
ANALYTICAL PERFORMANCE OF A (BIO)SENSOR

Definition

Biosensors and electronic biochips: Tools to perform bioanalytics and other analytical and processing 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)
- Measurement of other molecules in blood (drugs, pollutants)
- Characterization of binding kinetics
- Cell content. Detection of specific cells. Isolation of cells for further analysis
- Cell manipulation
- Cell electroporation

Biosensors

- 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

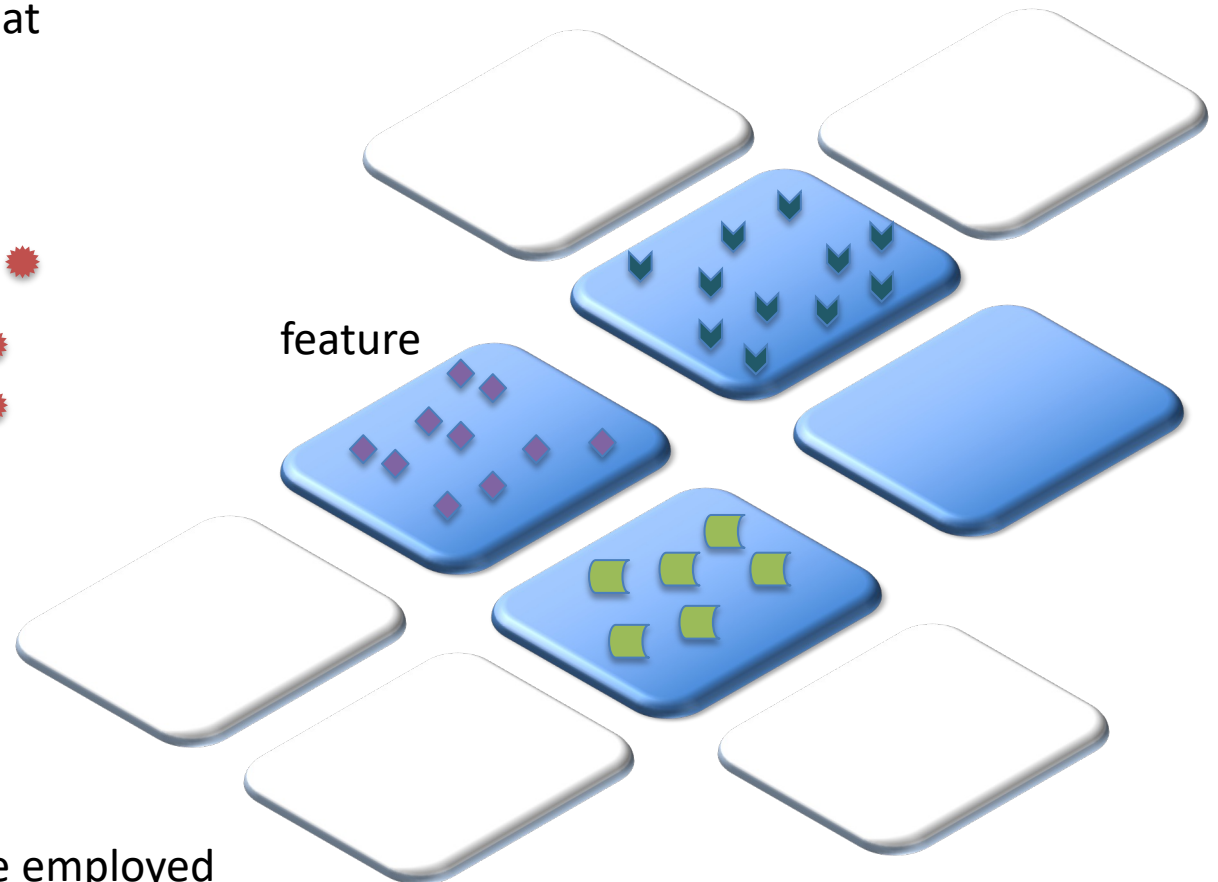
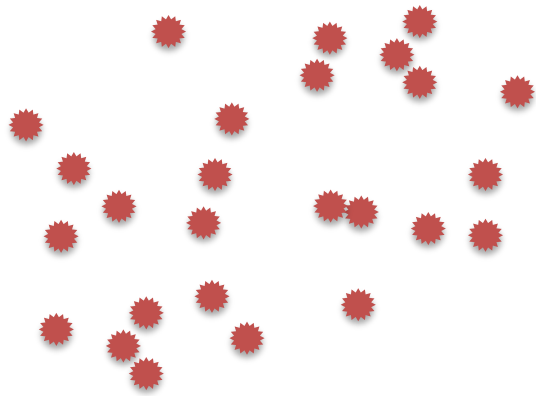
Detection of molecules on surface.

Ligand binding assays

● Target molecule

▼ Receptor (immobilized on surface)

In volume, free-floating at concentration C



Always focus on:

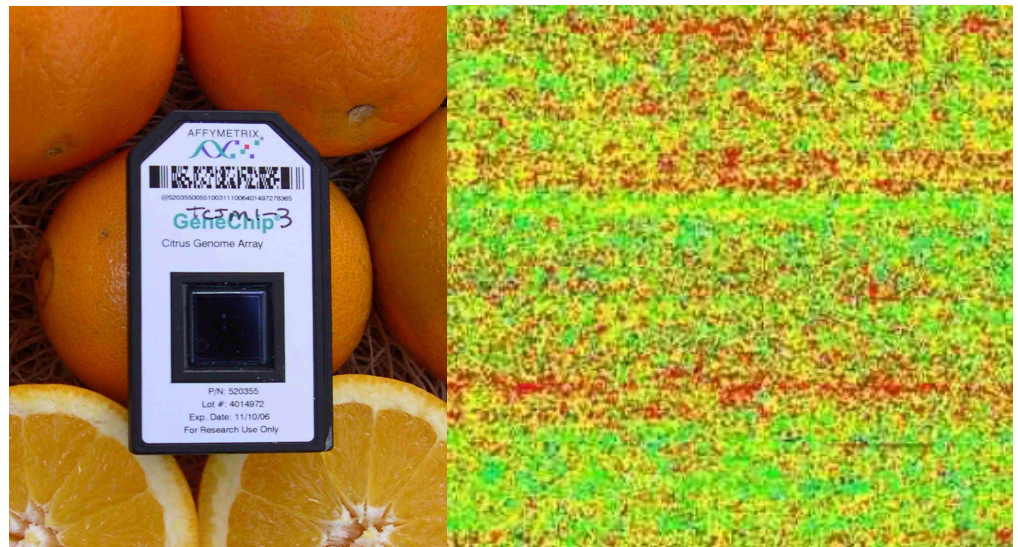
- Concentration AND volume employed
- Volume with respect to feature dimension

Biochips (passive, no electronics)

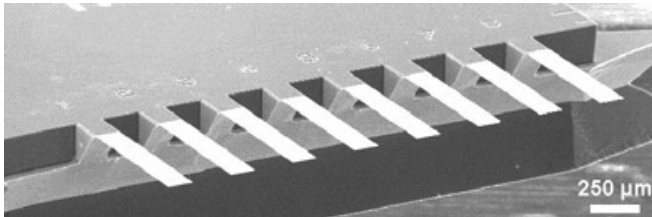
Advanced platforms to support genetic research and new diagnostics approaches

Microarrays

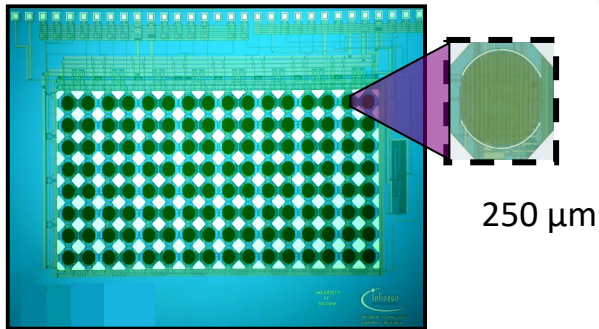
- Can test a whole genome at once
- 500.000 sites per centimeter square.
- Based on photolithography (founders where EE..)



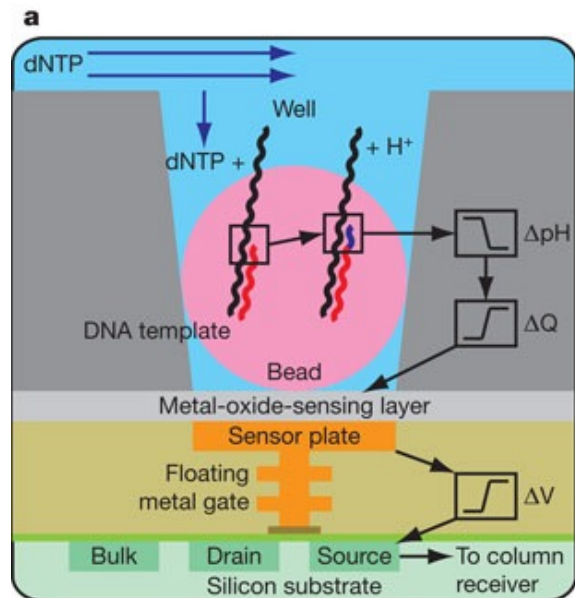
Electronic biochips



Microcantilevers on chip.
Originally developed at IBM Switzerland



DNA detection on CMOS chips. Infineon
and University of Bologna

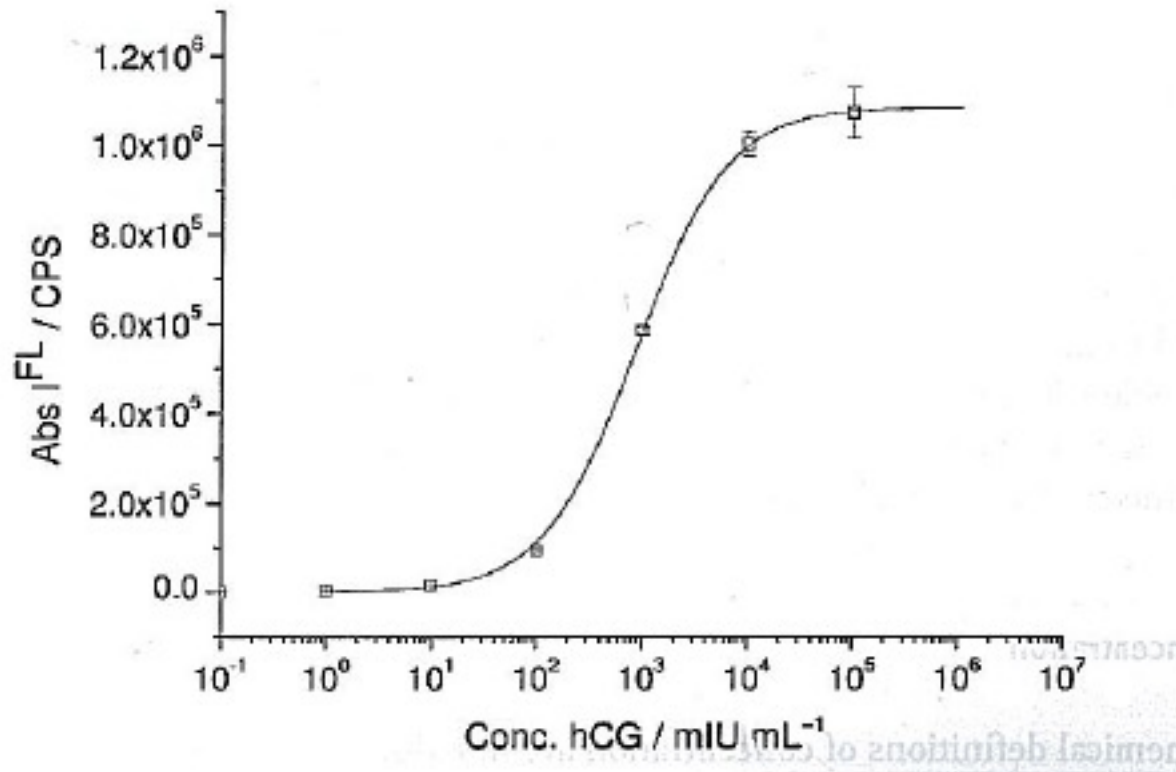


JM Rothberg *et al.* *Nature* **475**, 348-352 (2011) doi:10.1038/nature10242

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



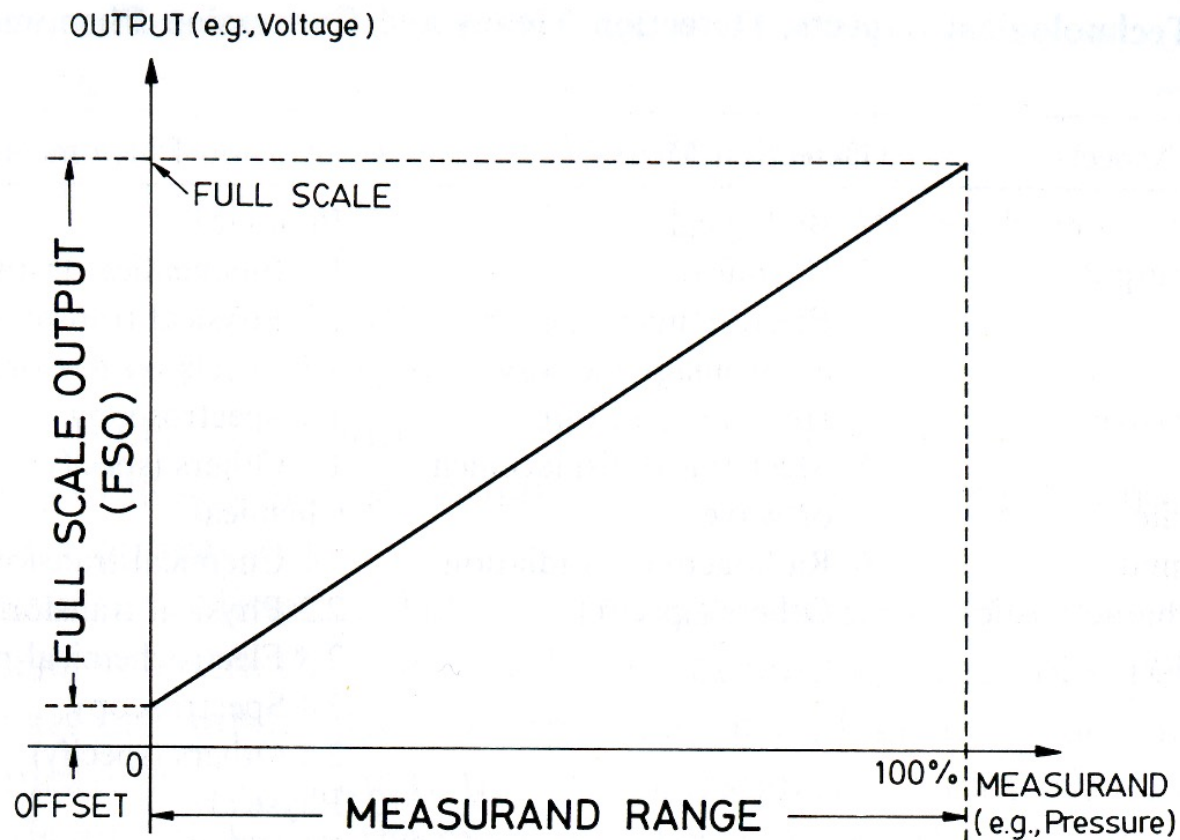
Units:

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

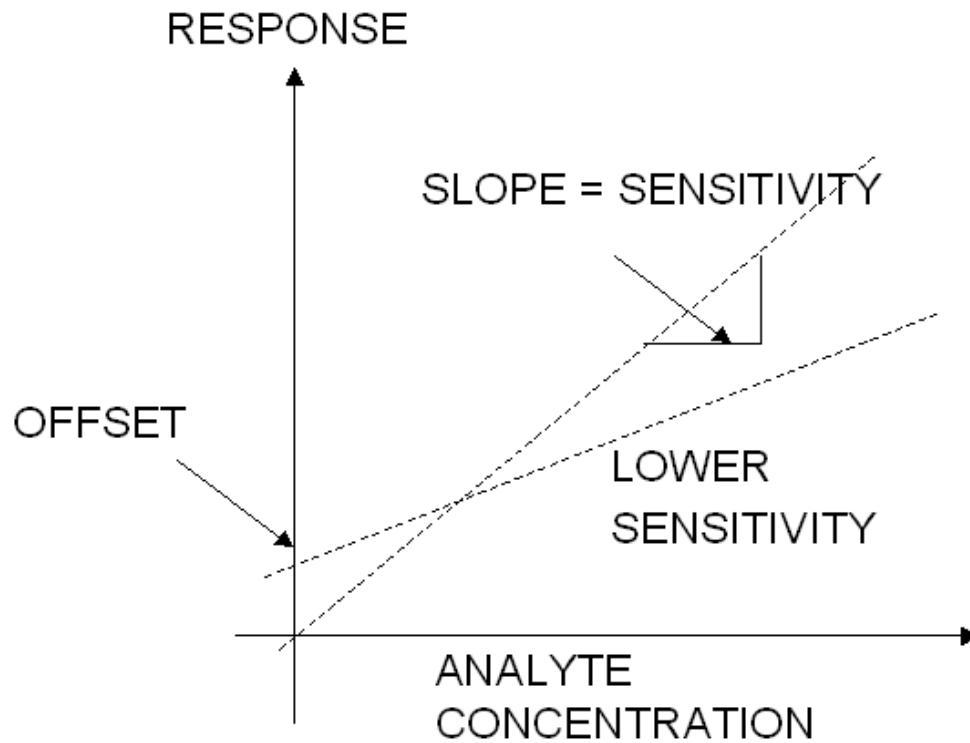
hCG: hormone, human chorionic gonadotropin

Output-measurand relationship

Sensor featuring 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

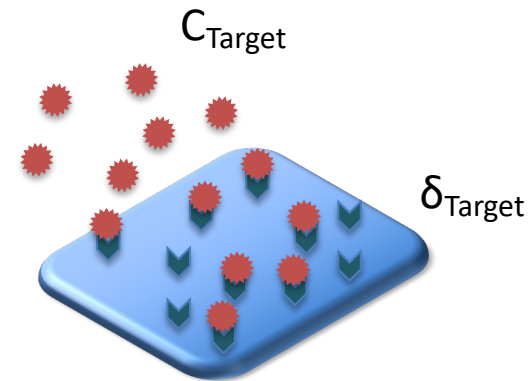
▼ Probe (immobilized on surface)

Signal in response to a change in the surface density of target molecules.

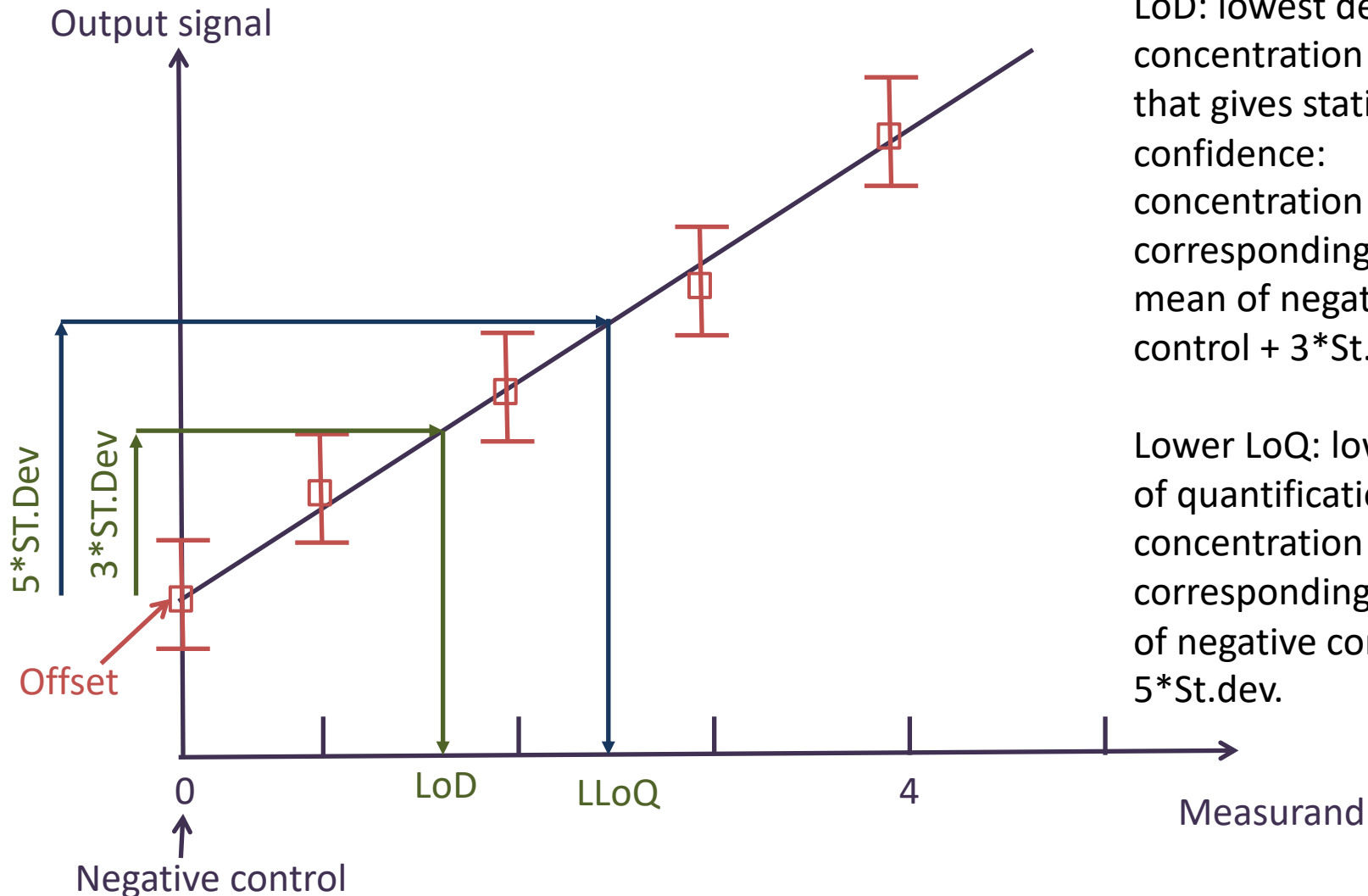
$$\Delta S_{\text{OUT}} / \Delta \delta_{\text{Target}}$$

Signal in response to a change in the concentration of target molecules.

$$\Delta S_{\text{OUT}} / \Delta C_{\text{Target}}$$



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 + $3 \cdot \text{St.dev.}$

Lower LoQ: lower limit of quantification: concentration corresponding to: mean of negative control + $5 \cdot \text{St.dev.}$

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 $2 * \text{St.Dev.}$

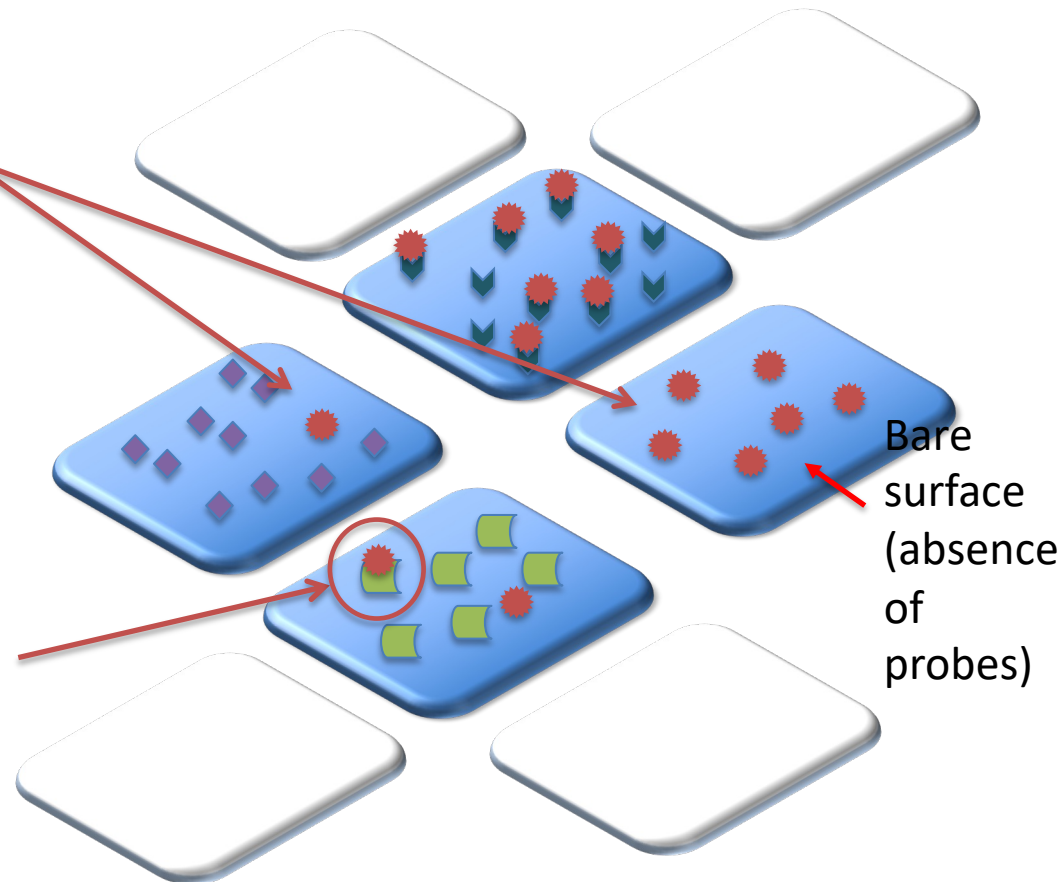
Analyte selectivity

● Target molecule

▼ Probe (immobilized on surface)

Unsuitable **passivation** of the surface

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



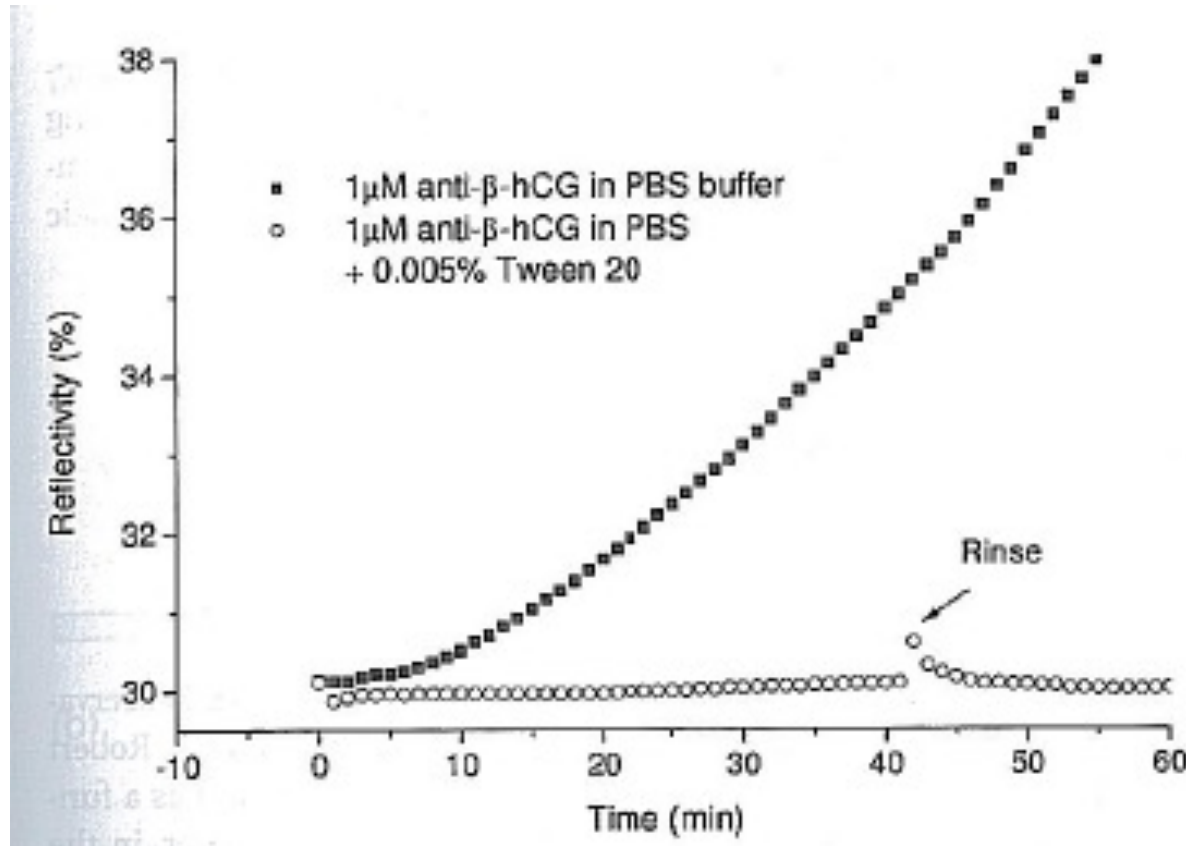
Selectivity - Specificity

- **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:

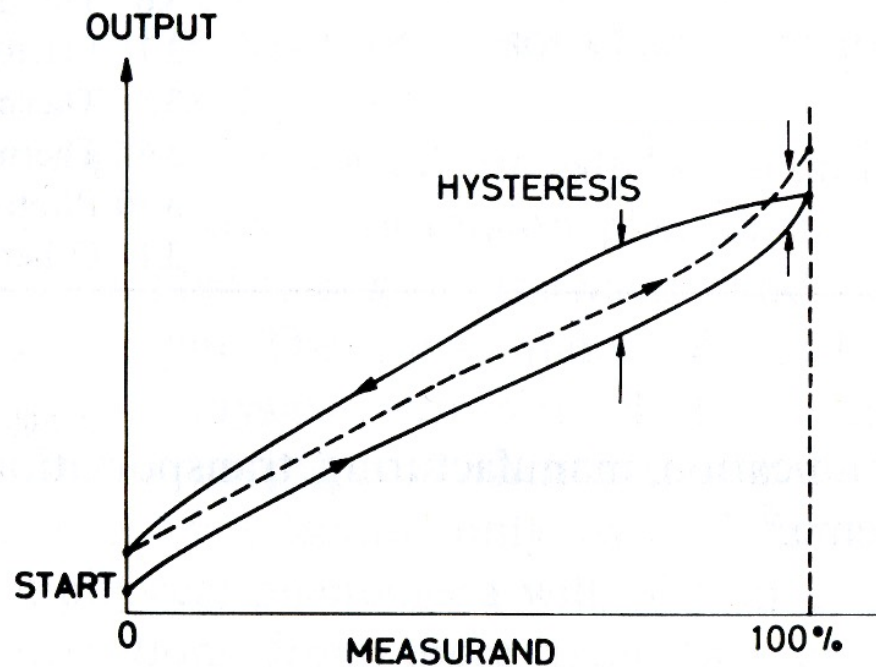
Repeatability: the ability of a sensor to reproduce output readings when the same measurand is applied to it consecutively

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

Reproducibility: 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

- 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.