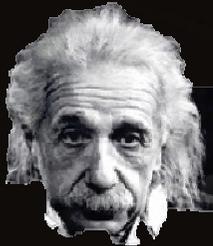


# “La llum a la nanoescala: des de Maxwell a la detecció precoç del càncer”

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Nanobiosensors & Bioanalytical Applications Group  
Institut Català de Nanociència i Nanotecnologia (ICN2)  
CSIC & CIBER-BBN  
Barcelona, Spain



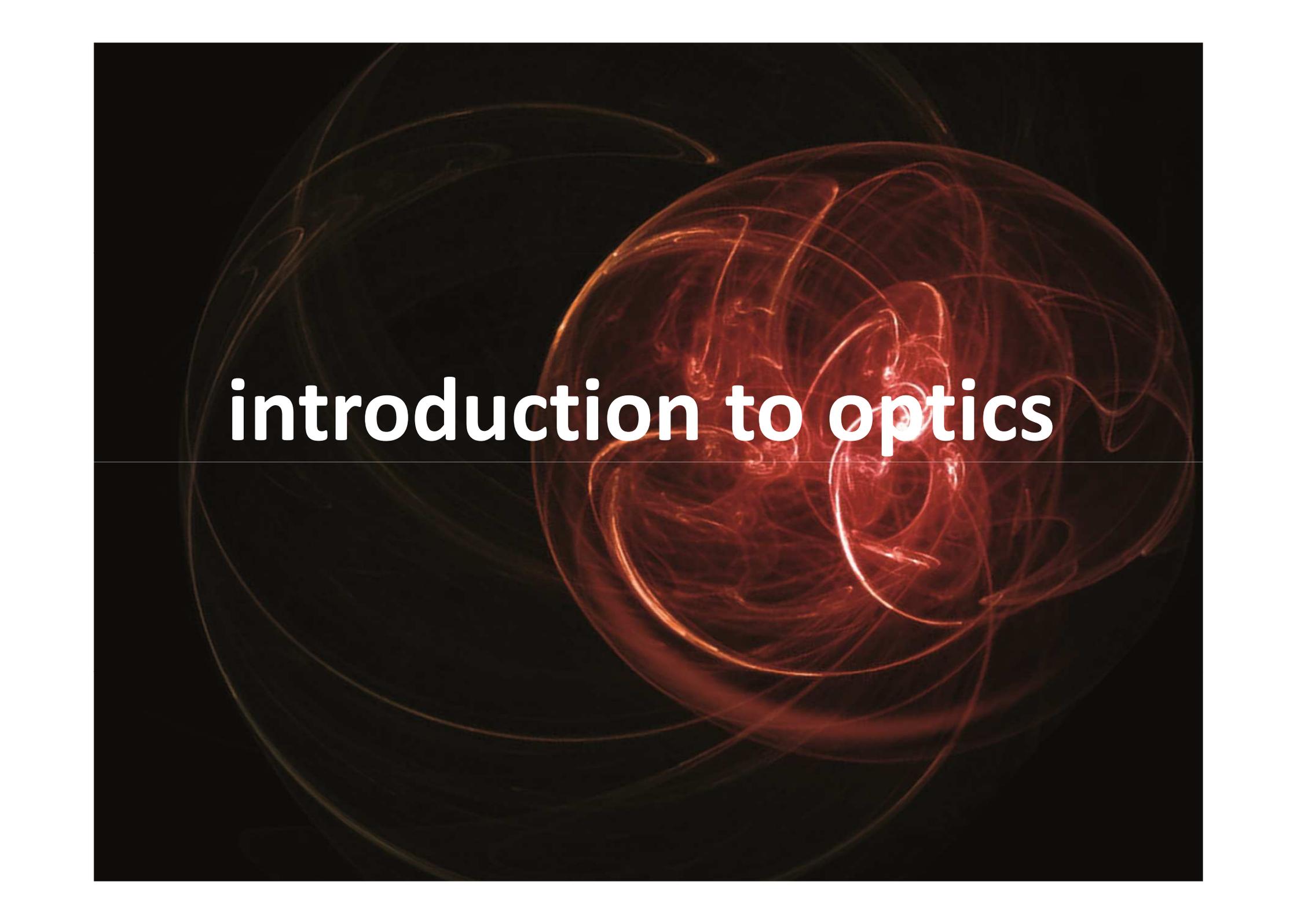
**DISSABTES  
DE LA FÍSICA 2014-15  
15a EDICIÓ**

DEL 14 DE FEBRER AL 7 DE MARÇ DE 2015

# Summary



- 1. Introduction to Optics.**
- 2. The rise of Nanotechnology.**
- 3. Optical Nanobiosensors.**
- 4. Chemistry, Biology and Bioapplications.**



# introduction to optics

# Introduction to optics

**Optics** involves the study of the interaction of light with matter.

## Eyeglasses: Italy (1286)



Salvino D'Armato is credited with inventing the first wearable eye glasses.

Nimrud lens (3000 year old piece), Assyrian palace, Iraq.



## Mirrors: Mexico (1530)



Highly-polished volcanic glass. Treasure brought to Europe by Cortés after the conquest of Mexico.



## Telescope: Germany (1608)

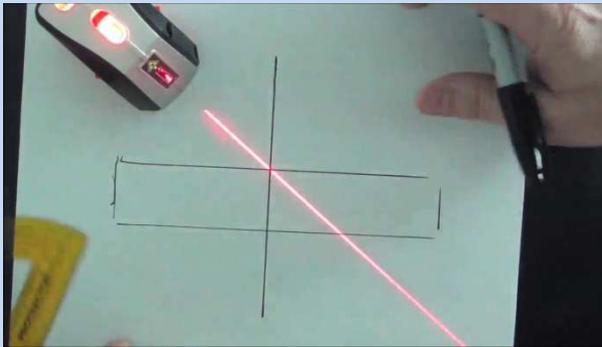


Galileo Galilei (1600) was the first to use it to observe celestial objects and record his discoveries.



# Introduction to optics

## Law of refraction: Snell, 1621

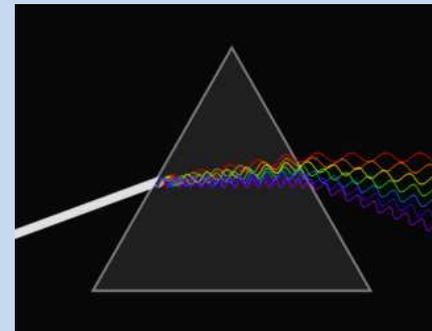


Law employed to calculate the refraction angle of the light in cross a medium with a different refractive index that the initial medium.



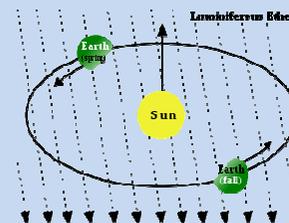
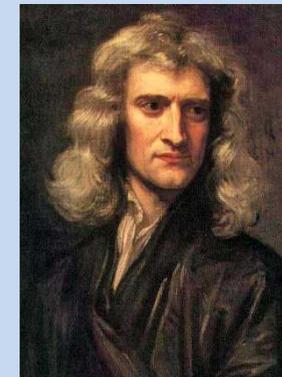
$$\frac{n_i}{n_r} = \frac{\sin \theta_i}{\sin \theta_r}$$

## Isaac Newton, 1671



Newton observed that colour is a property of light rather than the objects.

Newton argued that light is composed of particles or *corpuscles*, but he had to associate them with waves to explain the diffraction of light.



Existence of the **ether** to transmit forces between particles and the medium for the propagation of light.

# Introduction to optics

**Diffraction: Grimaldi (1665), Young (1803), Fresnel (1815)**



**Brian Greene: What's beyond the double slit experiment?**

# Introduction to optics

Maxwell equations (1864)

And God said

$$\nabla \cdot \mathbf{E} = \frac{\rho}{\epsilon_0}$$

$$\nabla \cdot \mathbf{B} = 0$$

$$\nabla \times \mathbf{E} = -\frac{\partial \mathbf{B}}{\partial t}$$

$$\nabla \times \mathbf{B} = \mu_0 \mathbf{J} + \mu_0 \epsilon_0 \frac{\partial \mathbf{E}}{\partial t}$$

and then there was  
"Light"



**Ampère:** existence of an electrodynamic molecule (electron)



**Faraday:** relationship between magnetism and electricity



Maxwell (1831– 1879)

Permittivity:  $\epsilon$

Permeability:  $\mu$

$$c = \frac{1}{\sqrt{\epsilon\mu}}$$



# The rise of Nanotechnology

# "NANOTECHNOLOGY"

Discipline involving the design, fabrication and application of materials and systems at the atomic and molecular scale, rendering in new properties and functions due to the size.



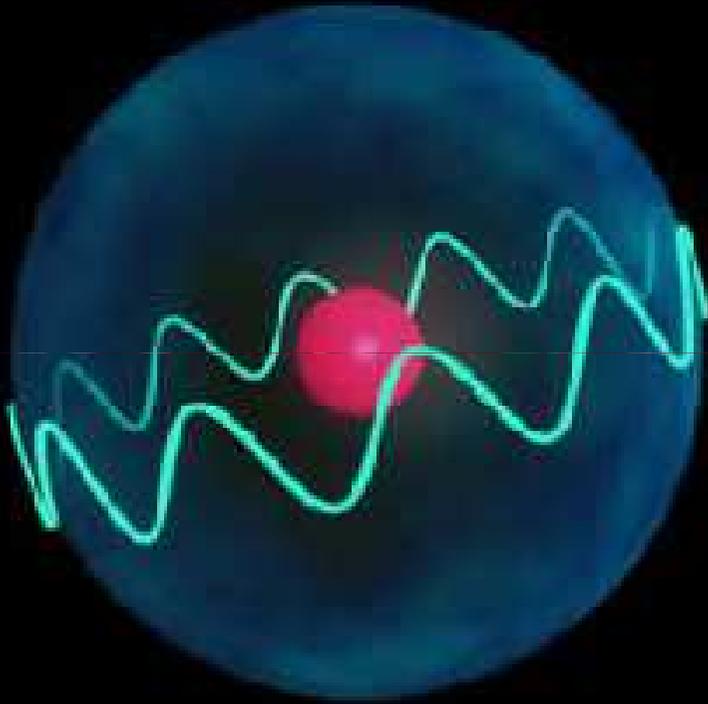
At least one dimension sized from 1 to 100 nm, showing new properties (physical, chemical or biological)

- Quantum effects
- Mechanical, electrical, magnetic properties
- Interfacial properties
- Enhancement of biological processes

All type of industrial applications: electronics, energy, medicine, transport,...



# A matter of size

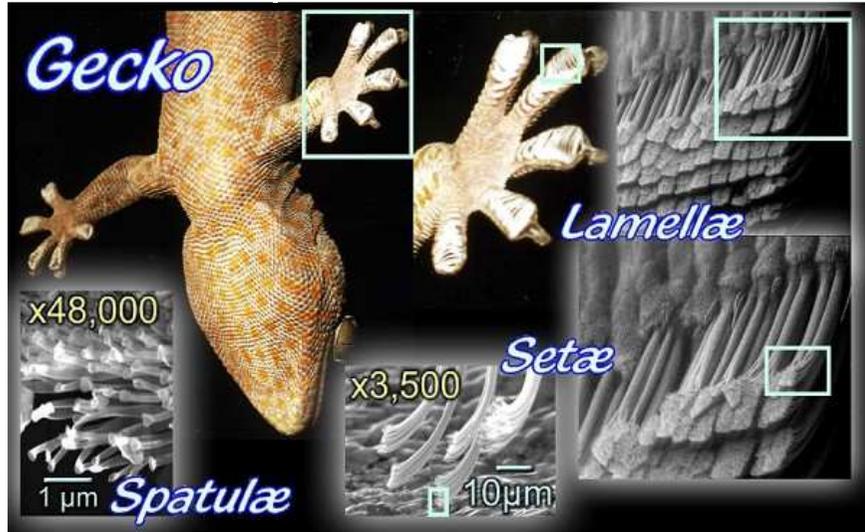


0.1 nanometre

Hydrogen atom

# Is really new the Nanotechnology?

## In Nature.....



Van der Waals and capillarity forces allow a strong attachment



**Gecko Tape**  
U. Manchester



New Scientist, Mayo 2003:  
"Synthetic gecko hairs promise walking up walls"

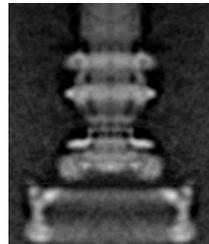
Colours in Nature

## Photonic Crystals: periodic nanostructures

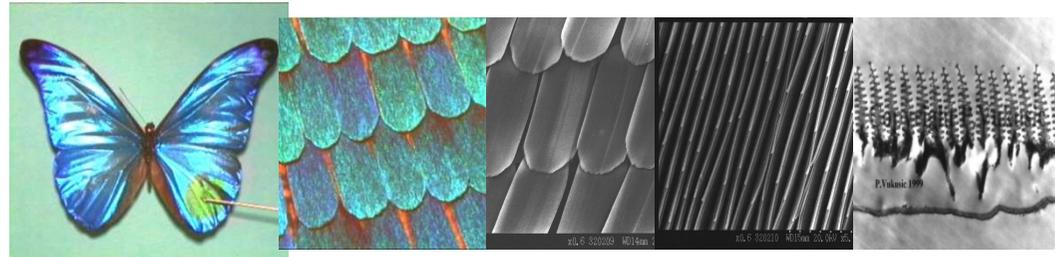
Biological molecules are the most sophisticated **Nanometric machines**



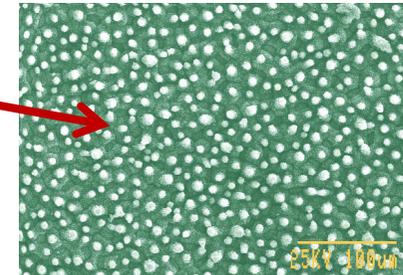
Ribosome



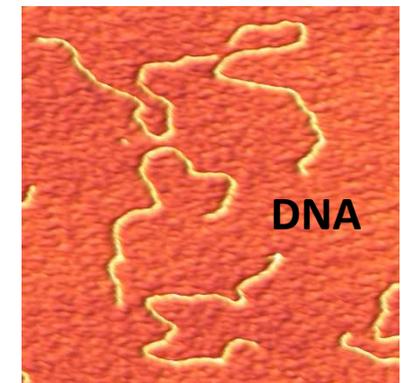
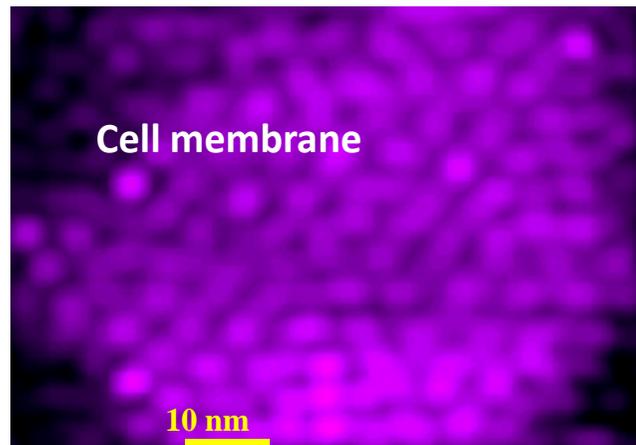
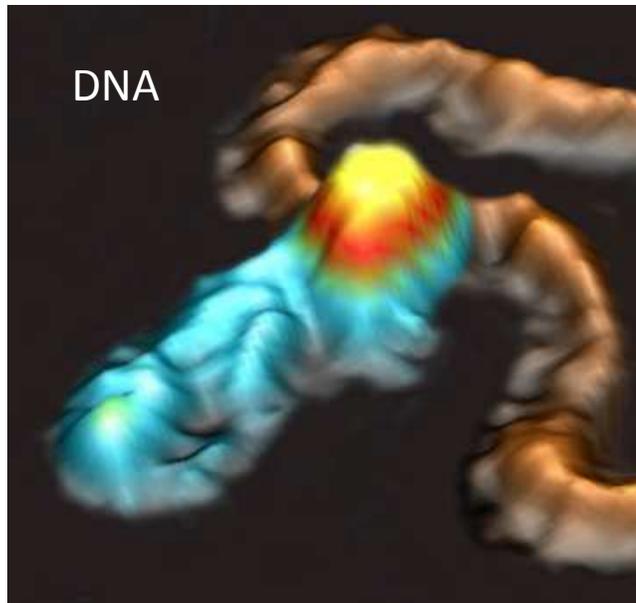
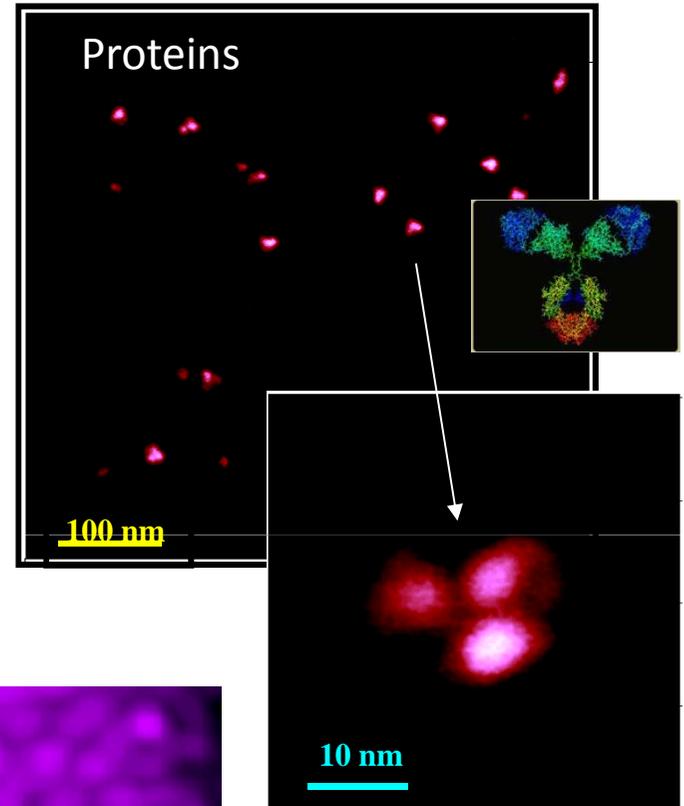
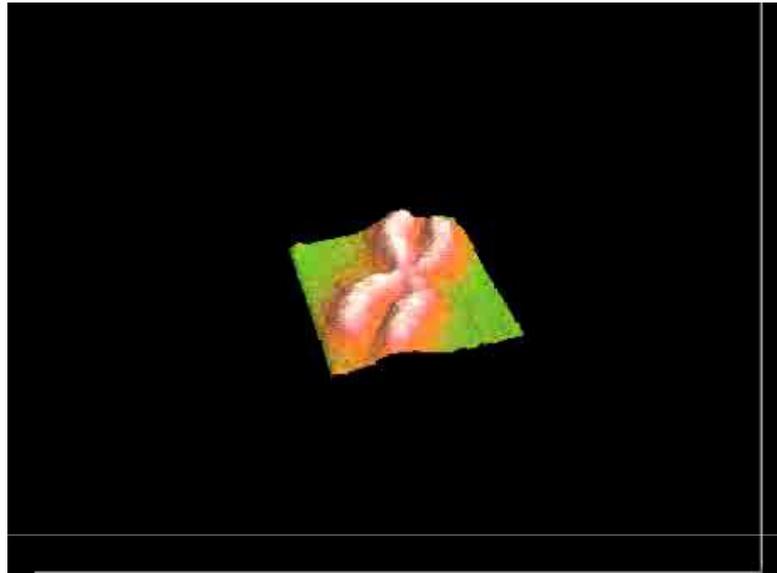
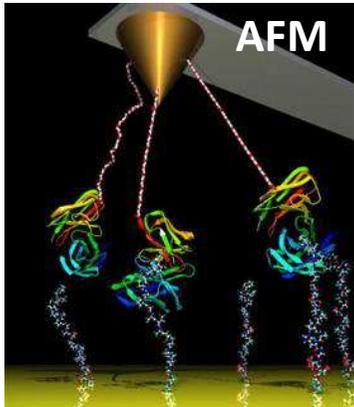
bacterial flagellum



## Lotus leaf self-cleanness



# Looking at biomolecules....



# Nanomedicine

**Nanomedicine** uses nano-sized tools for the **diagnosis, prevention and treatment of diseases** and to gain increased understanding of the complex underlying pathophysiology of disease. The ultimate goal is improved quality of life.

## Nanomedicine

### Nanodiagnostics

Nanobiosensors, Imaging tools

### Nanotherapy

Drug delivery  
Nanotherapy for selective destruction

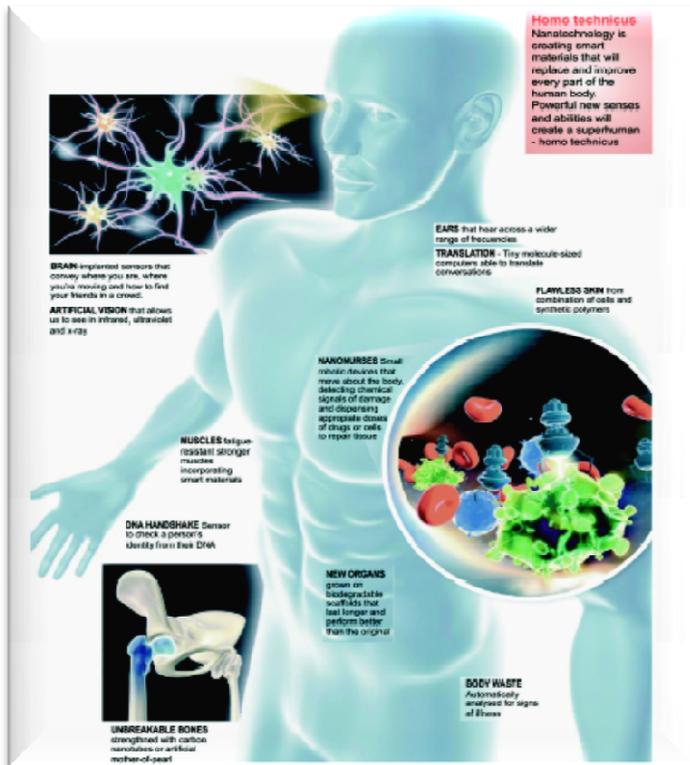
### Regenerative Medicine

Genetic Therapy  
Cell Therapy  
Tissue engineering

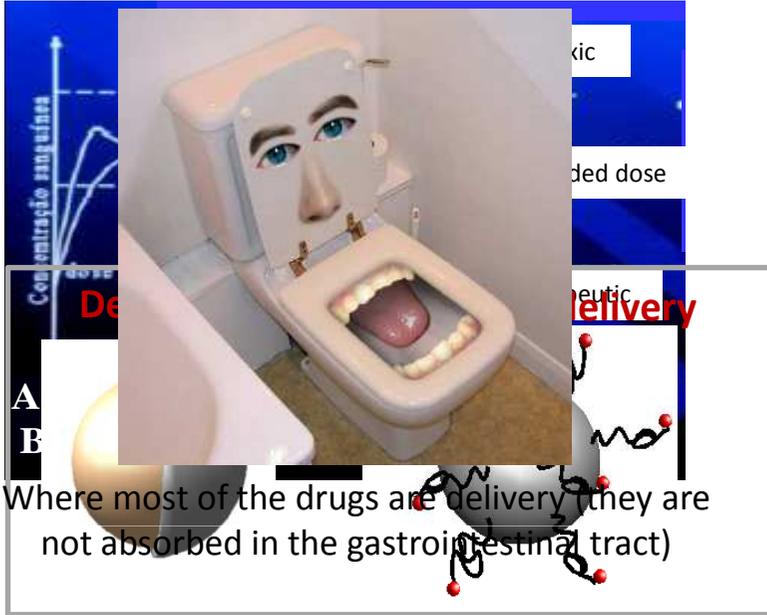


**To improve prevention, diagnosis and therapy in Human Health**

**(“find, fight, follow”)**

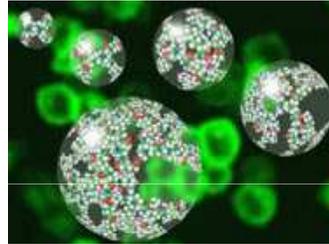


# Targeted drug delivery

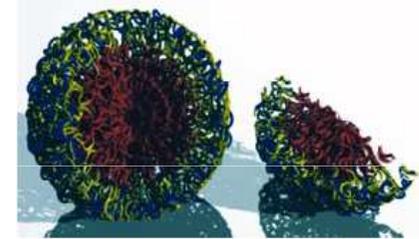


Nanodrug delivery systems can improve the adsorption of the drug and can deliver the drug specifically in the illness without affecting the healthy areas.

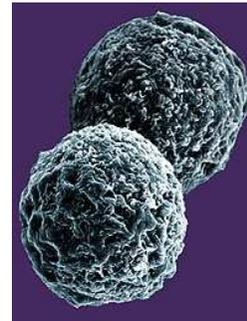
## Nanosystems for drug delivery



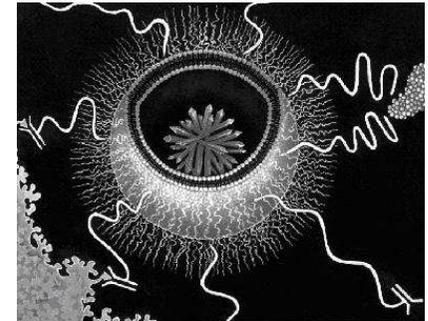
Nanoparticles



Dendrimers



Polymeric nanocapsules



Liposomes

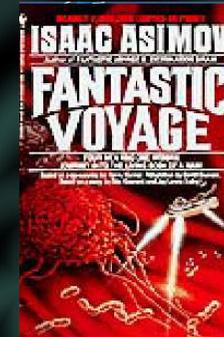
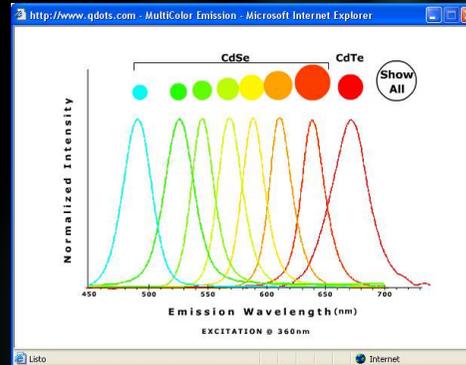
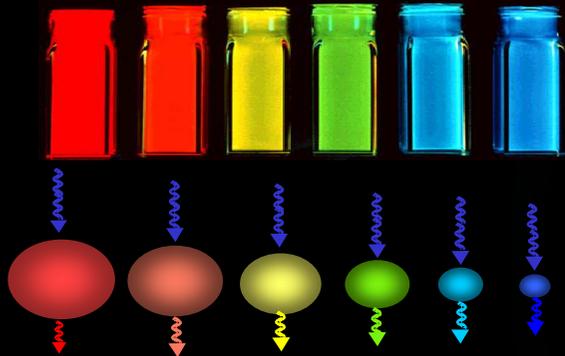
Improving solubility and average time of efficiency

Improving specificity and efficiency. Decrease side effects.

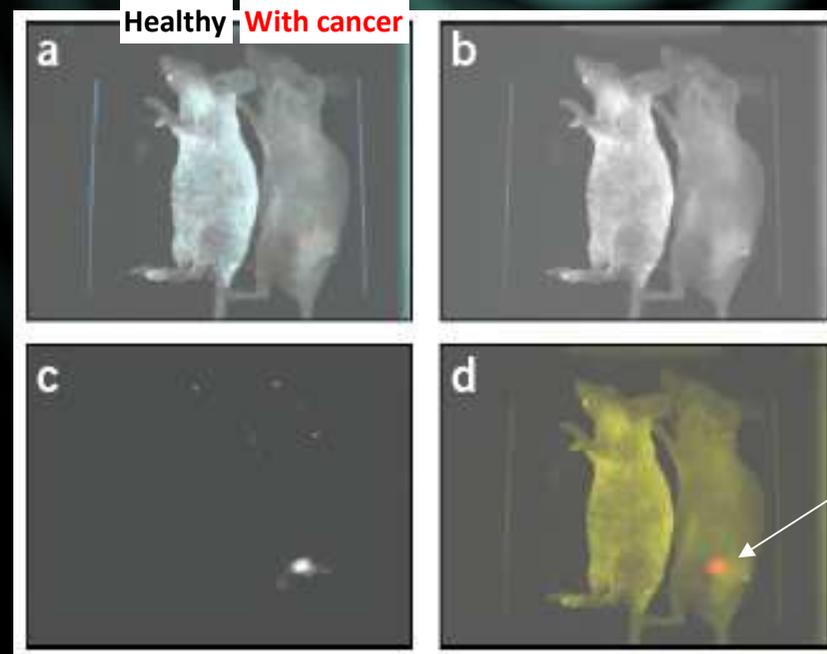
# Nanodiagnosics:

Early detection of cancer cells

## Quantum dots



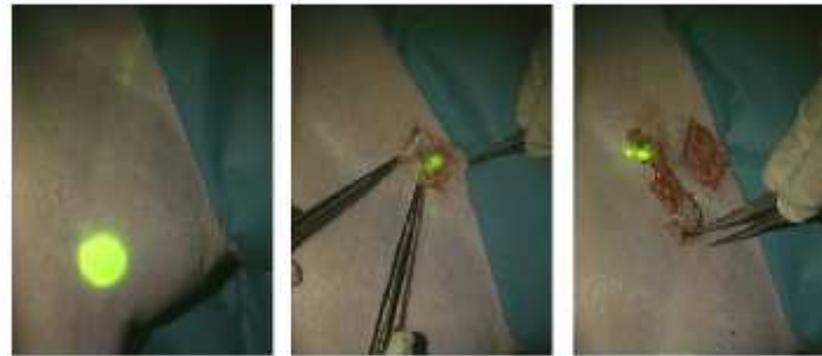
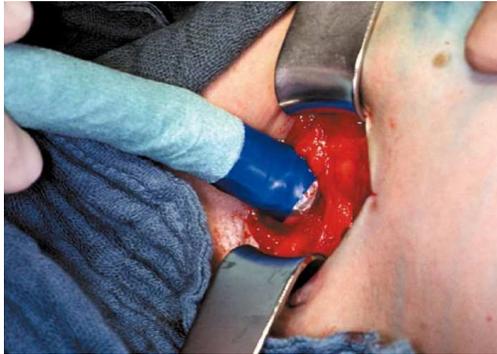
FANTASTIC VOYAGE (USA, 1966)



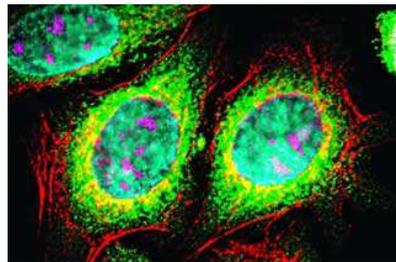
Prostate cancer cells

# Nanodiagnostics with quantum dots

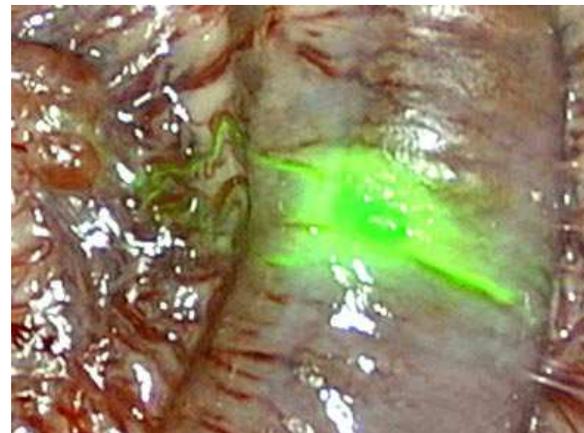
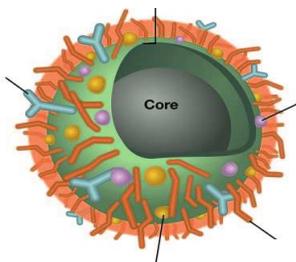
Intra-Operative Imaging: Sentinel lymph node evaluation and tumor extent



Quantum Dot Nanoparticle Fluorescence.  
Visualization through 1 cm of tissue



Laboratory diagnostics



Quantum dot labeling  
of mouse colon cancer

# Diagnostics & Therapy using nanoparticles

## Multifunctional nanoparticles

Magnetic  
Metallic (gold)  
Semiconductor

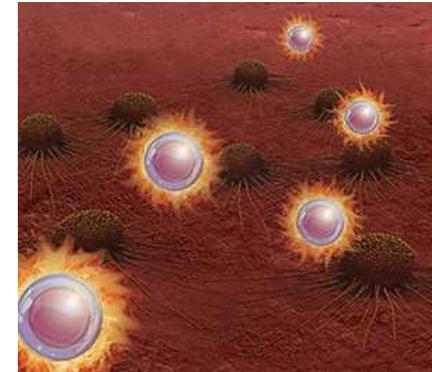
+

## Energy sources

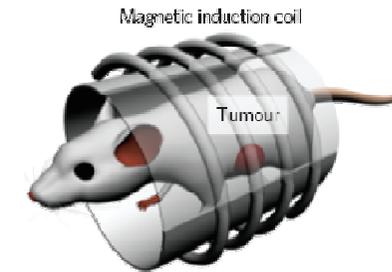
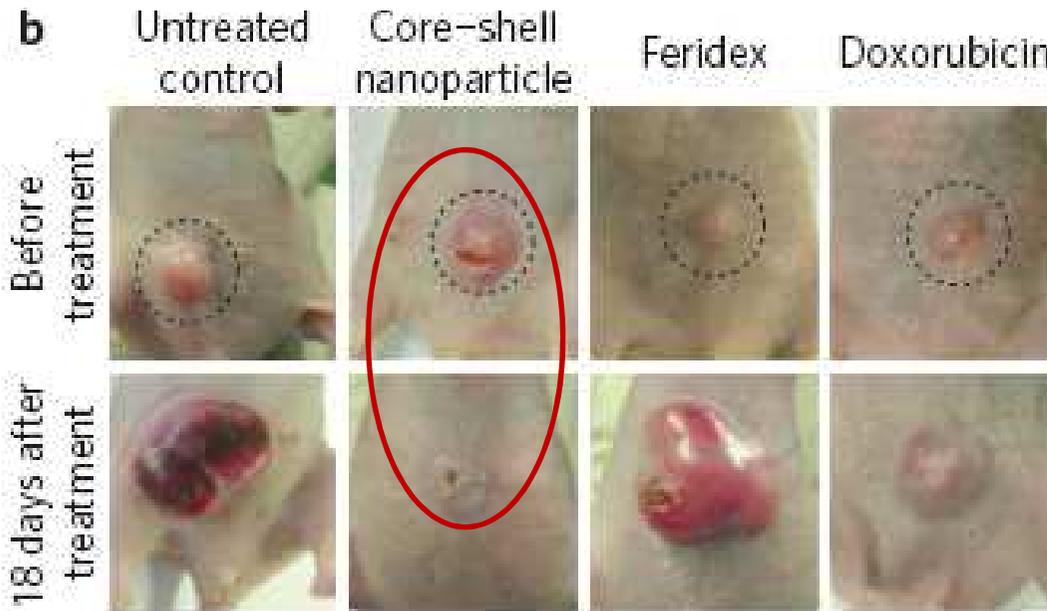
Magnetic fields  
Microwaves  
Lasers  
Ultrasounds



## *in-vivo* treatment of tumors by hyperthermia

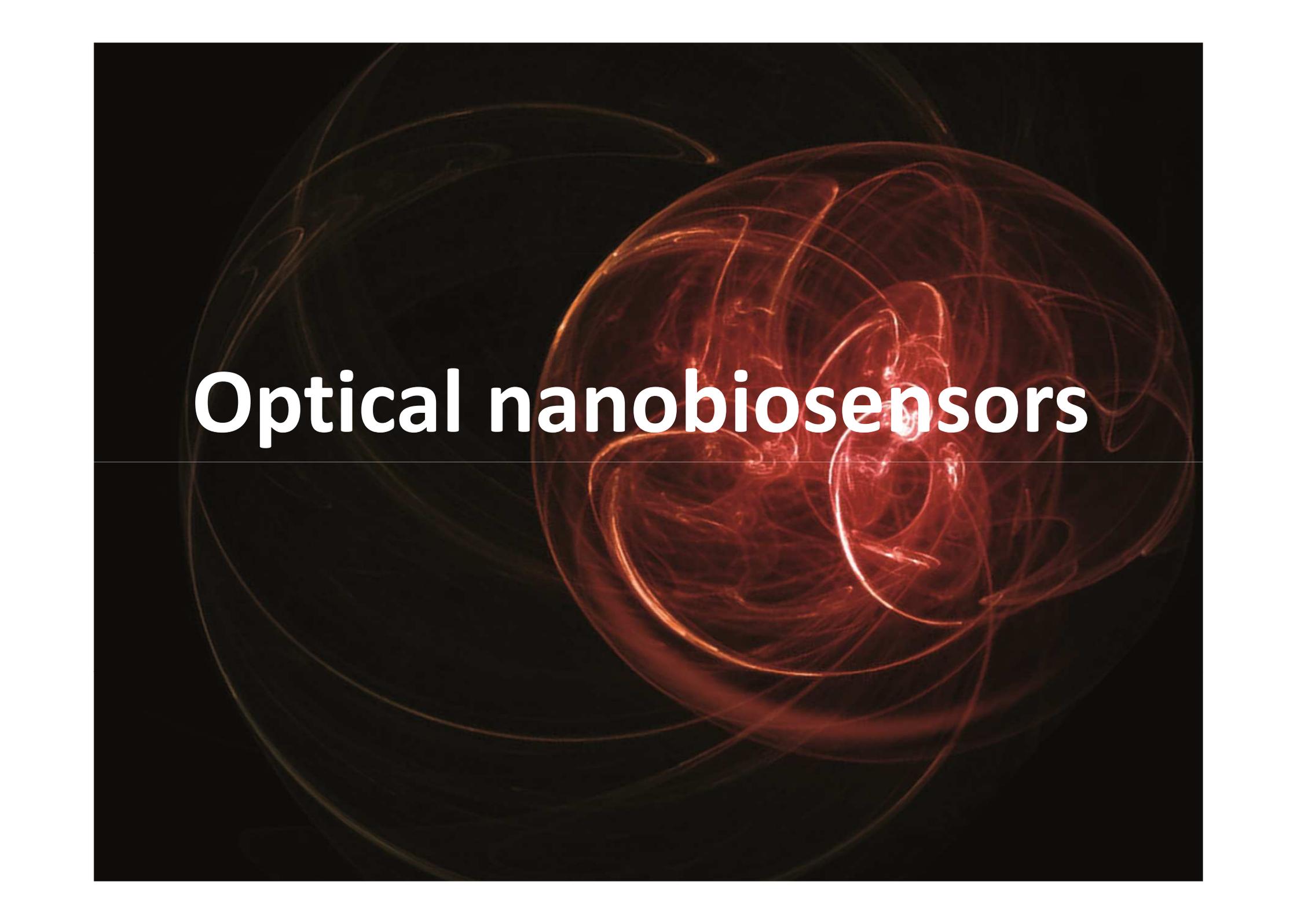


**Hyperthermia**  $T \approx 41-45^\circ \text{C}$  combined with radio/chemotherapy.  
Localised cancer treatment, more effective with less side effects



Compatible with imaging by NMR

Korea Research Institute of Bioscience and Biotechnology (KRIBB)



# Optical nanobiosensors

# DIAGNOSTICS: today and tomorrow

## Clinical laboratory



- Limited to centralised labs
- Trained personnel, time-consuming
- Expensive instrumentation
- Not available to everyone

*(resource-constrained settings)*

## Point-of-care device (POC)



- Suitable for diagnostics in the field and in-situ
- Fast, label-free, high sensitivity, low-cost
- Enable permanent deployment and unattended operation



sample



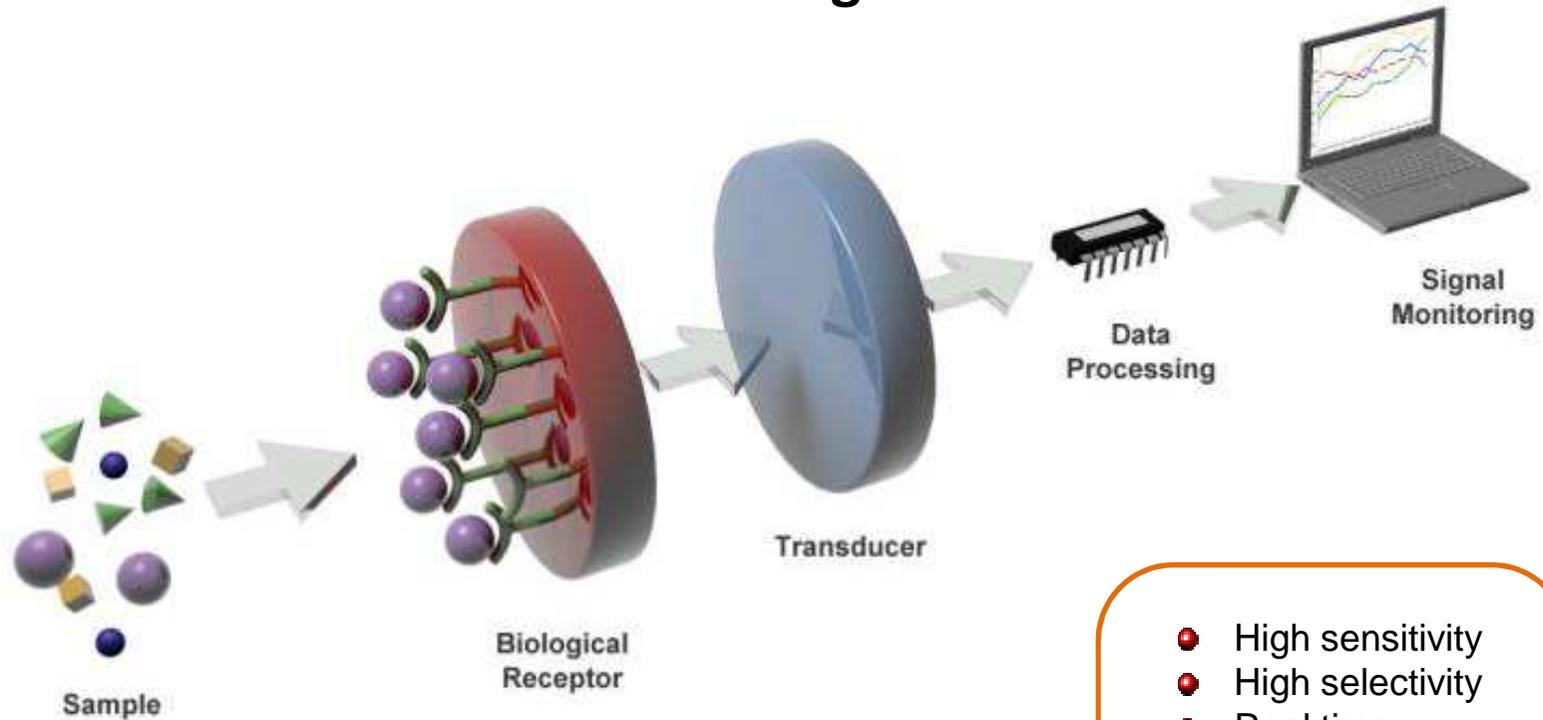
biosensing evaluation



treatment

# Biosensor device

## Biosensor device for diagnostic



- High sensitivity
- High selectivity
- Real time
- High reliability
- Reproducibility
- Multiplexing
- Low cost
- Easy to use

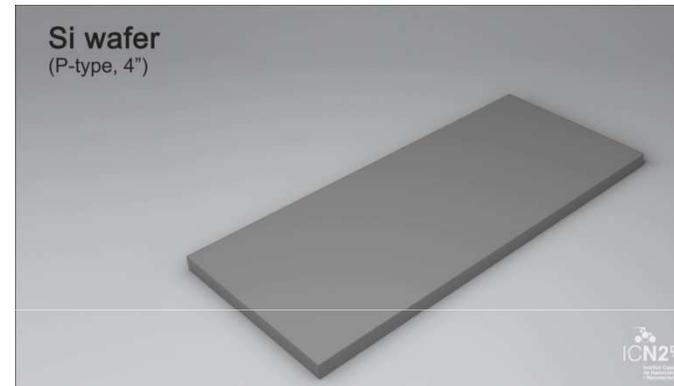
# Optical nanobiosensors

## FABRICATION OF NANOSTRUCTURES

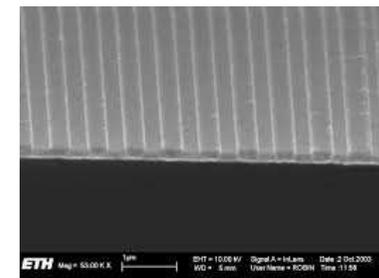
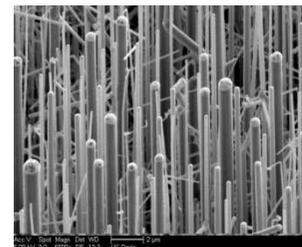
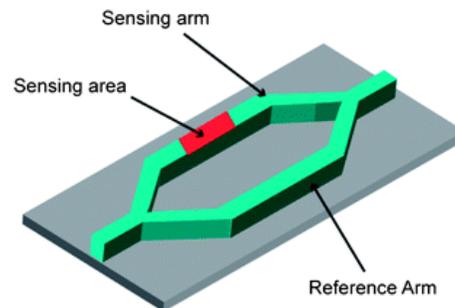
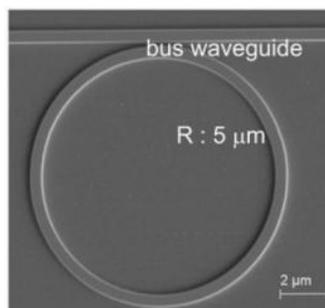
### Cleanroom facilities



### Fabrication process

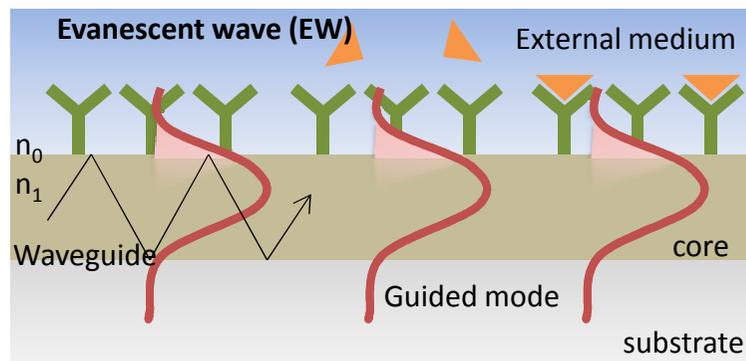
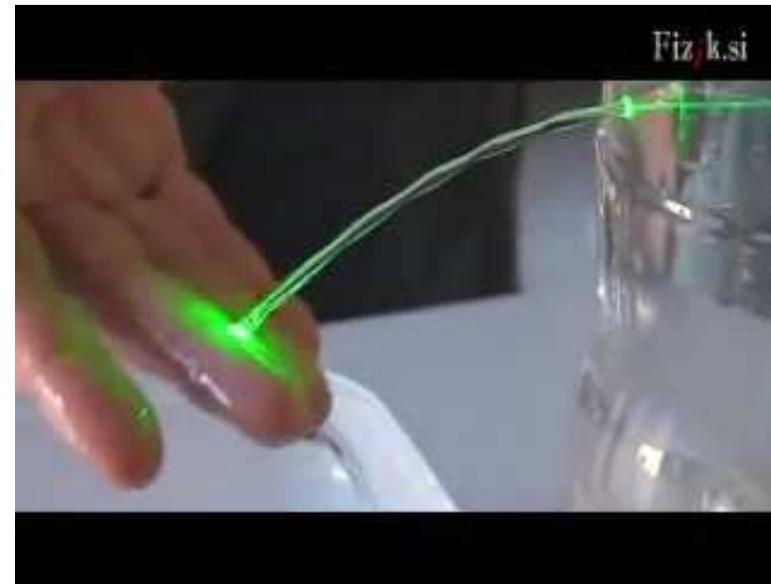
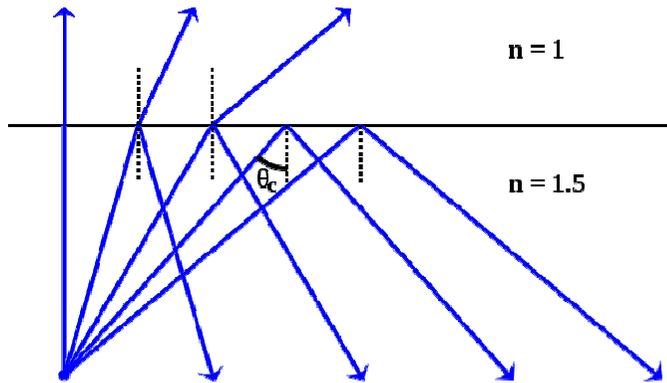


## Optical nanobiosensors

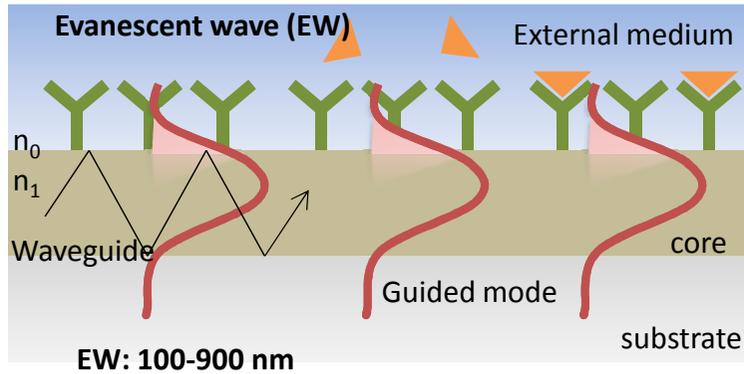


# Optical nanobiosensors

## Light in a waveguide: Total Internal Reflection



## Photonic Biosensors based on EW detection

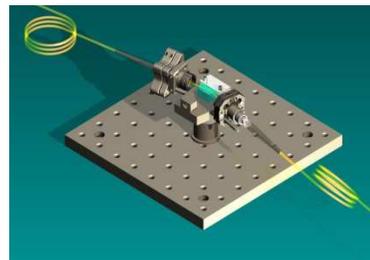


- Ⓒ High selectivity and sensitivity:  
Avoiding purification and amplification steps
- Ⓒ Label-free detection:  
Fast and user-friendly analysis
- Ⓒ Small size:  
Mass production (wafer-level) reducing cost of the analysis  
POC device

## Transducers developed at the group

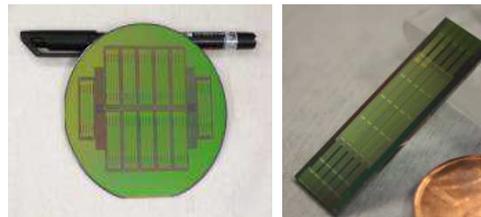
Plasmonics (SPR) and LSPR

**LOD: ng/mL**

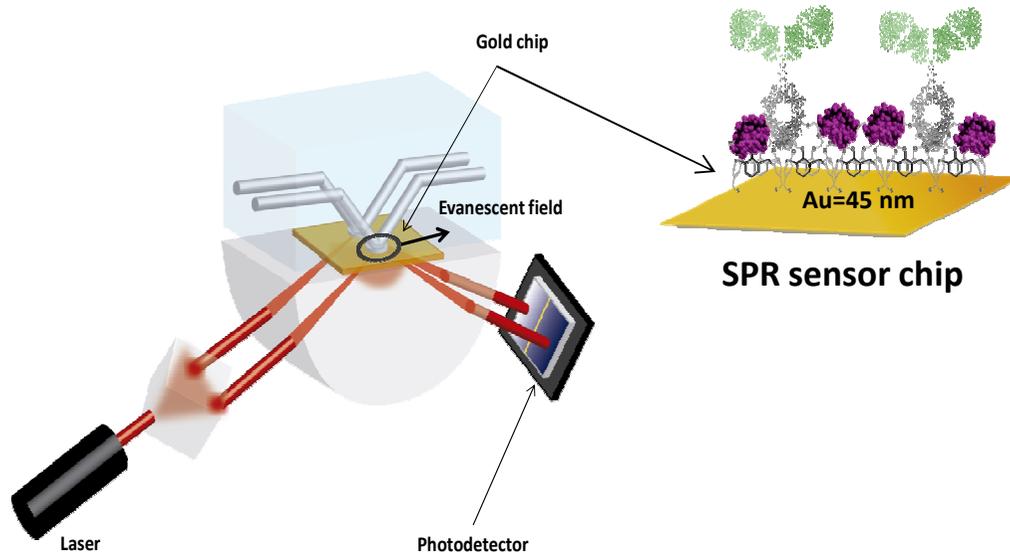


Interferometric Waveguide Biosensor

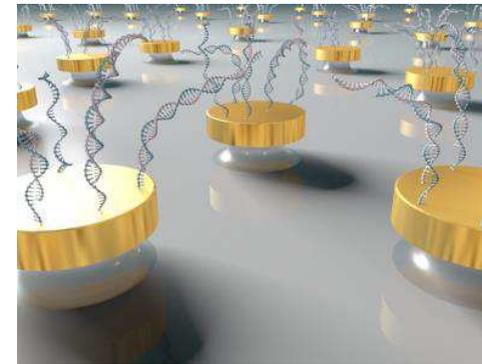
**LOD: pg/mL**



## Surface Plasmon Resonance Sensor (SPR)



LSPR Localized  
plasmonic modes



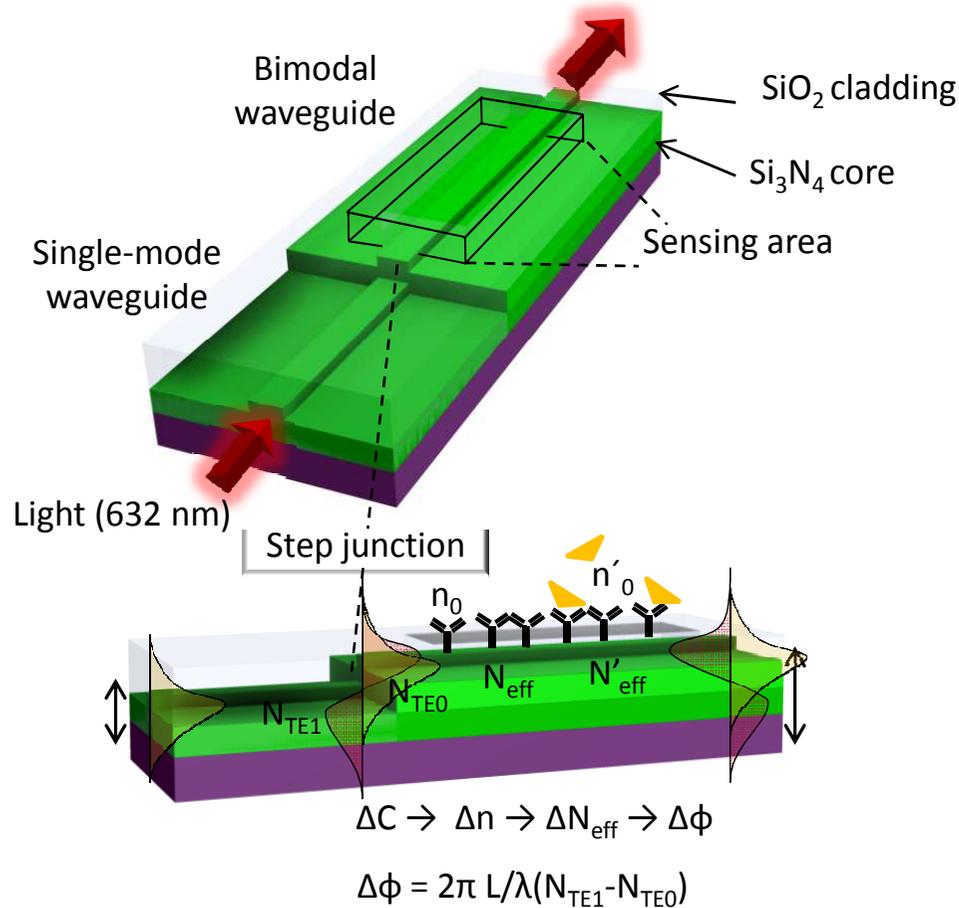
Most developed and commercially available optical biosensor



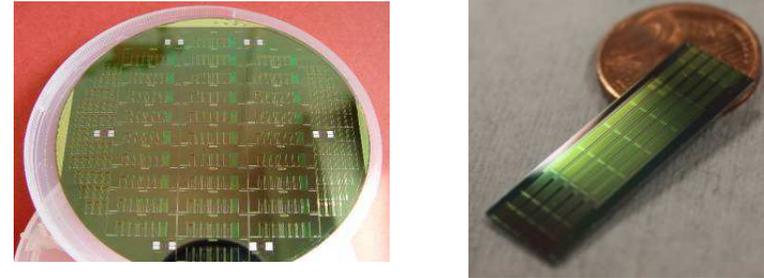
More than 20 companies worldwide: **Biacore**, Horiba, Biosensing Instruments, Sensia, Analytical systems, Reichert, Moritex...

# Bimodal Waveguide Interferometer

Working principle:  
two-mode interferometry

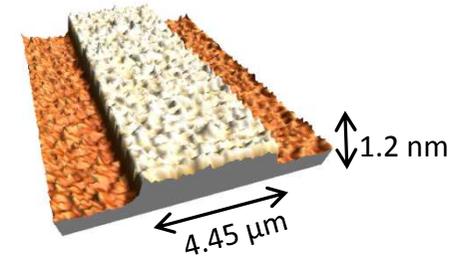


## Fabrication



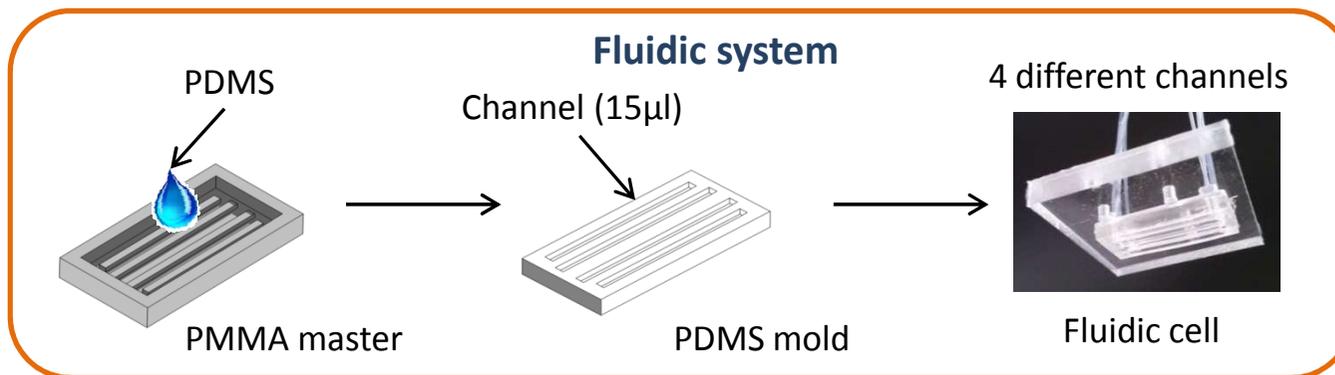
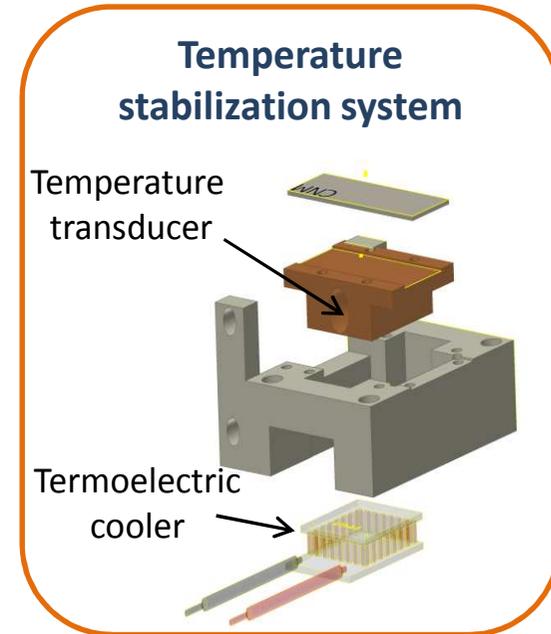
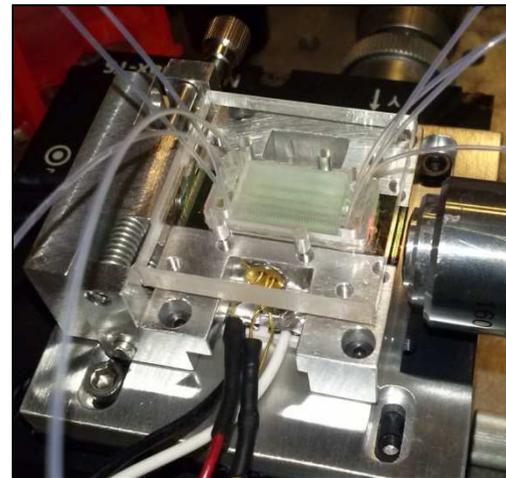
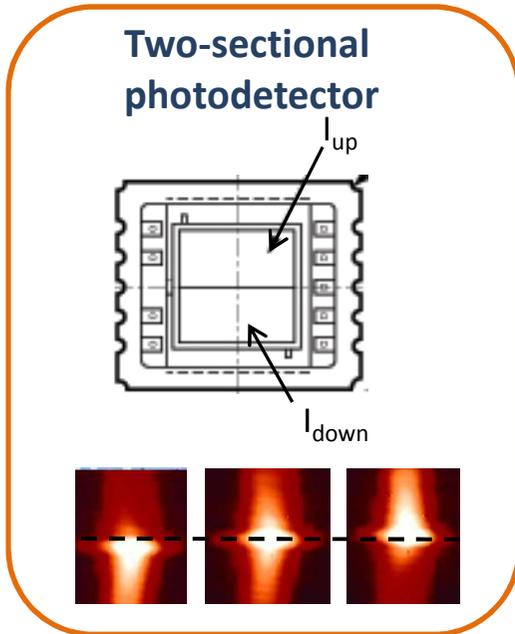
Standard fabrication at Clean Room facilities.

## Nanometric rib waveguide

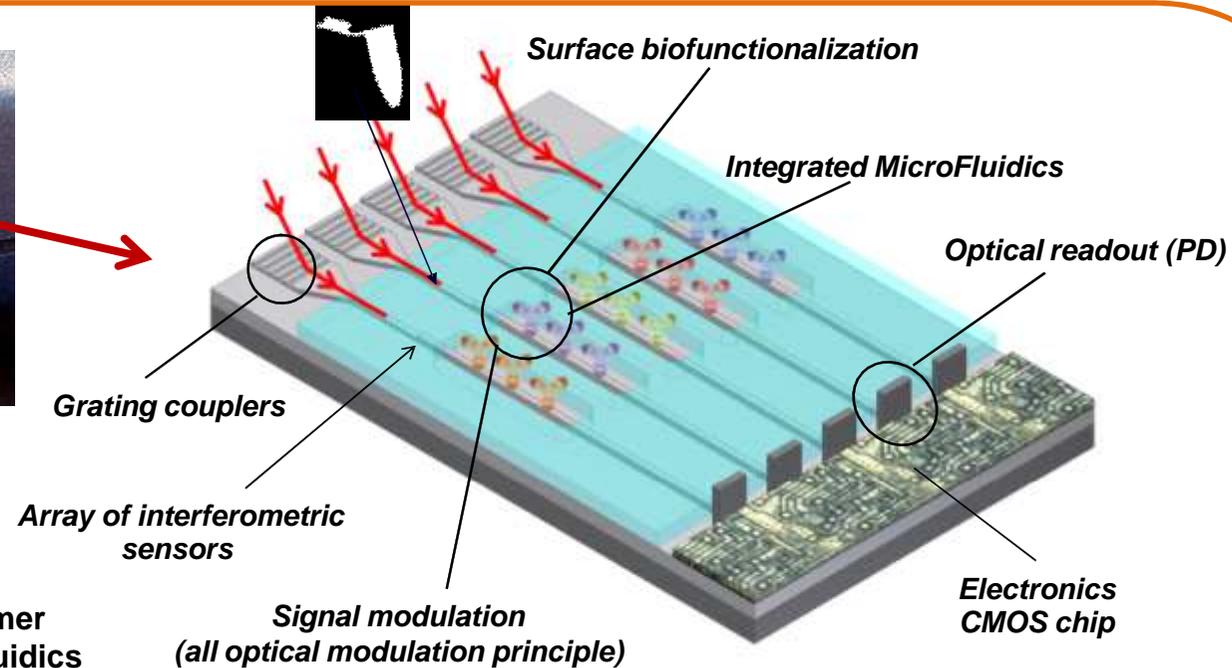


Structural characterization by AFM

# Lab system for the BiMW biosensor



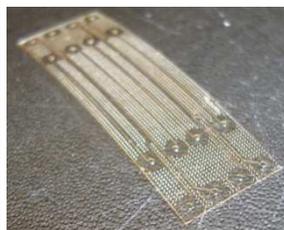
## BiMW integration for Point-of-Care devices



Sensor chip  
(Si technology)

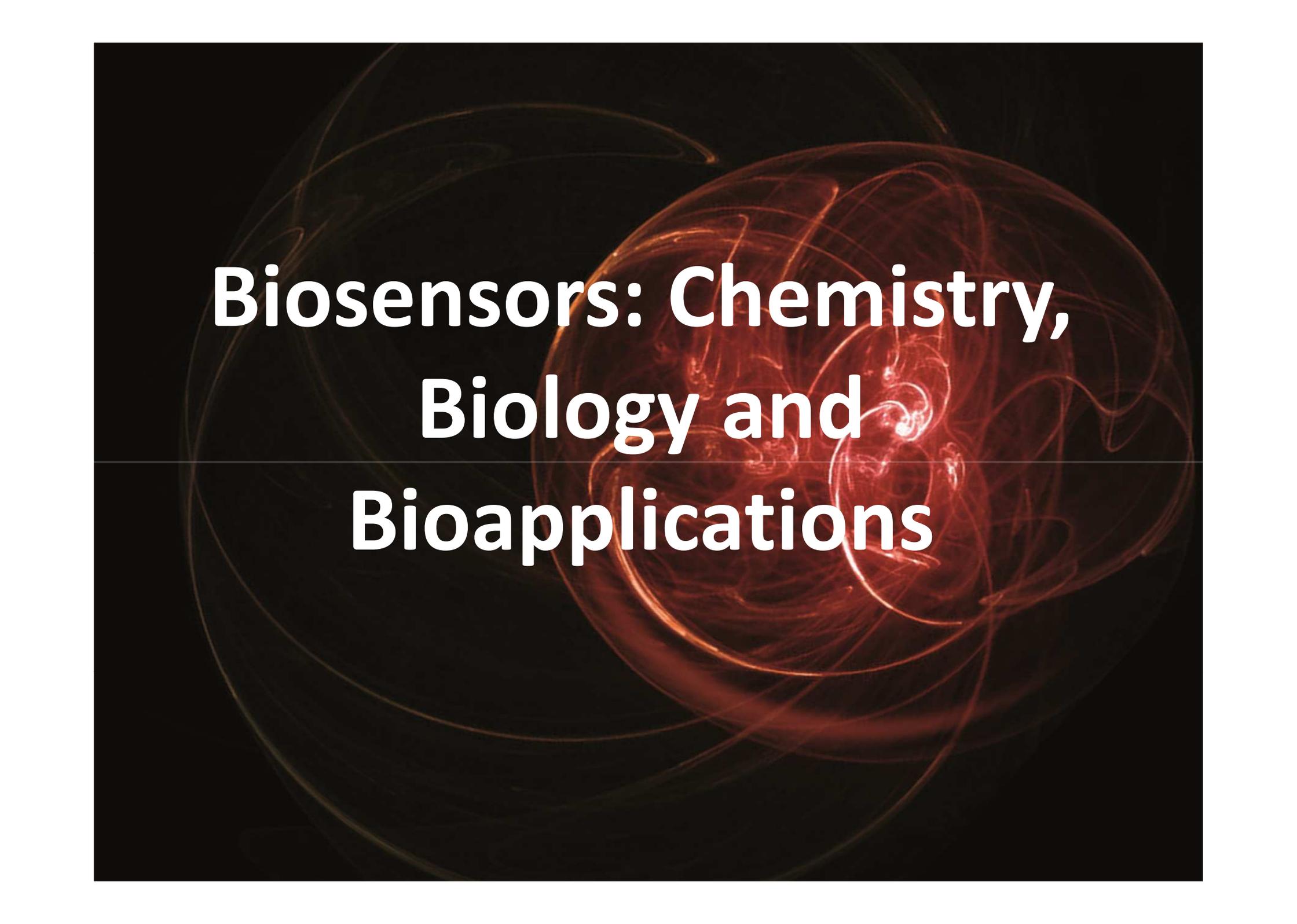


Polymer  
microfluidics



- One of the most sensitive label-free existing biosensors (fM)
- Standard microelectronics technology
- Miniaturization, integration & mass-production
- Truly portable POC

EP2278365 (Granted 2014), PCTES08070142 (Granted 2013), CA2693423, CN102077124, US20110102777 (Granted 2012), JP2011519071. **TECHNOLOGY TRANSFER TO INDUSTRY IN APRIL 2014.**

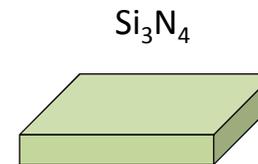


**Biosensors: Chemistry,  
Biology and  
Bioapplications**

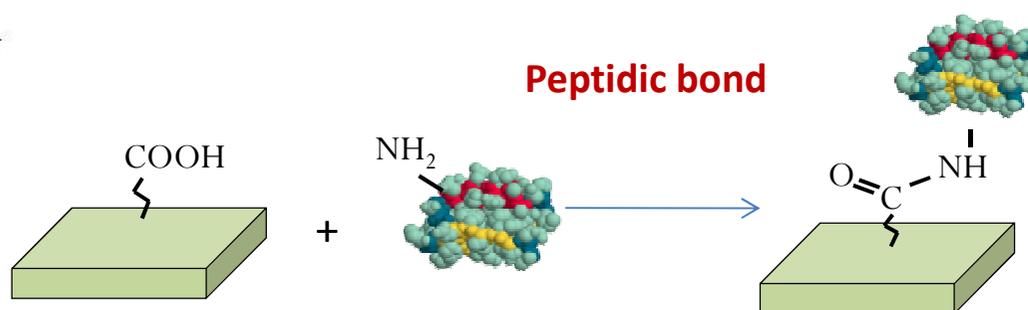
# Biosensors: Chemistry

## Surface biofunctionalization: the requirement of functional groups

Functional Group	Structure	Compound Classification
Halogen (-X is F, Cl, Br, I)	R-X	Halocarbon
Hydroxyl (-OH)	R-OH	Alcohol
Carboxyl	$\begin{array}{c} \text{O} \\ \parallel \\ \text{R}-\text{C}-\text{OH} \end{array}$	Carboxylic acid
Carbonyl (-C=O)	R-C=O	Aldehyde
	$\begin{array}{c} \text{O} \\ \parallel \\ \text{R}-\text{C}-\text{R} \end{array}$	or Ketone
Ester	$\begin{array}{c} \text{O} \\ \parallel \\ \text{R}-\text{C}-\text{O}-\text{R} \end{array}$	Ester



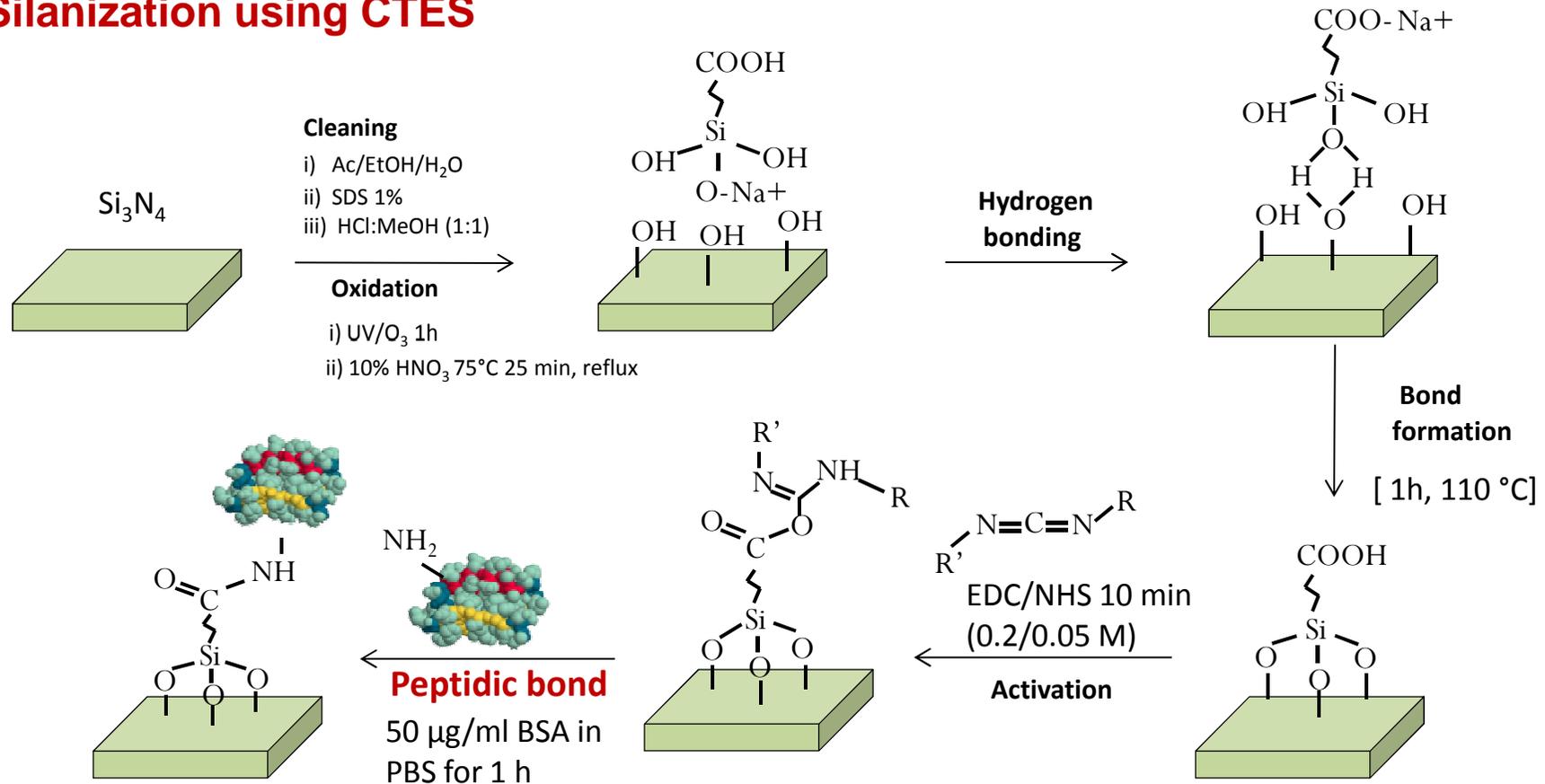
Transducer surface: very inert



# Biosensors: Chemistry

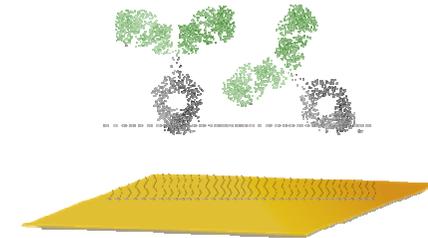
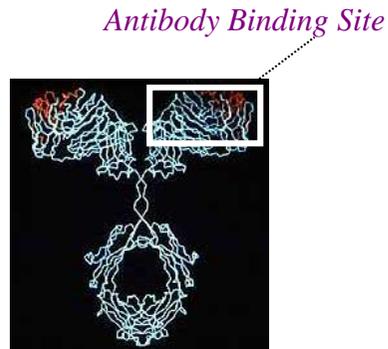
## Biofunctionalization of SiN surfaces

### Silanization using CTES



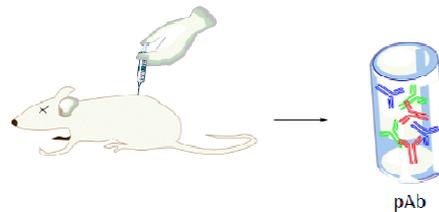
# Biosensors: Biology

## Selective bioreceptors for detection of proteins: the antibodies

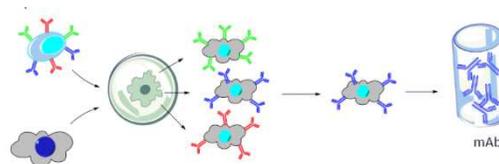


- Random orientation
- One step
- Ab chemically modified
- Stable

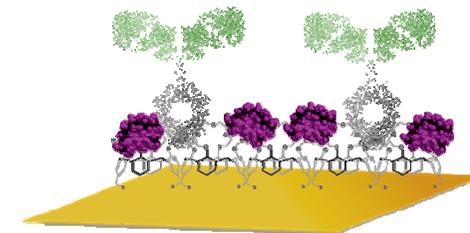
### Polyclonal antibodies



### Monoclonal antibodies



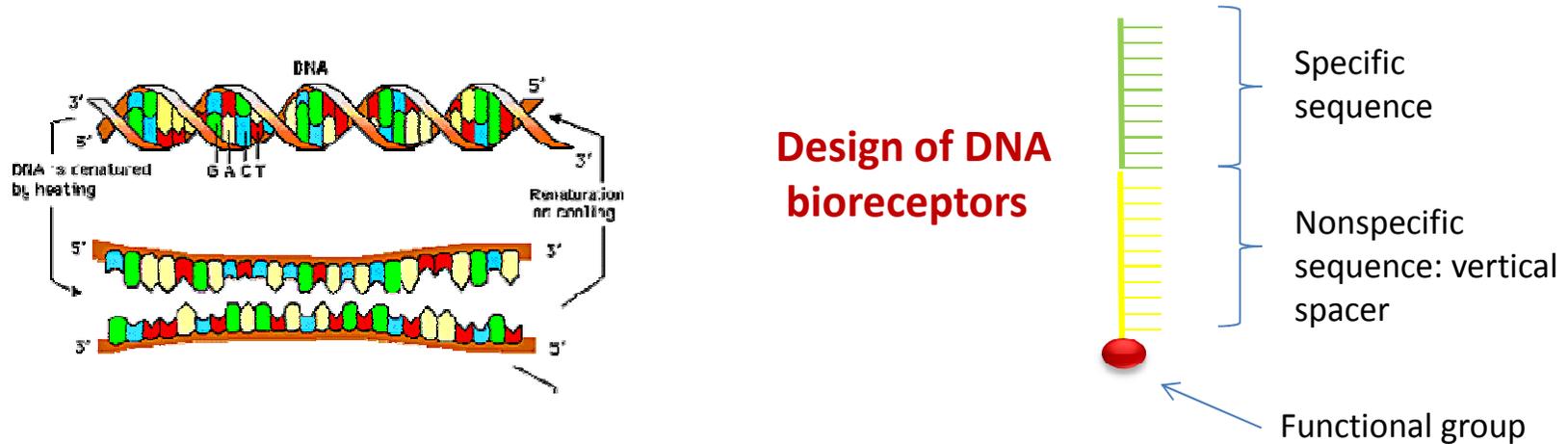
For high sensitive immunoassay **highly purified monoclonal antibodies** are usually preferred.



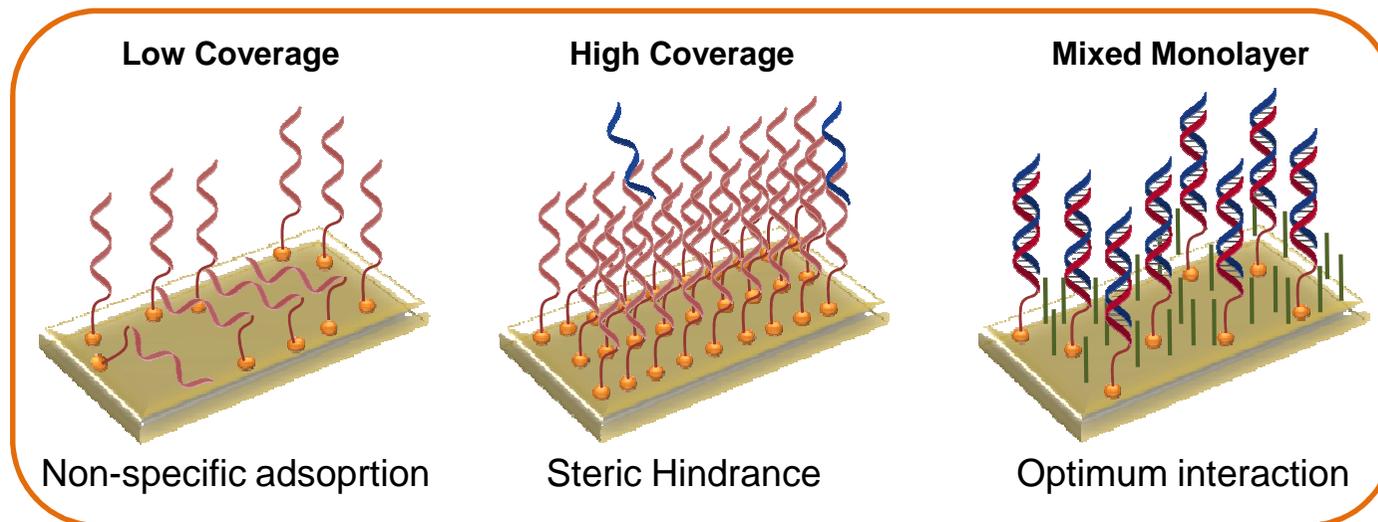
- Oriented binding
- More than one step
- Immobilization stability (?)

# Biosensors: Biology

## Selective bioreceptors for detection of nucleic acids: the complementary strand

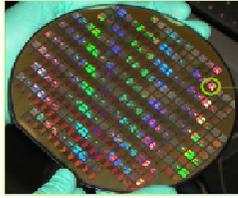


### Importance of the DNA receptor coverage on the surface



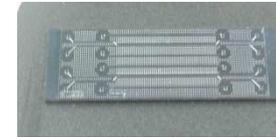
# Biosensors

## From the transducer to the bioapplication



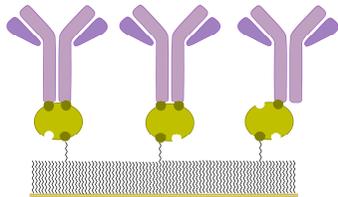
**Device physics and engineering:**  
modeling, fabrication and characterization

**Sample handling:**  
Microfluidics  
Pretreatment chambers



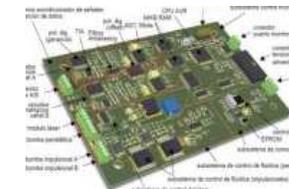
**Surface biofunctionalization:**

Modification of the surface  
Bioreceptor  
Nonfouling surfaces

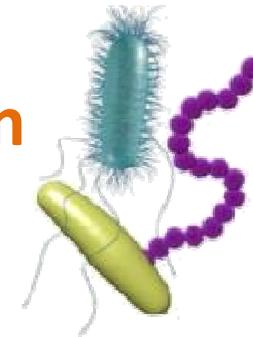


**BIOSENSOR**

**Electronics:**  
Software&hardware



**Bioapplication**



# Biosensors: Bioapplications

## Biological Samples for Diagnosis Assay



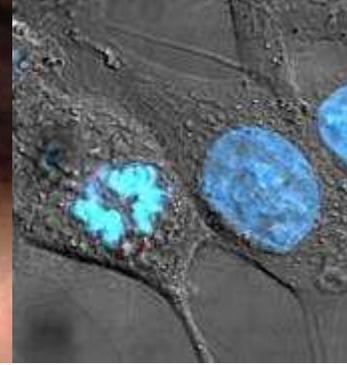
Blood/Serum



Urine



Tears



Cells



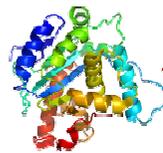
### Oligonucleotides



DNA  
RNA

- Circulating cell-free DNA
- microRNA contained in exosomes

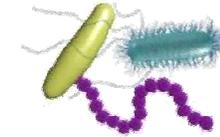
### Proteins



Hormones  
Antibodies

- Protein concentration in serum:  $\mu\text{g/mL}$
- Protein concentration in urine:  $\text{pg/mL}$

### Bacteria

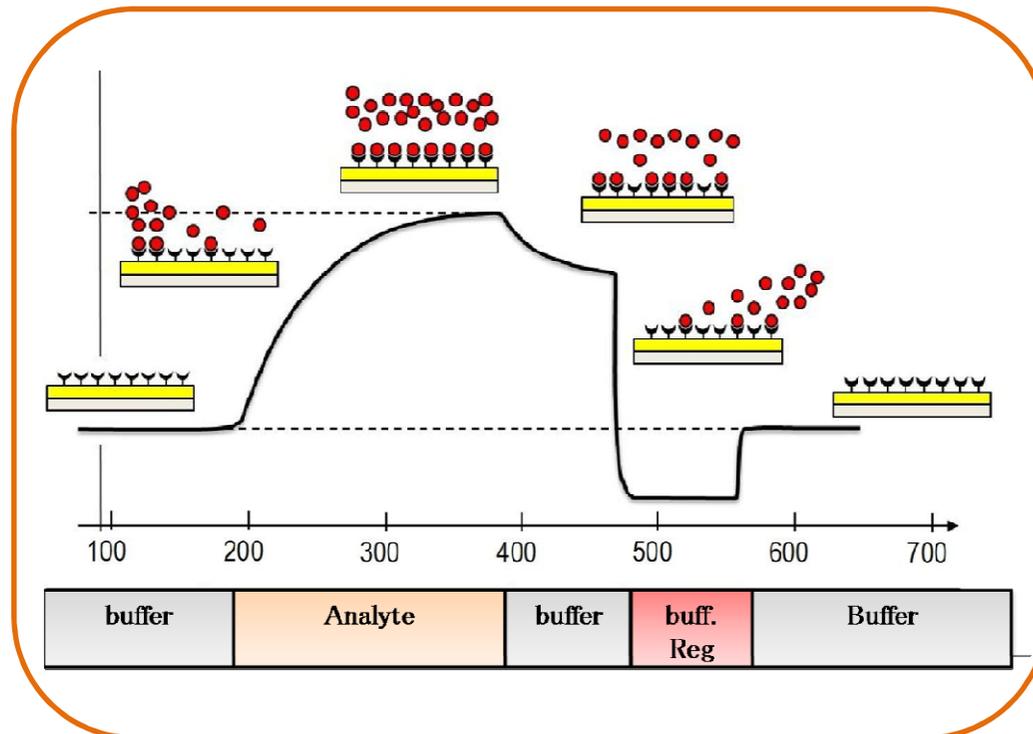


- Specific membrane antigen

**Biomarkers**

# Biosensors: the signal

## Optical biosensing: label-free evaluation in real samples

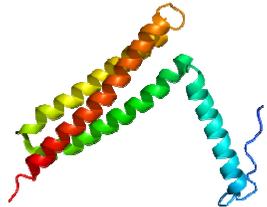


### Dealing with non-specific adsorption:

- ❖ Development of super-high blocking buffers.
- ❖ Dilute the sample in the buffer (50%).
- ❖ Development of high non-fouling surface chemistries.

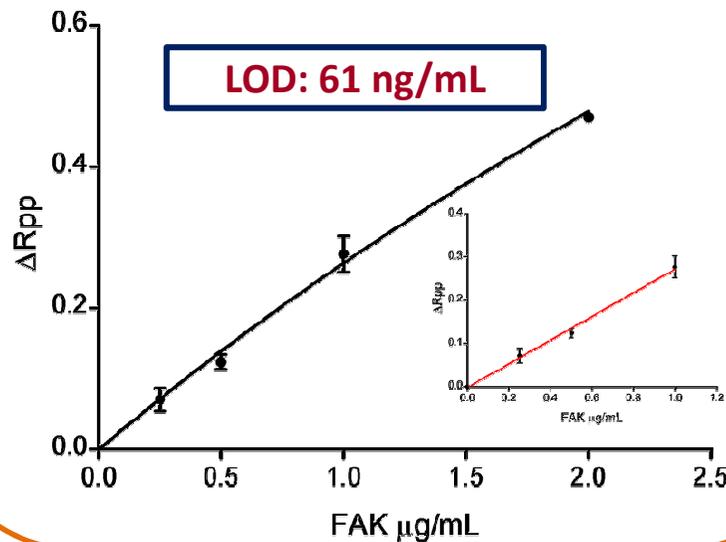
# Detection of protein cancer biomarkers

## Focal Adhesion Kinase (FAK)



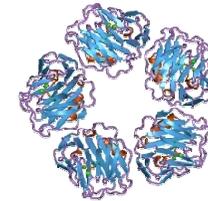
It is found in focal adhesions that form among cells attaching to extracellular constituents. Important key in cell migration.

Overexpression of FAK leads to inhibition of apoptosis and an increase in the prevalence of metastatic tumors.

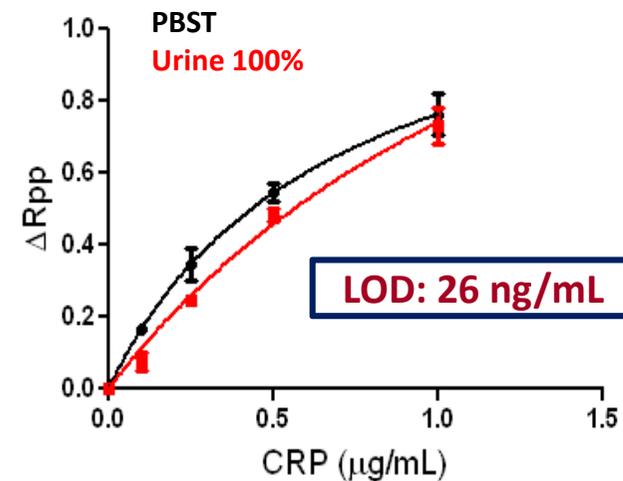


## C-Reactive Protein (CRP)

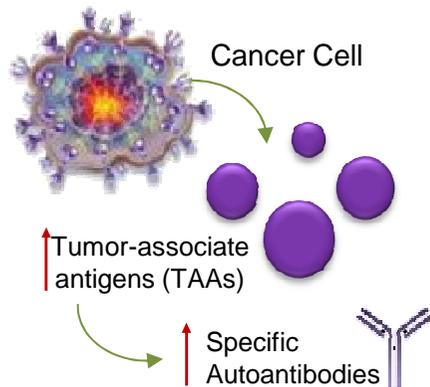
Levels rise in response to **inflammation**. Target role in cardiovascular disease and cancer.



Concentration in healthy serum <math><10 \mu\text{g/mL}</math>  
Pregnant, mild inflammation and viral infection: 10-40  $\mu\text{g/mL}</math>  
Active inflammation: 40-200  $\mu\text{g/mL}</math>$$



# Autoantibodies detection for early colon cancer diagnosis



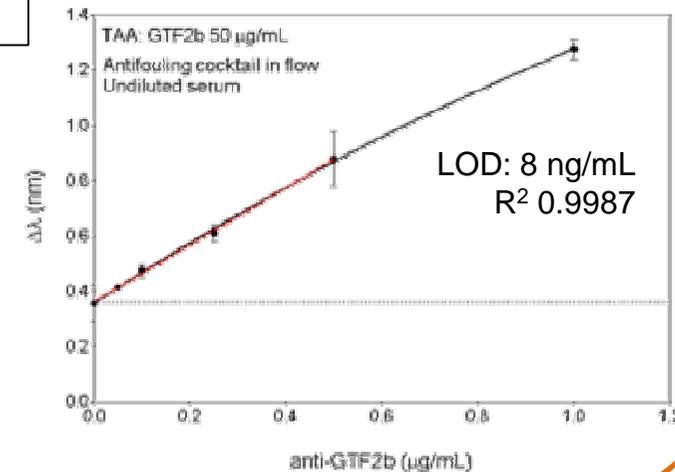
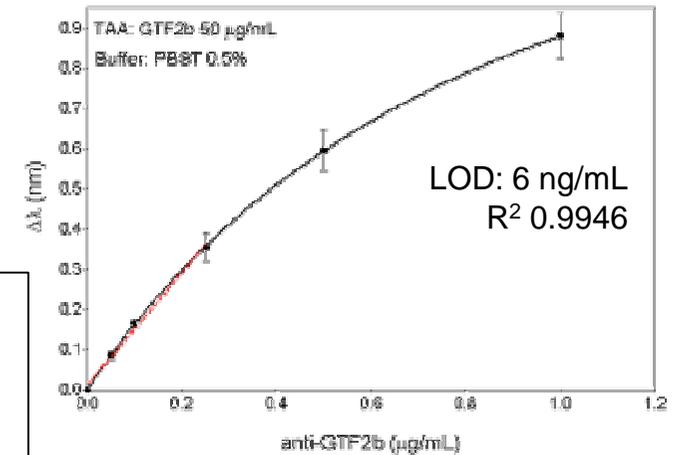
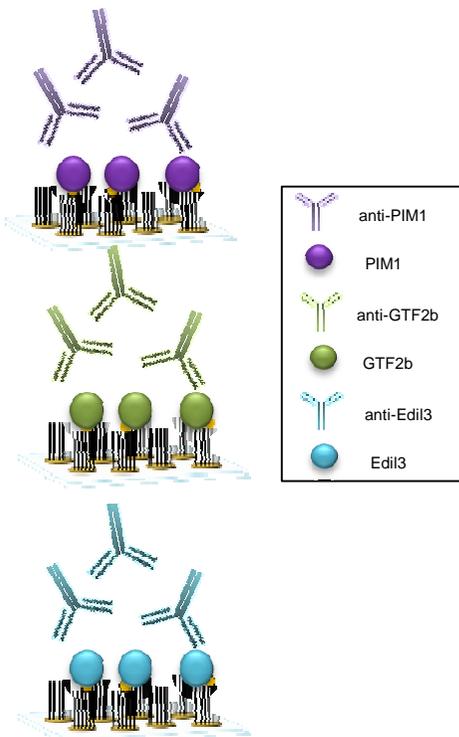
Autoantibodies are produced before adenoma formation, and they increase according to tumour progression.

## Colorectal Cancer TAAs

- **PIM1** MW: 33 kDa
- **GTF2b** MW: 34.8 kDa
- **Edi3** MW: 54 kDa

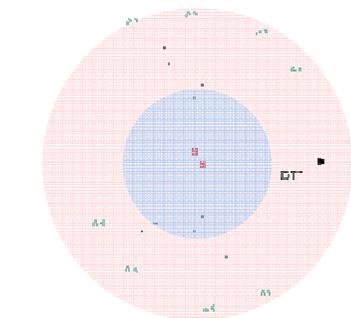
## Biosensor Results

### Direct immunoassay



# Dendrimer-based allergy diagnostic platform

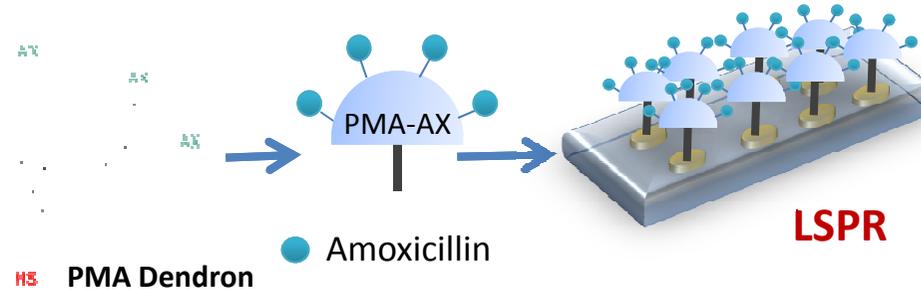
## Label-free strategy based on dendrimers as carrier molecule



PMA Dendrimer

### Undiluted serum

- Surface passivation: PLL-g-PEG
- High coverage of gold surface: SH-PMA-AX
- Antifouling cocktail: PBS + 2% Tween 20 + 2% Serum



HS PMA Dendron

● Amoxicillin

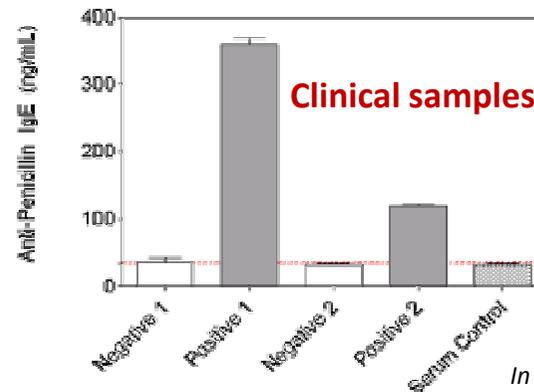
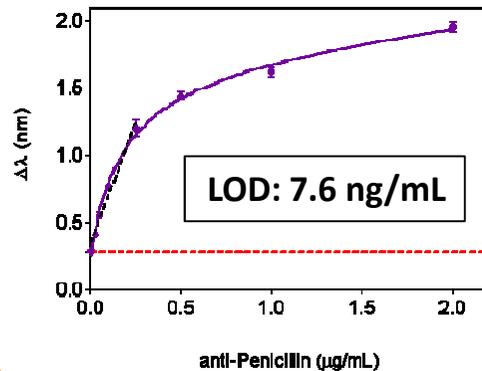
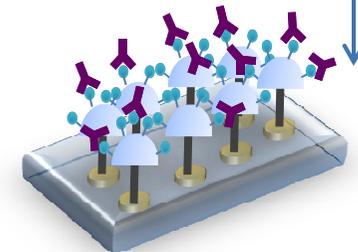
LSPR

IgE Serum Levels: [100-1000 ng/mL]  
IgE in patients > 290 ng/mL

## Determination of IgE in serum



IgE patient



In collaboration with E. Inestrosa (Univ Málaga and BIONAND)

# Gluten detection in celiac patients

## Celiac Disease Monitoring

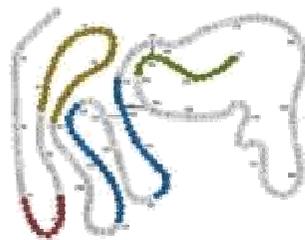


GLUTEN FREE

Usual ingestion of small quantities of gluten can lead to serious injuries in Celiac Disease patients

## Gliadin 33-mer Peptide

LQLQPFQPQLPYQPQLPYQPQLPYQPQPF



Resistant to digestion process  
Detectable in urine or faeces

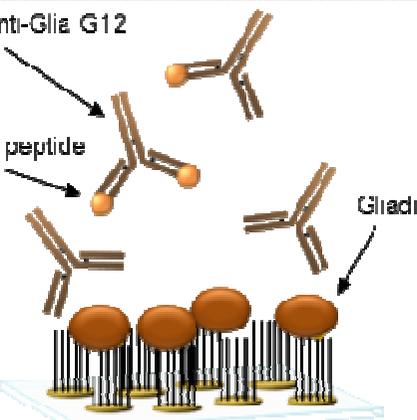
## Nanoplasmonic Biosensing

mAb anti-Glia G12

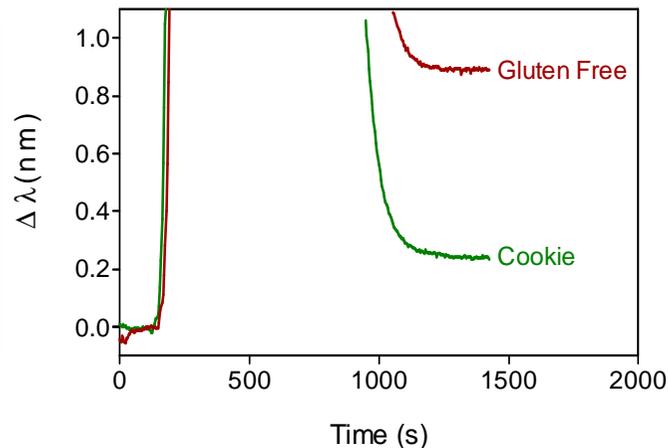
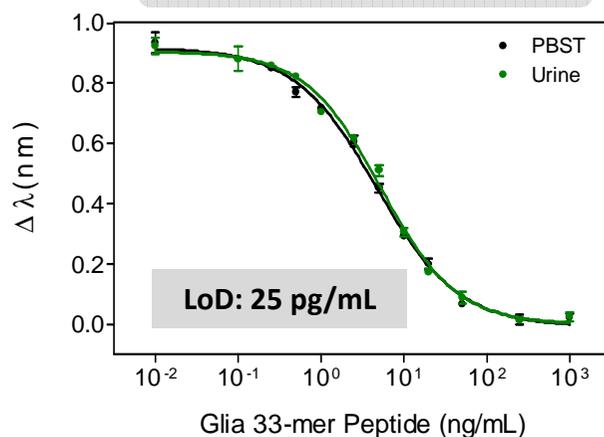
33-mer peptide

Gliadin

LSPR



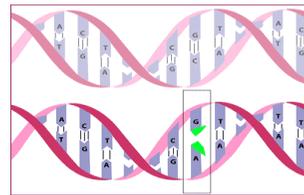
## 33-mer Detection in Urine



In collaboration with BIOMEDAL, SL (Sevilla)

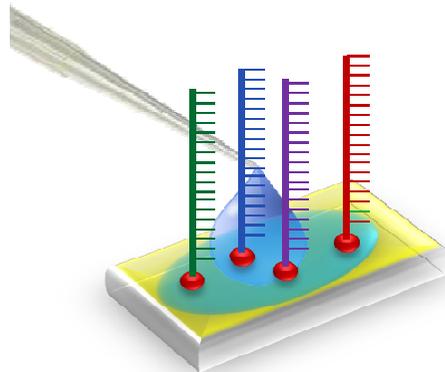
# Single-point mutations for early cancer diagnostics

## Detection of Single Point Mutations related to inherited breast cancer gene BRCA-1



Sequence with a mismatch

*Ex-situ* immobilization of the SH-DNA sequences

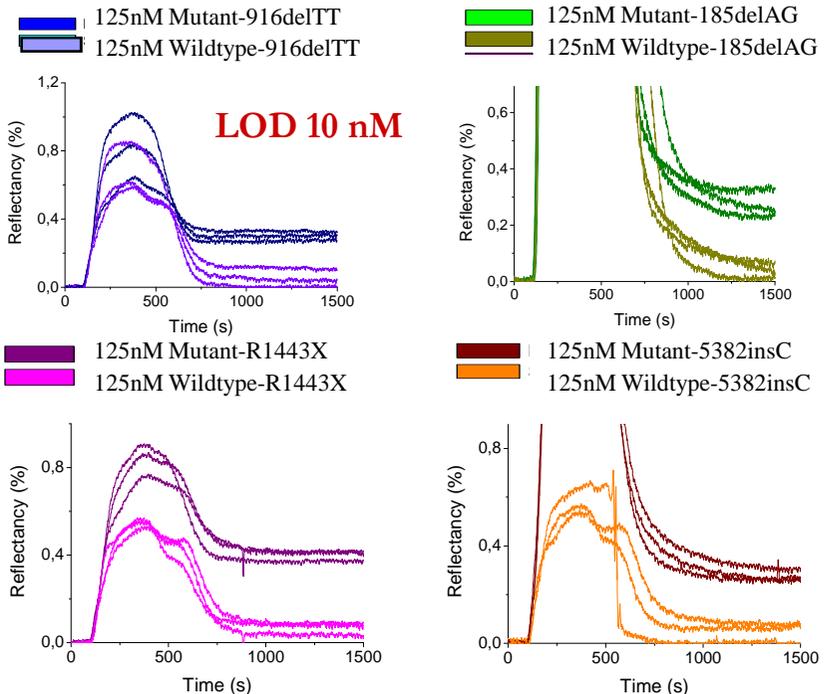


### Control of DNA-receptor monolayer

Lateral spacers: DNA spacers

Vertical spacers: insertion sequence of 15 thymidines

### Mismatch screening on PCR targets



Hybridization on SSC 5X and 5% Formamide

Anal Bioanal Chem. 2009. 393(4):1173-82

PCCP 2010, 12: 3301-3308

Eur Biophys J. 2010 Sep;39(10):1433-44.

# Enhancing sensitivity using the BiMW device

## BiMW

LOD: 8 pg/mL, 160 fM

IC<sub>50</sub>: 35 ng/mL

Linear range: 680 pg/mL- 4 µg/ml



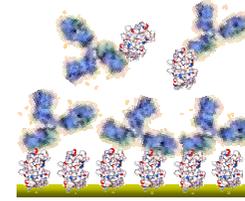
## SPR

LOD: 4 ng/mL, 200 pM

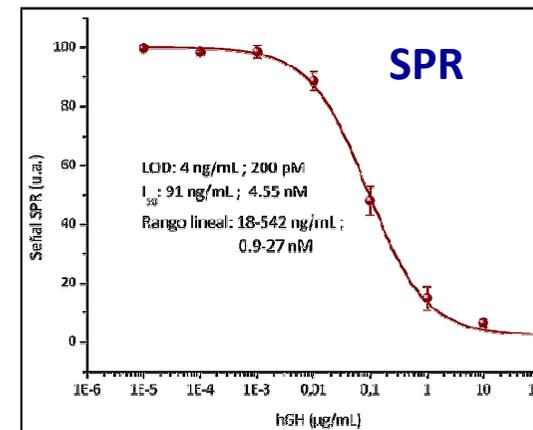
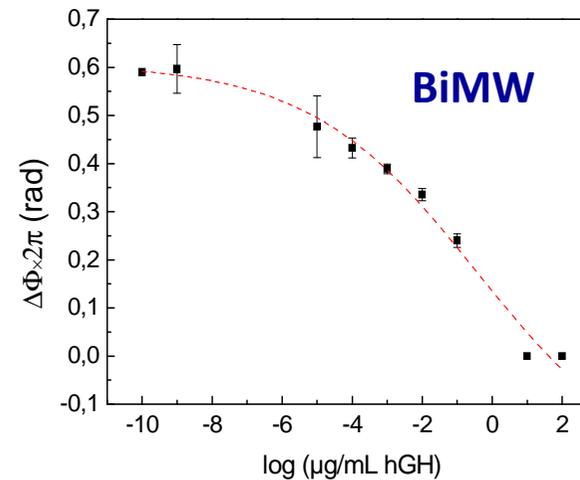
IC<sub>50</sub>: 91 ng/mL

Linear range: 18-542 ng/ml

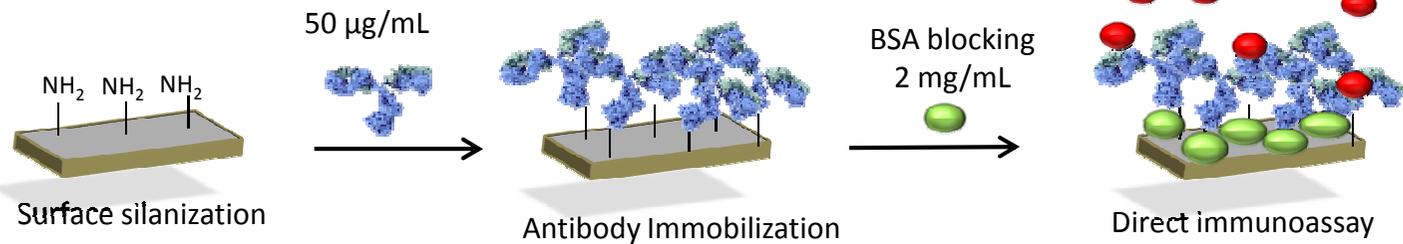
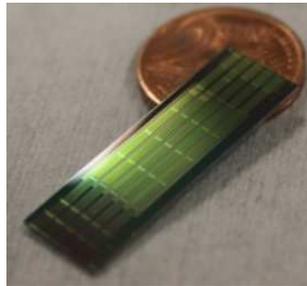
- The total assay time has been reduced to **13 min**
- The LOD has been improved **1000 times**
- The linear range has been widely increased



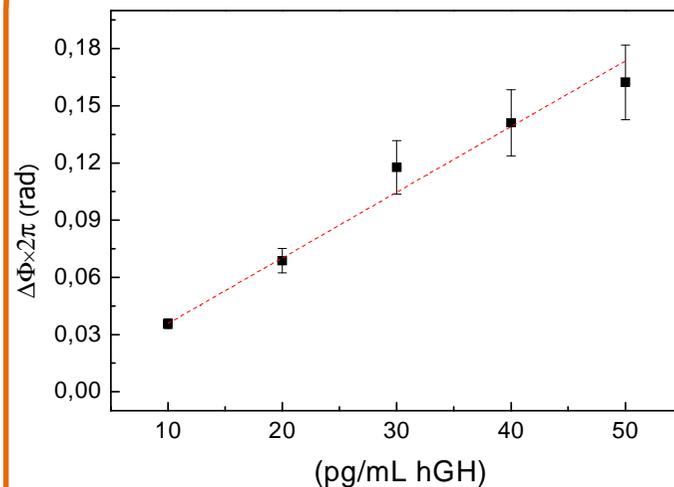
Inhibition  
immunoassay



# Detection of the Human Growth Hormone (hGH)

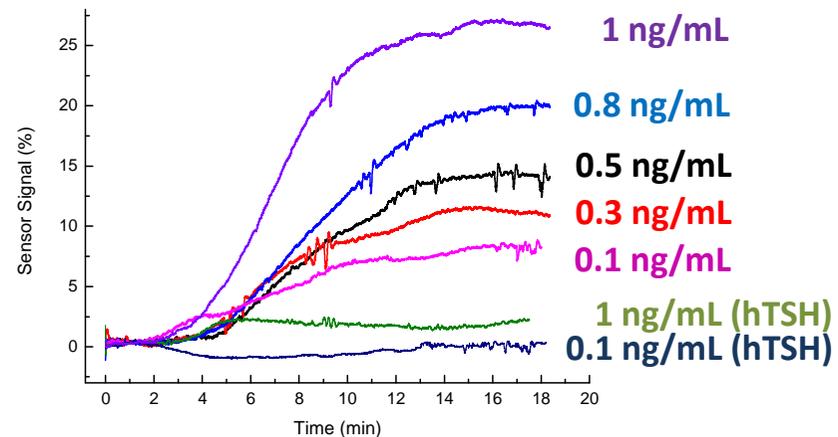


## Direct immunoassay: calibration curve



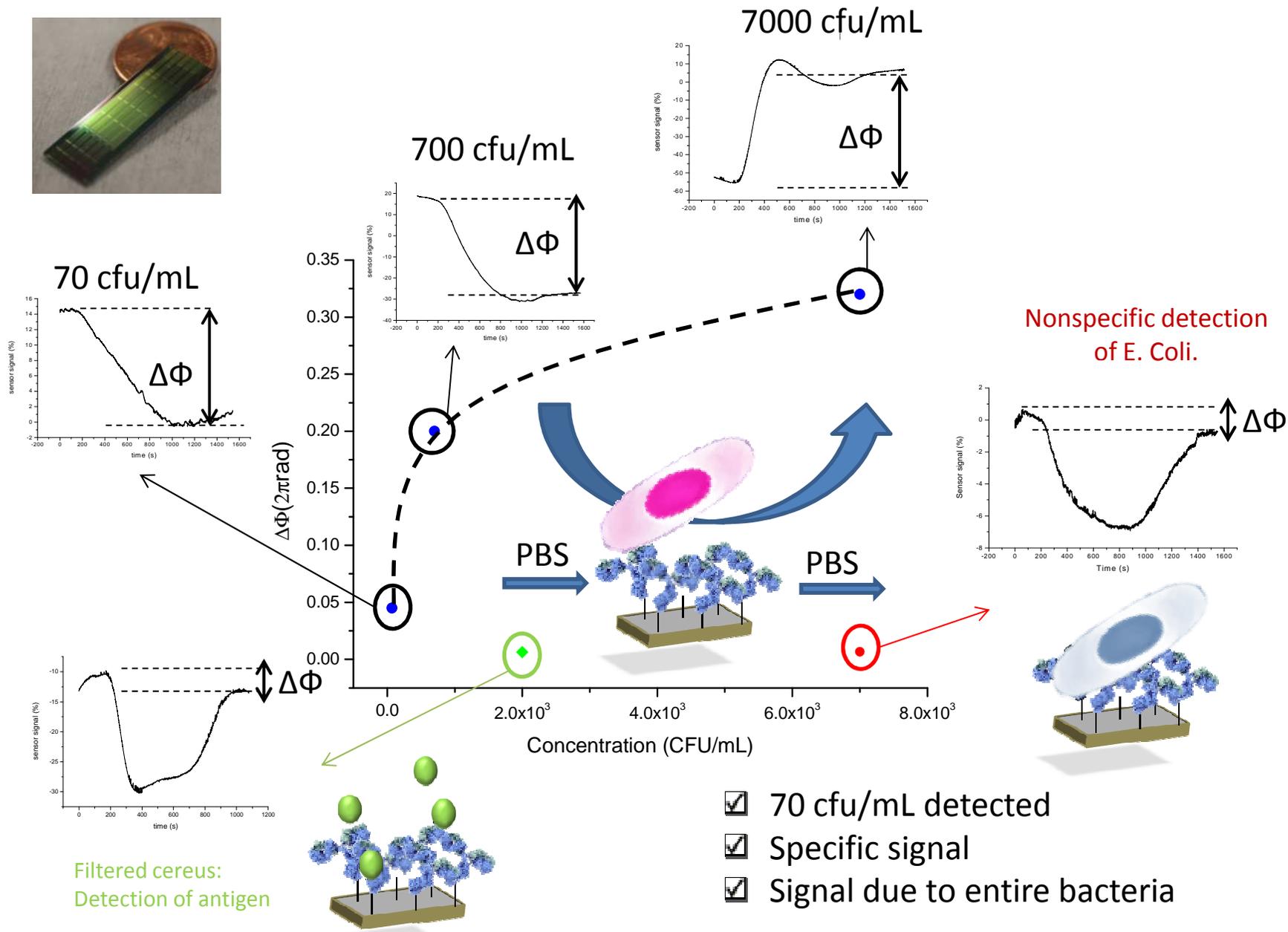
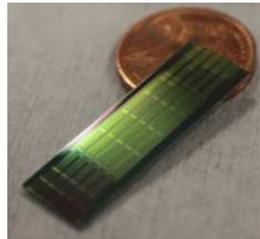
**LOD : 0.13 pg/mL (in buffer)**

## Detection in 100 % urine



**LOD : 3 pg/mL (in urine)**

# Detection of Bacillus Cereus by a direct immunoassay



# BiMW Lab-on-chip for direct bacteria detection

## Early Identification of liver diseases and infections in intensive care units

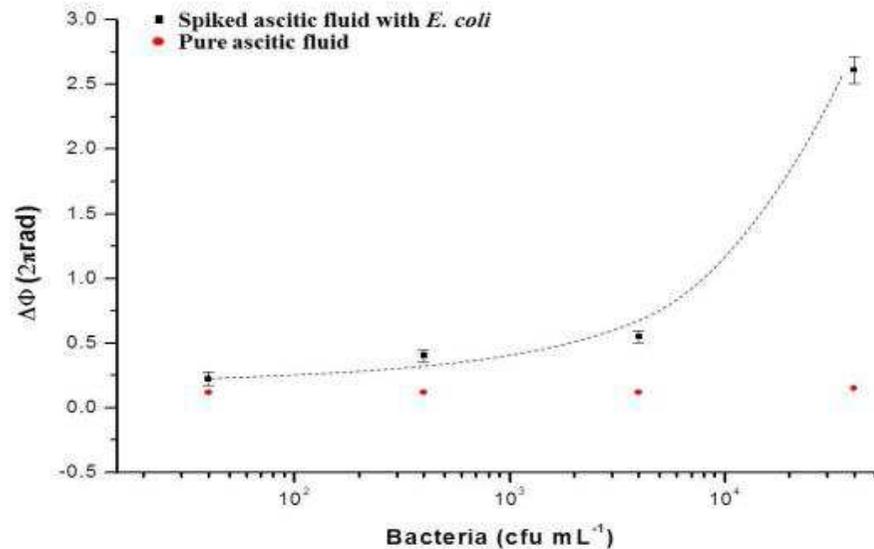
- 1.- Identification and quantification of bacteria
- 2.- Detection of the antibiotic resistant gene sequences



- Escherichia Coli
- Coagulase-negative staphylococci spp
- Pseudomona Aureginosa
- Staphylococcus aureus



### Direct detection of E. Coli in patients' ascitic fluid



**Detection limit: 40 cfu.mL<sup>-1</sup>**

### THEME [OCEAN 2013.1]

**Biosensors for real time monitoring of biohazard and man made chemical contaminants in the marine environment**

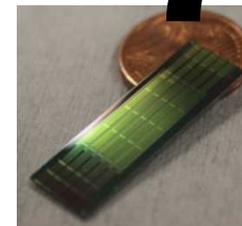
Project title: **Biosensors, Reporters and Algal Autonomous Vessels for Ocean Operation (BRAAVOO)**



The BRAAVOO concept: (i) standalone biosensor modules that are embedded in (ii) a data buoy, and (iii) an autonomous unmanned surveying vessel.

#### Analytes in sea water

- Organo-halogenated compounds  
**Pentabromodiphenyl ether (BDE-47)**  
*(Negotiation with Abraxis, Inc)*
- Marine aquaculture antibiotics  
**Ampicillin, Tetracycline**
- Antifouling paint booster biocides  
**Irgarol 1051**
- marine toxin biohazards from algal blooms  
**Domoic acid, Okadaic acid**



# Development of low-cost point-of-care test for Diagnose Disease in the Developing World



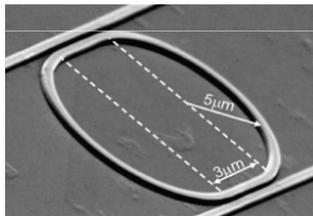
## Tuberculosis detection: POCKET PROJECT

### Objective:

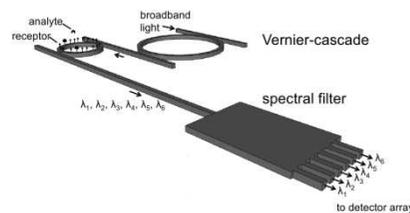
The development of a low-cost and high accuracy point-of-care urine test for the detection of tuberculosis by a direct and label-free immunoassay.



### Photonic transducer



### Integrated Device



### Biological system

Lipoarabinomannan (LAM)/antiLAM IgG  
Ag85 complex proteins/antiAg85 IgG

Real Samples evaluation in Africa and Asia



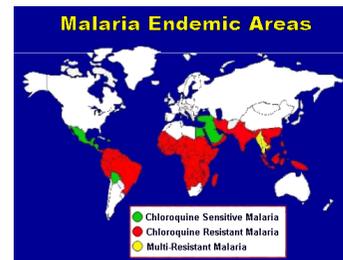
## Malaria detection



### Objective:

The development of rapid test for the detection of malaria by the expression of aldolase isoenzymes.

### Malaria Endemic Areas



### The parasite: plasmodium



### Biological system

pAldo from *p. falciparum*  
pAldo from *p. vivax*  
anti pAldo



NATIONAL  
BIOPRODUCTS  
INSTITUTE

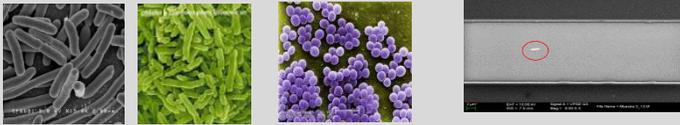
# Bioapplications in progress

## Implementation of a photonic lab-on-a-chip platform for early identification of liver diseases and infections in intensive care units



- 1.- Detection of bacteria in ascites liquid using the BiMW
- 2.- Detection of the antibiotic resistant gene sequences

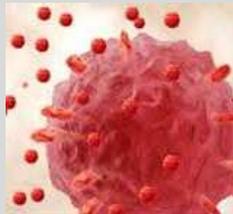
- Escherichia Coli
- Coagulase-negative staphylococci spp
- Pseudomona Aureginosa
- Staphylococcus aureus



## Detection of exosomes and RNA contained for personalized cancer therapy



- 1.- Quantification of exosomes by an immunoassay using BiMW
- 2.- Detection of mutations in RNA derived from oncogene expressed in glioma



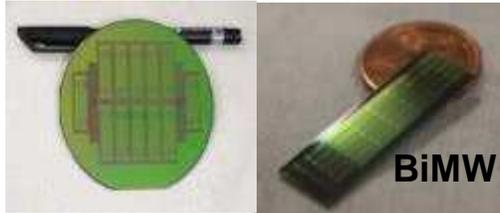
Oncogene	Alteration presented
EGFR.....	Amplification
TP53.....	Mutation
PTEN.....	Mutation

# nanoB2A Group

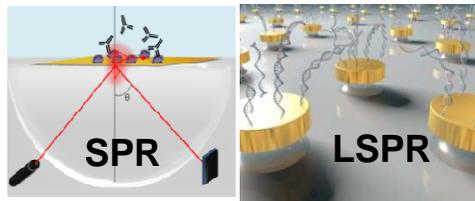
## From the lab to the market

### Proprietary Technological platforms

#### Integrated Interferometers



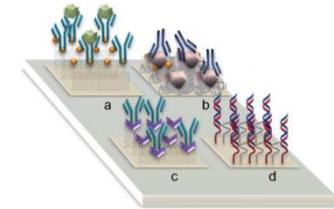
#### Plasmonics



- Design, optimization, fabrication and characterization of the devices

### Biofunctionalization protocols

- Tailor-made functionalizations for each applications
- Non-fouling surfaces for real sample analysis
- Oriented bioreceptor strategies for enhancing sensitivity



### Real Sample Evaluation

#### Clinical

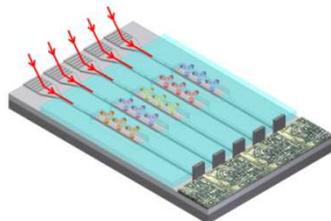


#### Environmental



### Operational prototypes

#### Full integration in portable point-of-care platforms



- Microfluidics
- Software and Electronics
- In-Out Coupling of light
- Modulation system
- Pretreatment chambers: microsonicator channel, blood filter unit, etc

### Technology transfer

- Packaging and storage of biofunctionalized chips
- Development of microfluidics at wafer level compatible with biofunctionalization
- Patterning/spotting of bioreceptors at wafer level

- SENSIA, SL (2004) (Sold in 2012)
- BiMW tech transfer to Industry (2014)

- **Nanobiosensors and Bioanalytical Applications Group (CIN2-CSIC)**

ers, molecular biologist, biotechnolo

- **Finantial funding from:**

**M. Botín Foundation**

- **Spin-offs:**  
**SENSIA, SL**  
**BIOD, SL**



<http://nanob2a.cin2.es>

