DNA Detection by Solid-State UV Sensors

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State-of-the-art DNA sensors

Labelleld techniques

labelling imposes extratime and cost demands; **can interfere** with the molecular interaction (false negative); **background** binding(positive negative)

Fluorescence Microarrays

- high-density (390000sites/cm^2 for in-situ synthesis of short oligonucleotides)
- very high-sensitivity (1 pM single-molecule-fluorescence)

Electrochemical Biosensors

- low-density
- very-high sensitivity (10 pM)
- some drift issues

degree of development: Research and laboratory applications

Label-free techniques

Surface Plasmon Resonance

Mass sensors (QCM, SAW and cantilevers)

- very limited number of sites: problems of addressability and implementation
- good sensitivity (1 nM SPR), (QCM 50 nM)
- no background signal, no sample pollution…

degree of development: Research and laboratory equipment (QCM and SPR)

Role of Technology and Electronics

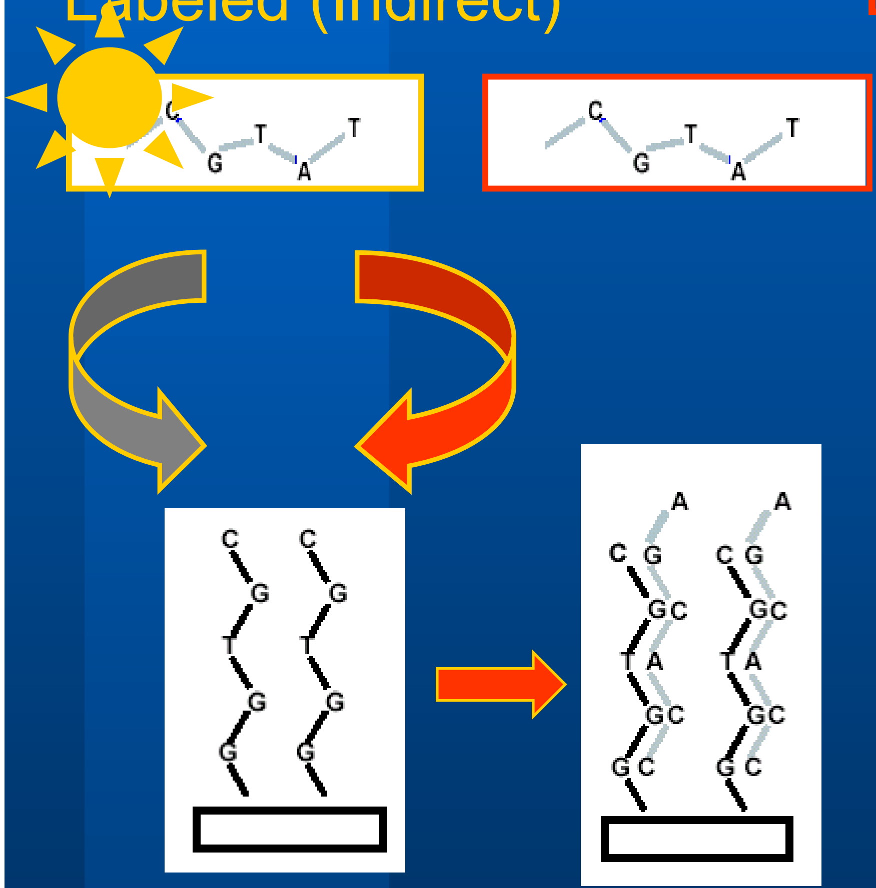
- Microfabrication and Photolithography
  - High-density patterns for molecular sites
  - Integration of microfluidic, thermal and mechanical functions on chip
- Semiconductor sensors (solid-state devices) for electronic transduction of molecular interactions
- Realization of measurements systems (embedded, SoB, SoC…) for signal detection, conditioning, processing

Can lead to

High-parallel, High-perfomance, Mass-Produced DNA sensors

Our Approach. Label-free Techniques

Labeled (Indirect)



Label-free (Direct) Techniques

1- Electrical technique

Changes in the capacitive behavior of an electrode/solution bio-sensing interface

Electronics: development of a measurement system based on integrable capacitive measurement circuit

Microfabrication: test of the technique on microfabricated electrodes

2 – Optical Technique

molecular UV-absorbance

Semiconductor sensors:

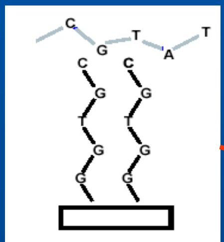
- ‘ad hoc’ high-sensitive amorphous silicon photodiodes
- Non-volatile memory cells

Charge-Based Capacitance Measurement Circuit

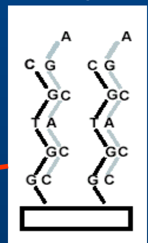
Advantages vs. standard complex impedance meters

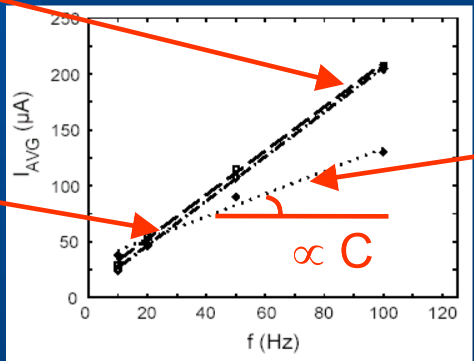
- very simple, easily integrable
- the analog part can be reduced to an I/V and an A/D converter

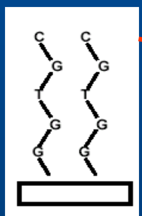
Non Complementary



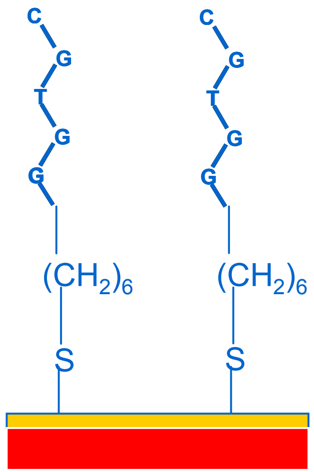
Complementary





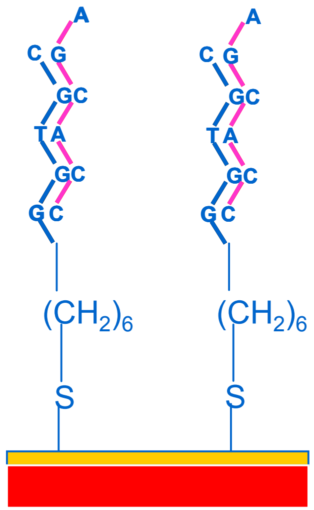


Probes



Thickness
2 nm

Permittivity
1.9



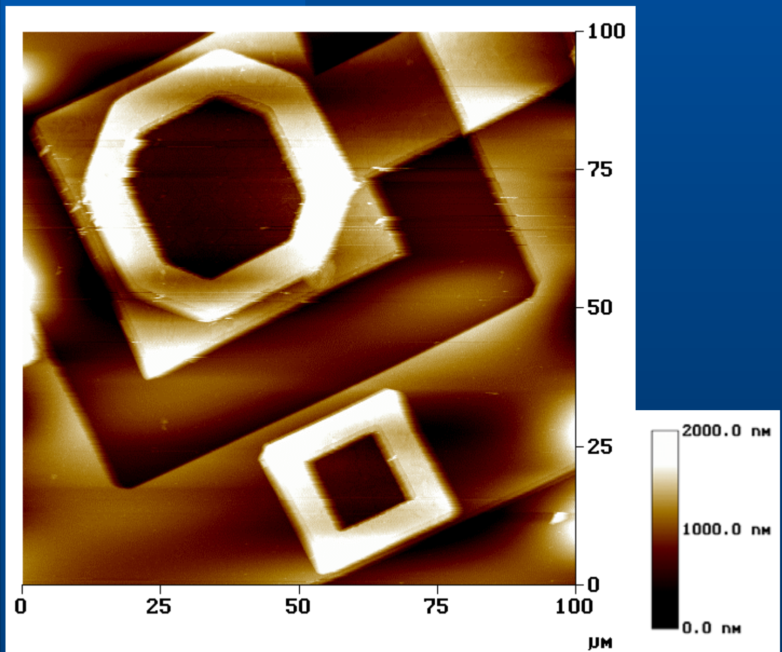
Thickness
5.5 nm
[Kelley et al., 1998]

Permittivity
2.5
[Peterlinz et al., 1997, Yang et al., 1997].

Microfabricated electrodes

Challenges:
1- Stability of electrode capacitance in solution
2- Adapted passivation
3- Integration with microfuidics

Total measured capacitance (2×10^{-3} μm^2): between 100 pF and 1 nF



Sample	**Percentage decrease of Capacitance after sample deposition and rinsing**
Complementary sequence	37.7
Complementary sequence	18.3
Complementary sequence	29.5
Non Complementary Sequence	-5.2
DNA-free sample	-6.2
DNA-free sample	-3.7
DNA-sample on bare electrode	1.8

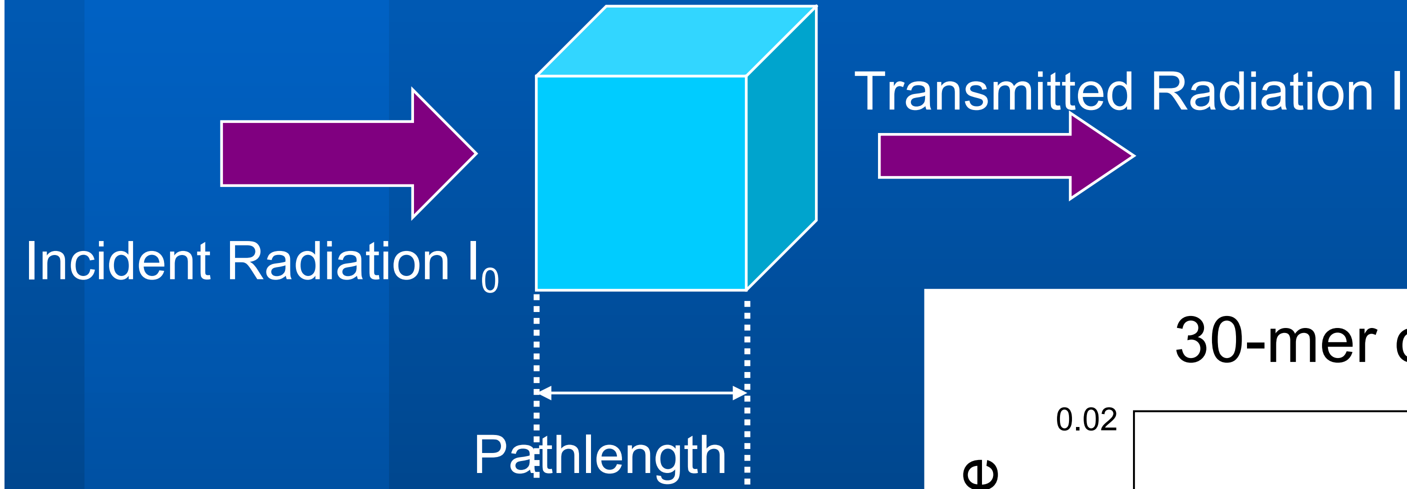
UV molecular absorbance

- UV spectroscopy for molecular quantification and detection of **molecular interactions**
- Amorphous Silicon UV sensor

In collaboration with Università La Sapienza di Roma
G. De Cesare, D. Caputo, A. Nascetti

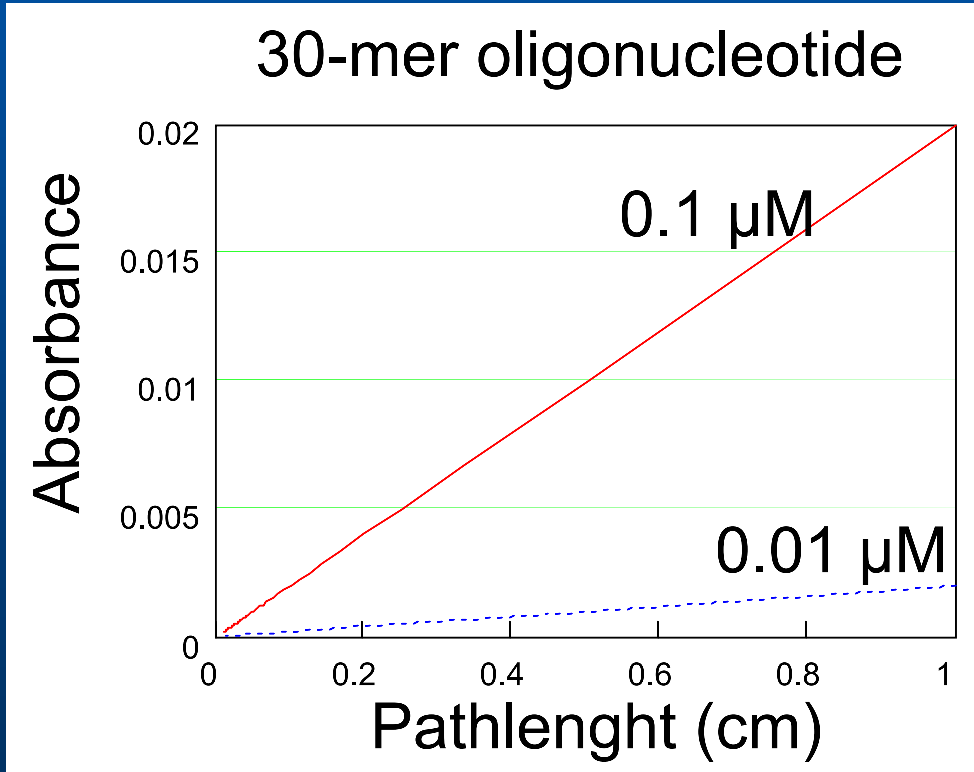
- Non-volatile single-poly memory cell

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

Lamber-Beer law:

$$A = \varepsilon c l$$

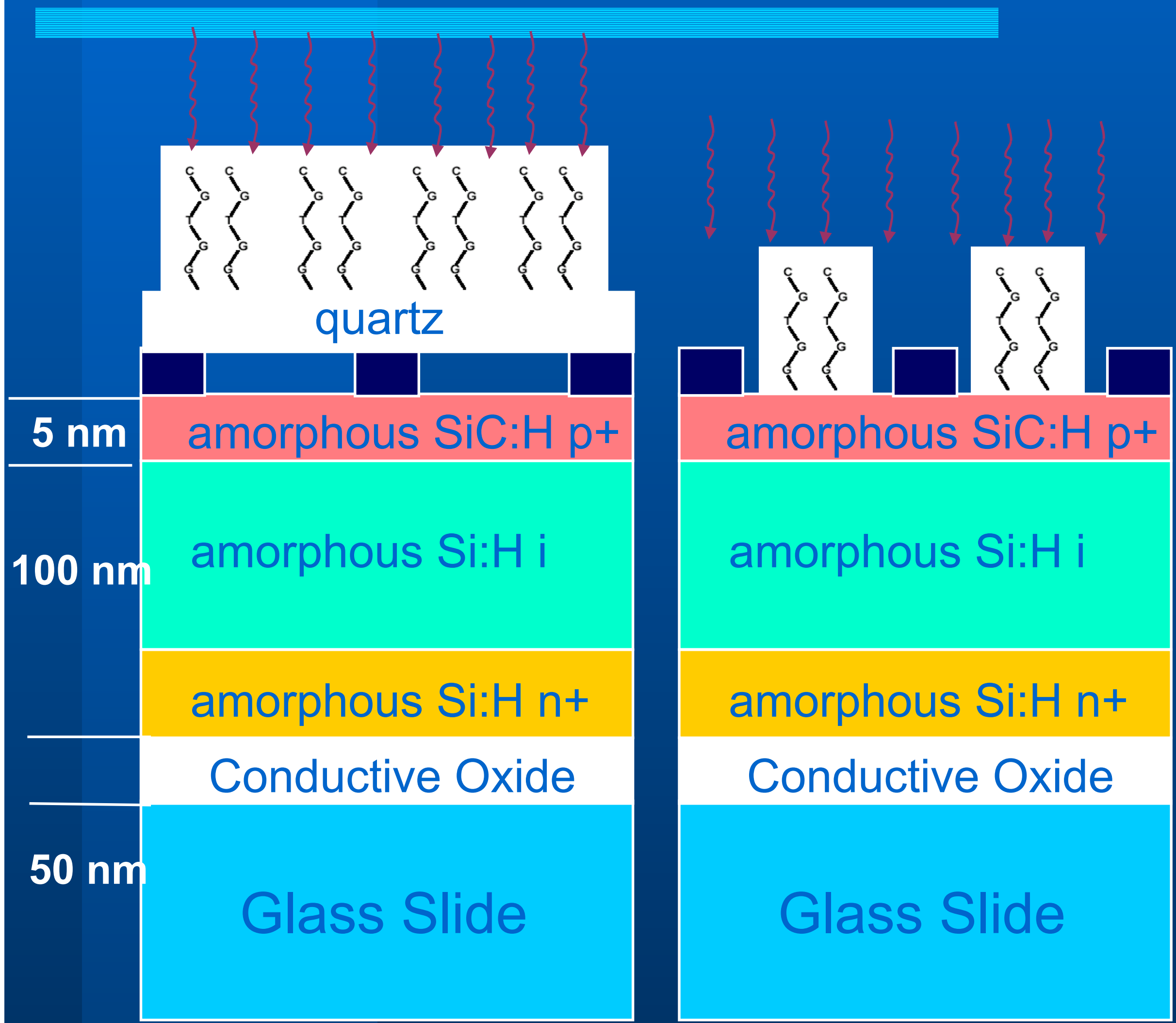
ε, molar extinction coefficient [L mole^{-1}cm^{-1}], C concentration [M], l pathlength [cm]

is a function of wavelenght and of molecular species in solution

Fabrication of Amorphous Silicon devices for UV sensing

- Amorphous silicon is a low-cost material, low-temperature deposition process (PECVD (Plasma Enhanced Chemical Vapour Deposition)
- Deposition process is compatible with several low-cost substrates on which a conductor film can be pre-deposited
- Can be fabricated on substrates of any size
- The device implementation can be tuned to improve resolution or selectivity or in the UV range.

System implementations

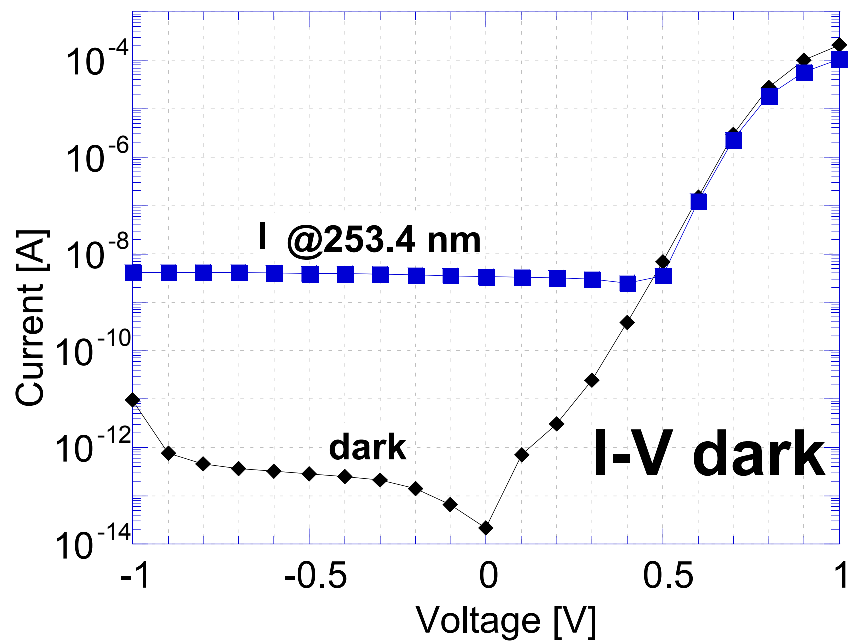


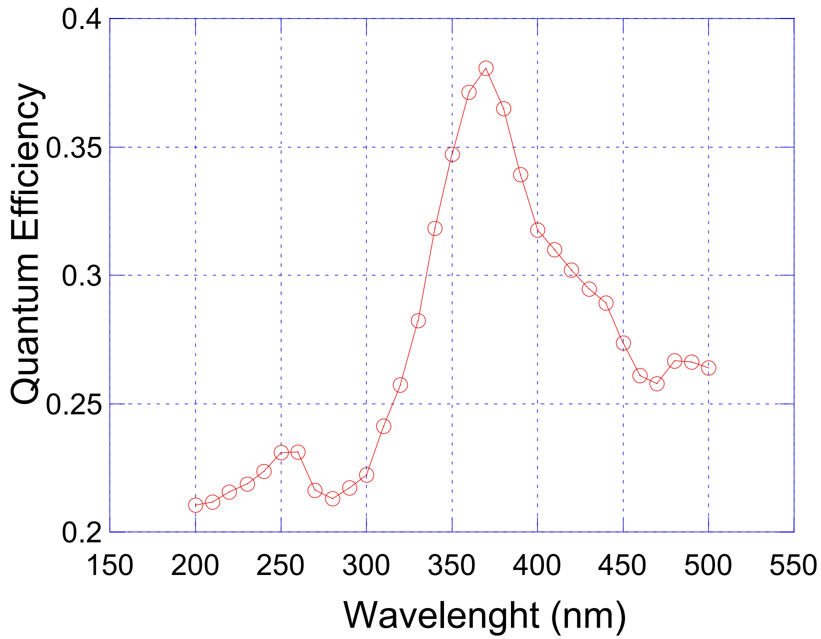
Selectivity in the UV-range is penalized to ensure low dark currents by the following technological choices:

1- wide intrinsic region
2- pure Si intrinsic region

I-V Characteristics

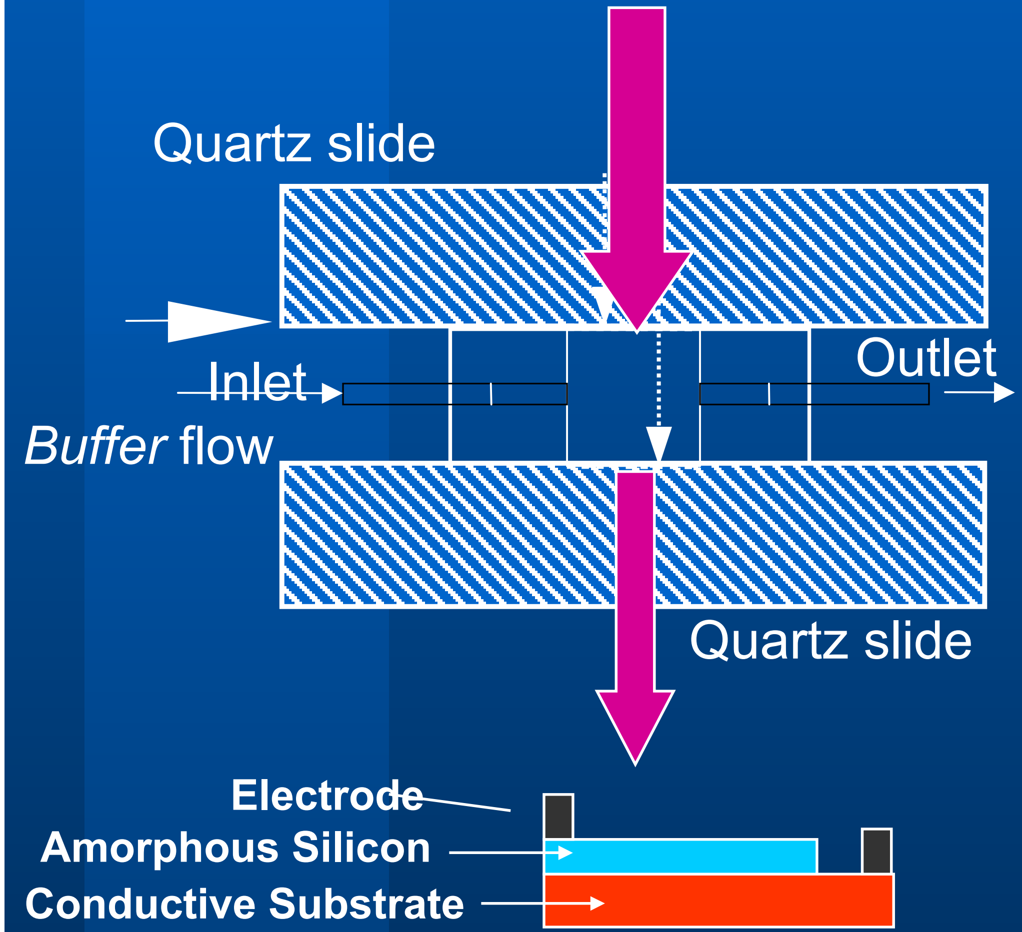
I-V light @253.4 nm and 0.5µW incident radiation

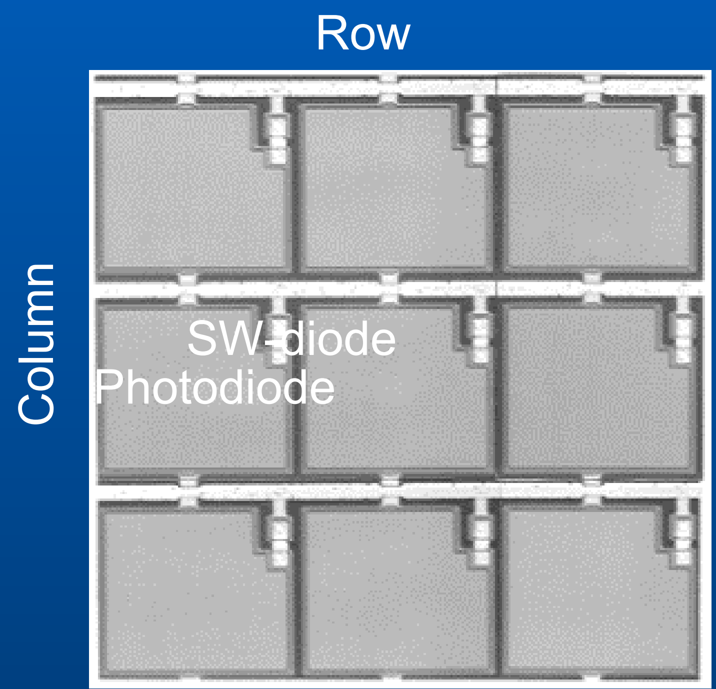




Responsivity @253.4 nm: 45mA/W

Experimental Setup



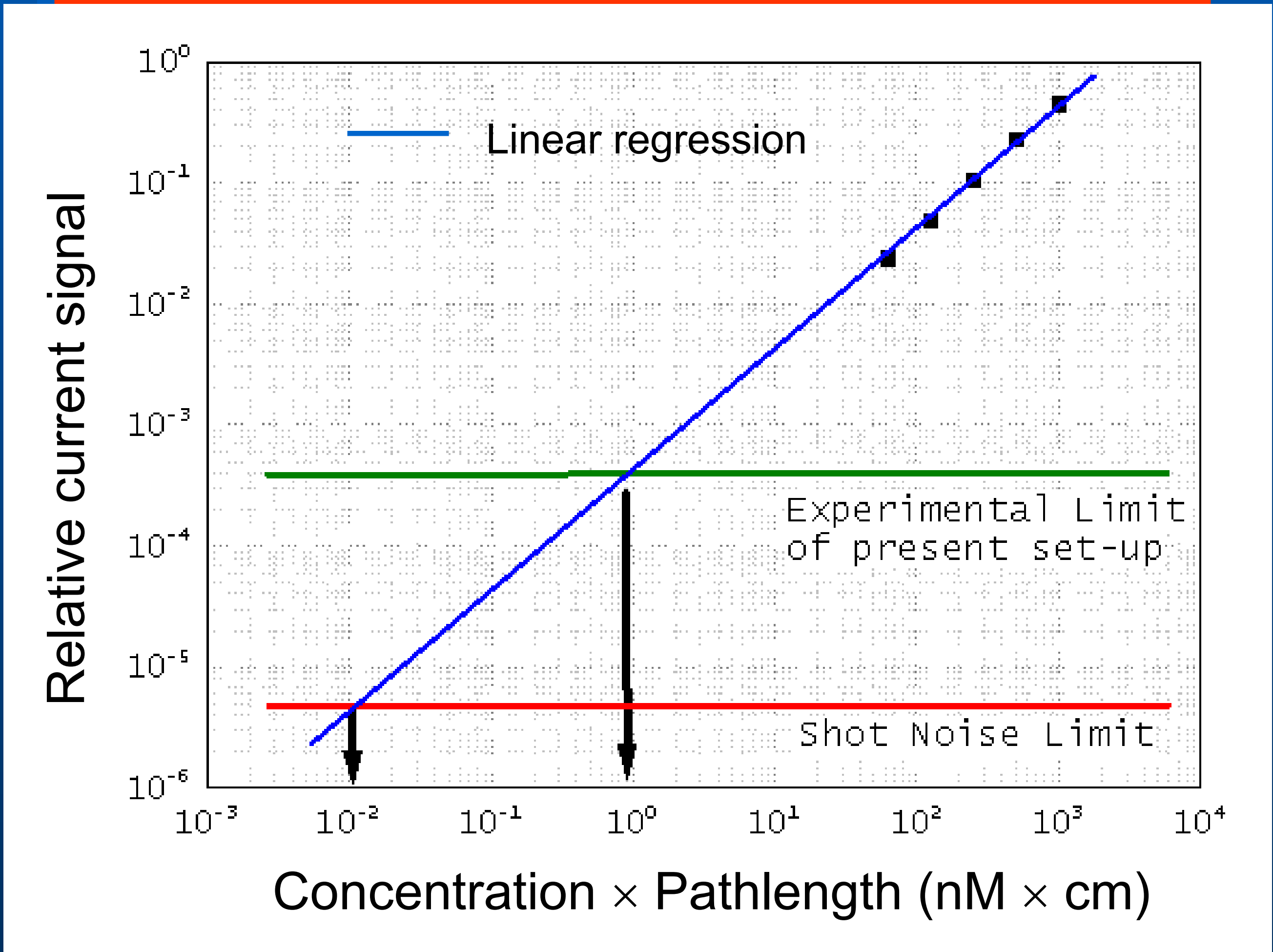


Array

Amorphous Silicon UV sensor

Relative current signal corresponding to DNA samples with different absorbances

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



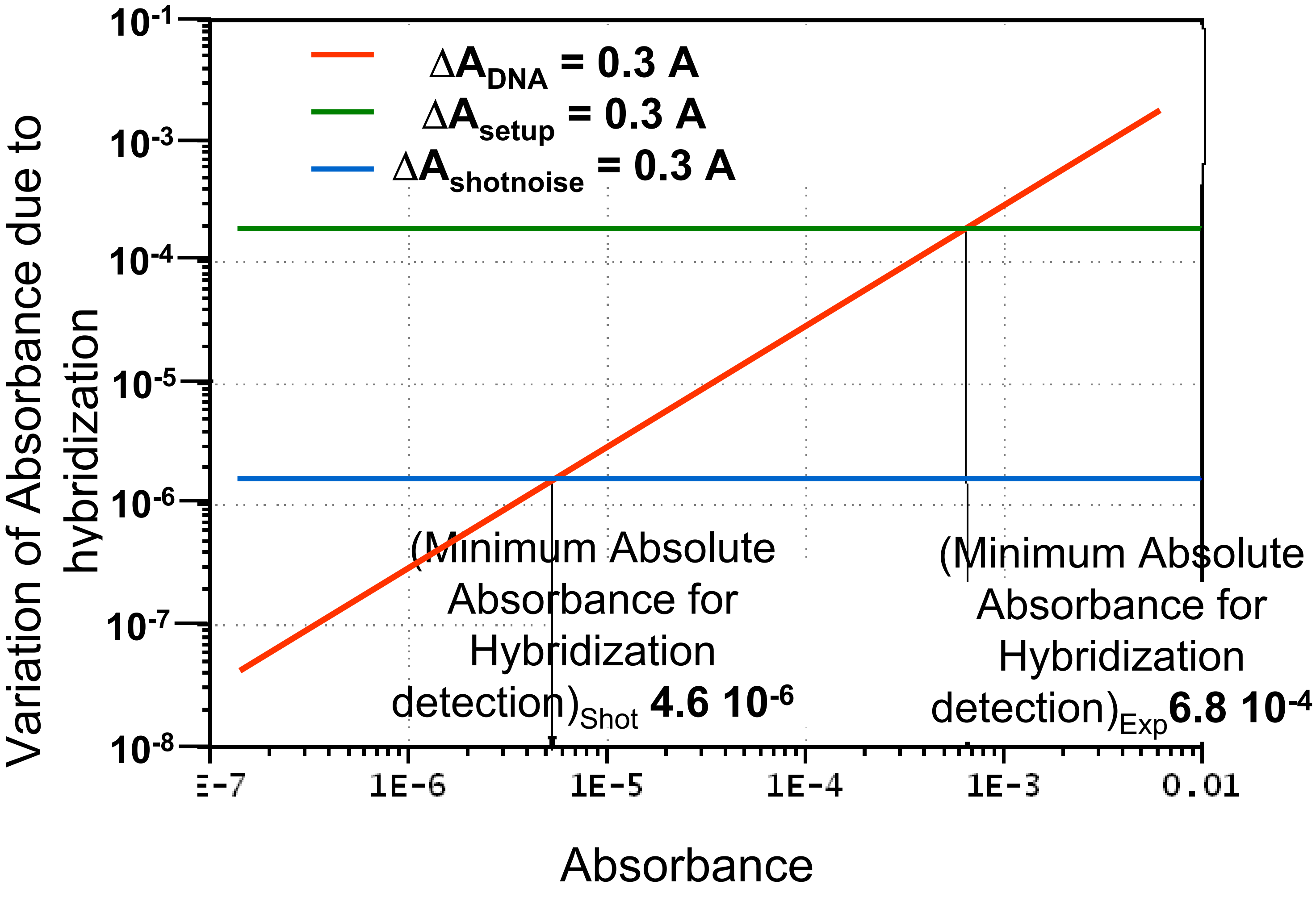
The relative current signal is derived from the measured current of the UV sensor as follows:

$(I_{BUFFER} - I_{DNA}) / I_{BUFFER}$

I_{DNA} with oligonucleotides

I_{BUFFER} buffer solutions without oligonucleotides

Minimum Absorbance for Hybridization detection



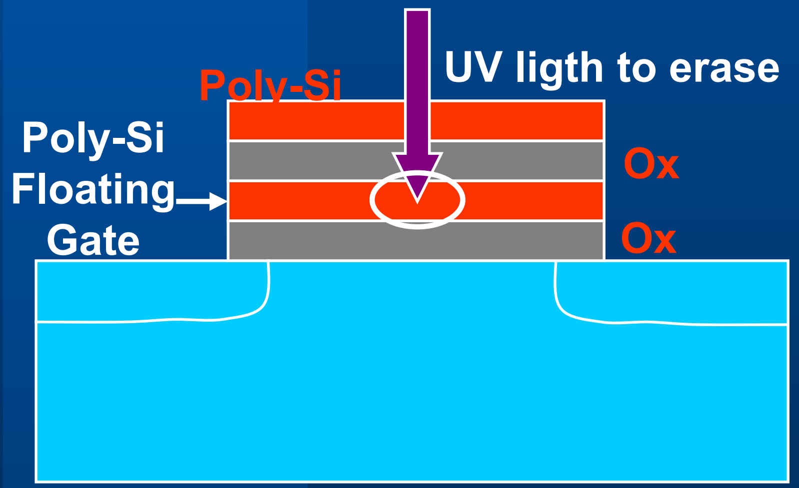
Amorphous Silicon Devices

- Minimum absorbance to resolve hypochromic effect (detect DNA hybridization)
  - theoretical: Amin = 4.6×10^{-6}
  - experimental setup : **Amin = 6.8×10^{-4}**, which corresponds to 2.43 nM concentration for 1 cm pathlength and a 30-mer oligonucleotide (suitable for PCR-amplified strands)
- Eximated Absorbance of immobilized DNA layers: $A \approx 10^{-3} \Rightarrow$ DNA detection can be achieved with the present set-up

Non-volatile memory Cell

Standard EPROM cell

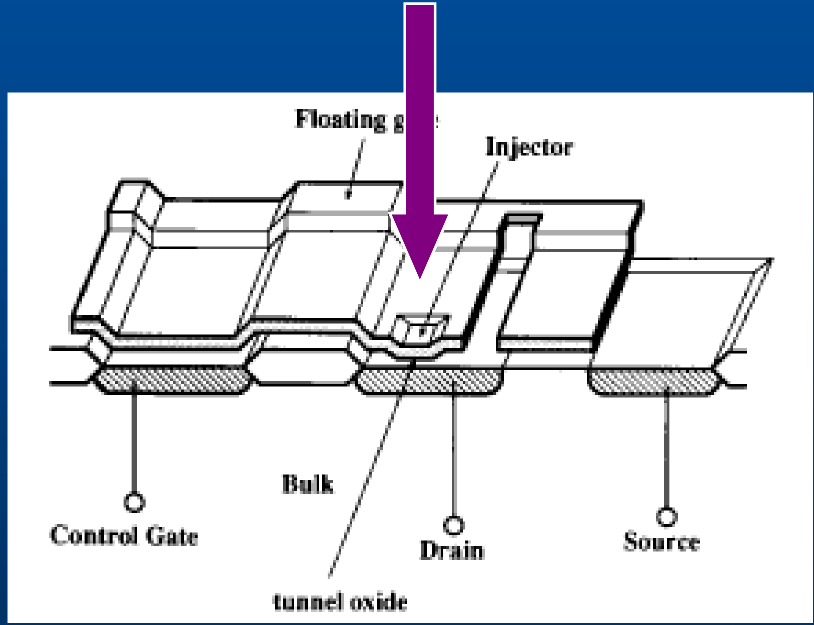
UV are used to lower the threshold voltage V_{TH} by extracting electrons previously injected into the Floating Gate



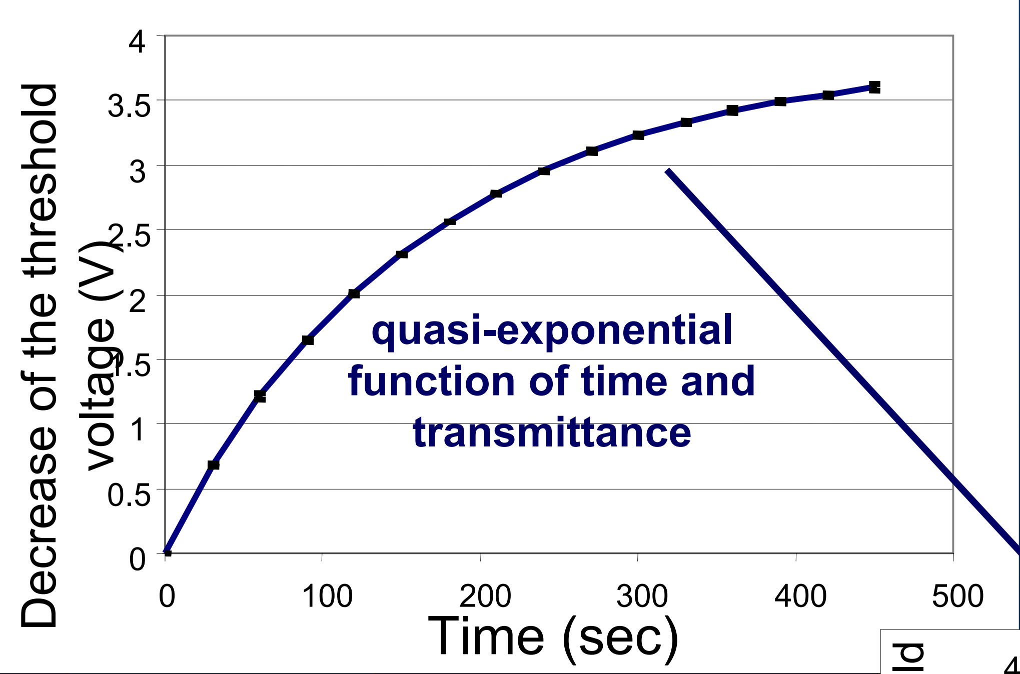
EPROM cell single-poly

better exposition to UV ligth

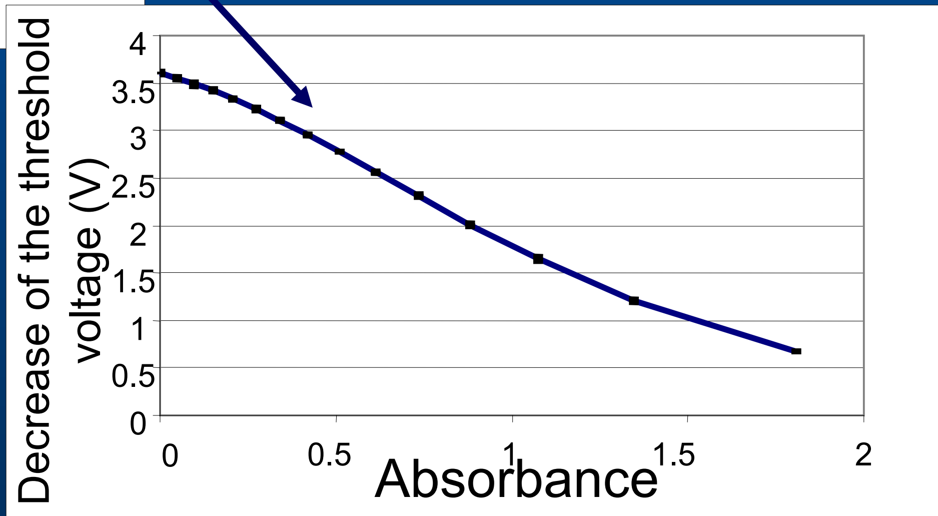
- extended floating gate surface
- exposed floating gate



Memory Cell Characterization



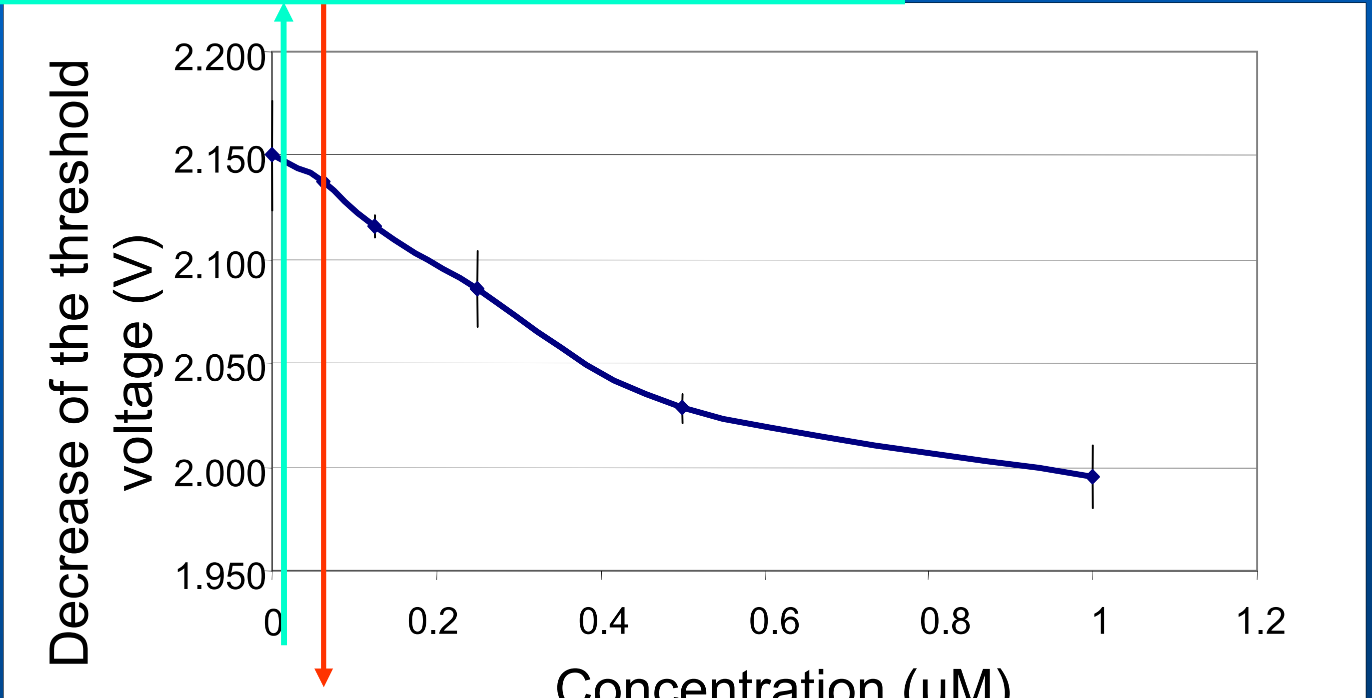
Experimental characterization of the memory cell



Expected trend of ΔV_{TH} vs Absorbance (molecular concentration)

Experimental Results

Expected concentration for a DNA layer 10 nM



Minimum measured concentration 60 nM

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

Comparison between Amorphous Silicon Detectors and EPROM memory cell

EPROM memory cell	Amorphous Silicon Detectors
▪ easy implementation of high-density chips ▪ high-cost implementation/materials ▪ good sensitivity in the nanomolar range	▪ high-density and large surface ▪ low-cost implementation materials ▪ high resolution on the sub-nanomolar range with enhanced stability in integrated set-up

Grazie!!