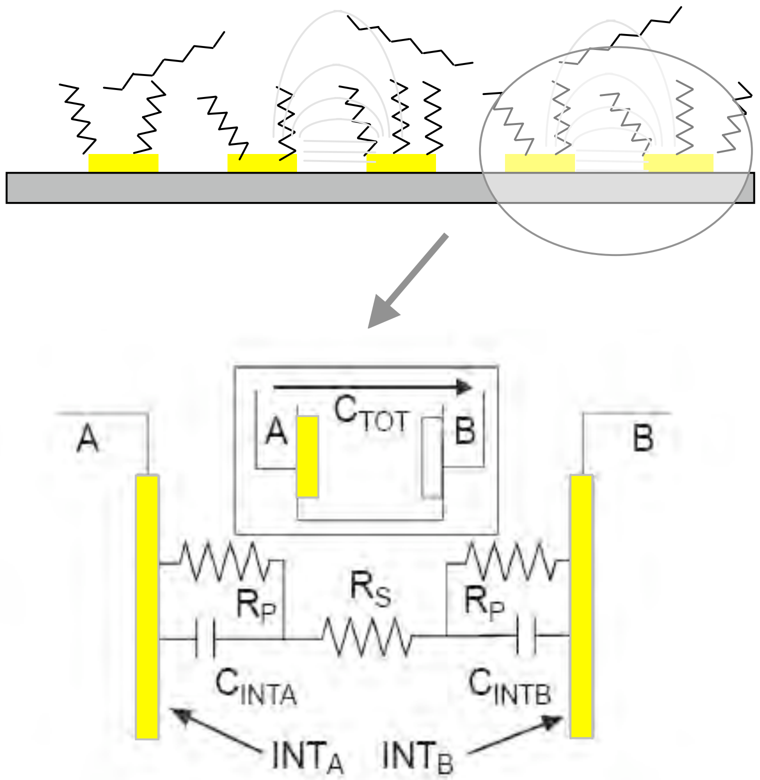
Gold electrodes – Integration

1 – Macroelectrodes
2 - Microelectrodes on a passive silicon substrates (STMicroelectronics)
3 – Microelectrodes on an active silicon chip integrating addressing circuitry. Analog output is provided and measured externally (Infineon Technologies)
4 – Microelectrodes on an active silicon chip integrating addressing circuitry and distributed measurement circuit (fully-digital interface – higher array density) (Infineon Technologies)

Gold electrodes on a silicon chip

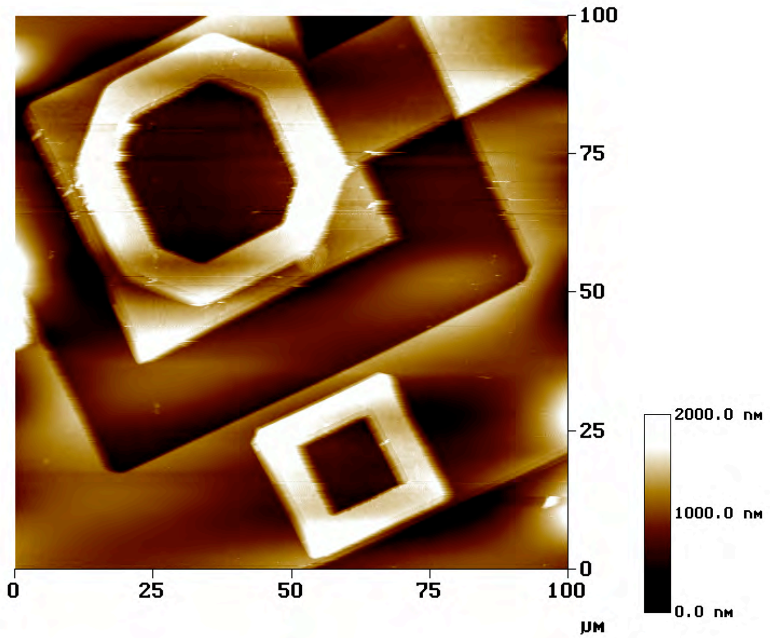
Gold Electrodes
exposed on the surface of the biochip





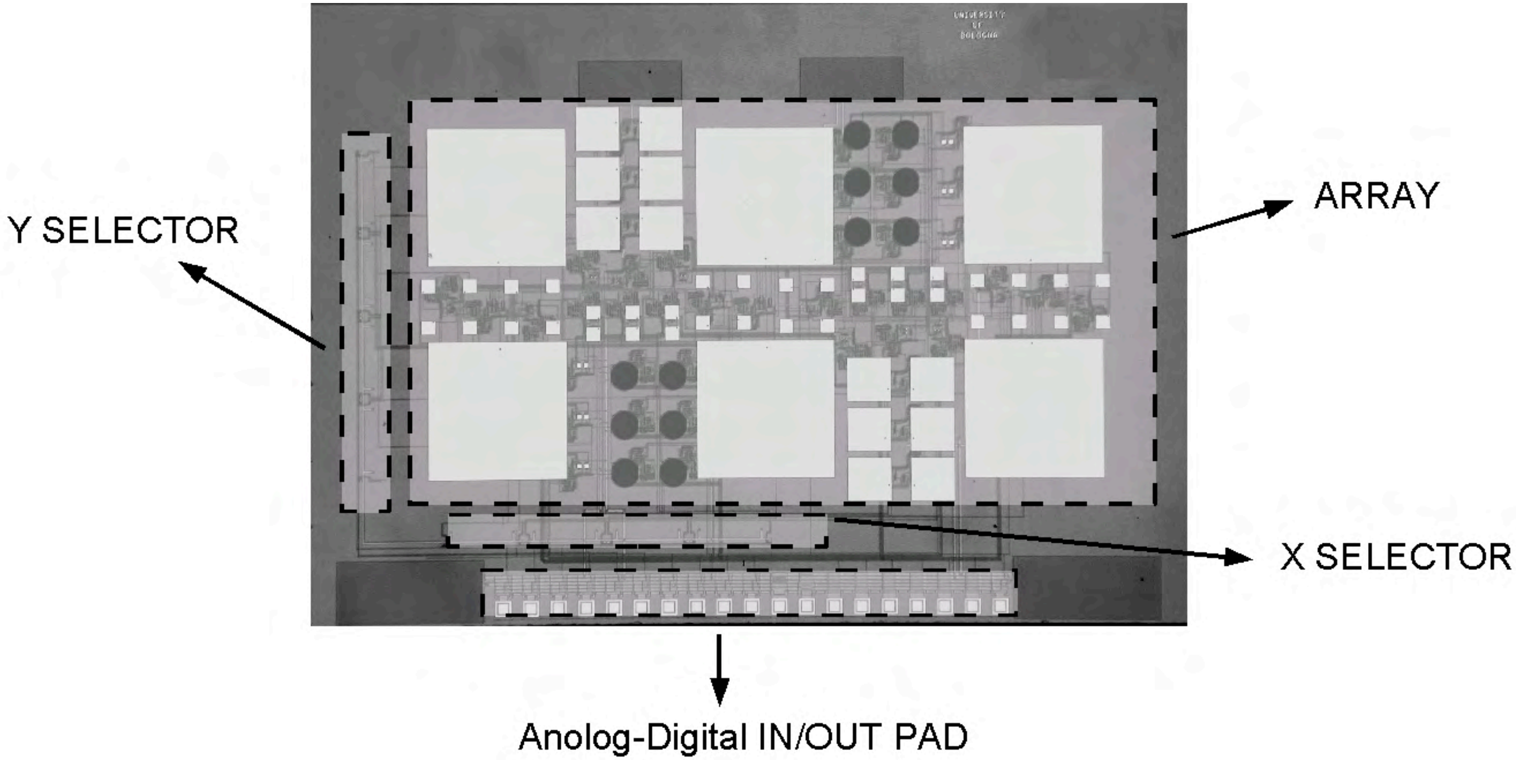
2 - Microelectrodes on a passive silicon substrates (STMicroelectronics)

Total measured capacitance ($2 \times 10^3\ \mu m^2$): between 100 pF and 1 nF



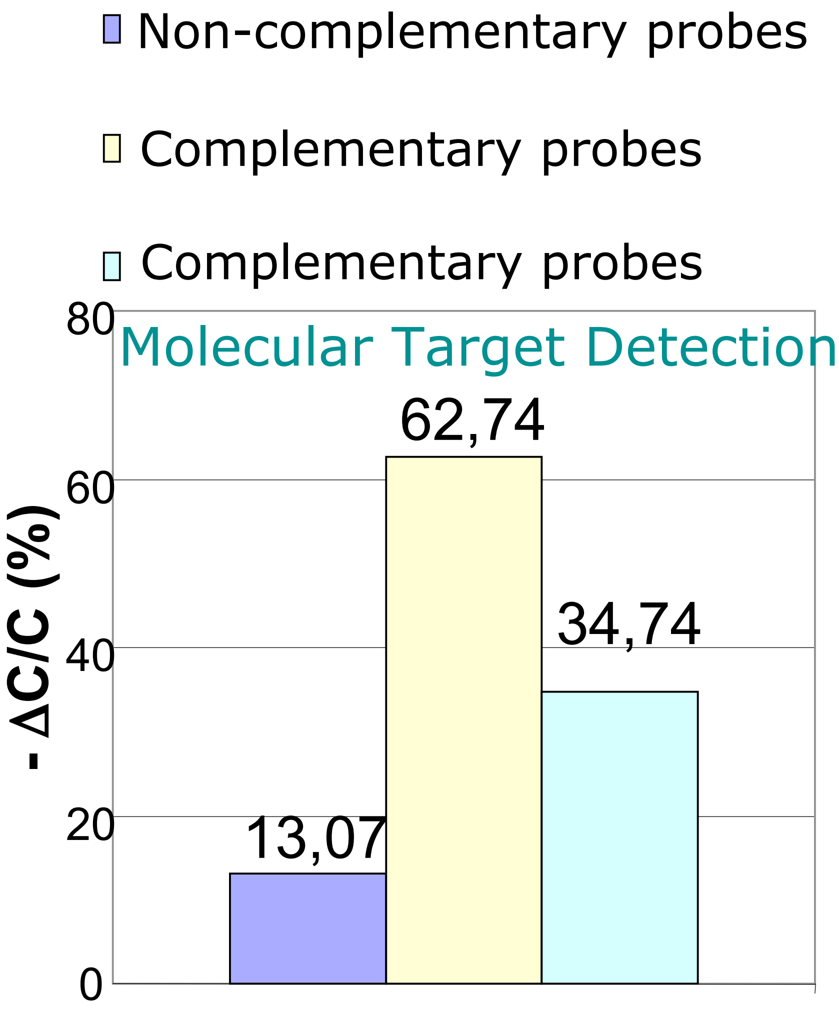
Sample	Percentage decrease of Capacitance after sample deposition and rinsing
Complementary sequence	25.5 ± 5.33
Non Complementary Sequence	-5.2 ± 2.2
DNA-free sample	- 4.95 ± 2.22

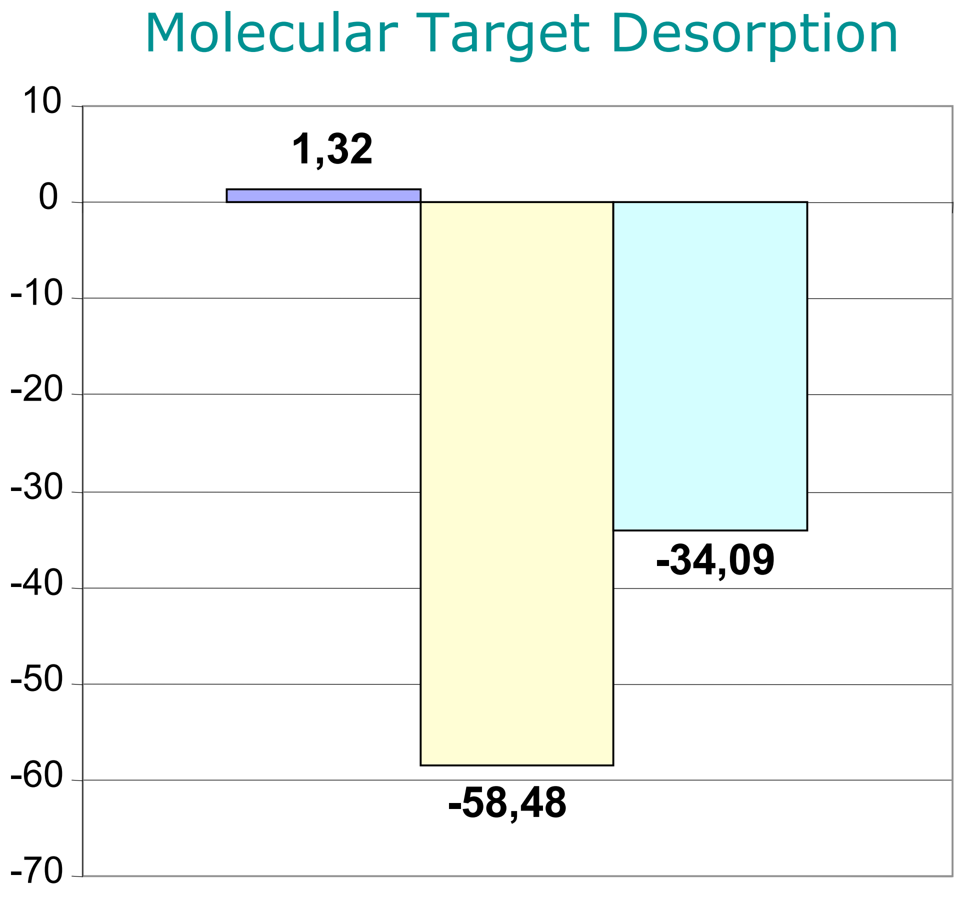
3 – Microelectrodes on an active silicon chip



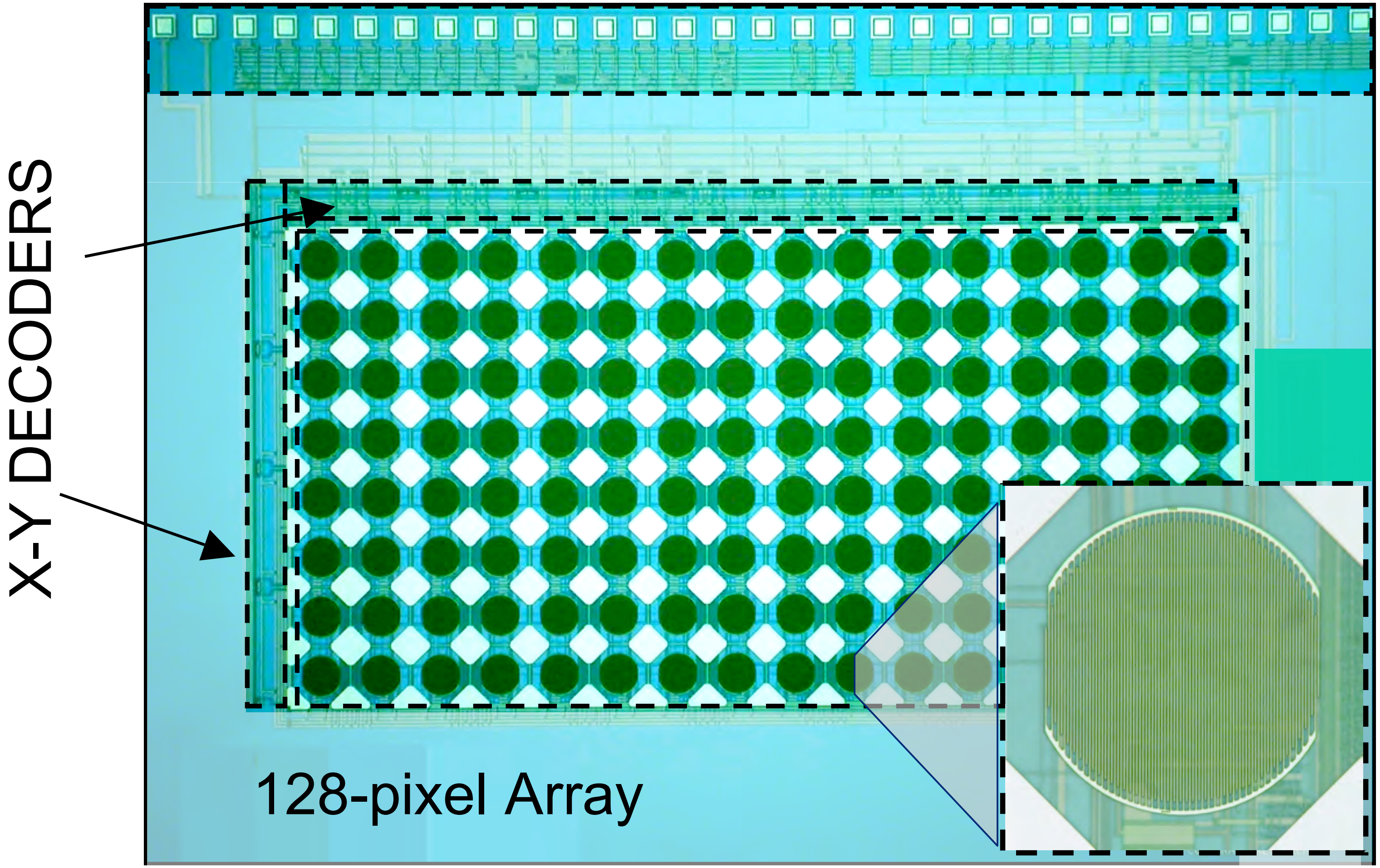
- **CBCM technique**
- Variable electrodes surfaces (1 mm^2-0,0001 mm^2)
- Analog output signal (current)

3 – Microelectrodes on an active silicon chip
Results

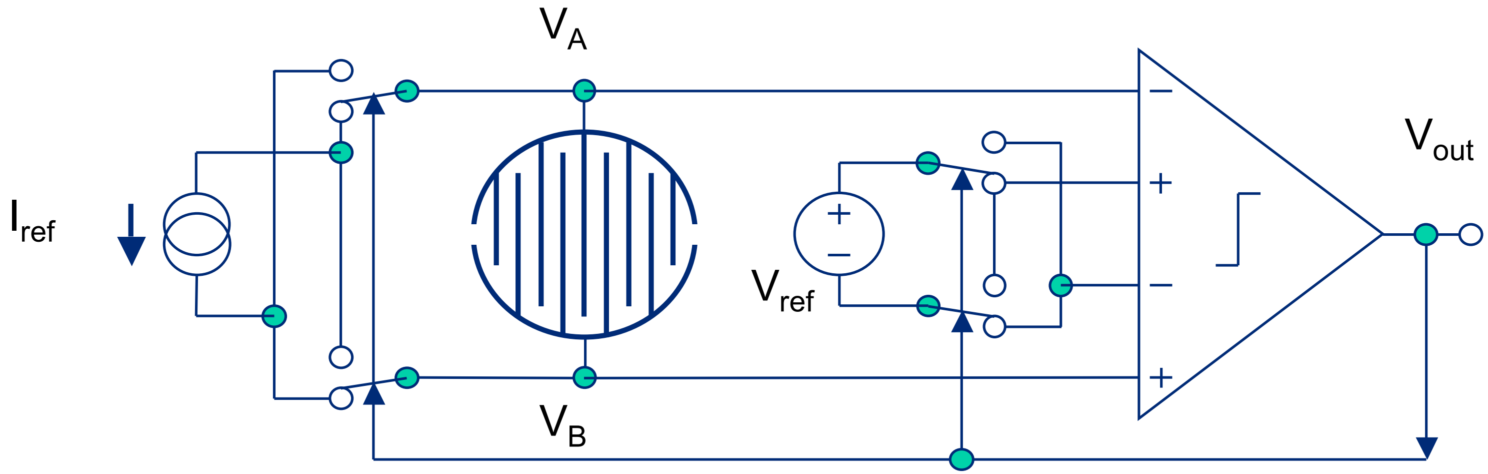


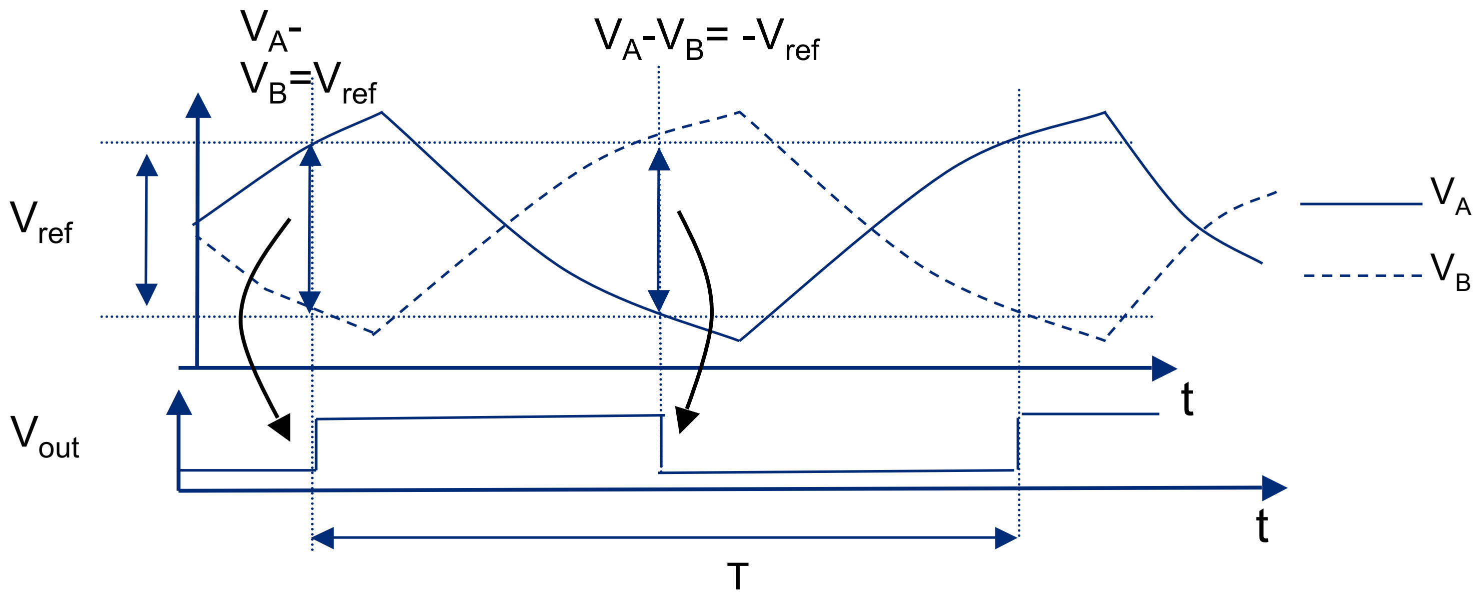


4 – Microelectrodes on an active silicon chip

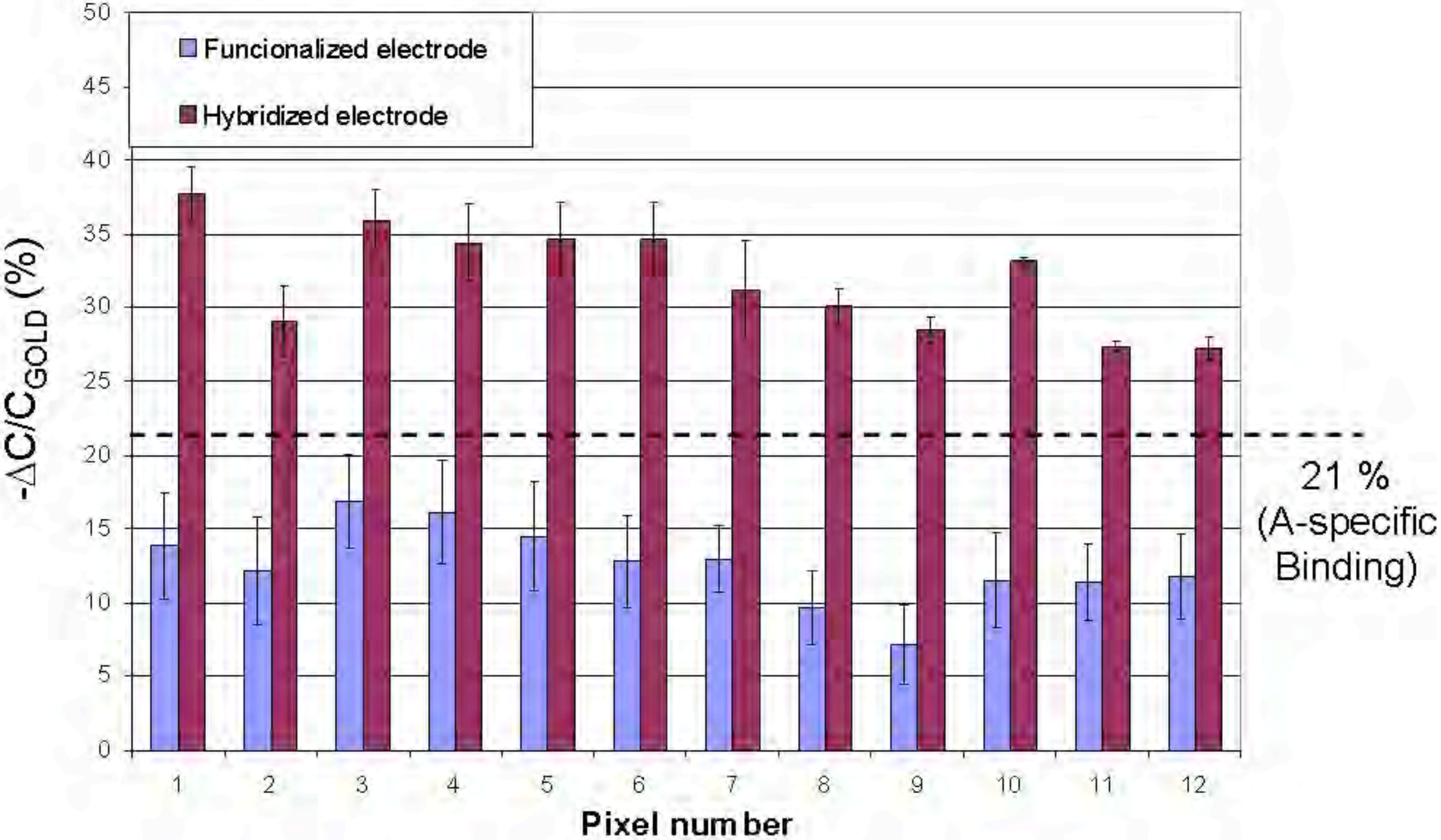


Distributed Measurement Circuit





4 – Microelectrodes on an active silicon chip

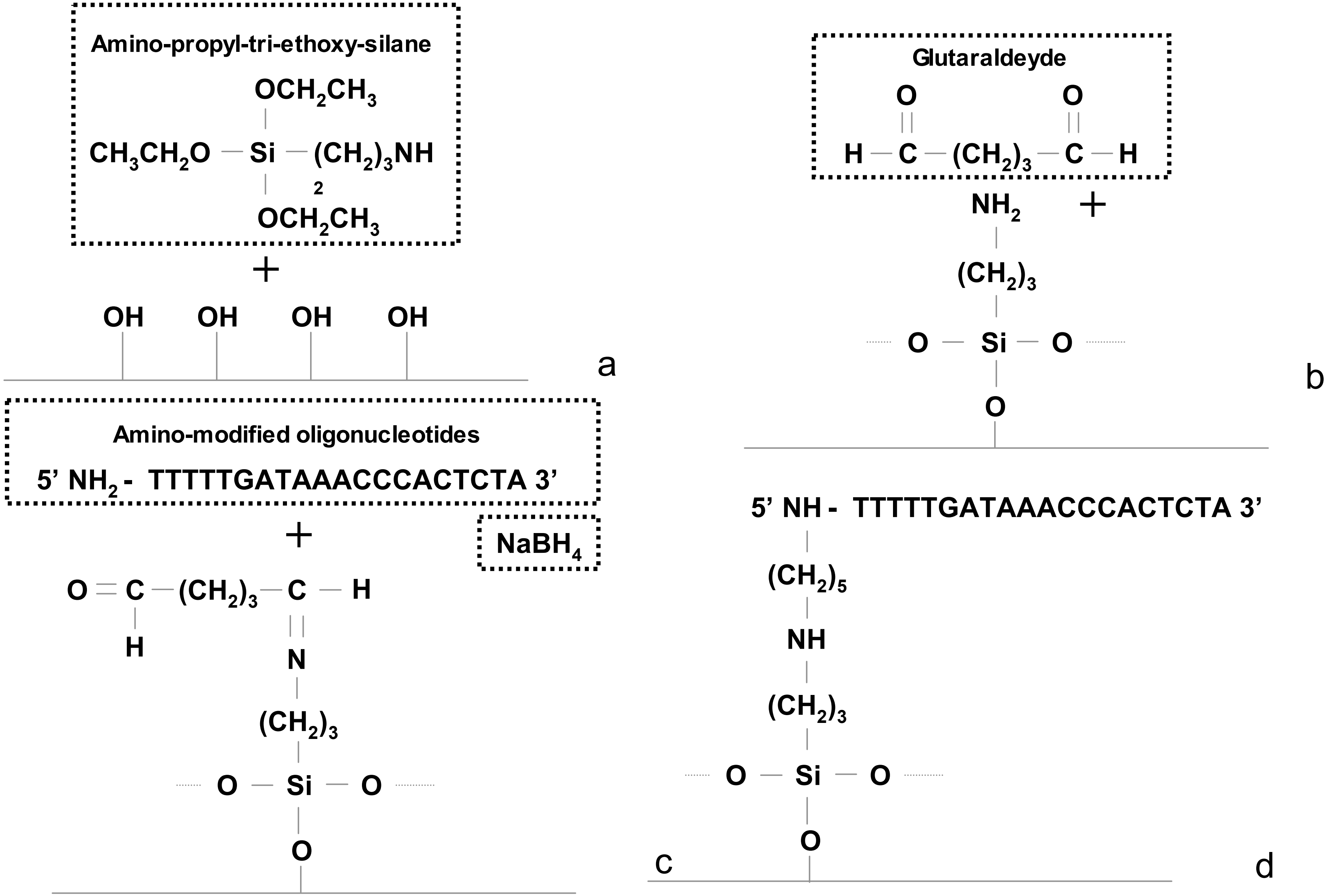


Detection Principle based on changes of
Conductive Substrate /solution interface **impedance**

Substrate-linker layer
Conductive oxide substrates modified
with silane linkers

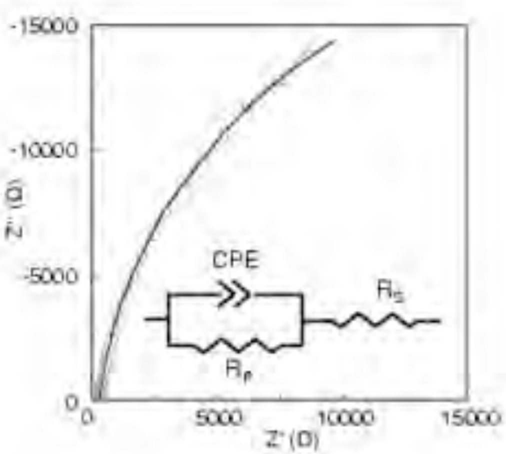
Transducer
Capacitance measurement circuit

Substrate-linker layer



Results

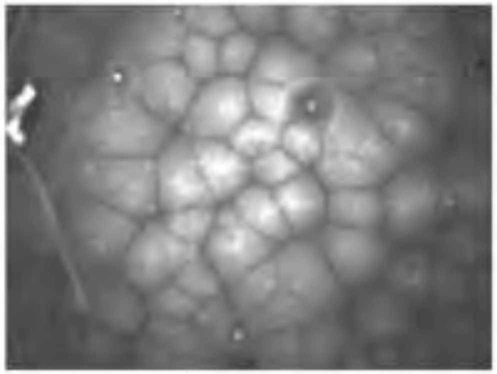
Transparent and conductive oxides: **$CdIn_2O_4$ SnO_2 ITO**
(in collaboration with LMGP-Grenoble)



	CPE-T (µF)	CPE-P
Single-strands (P1)	9.08 ± 0.07	0.9222 ± 0.0022
Double-strands (P1+T1)	6.33 ± 0.03	0.9495 ± 0.0011

Fluorescence images to demonstrate specificity of oligonucleotide detection on conductive oxides

Probes P1 + Targets T1



Probes P2
Non-complementary

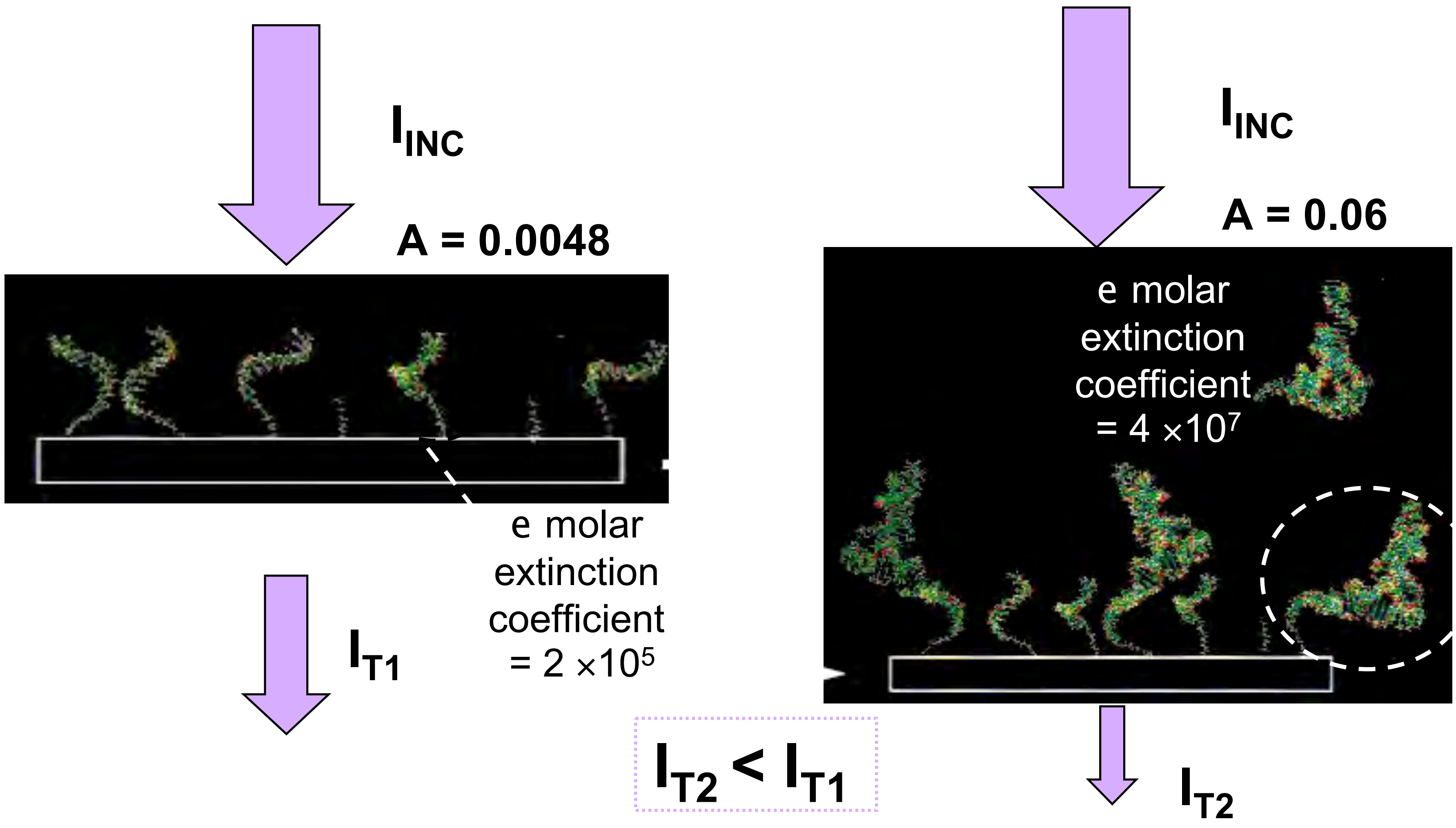
Detection Principle based on changes of
Molecular UV-**absorbance**

Substrate-linker layer
Quartz substrate modified with silane-based (amine termination) linkers

Transducer
UV **photodetectors**

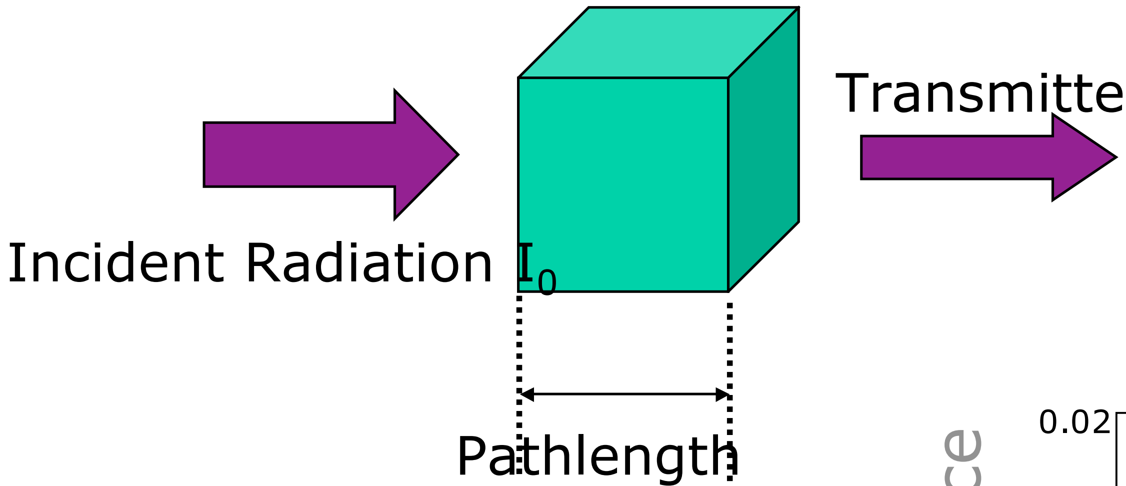
Detection Principle based on changes of

Molecular UV-absorbance



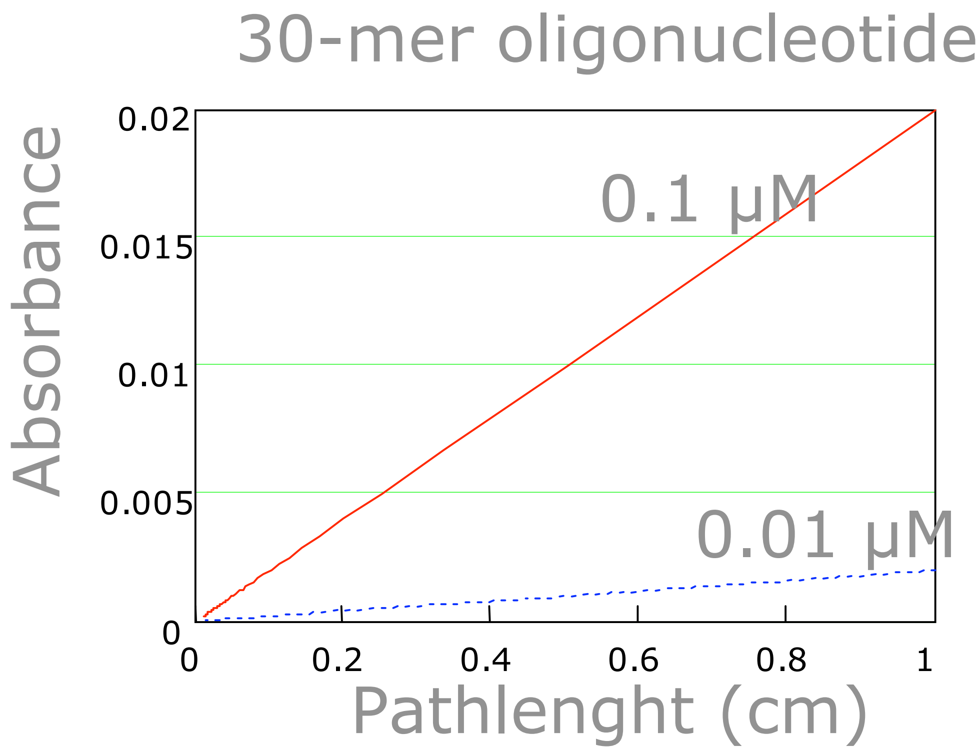
Each detected target molecule is 100-100 times more absorbent than its probe

Molecular UV-absorbance



Transmittance
$T = I/I_0$

Absorbance
$A = \ln (I_0/I) = \ln (1/T)$



Absorbance

The measurement of absorption of ultraviolet by species in solution provides one of the most widely used methods of quantitative analysis available in analytical laboratory

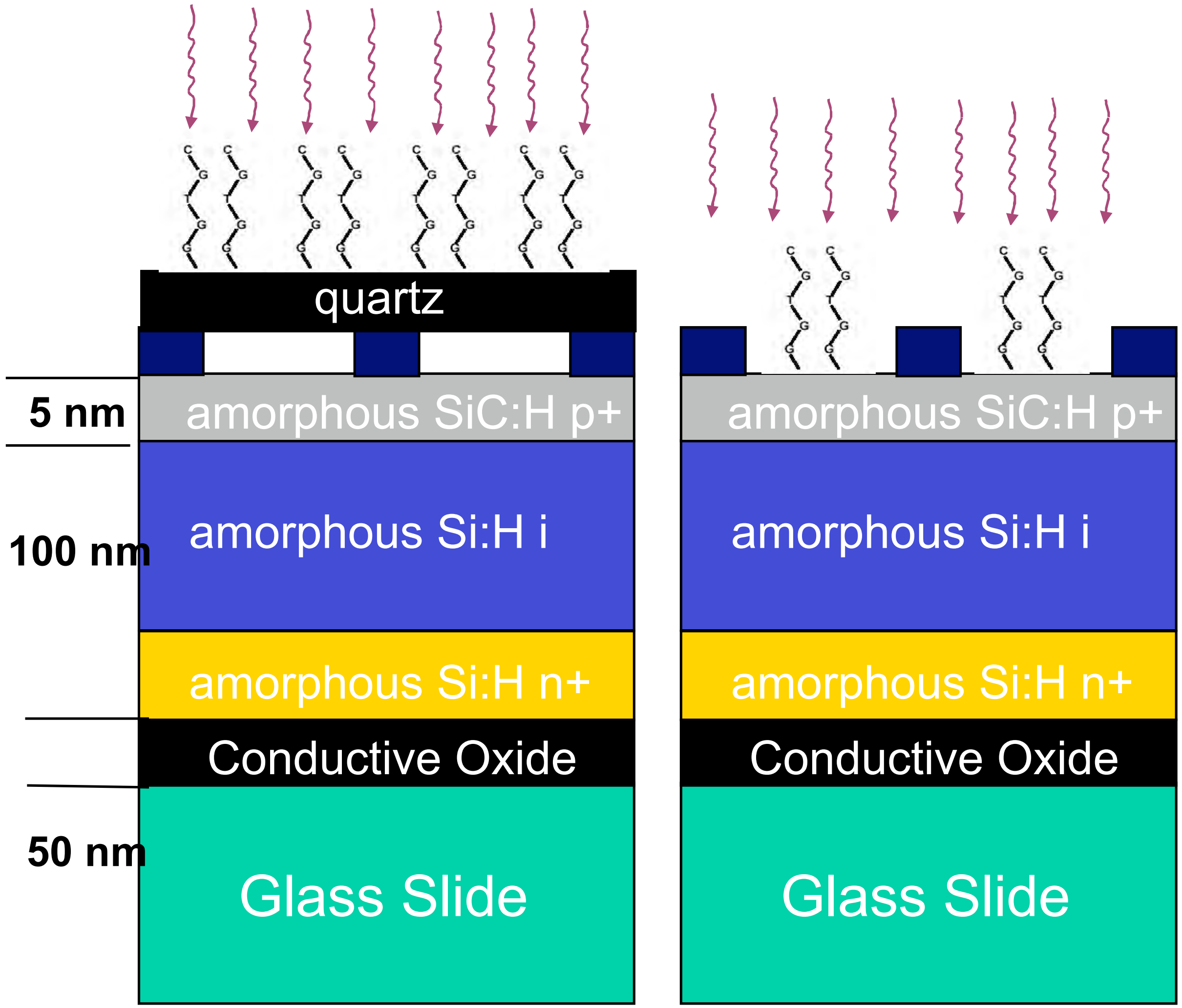
Lambert-Beer law:

$$A = \varepsilon \, c \, l$$

ε, molar extinction coefficient [L mole^{-1}cm^{-1}], C concentration [M], l pathlength [cm]

is a function of wavelenght and of molecular species in solution

Transducer a:Si photodiodes

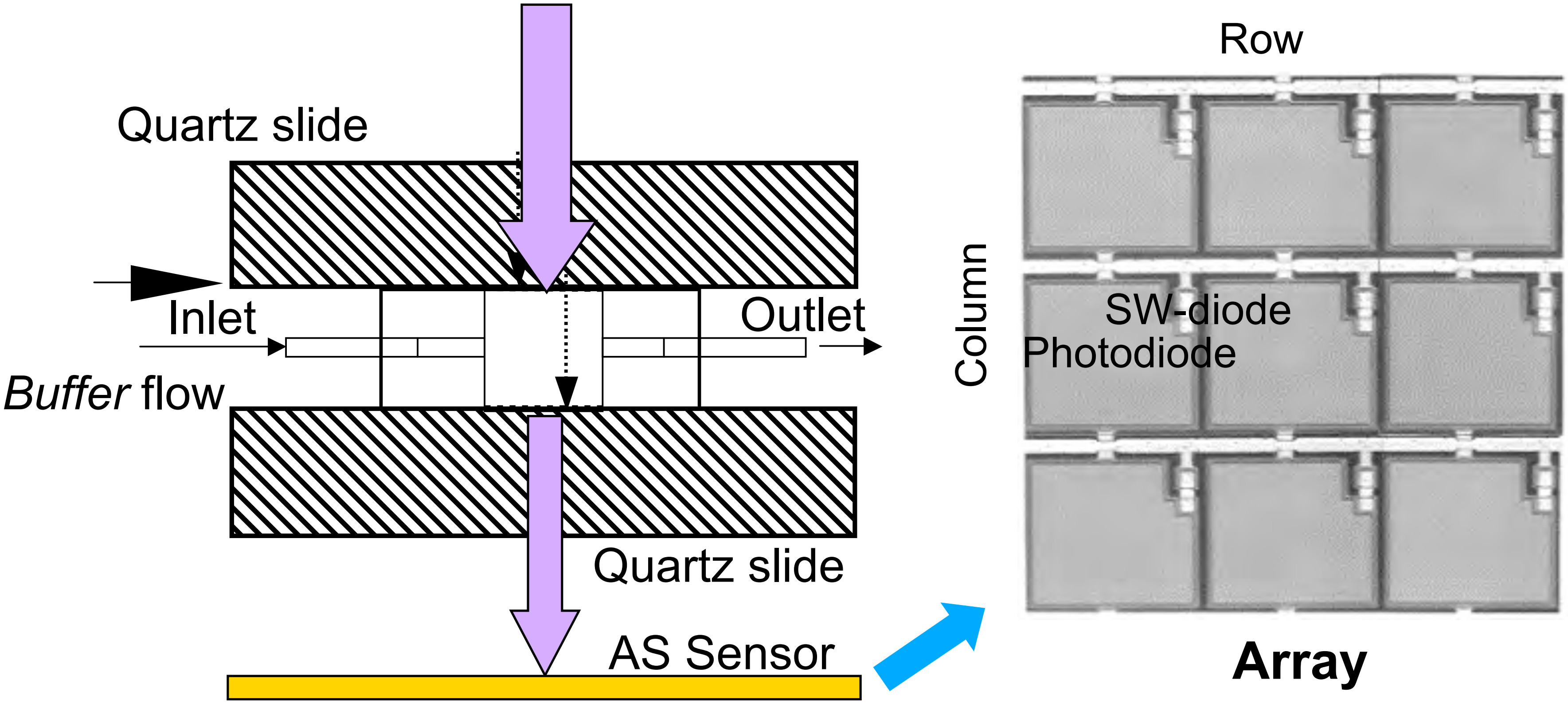


Selectivity in the UV-range is penalized to ensure low dark currents by the following technological choices:

1- wide intrinsic region
2- pure Si intrinsic region

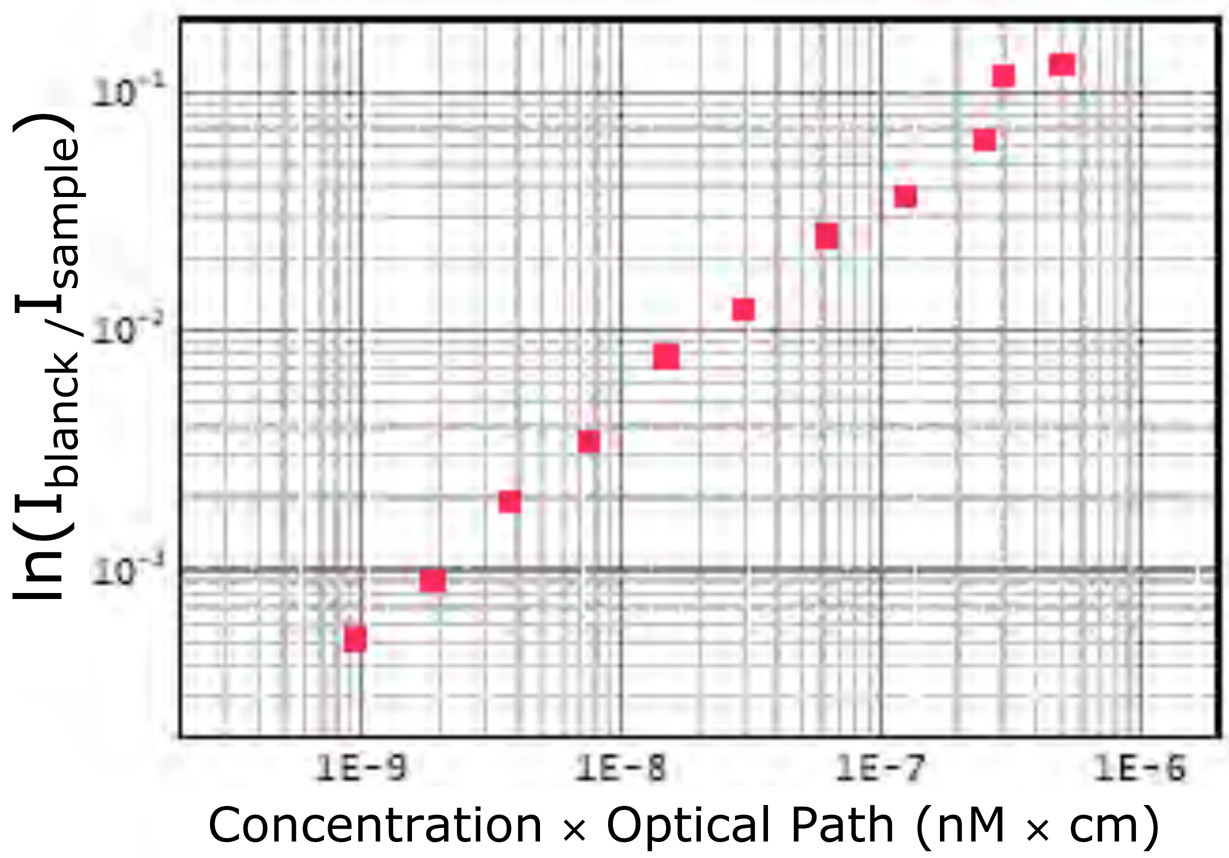
Transducer

Amorphous Silicon UV photodetector

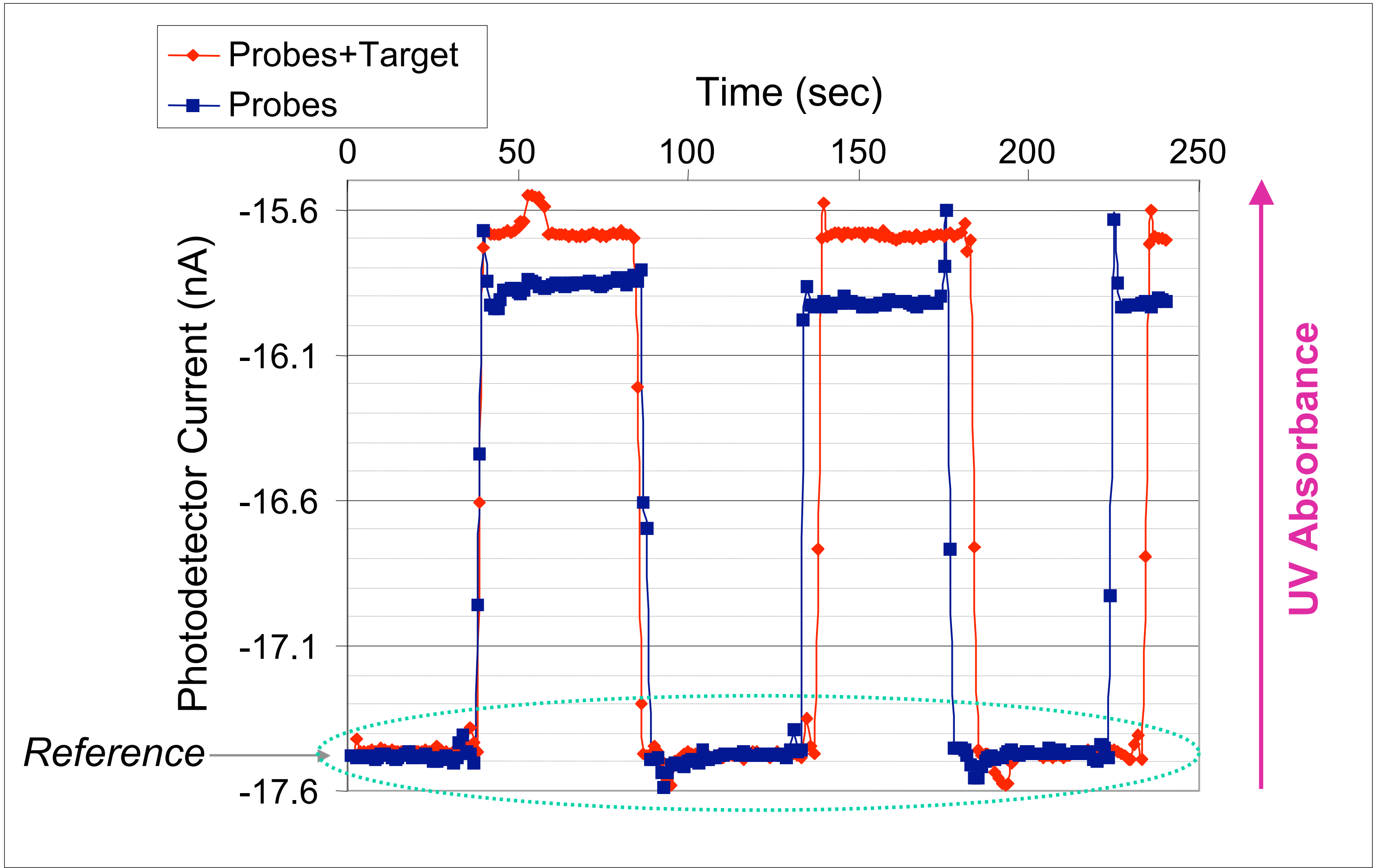


Resolution (Minimum UV-absorbance A):
10^{-4} - 10^{-3}

Photodetector response to DNA samples absorbance



Detection of molecular layers



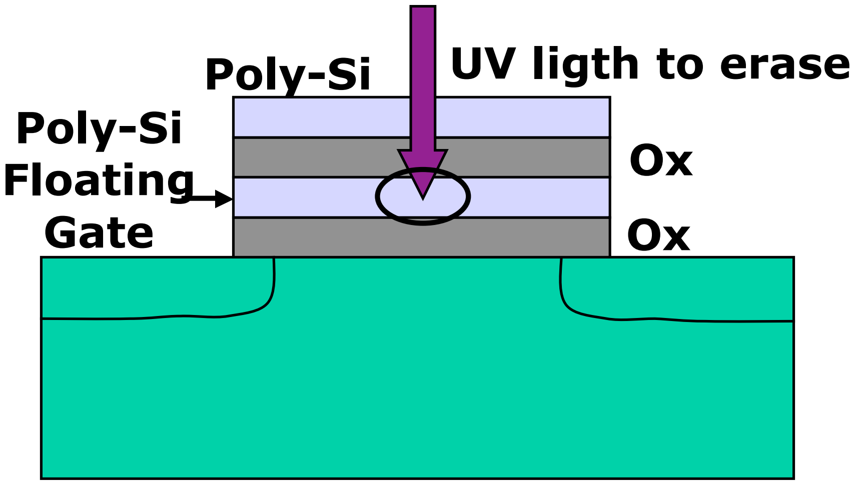
Probes:
25-mer single-stranded oligonucleotides

Targets:
linearized and denaturated pBR 322
4162-mer
(nM concentration)

Transducer Non-volatile memory cells

Standard EPROM cell

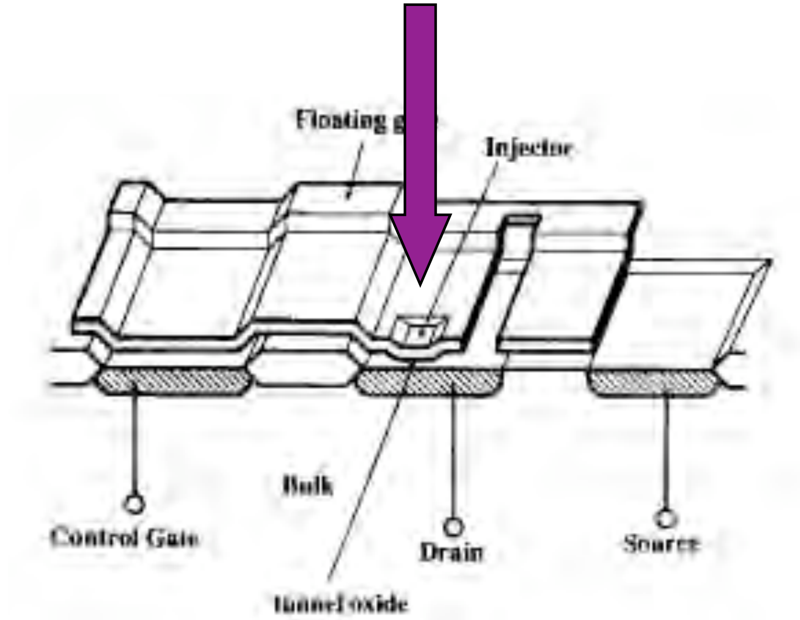
UV are used to lower the threshold voltage V_{TH} by extracting electrons previously injected into the Floating Gate



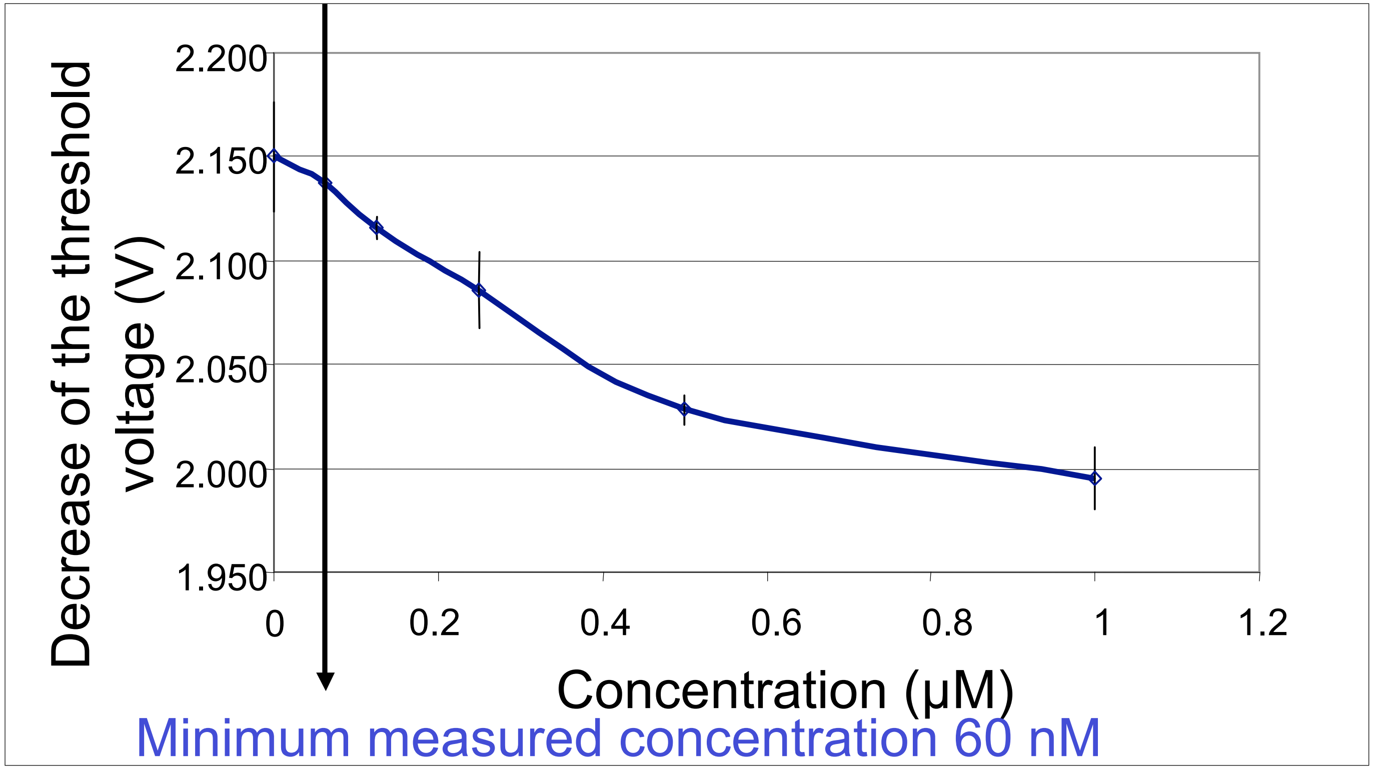
EPROM cell single-poly

better exposition to UV ligth

- extended floating gate surface
- exposed floating gate



Experimental Results



DNA in buffer solution (bulk) 30-mer; ε = 280700(L/mole*cm);
MW 9208 (g/mole)   Buffer TAE Mg2+

Non-volatile memory cell

- easy implementation of high-density chips

Amorphous Silicon Detectors

- can be deposited large surface
- low-cost implementation materials
- high-resolution

Technology challenge
Heterogeneous integration

Technology	Function	Material
Microfluidics	Sample handling	Biocompatible materials (PDMS, Plexiglass)
Surface chemistry Bio-Chemistry	Bio-functionalization	Biochem. materials & surface chemistry
MEMS	Sensors & RF components	Bio- & Si-BIOL Compatible materials
Microelectronics	Processing & Digital communication	Silicon technology