# ANALYTICAL PERFORMANCE OF A (BIO)SENSOR

# Definition

**Biosensors and electronic biochips:**
Tools to perform **bioanalytics and other analytical tasks such as:**

- Measurement of physiological parameters in fluids
- Determination and quantification of the DNA/RNA content in a sample (viral DNA, miRNA, expression analysis, forensic applications, SNPs)
- (DNA sequencing)
- Measurement of other molecules in blood (drugs, pollutants)
- Characterization of binding kinetics
- Cell content. Detection of specific cells. Isolation of cells for further analysis

# Definition

**Biosensors and electronic biochips**

Working rationale:

- The signal is induced involving a biological element or by interaction with biological matter.
- The signal is transduced into an electrical signal by sensing-surfaces or sensing-devices placed in close proximity of the biological element/matter

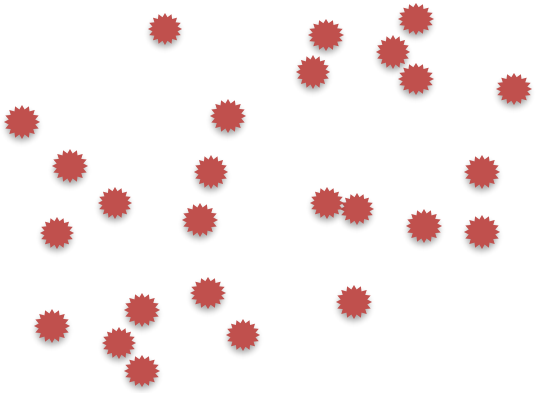
***In this course, I will use "biosensing system" when referring to both biosensors and electronic biochips***

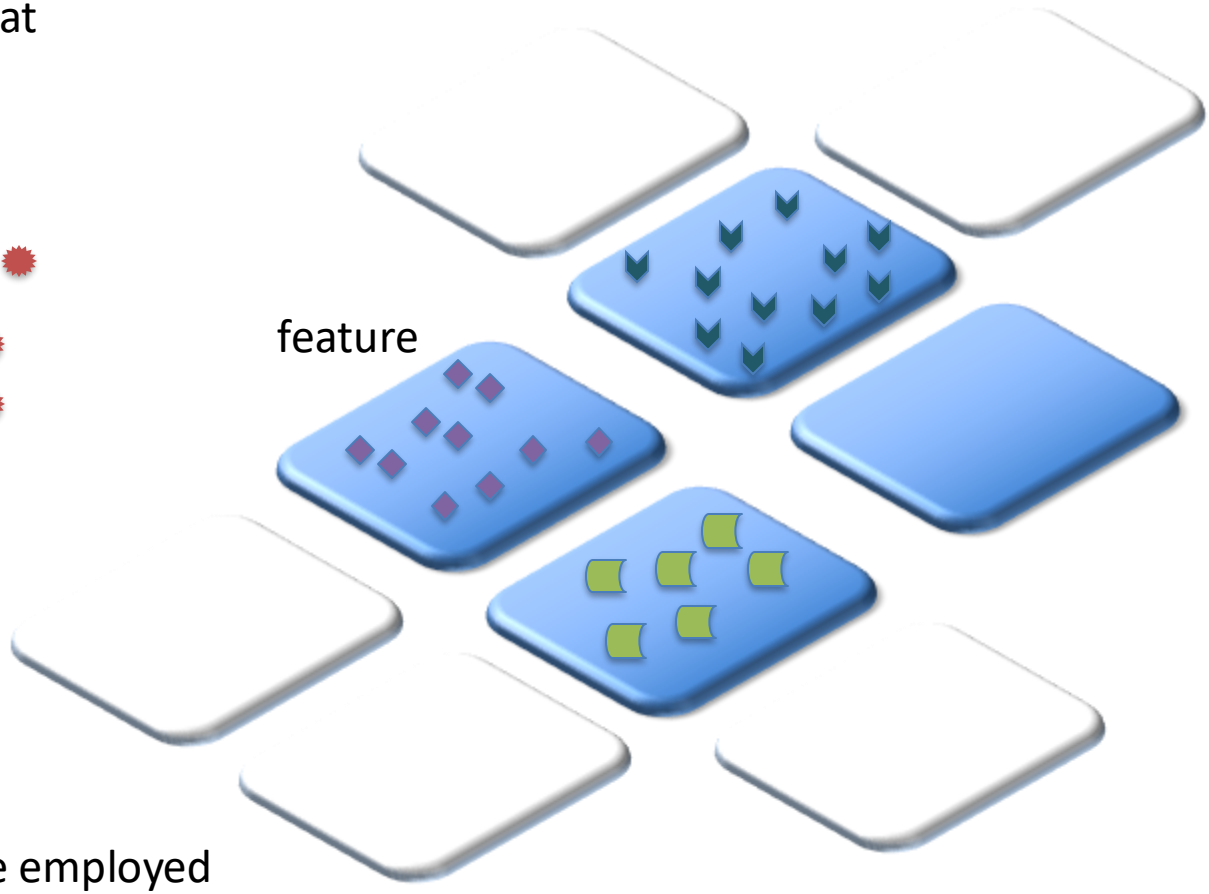
# Detection of molecules on surface. Ligand binding assays

Target molecule

Receptor (immobilized on surface)

In volume, free-floating at concentration C






Always focus on:

- Concentration AND volume employed
- Volume with respect to feature dimension

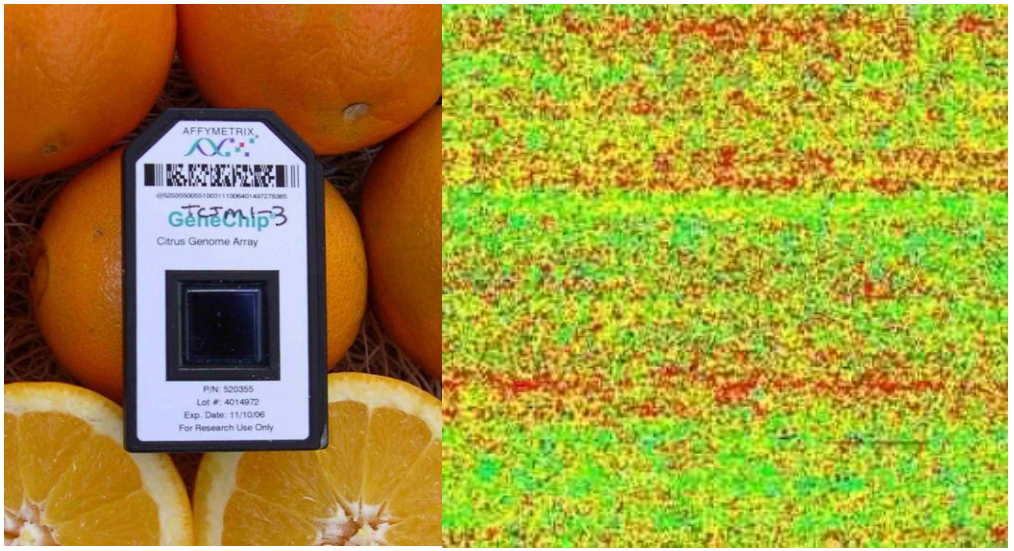
# Biochips

## Advanced platforms to support genetic research and new diagnostics approaches

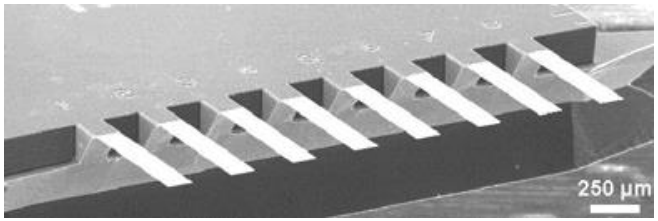
Affymetrix (CA).
Can test a whole genome at once

500.000 sites on one 1 centimeter square .
Implemented by photolithography (founders where EE..)

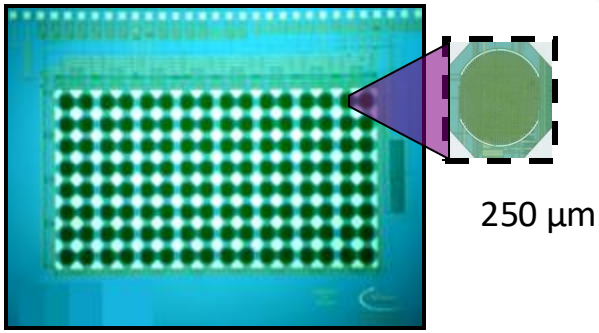
Microarrays



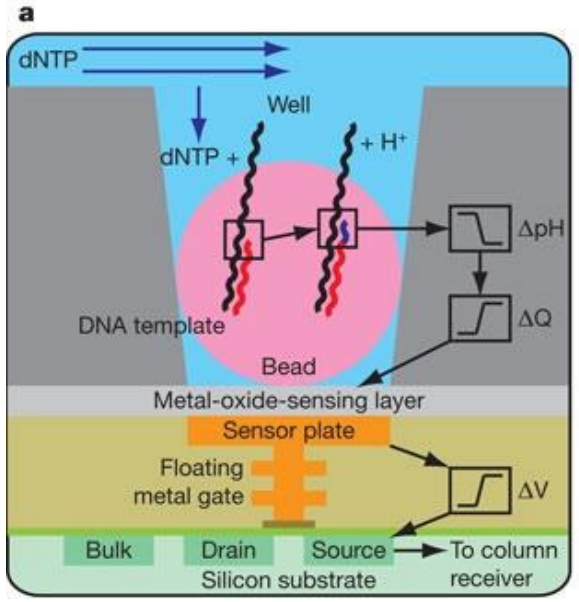
# Electronic biochips




Microcantilevers on chip.
Originally developed at IBM Switzerland




DNA detection on CMOS chips. Infineon and University of Bologna

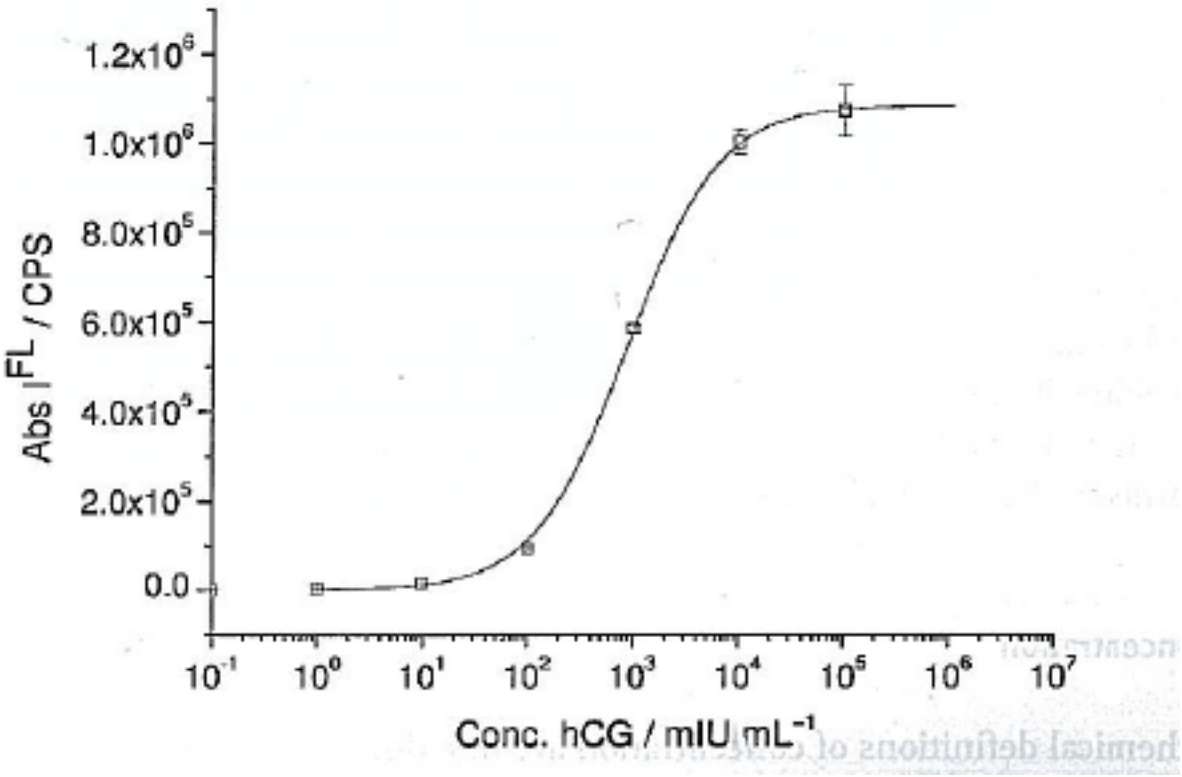



JM Rothberg *et al. Nature* **475**, 348-352 (2011) doi:10.1038/nature10242

# Possible qualifications of ligand binding assays

- Label (-based) sensing
  - label-based
  - Label-free
  - Quasi label-free
- Real-time sensing
- Bulk sensing, surface sensing

# Calibration curve



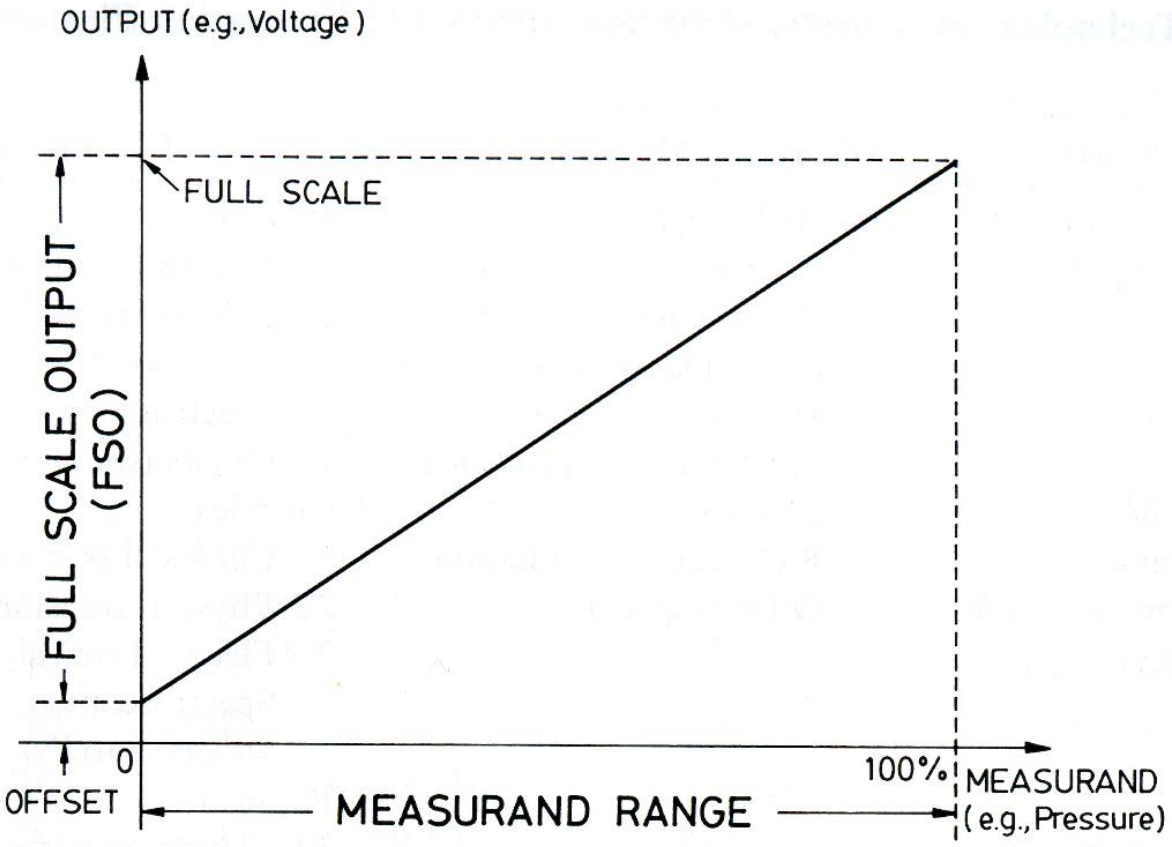

Units:

CPS, counts per seconds, number of photons per seconds detected by the fluorescence detector

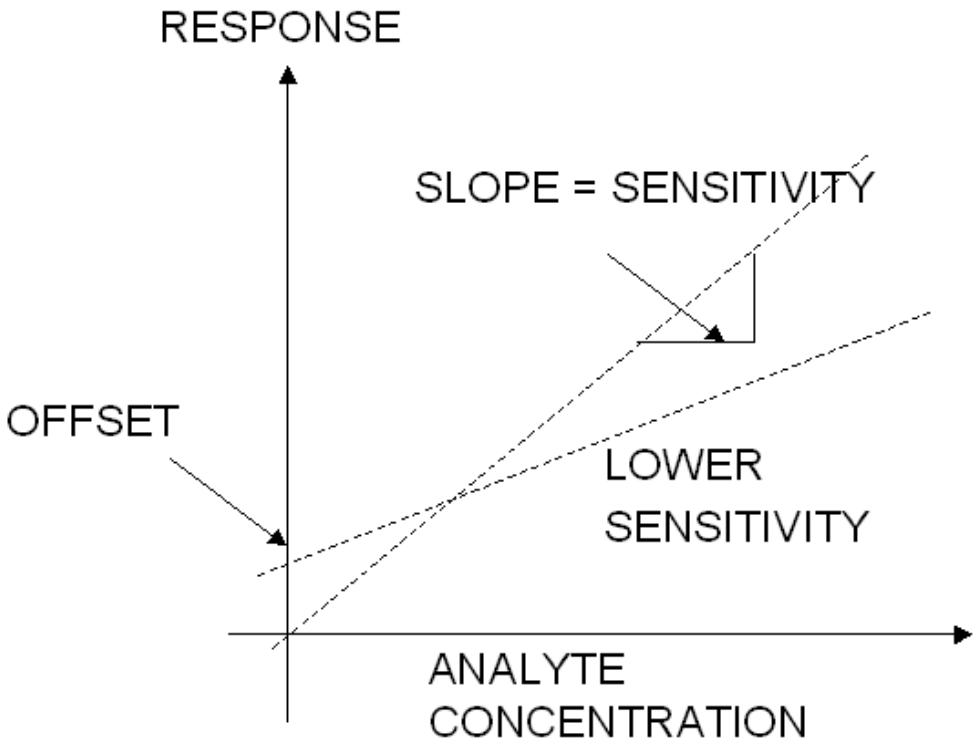
hCG: hormone, human chorionic gonadotropin

# Output-measurand relationship

sensor characterized by a linear-output and an offset



# Sensitivity




**Linearity of the sensor:** The closeness between the calibration curve and a specified straight line. It is measured as the maximum deviation of any calibration point from a specified straight line, during any one calibration cycle and is generally expressed in percentage

- mol $dm^{-3}$
- mol $L^{-1}$
- mg $ml^{-1}$
- IU $ml^{-1}$

Units of concentration

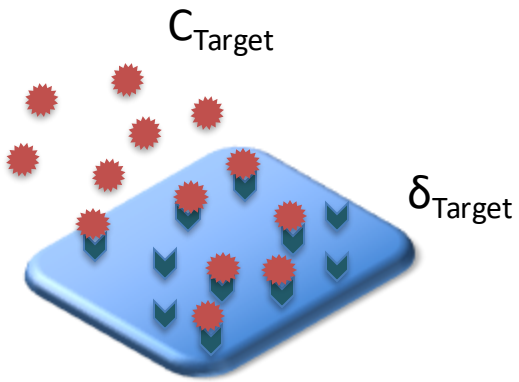
# Sensitivity: two ways to express it

Target molecule

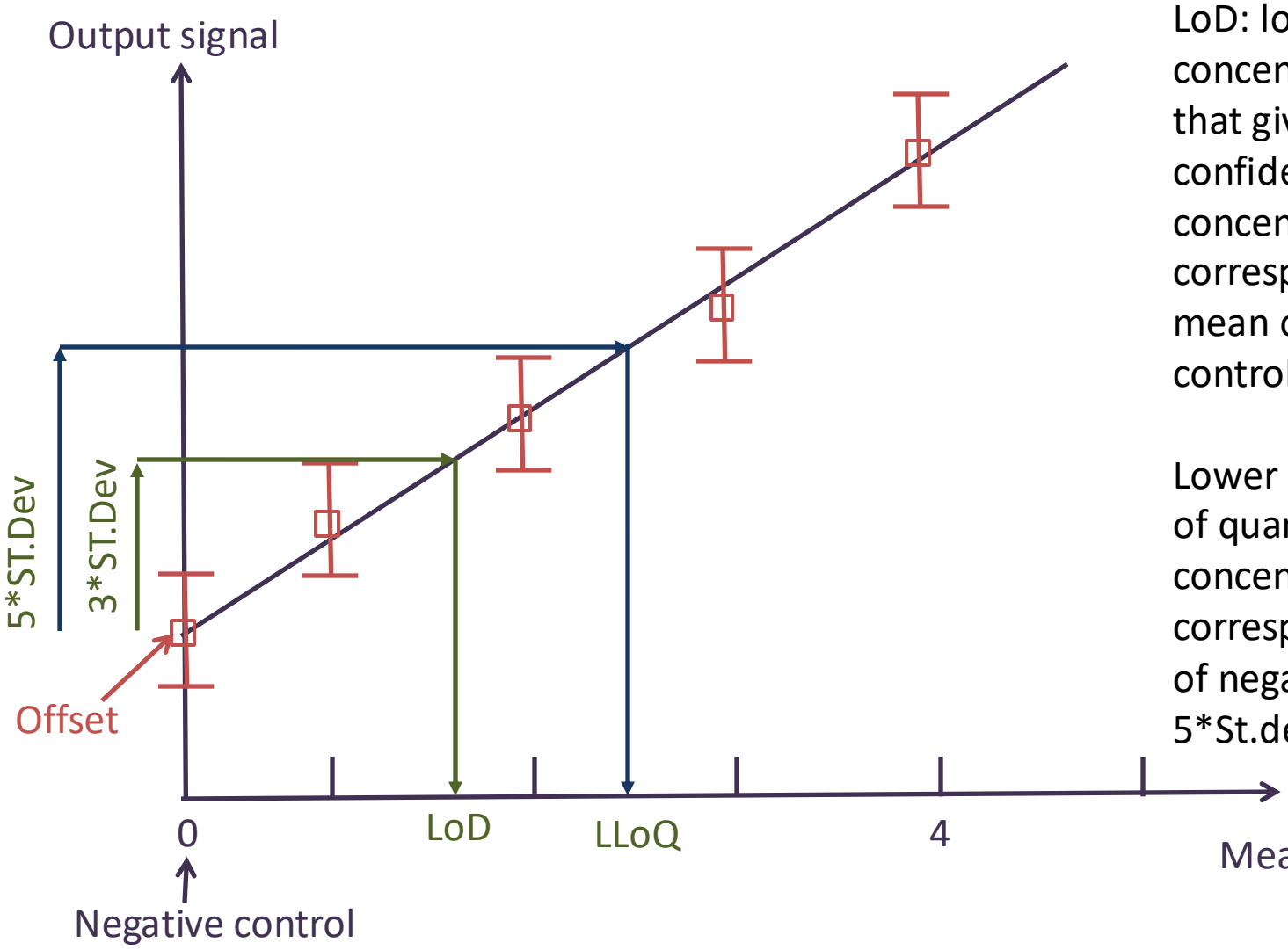
Probe (immobilized on surface)

Signal in response to a change in the surface density of target molecules.
$\Delta S_{OUT}/\Delta \delta_{Target}$

Signal in response to a change in the concentration of target molecules.
$\Delta S_{OUT}/\Delta C_{Target}$



# Limit of detection (LoD) and Limit of Quantification




LoD: lowest detectable concentration of analyte that gives statistical confidence: concentration corresponding to the: mean of negative control + 3*St.dev.

Lower LoQ: lower limit of quantification: concentration corresponding to: mean of negative control + 5*St.dev.

# Resolution

- **Resolution**: the minimal change of the measurand value necessary to produce a detectable change at the output.

- Can be calculated in a given measurand point as the change of measurand needed to induce a change in signal equal to 2*St.Dev.

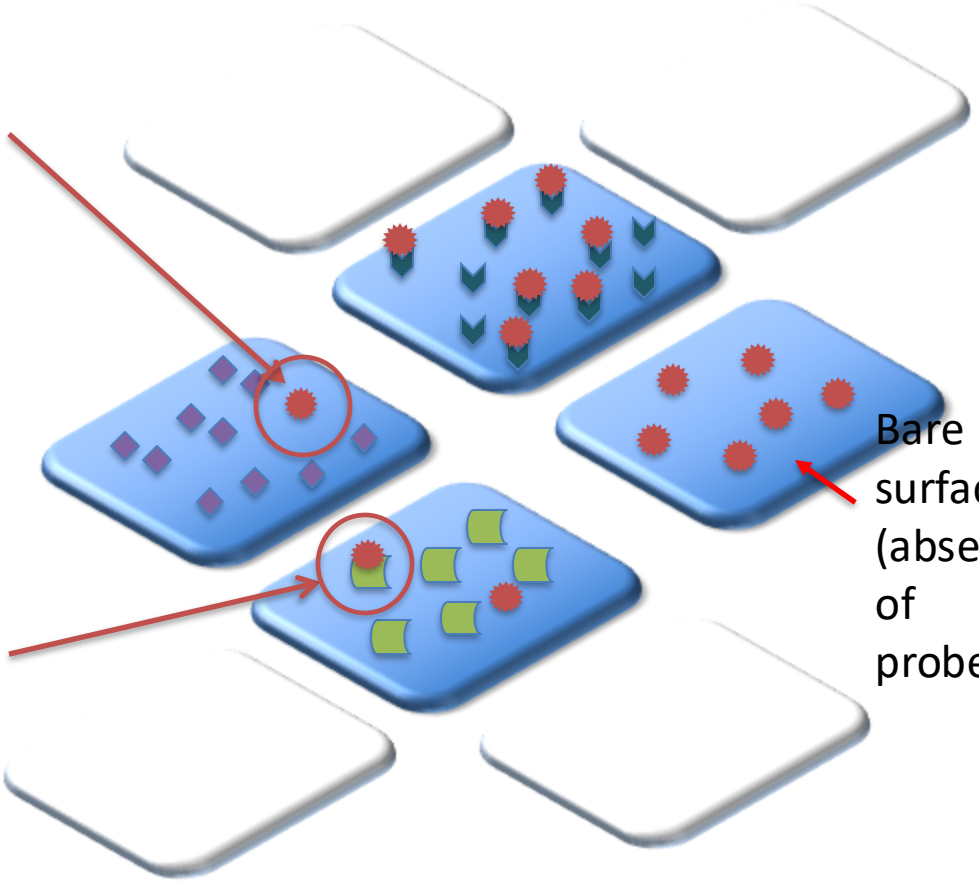
# Analyte selectivity

Target molecule

Probe (immobilized on surface)

Non suitable **passivation** of the surface

Insufficient **selectivity** of the receptor
(or excess of non specific molecules)



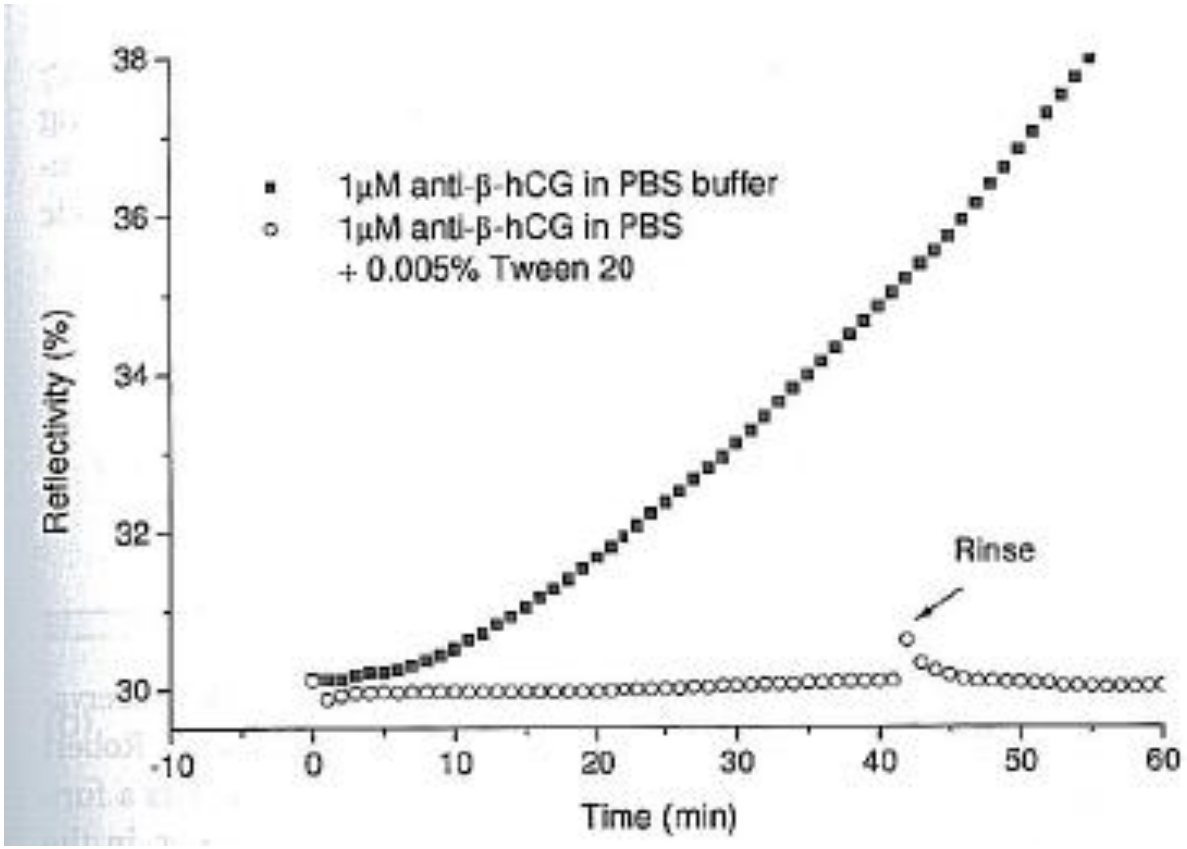
# Selectivity - Specificity

- **Selectivity** refers to the extent to which a method can determine particular analytes in mixtures or matrices without interferences from other components. Selectivity can be graded
- IUPAC :"*specificity is the ultimate of selectivity". No component other than the analyte contributes to the result.*

# Prevention of non specific adsorption. Example

Eg: employment of a surfactant

Measurement unit for optical detection: SPFS




surface plasmon field-enhanced fluorescence spectroscopy (SPFS)

Surface Plasmon Fluorescence Measurements of Human Chorionic Gonadotrophin: Role of Antibody Orientation in Obtaining Enhanced Sensitivity and Limit of Detection Margarida M. L. M. Vareiro,, Jing Liu,, Wolfgang Knoll,, Kris Zak,, David Williams, and, A. Toby A. Jenkins*Analytical Chemistry 2005 77 (8), 2426-2431*

# Repeatability - reproducibility

- Repeatability is the variability of the measurements obtained in the same conditions of measurement over a short period of time
- For sensors:

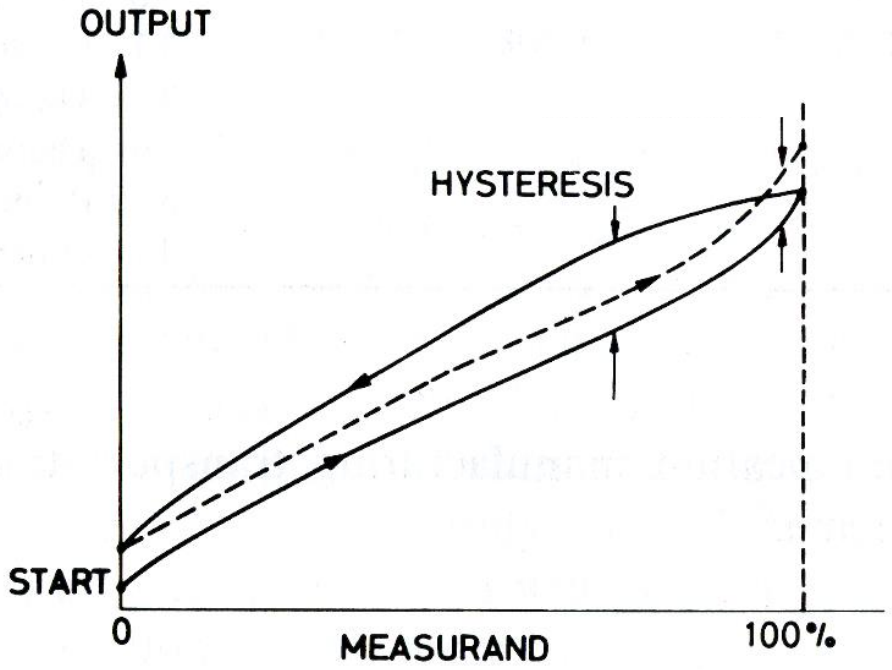
**Repeatability**: the ability of a sensor to reproduce output readings when the same measurand is applied to it consecutively

- Reproducibility is the variability of the measurement system caused by differences in the conditions of measurement

**Reproducibily:** refers to suitability of an experiment to be reproduced by someone else.

# Hysteresis

**Hysteresis:** the maximum difference in output, at any measurand value, when the value is approached first with an increasing and then decreasing the measurand.



# Precision and accuracy

- Important parameters but mostly used improperly:
  - Accuracy: closeness of the agreement between the average of the results of a measurement and the nominal value of the measurand
  - Precision: variability of the measurements (can be expressed as the standard deviation obtained under repeatability conditions)

# Output-measurand relationship

**Measurand range**

The value of the measurand over which the sensor is intended to measure, specified by upper and lower limits (typically, linear range)

**Offset**

The output of a sensor, under room temperature condition with zero measurand applied

**Sensitivity**

The ratio of the change in sensor output to the change in the value of the measurand

**Full-scale output (FSO)**

Algebraic difference between the end points of the output. The upper limit of sensor output over the measurand range is called the full scale (FS)

# Sensor Characterization

- Speed of response (the time at which the output reaches 63% (i.e. 1/e) of its final value in response to a step change in the measurand

- Output format
  - analog output (continuous function of the measurand)
  - digital output (represents the measurand in form of discrete quantities coded in some system of notation)

# Sensor Characterization

- **Ambient conditions allowed** (may have profound effects on sensor operation. temperature, acceleration, vibration, shock, ambient pressure, moisture, corrosive materials, electromagnetic field)

- **Operating life**. The minimum time over which the sensor will operate without changing performance characteristics beyond specified tolerance

- **Stability:** the ability of a sensor to maintain its performance characteristics for a certain period of time

# Overload characteristics

- **Overload:** maximum magnitude to be applied to a sensor without causing a change in performance beyond specified tolerance.

- **Recovery time**: amount of time allowed to elapse after removal of an overload condition before the sensor again performs within the specific tolerance.