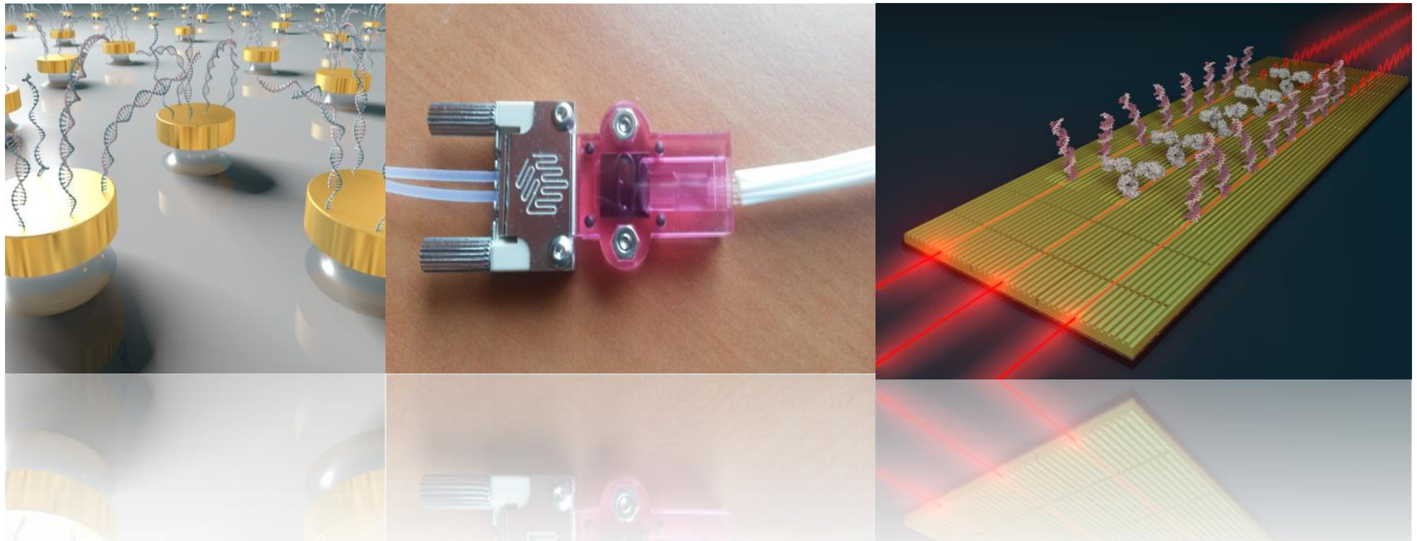
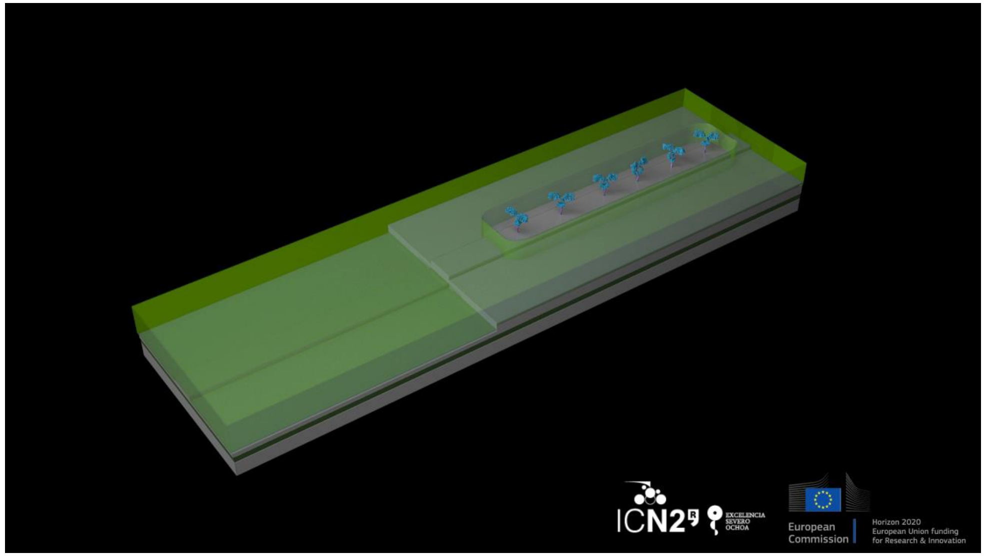
Nanophotonic biosensors for ultrasensitive and label-free diagnostics at the Point-of-need





Prof. Laura M. Lechuga
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CSIC, BIST & CIBER-BBN
Barcelona, Spain

@NanoB2A_group

nanob2a.icn2.cat

Clinical Diagnostics: The Problem

Lessons learned from COVID-19 Pandemic

Centralized Diagnostics

In Europe, 1 Million cancer cases have gone undiagnosed due to the Pandemic

Long times to get a PCR result…..

Long lines to get a PCR analysis…..

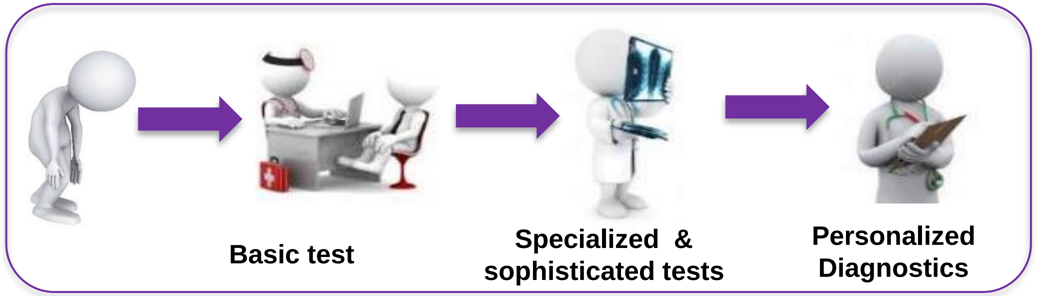
Test COVID-19

Pandemic has raised awareness of in-home rapid testing amongst patients. Home testing existed for diabetes, pregnancy and HIV, but these were used by specific population groups.

Impact of COVID-19: More, better, faster growth in POC rapid testing

Clinical diagnostics: Problem & Solution

Centralized Diagnostics



- ELISA
- PCR
- Microbiology culture
- Chromatography
- Mass spectrometry
- Imaging techniques

Excellent lab diagnostics techniques but….

- Time consuming
- High Sample volume
- Trained personnel
- Lab installations
- Bulky/expensive instrumentation

Main Goal in Diagnostics (…..*in the post-pandemic times…*)

Drop of sample

Abbott's FreeStyle Libre

Point-of-care (POC) device

Pesonalised Treatment

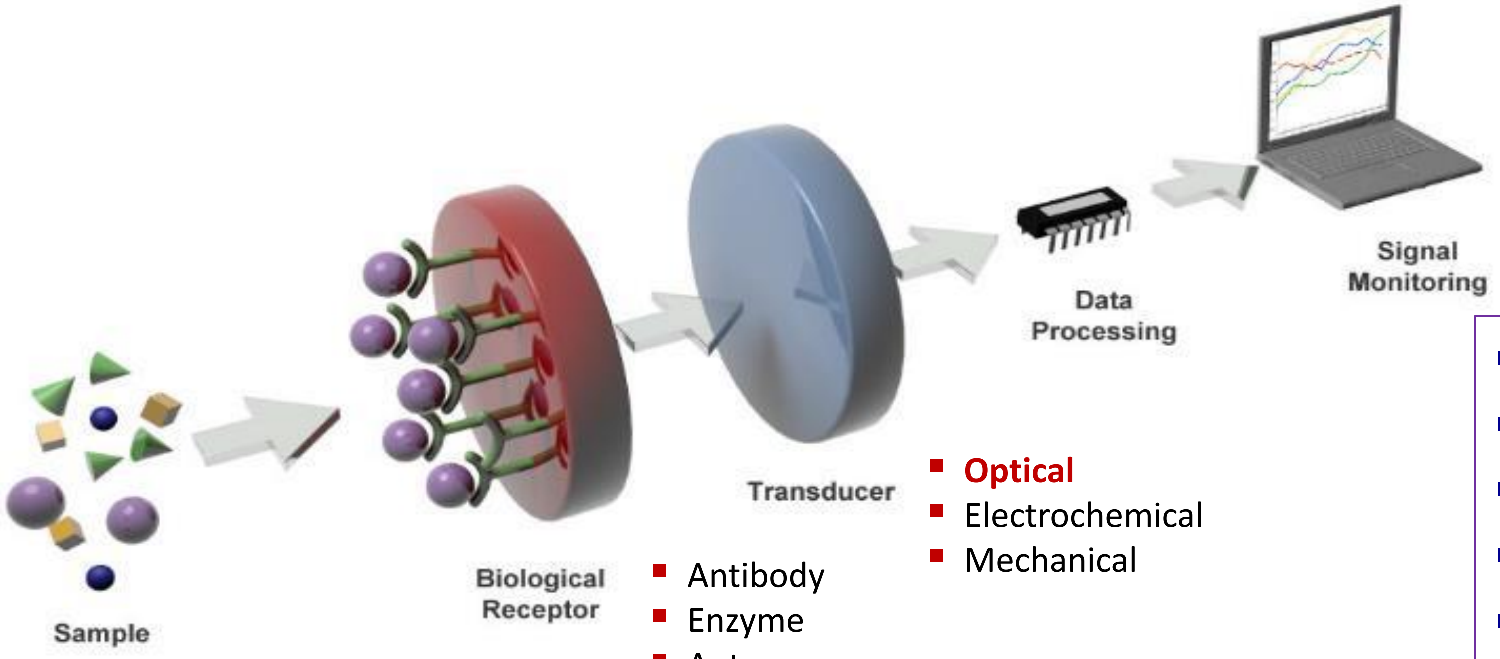
- Easy diagnostics
- High sensitivity and Fast
- Reliability and Quantitative
- Multiplexing capabilities
- User-friendly/minimum operation
- Minimum sample
- Competitive cost

BIOSENSORS provide the possibility to create <u>**POINT-OF-CARE**</u> devices containing the functionalities of an analytical laboratory

Decentralized Diagnostics

Same day test-to-treatment solution

BIOSENSOR DEVICE



- Antibody
- Enzyme
- Aptamer
- Oligonucleotide

- Optical
- Electrochemical
- Mechanical

- FAST
- DIRECT
- LABEL-FREE
- HIGH SENSITIVITY
- LOW SAMPLE VOLUME

Glucose biosensor

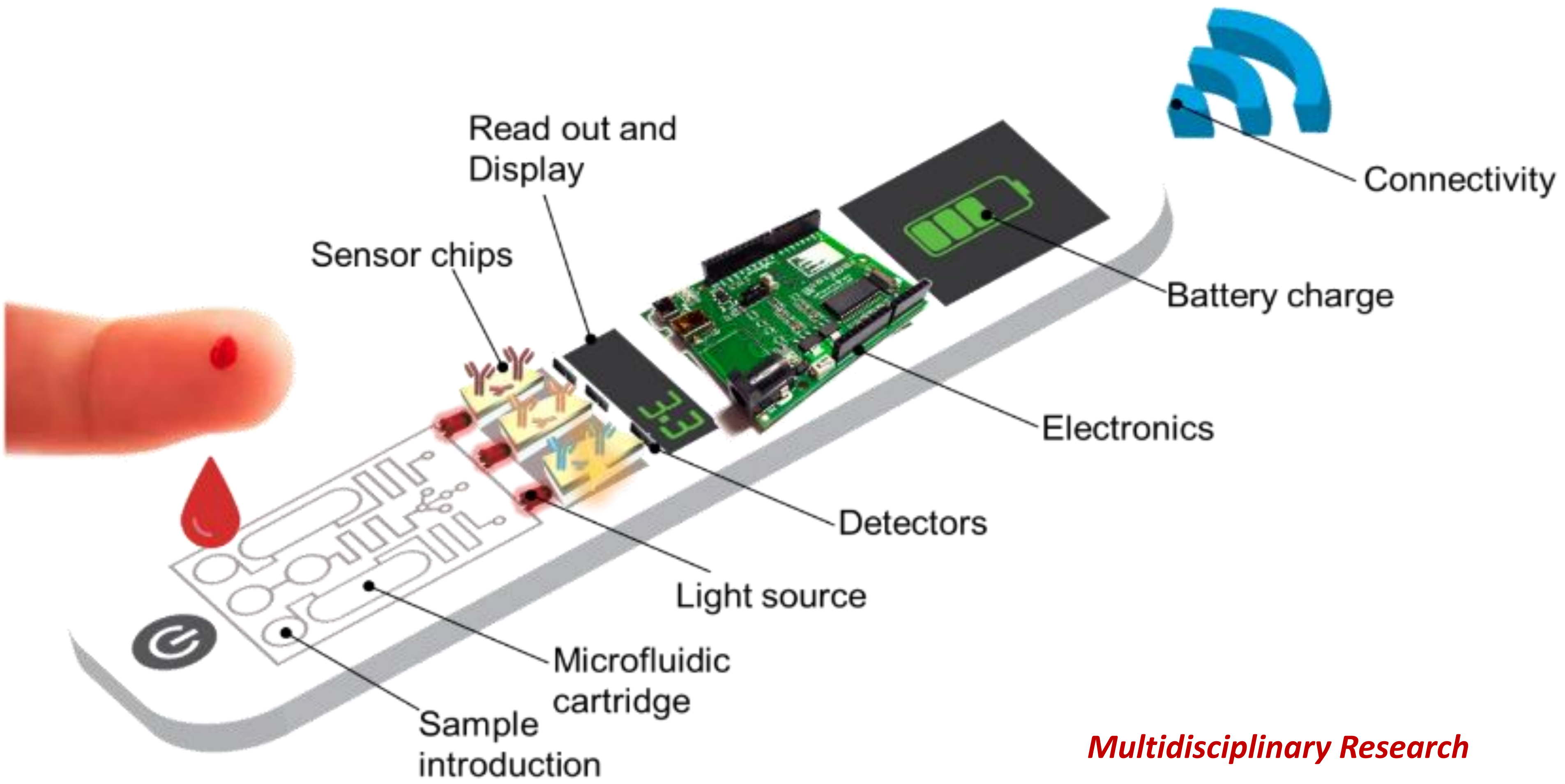
Abbott's FreeStyle Libre

Pregnancy Test

Test COVID-19

i-STAT biosensor

Point-of-Care Biosensor



Multidisciplinary Research

Biosensor applications & Market

ICU

Emergency room

Ambulances

Family doctor´s offices

Rural clinics

Nursing homes

Home testing

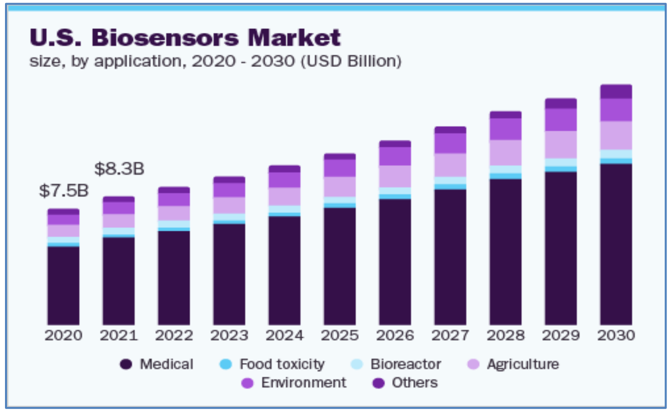
Pharmacies

Global biosensor market

USD **24.9 billion** in 2021

Annual growth rate (CAGR):

8.0% from 2022 to 2030



Making testing available to anyone who needs it

47% world population has not access to the diagnosis of common diseases

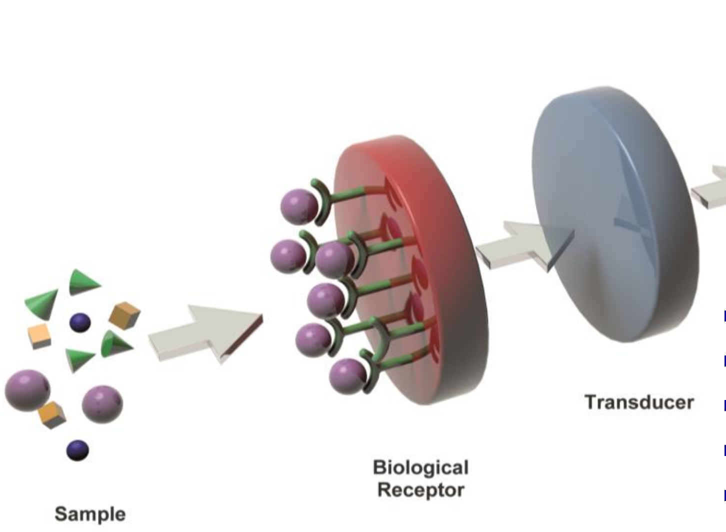
Environmental Control

Animal and livestock health management

Ocean Control

Food and farming control

Biosensor devices for POC diagnostics

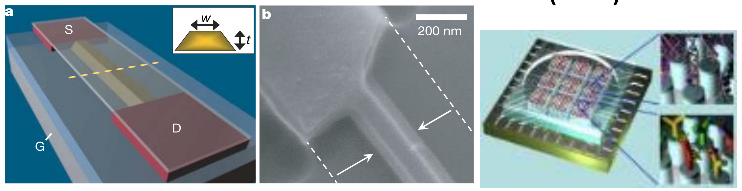


- FAST
- DIRECT
- LABEL-FREE
- HIGH SENSITIVITY
- LOW SAMPLE VOLUME

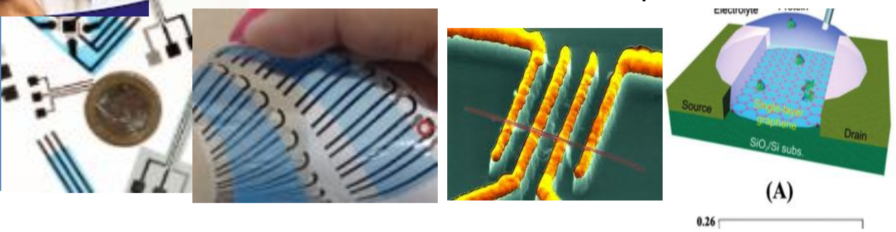
Electrochemical Biosensors

Glucose biosensor

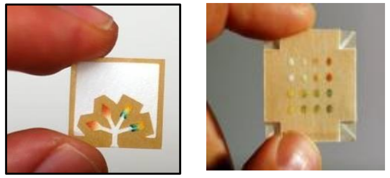
Silicon nanowires (FET)



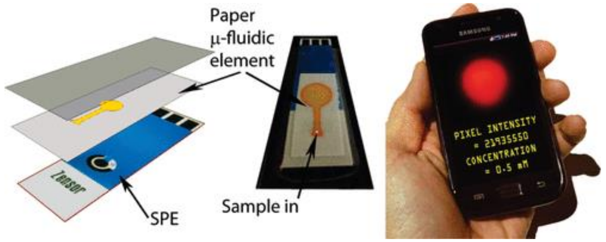
Carbon Nanotubes/Graphene



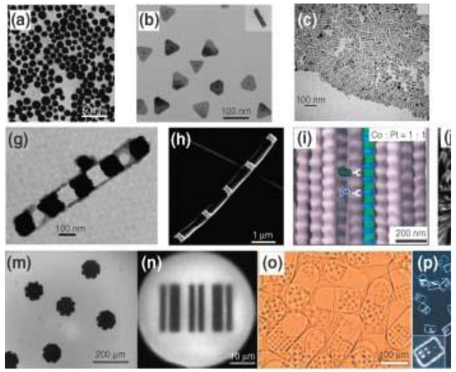
Microfluidic Paper-based Biosensors

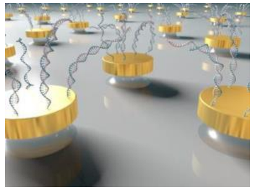


A 3-Cent
HIV Test

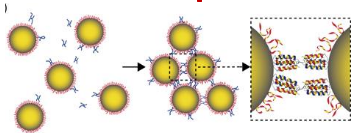


Biosensors based on Nanoparticles/Nanomaterials





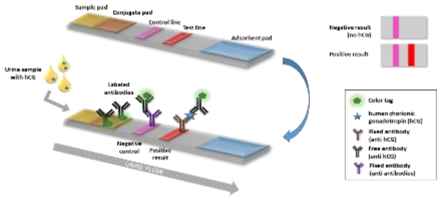
Au Nanoparticles



Lateral-Flow (LFA) based Biosensors

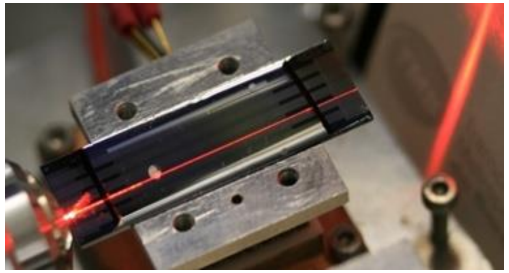
Pregnancy Test

Test COVID-19



PHOTONIC BIOSENSORS

Optical waveguide biosensors offer a unique opportunity for POC devices



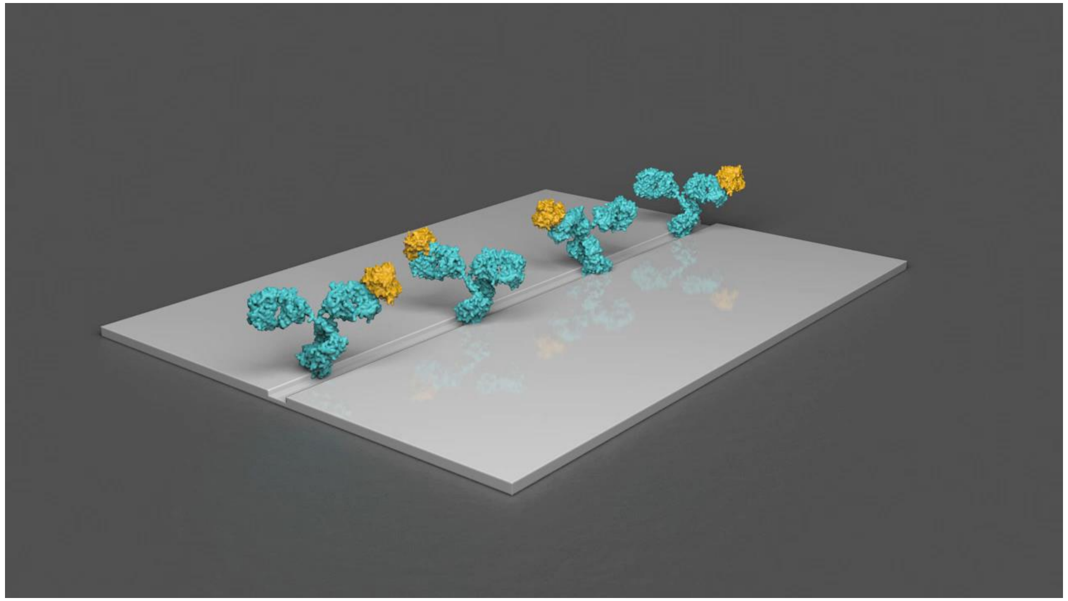
- Immunity to electromagnetic interferences
- **ULTRA SENSITIVITY**
- Miniaturization
- Integration in lab-on-a-chip
- Multiplexing
- **LABEL-FREE**
- **Real-time** analysis
- Quantitative information

BIOSENSOR OPTICAL PROBING

- **Phase change: refractive index change**
- **Amplitude Change**
  - **Absorption**
  - **Propagation**
- **Frequency Change**
  - **Nonlinear optical: 2nd harmonic/sum frequency generation**
  - **Raman**
  - **Fluorescence**
    - **Frequency domain**
    - **Time domain**

Evanescent Wave principle

refractive index change at the sensor surface



Binding event, ΔΓ

↓

$\Delta RI \sim (dn/dc)_{vol}\ \Delta\Gamma$

↓

Change of refractive index, Δn

↓

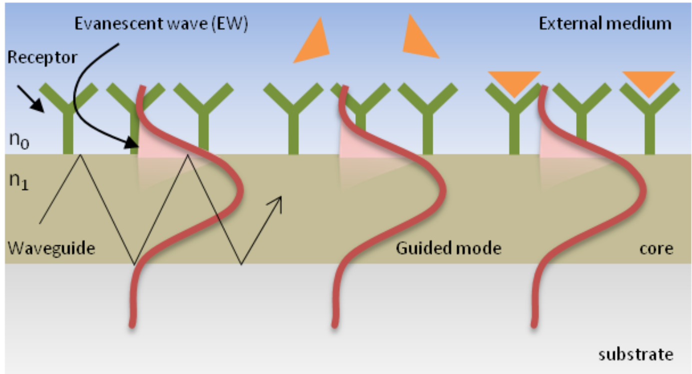
$\Delta p \sim \Delta\Gamma$

↓

Change of optical parameter, Δp

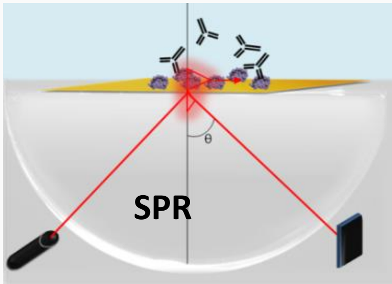
(Intensity,Phase, polarisation, resonant momentum,…)

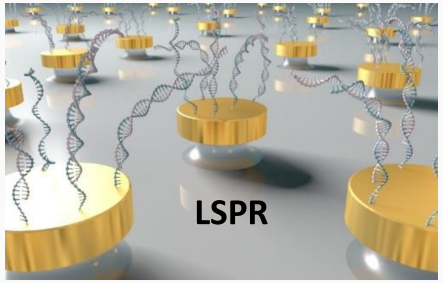
- Guided modes in dielectric media are not totally confined. EW sensing: 100-900 nm
- The part of the mode travelling "outside" the core "feels" whatever is "in the vicinity":
  High sensitivity, specially at the surface
- The analyte induces a local change of the refractive index. Direct measurement **(LABEL-FREE)**
- **Real-time** (binding can be continuously monitoring). Small volume of samples.
- Detection of **selective** biomolecular interactions. A **bioreceptor layer** is required.



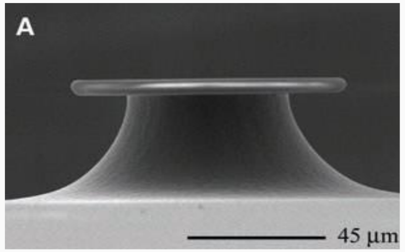
STATE-OF-THE-ART: PHOTONICS BIOSENSORS

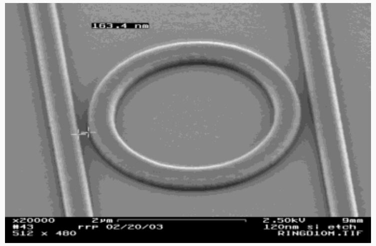
(Nano)Plasmonic sensors



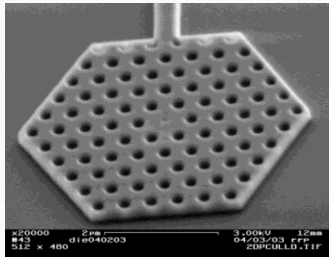


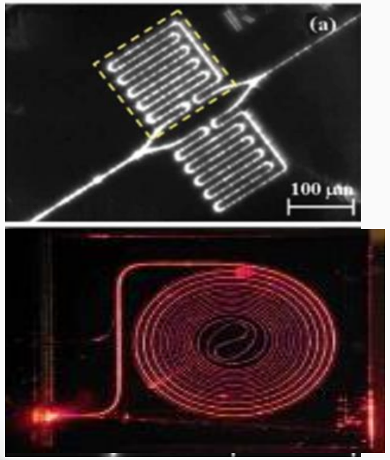
Microring resonators



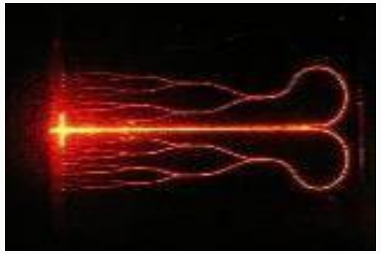


Photonic crystals

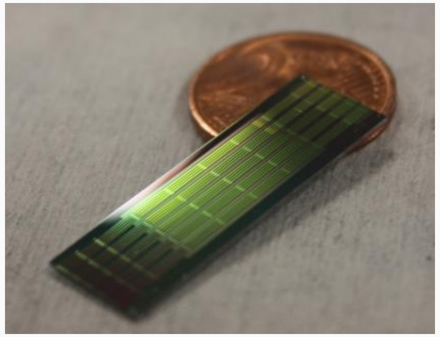




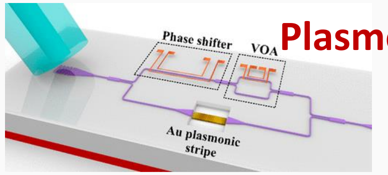
Silicon wires, slot, subwavelength WG

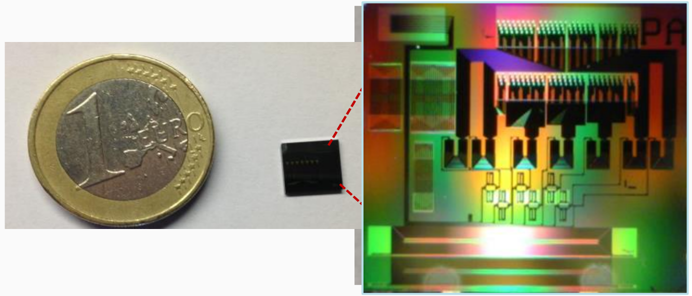


Interferometers



Plasmo-Photonics





Limit of detection: 10^{-6}-10^{-8} RIU

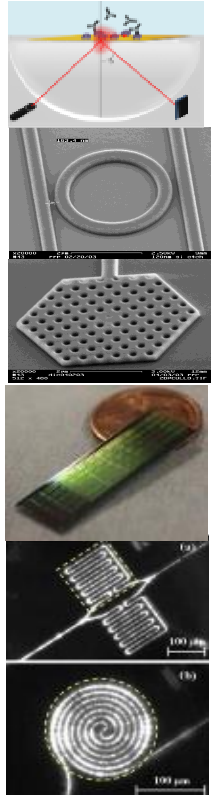
Laser & Photonics Reviews 6, 463-487 (2012)
Analytical Chimica Acta 806, 55-73 (2014)
Analytical Methods 8, 8380 – 8394 (2016)
Sensors 16(3), 285 (2016)
Nanophotonics 6,123–136, (2017)
Optics and Photonics News 31 (4), 24 (2020)
Optics Letters 45 (24), 6595 (2020)
J. Applied Physics 129 (11), 111102 (2021)

EW BIOSENSORS
- **(Nano)Plasmonics biosensors**
- **Silicon Photonics biosensors**
  - **Interferometric Architecture**
  - **Resonant Architecture**

PHOTONIC BIOSENSORS: Sensitivity comparision

STATE-OF-THE-ART: PHOTONIC BIOSENSORS

BIOSENSORS	Detection limit (pg/mm²)	Bulk Sensitivity (Δn, RIU)
Plasmonics (SPR, LSPR)	0.1-1	10^{-5}-10^{-7}
Grating couplers	0.3	2.10^{-6}
MZI interferometer	0.01-0.06	$1{\cdot}10^{-7}$-$2{\cdot}10^{-8}$
BiMW interferometer	0,01	$1{\cdot}10^{-8}$
Young interferometer	0.013-0.75	$9{\cdot}10^{-8}$-$9{\cdot}10^{-9}$
Microring resonators	1.5-3	5.10^{-6}-$7{\cdot}10^{-7}$
Photonic crystals-based	0.4-7.5	$\sim 10^{-5}$
Silicon wires-based	0.25	$2.\ 10^{-6}$
Slot waveguides-based	0.9-16	$\sim 10^{-6}$



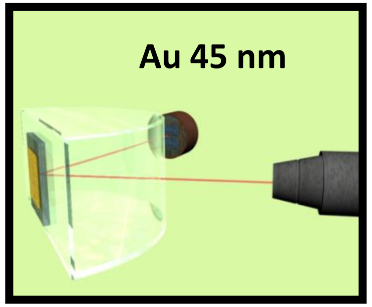
- **Plasmonics offer: simplicity, easy biofunctionalization protocols, relevant sensitivity, simple instrumentation**
- **Interferometers are the *most sensitive* ones**
- **SILICON PHOTONICS:**
  - **Ultrasensitivity**
  - **High multiplexing**
  - **Miniaturization, Integration, Portable**
  - **Mass production, Low cost, SINGLE USE**

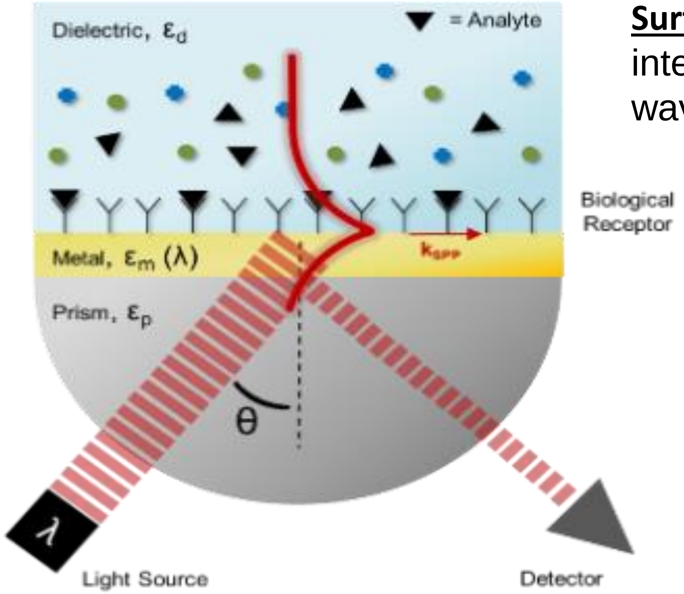
Laser & Photonics Reviews 6, 463-487 (2012)
Analytical Chimica Acta 806, 55-73 (2014)
Analytical Methods 8, 8380 – 8394 (2016)
Sensors 16(3), 285 (2016)
Nanophotonics 6,123–136, (2017)
Optics and Photonics News 31 (4), 24-31 (2020)

NANOPLASMONICS BIOSENSORS

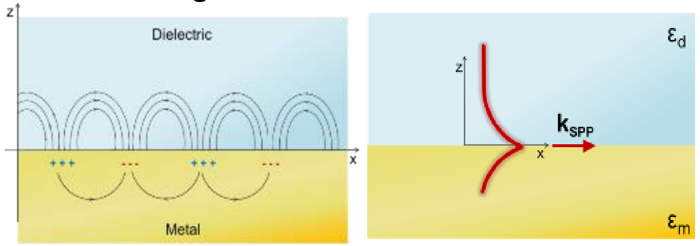
Surface Plasmon Resonance (SPR) Biosensor

SPR working principle



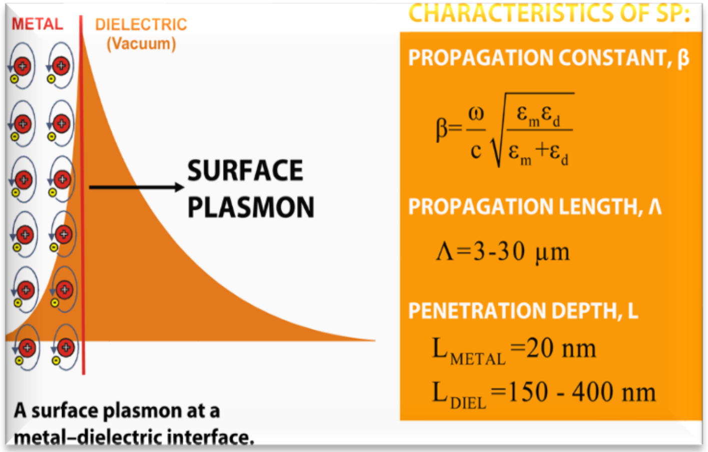


Surface plasmons: Collective oscillation of surface electrons at the interface of a metal and a dielectric that generates an evanescent wave sensitive to RI changes.



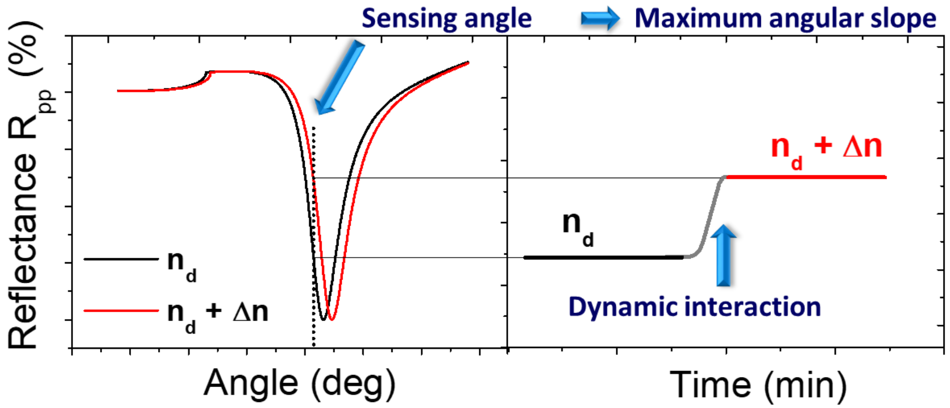
$$k_{spp} = \frac{2\pi}{\lambda} \cdot \sqrt{\frac{\varepsilon_m \cdot \varepsilon_d}{\varepsilon_m + \varepsilon_d}} = \frac{2\pi}{\lambda} \cdot \frac{n_m \cdot n_d}{\sqrt{n_m^2 + n_d^2}}$$

TM pol., $Re(\varepsilon_m) < 0$ y $Re(\varepsilon_d) < |Re(\varepsilon_m)|$

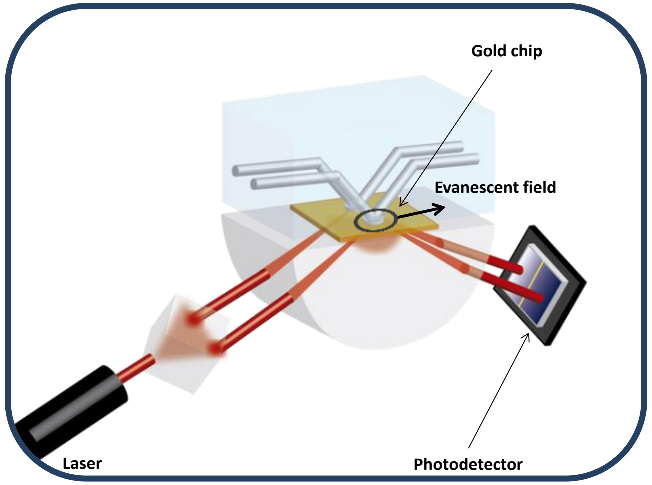


Adapted from J. Homola

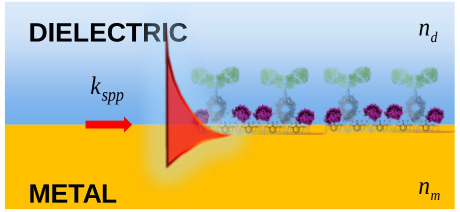
Principle of detection

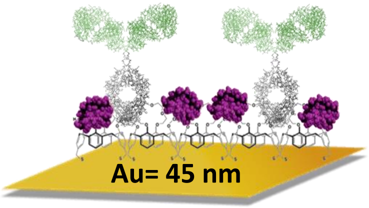


Surface Plasmon Resonance Biosensor (SPR)



SPR sensor chip





Penetration depth: 150-400 nm
Propagation length: 10-100 µm

$$k_{spp} = \frac{2\pi}{\lambda} \cdot \sqrt{\frac{\varepsilon_m \cdot \varepsilon_d}{\varepsilon_m + \varepsilon_d}} = \frac{2\pi}{\lambda} \cdot \frac{n_m \cdot n_d}{\sqrt{n_m^2 + n_d^2}}$$

TM pol., $Re(\varepsilon_m) < 0$ y $Re(\varepsilon_d) < |Re(\varepsilon_m)|$

Most developed and commercially available optical biosensor (more than **25 companies** worldwide, as BIACORE, BioNavis, Horiba)

(hundreds of thousands of citations…)

- Versatility: analysis of any type of biomolecular interaction
- Robustness and simplicity
- Sensitivity: LOD pM-nM (~10^{-6}–10^{-7} RIU)
- Affinity and kinetic studies
- Widespread technique and commercially available

POC- SPR Platforms

2-channels SPR

Tablet control

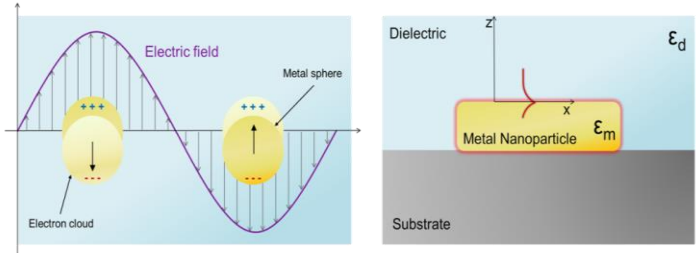
- **Complete in-house design and assembly**
- **Miniaturized & compact platforms**
- **User-friendly**
- **Gold Sensor chip production**

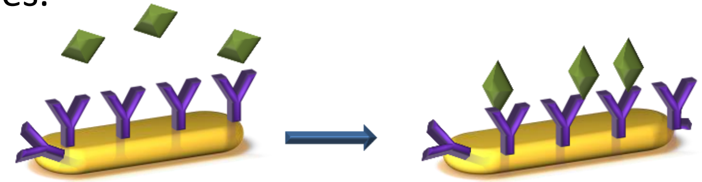
SPR Limitations

- ✖ Limited miniaturization
- ✖ Reduced multiplexed capabilities

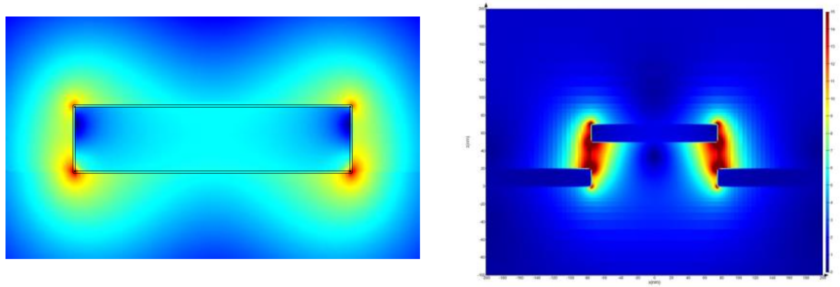
Localized Surface Plasmon Resonance (LSPR) Biosensor

Localized Surface Plasmon: Oscillation of surface electrons of metal nanoparticles (Ø < λ) inducing a dipolar field that generates an evanescent wave sensitive to RI changes.

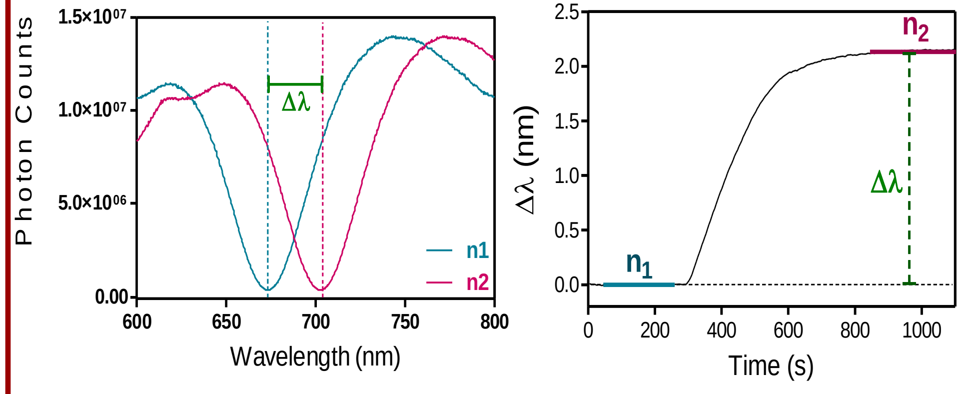




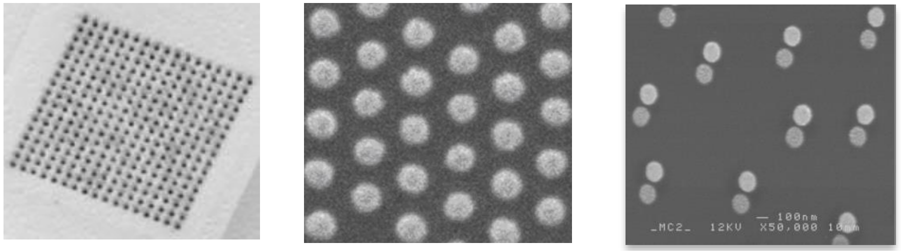
- **Evanescent Field Penetration:** 30-50 nm
- **Light Coupling:** not required
- **Size, shape and embedded medium:** resonance can be tuned, numerous sensor schemes
- High degree of **multiplexing**



Wavelength displacements (Δλ)



Nanoholes **Nanodiscs** **Nanodimers**



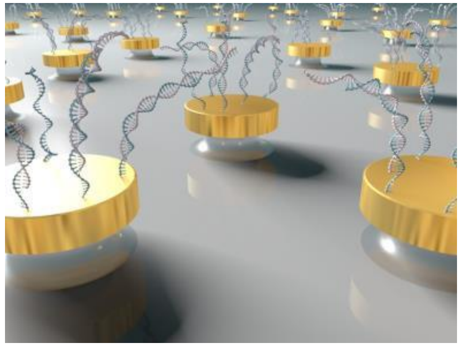
Recomended REVIEWS:

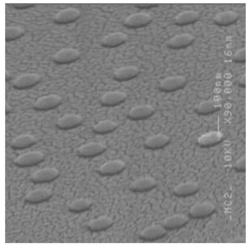
Lechuga et al.; Anal. Chim. Acta, 806, 55 (2014)

Lechuga et al.; Nanophotonics (2016)

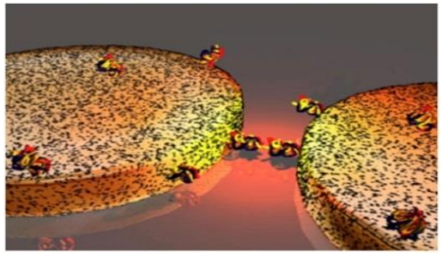
Localized Surface Plasmon Resonance (LSPR) Biosensor

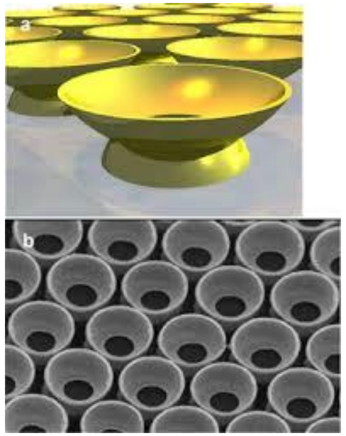
Au nanodisks

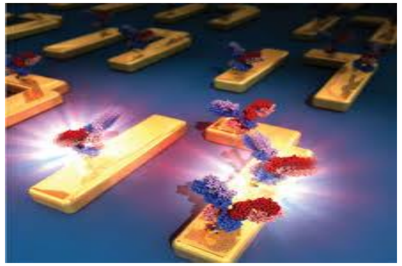




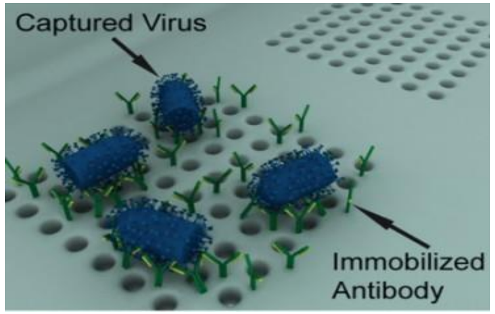
Nano-antennas



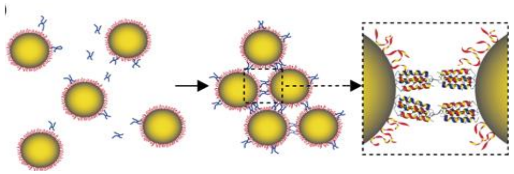




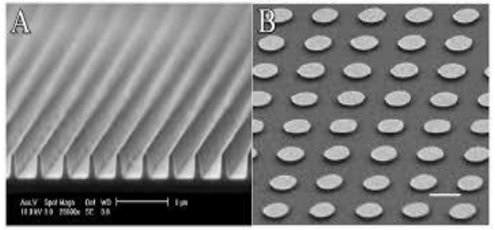
Nanoholes

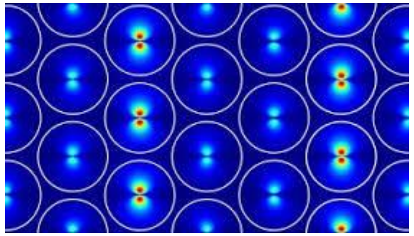


Au Nanoparticles

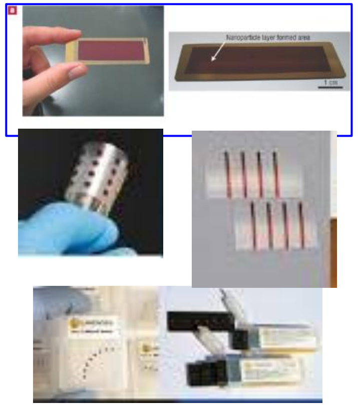


Au nanoslits

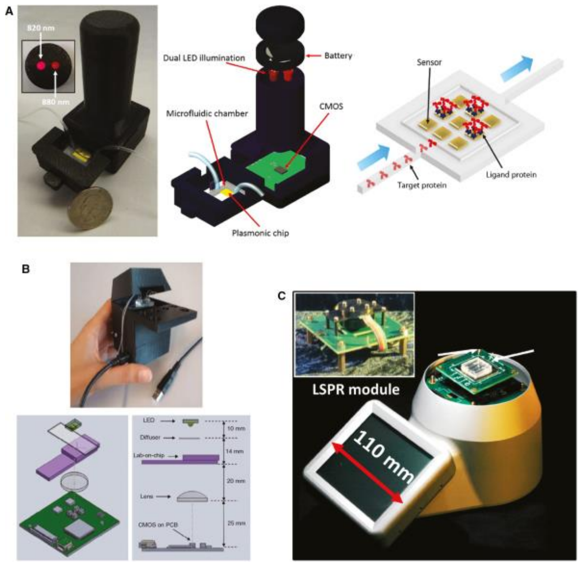




LAMDAGEN CORPORATION

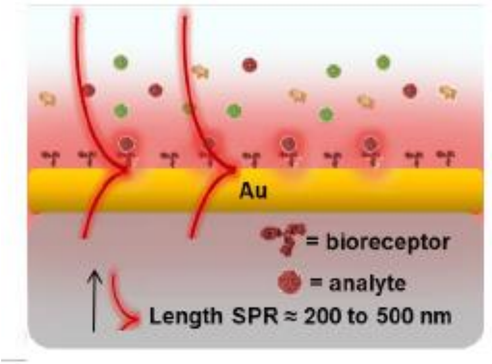


Insplorion



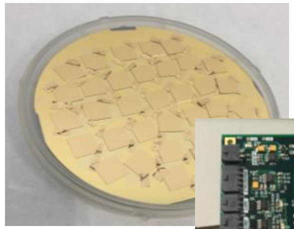
Plasmonics Biosensors POC @ Nanob2a Group

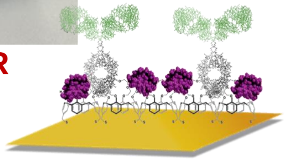
POC- SPR Biosensor



2-channels SPR

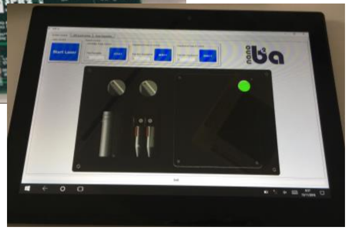
Gold sensor chips





Electronics

Tablet control

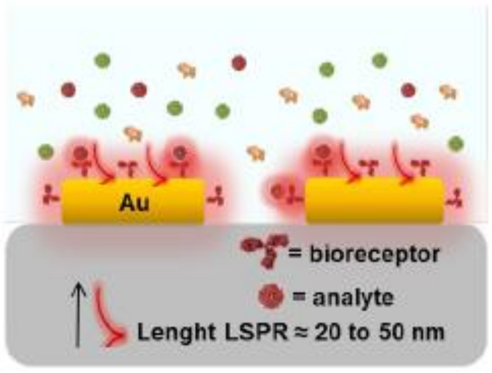


SPP Propagated plasmonic modes

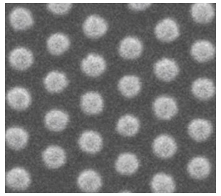
- LOD: 10^{-6} RIU (low ng/mL range)
- 2-channel biosensor
- Simple and reliable
- Well-know immobilization techniques
- Large effective sensing area

- **Fabrication of metallic sensor chips**
- **Complete in-house design& assembly**
- **Compact portable platforms**
- **User-friendly**

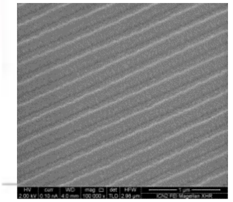
POC- LSPR Biosensor



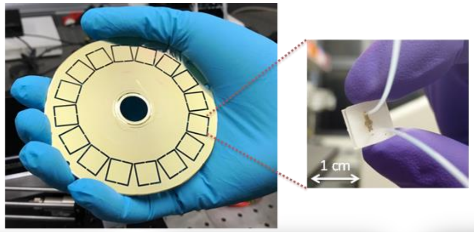
Metal Nanostructured sensor chips



Gold Nanodisks

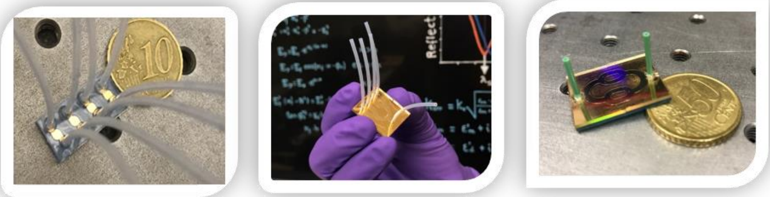


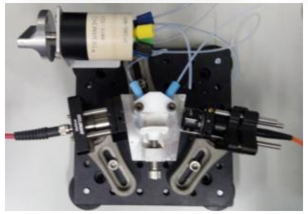
BlueRay-based Nanogratings



One channel

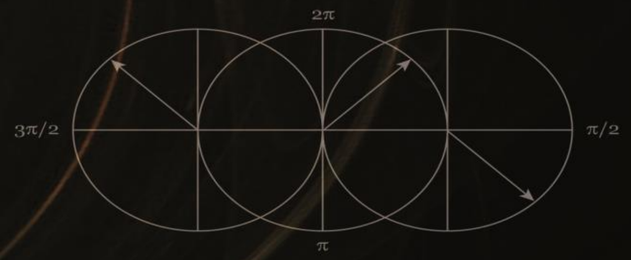
Multiplex





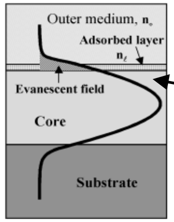
LSPR Localized plasmonic modes

- LOD: 10^{-6} RIU (low ng/mL range)
- Single- and four-channel prototypes
- Large multiplexing capability
- Fabricated in self assembled processes

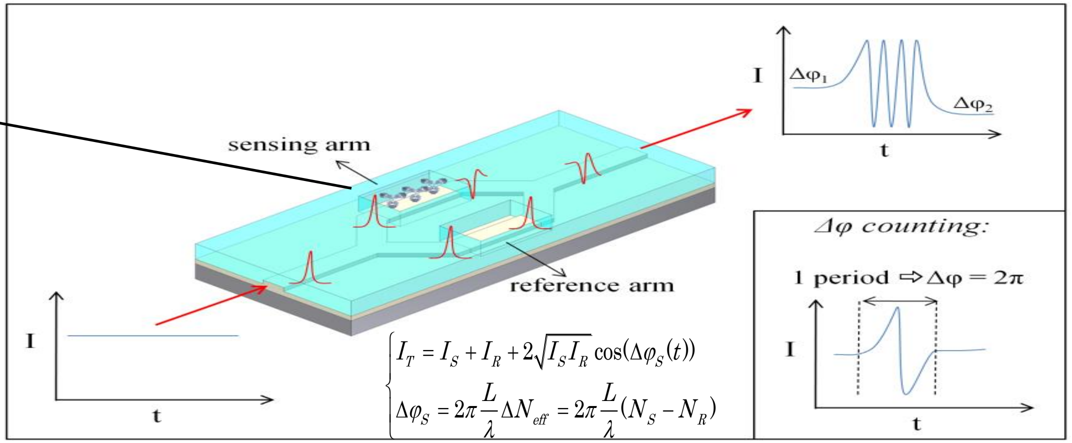


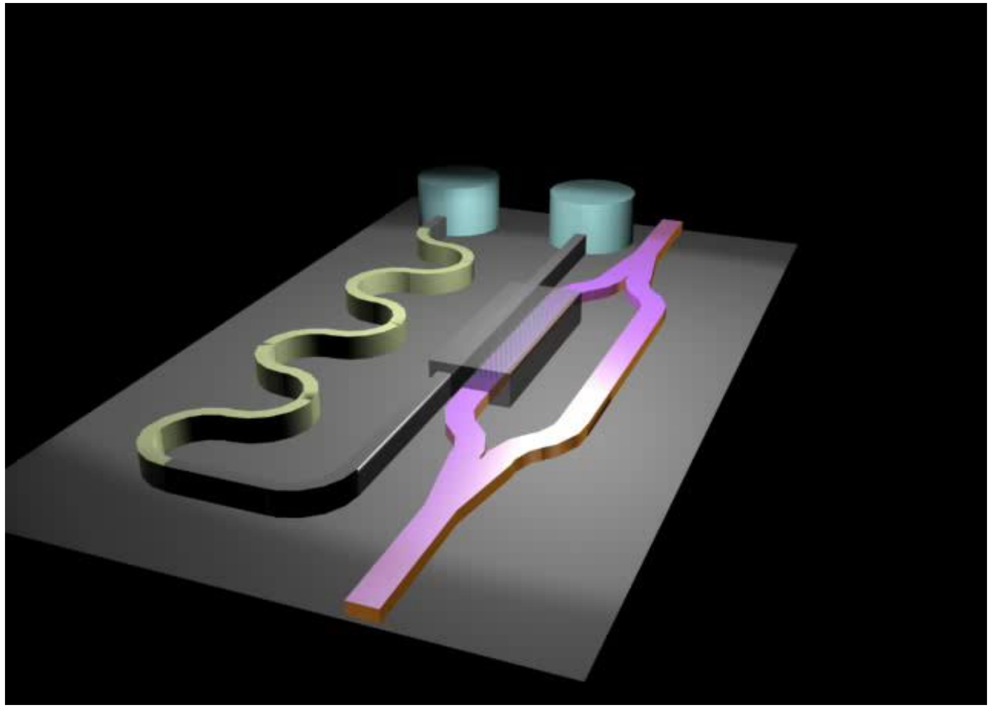
SILICON PHOTONICS BIOSENSORS

Mach-Zehnder Interferometer (MZI) biosensors



Evanescent wave detection

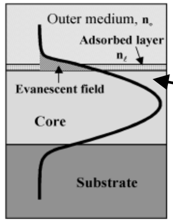




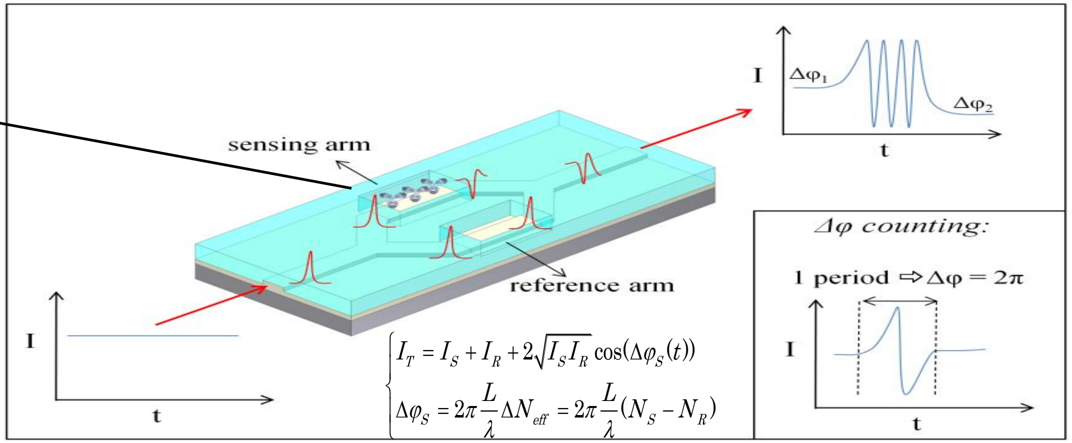
PRINCIPLE OF OPERATION

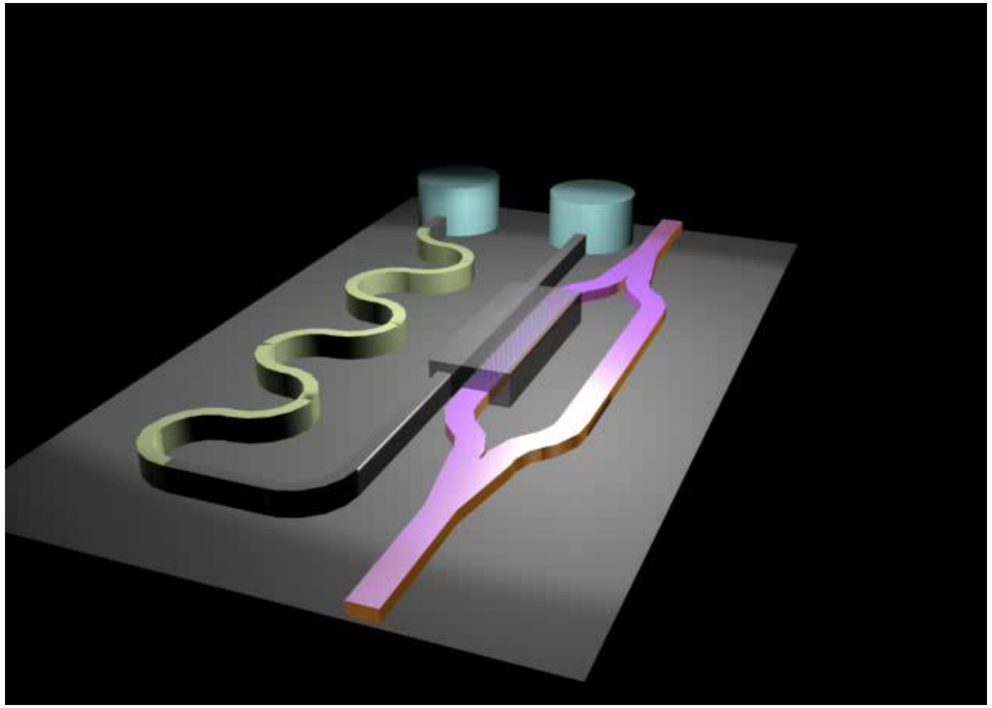
- **Monochromatic laser light is coupled** to the device
- One arm of the MZI is exposed to the analyte
- Specific **analyte probed by the evanescent field** of the guided modes, **causes a corresponding phase change** that is **measured as a change in intensity at the output**

Mach-Zehnder Interferometer (MZI) biosensors



Evanescent wave detection





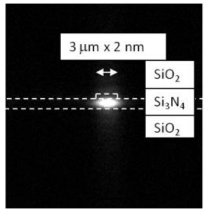
Design of a MZI biosensor

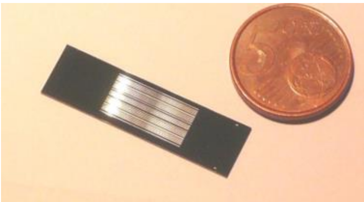
- **Single mode** behaviour
- High surface sensitivity

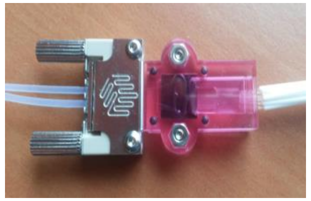
- Designed in **visible range** λ= 600-700 nm
- Designed in **Silicon Nitride**

LOD: 10^{-7} - 10^{-8} RIU

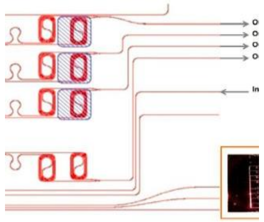
First integrated MZI biosensor was fabricated at CNM-CSIC, Barcelona (Spain) (1994)



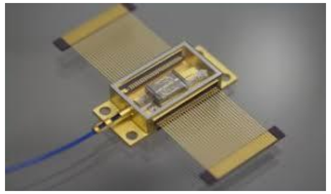




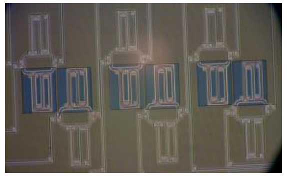
LioniX INTERNATIONAL



a-MZI

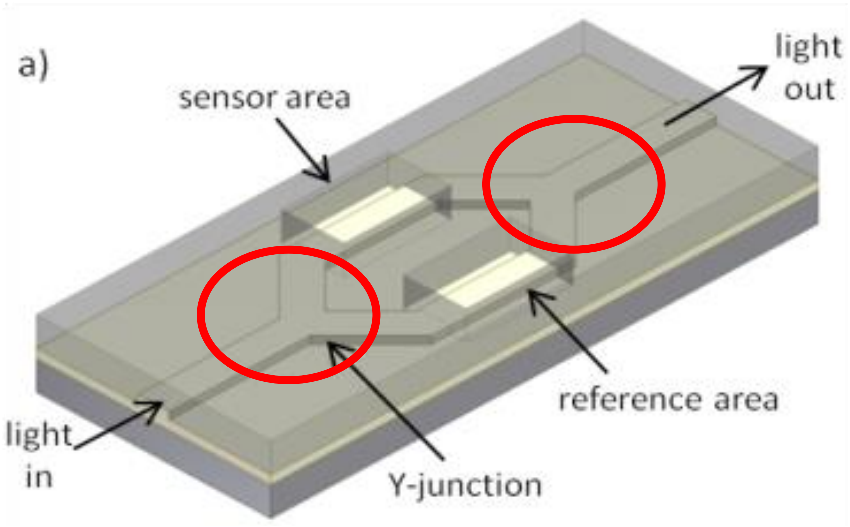


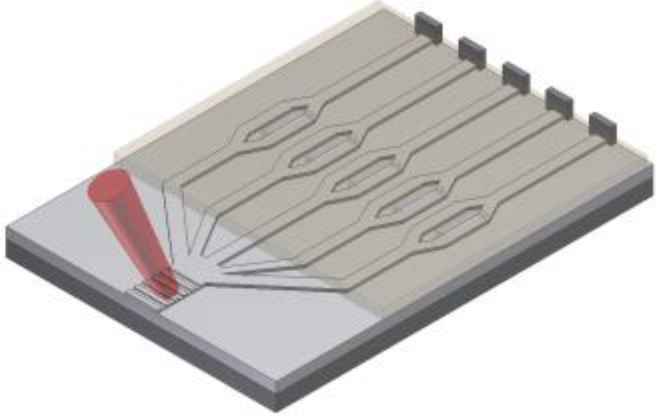
imec GHENT UNIVERSITY



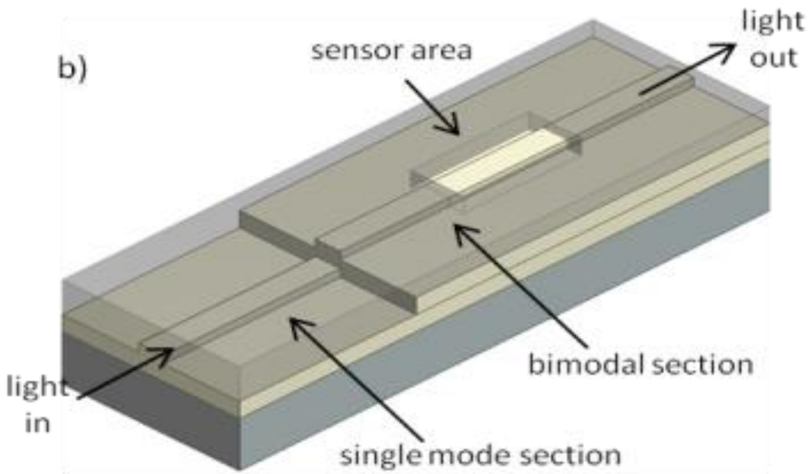
INTERFEROMETRIC BIOSENSORS

MACH-ZEHNDER INTERFEROMETER (MZI)





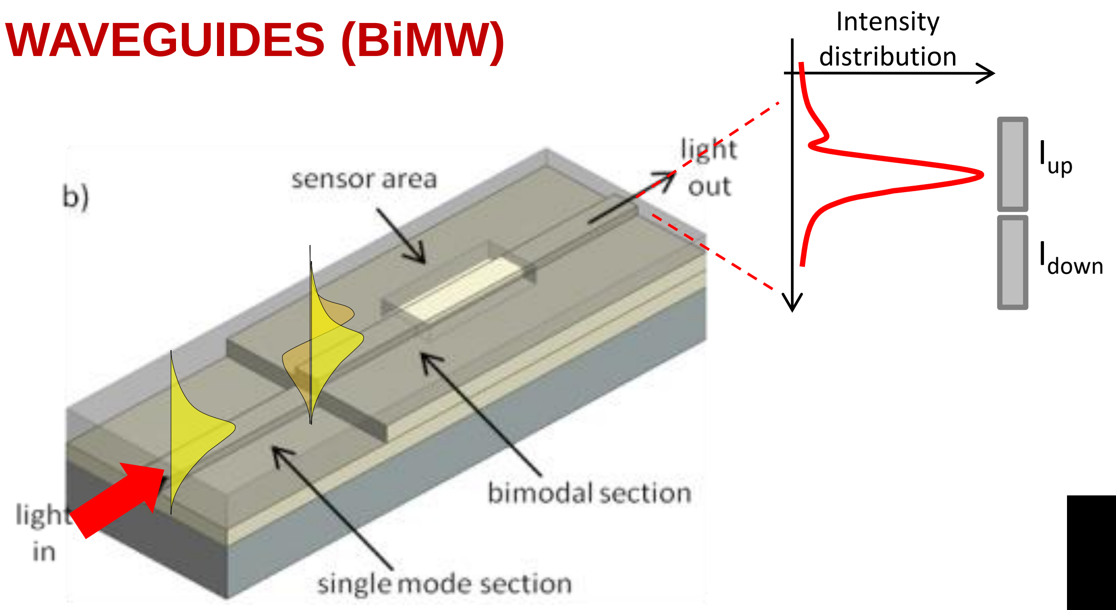
BIMODAL WAVEGUIDES (BiMW)



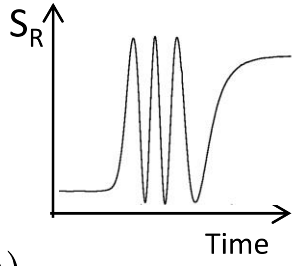
- EP2278365 (**Granted 2014**), PCTES08070142 (**Granted 2013**), CA2693423, CN102077124, US20110102777 (**Granted 2012**), JP2011519071

Bimodal waveguide interferometer (BiMW)

BIMODAL WAVEGUIDES (BiMW)



$$S_R(t) = \frac{I_{up} - I_{down}}{I_{up} + I_{down}}$$



$$S_R(\mathrm{t}) \propto V \cos\left(\Delta\varphi_S\left(t\right)\right)$$

$$\Delta\varphi_S = 2\pi \frac{L}{\lambda}(\Delta N_{TE10} - \Delta N_{TE00})$$

PRINCIPLE OF OPERATION

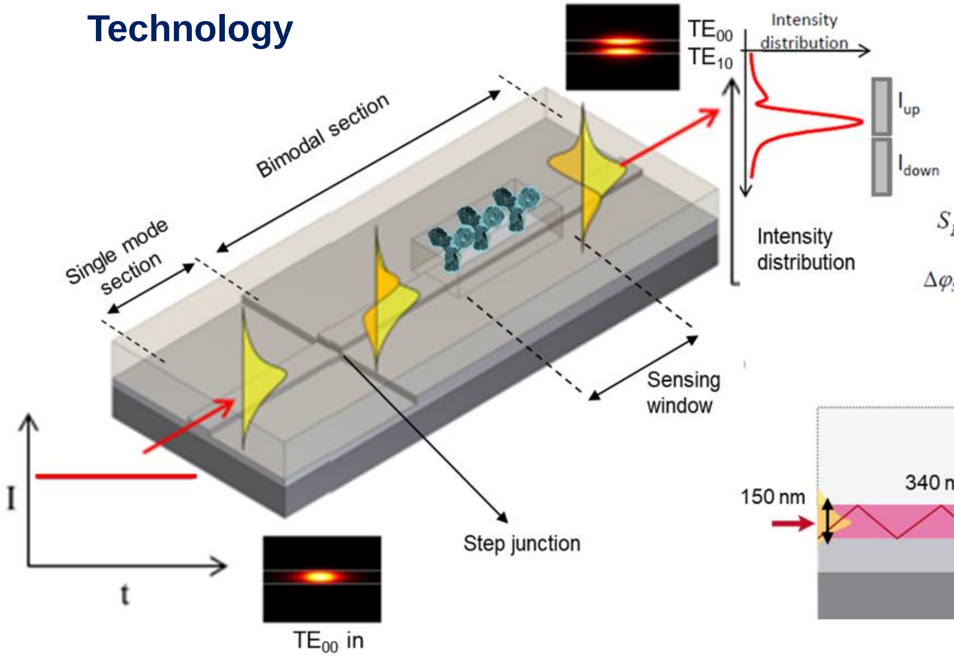
- Single channel waveguide interferometer
- Operated on interference of two light modes (fundamental and first order) of the same polarization
- No need anymore of Y-shape splitters (as in MZI or Young Interferometer)
- The modes propagate with different velocities and create an interference pattern at the exit, which intensity distribution depends on the refractive index of the cladding layer through the interaction with the evanescent field.

J. Lightwave Tech. 29(13), 1926-1930 (**2011**)

PCT/ES08/070142

Bimodal waveguide interferometer (BiMW)

Silicon Photonics Technology

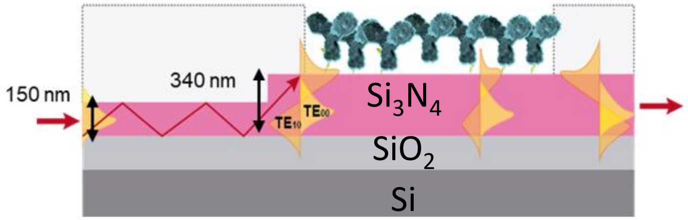


$$S_R(t) = \frac{I_{up} - I_{down}}{I_{up} + I_{down}}$$

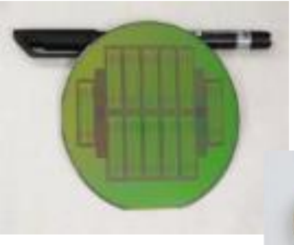
$$S_R(t) \propto V \cos(\Delta\varphi_S(t))$$

$$\Delta\varphi_S = 2\pi \frac{L}{\lambda}(\Delta N_{TE10} - \Delta N_{TE00})$$

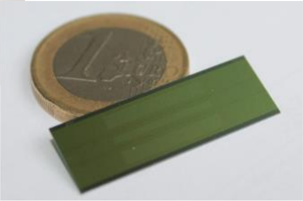
- **One of the most sensitive EW sensors**
- **A simple PIC sensor**
- **High Multiplexing capabilities**
- **Operating in the visible range**
- **Mass production (Clean Room foundries)**



- Si_3N_4 **150 nm** (single mode)/ **340 nm** (bimodal)
- rib depth: **1- 3 nm**
- Waveguide width ≤ 3µm

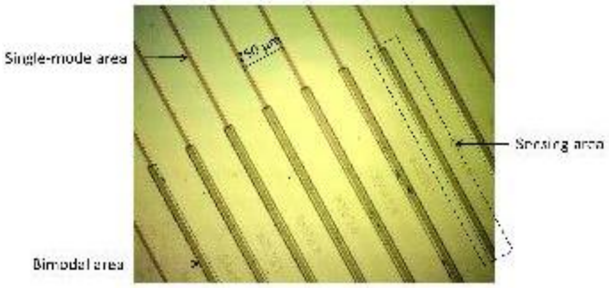


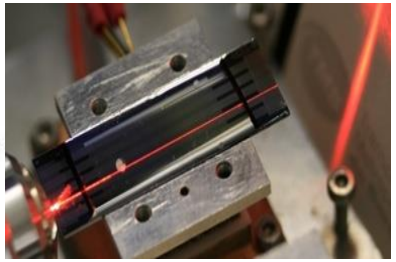
12 chips/wafer

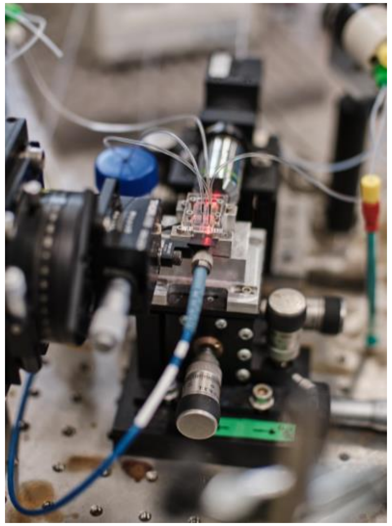


20 sensors/chip

Robust and Reproducible SiN technology

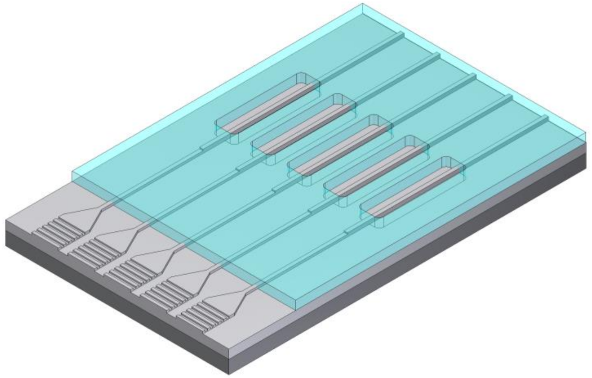


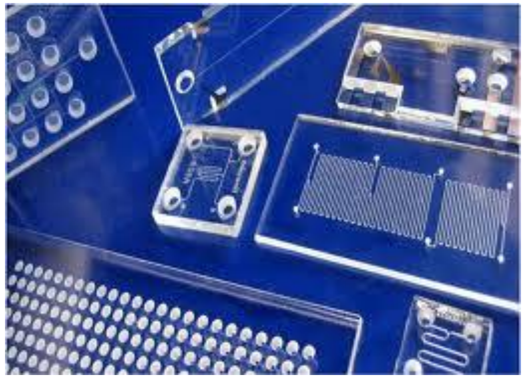




LOD: 10^{-8} RIU (low pg/mL range)

Microfluidics integration

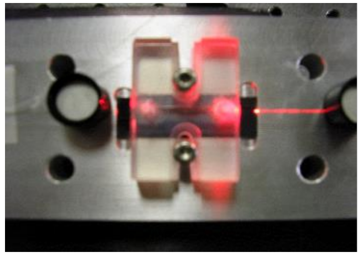


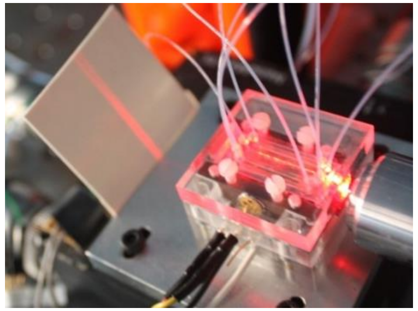


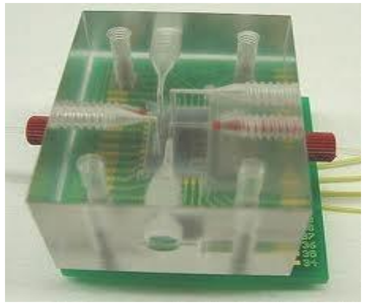
- Hermetic sealing
- No air bubbles
- Low cost (disposable)
- Affording multiplexing

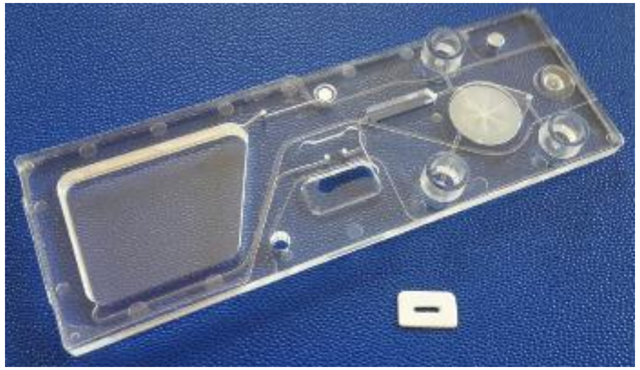
Available materials and technologies

- Silicon, glass, polymers (PDMS, PMMA, SU-8..), ceramics,…
- Micromachining, hot embossing, injection molding, casting, 3D printing..



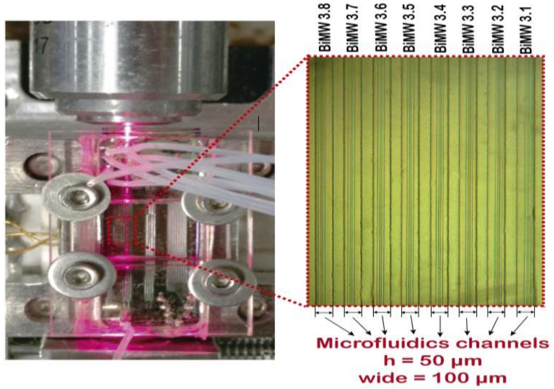




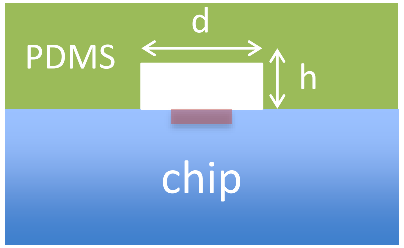


POINT-OF-CARE BIOSENSOR: microfluidics integration

- Hermetic sealing
- No air bubbles
- Low cost (disposable)
- Affording multiplexing

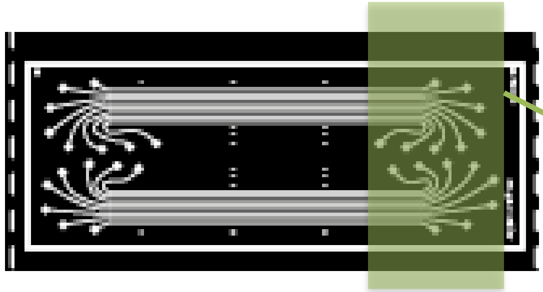


PDMS technology: **Independent flow cells for each sensor within the chip**



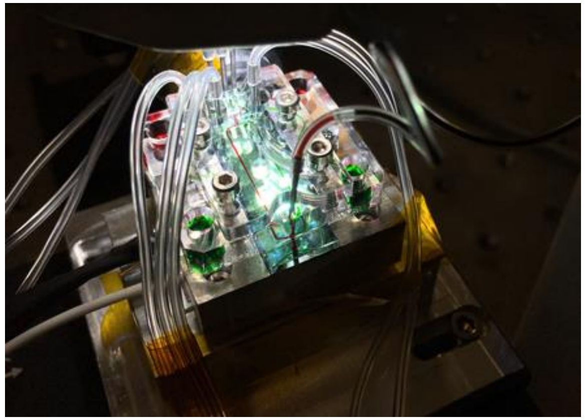
$d \rightarrow$ 50 – 150 µm
$h \rightarrow$ 20 – 100 µm

- **Width: 100 µm**
- **Height: 100 µm**
- **Pitch channels: 250 µm**

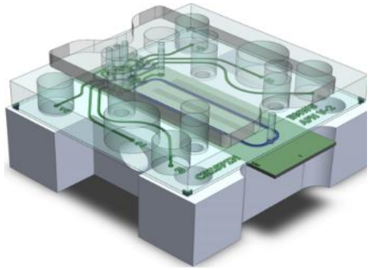


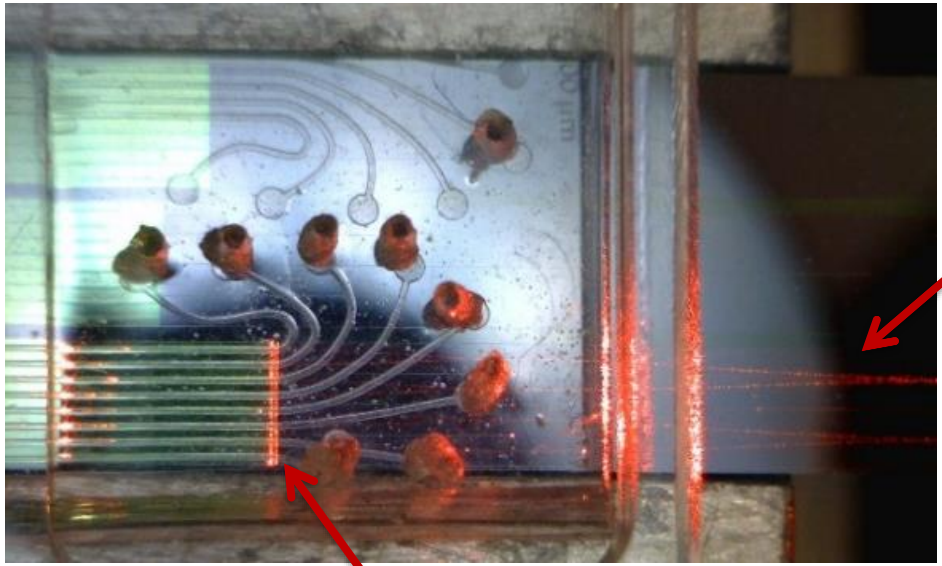
tubes

Automated on-chip fluid handling



integration of pneumatically actuated pumps and valves





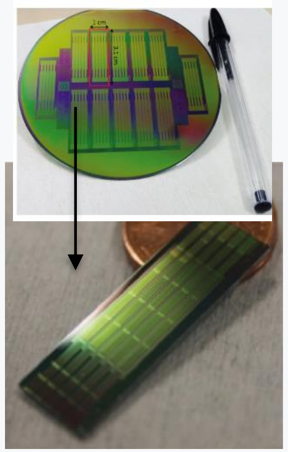
BiWG sensors

Individualized microfluidic channels

Lab on a Chip 17 (16), 2793-2804 (2017)

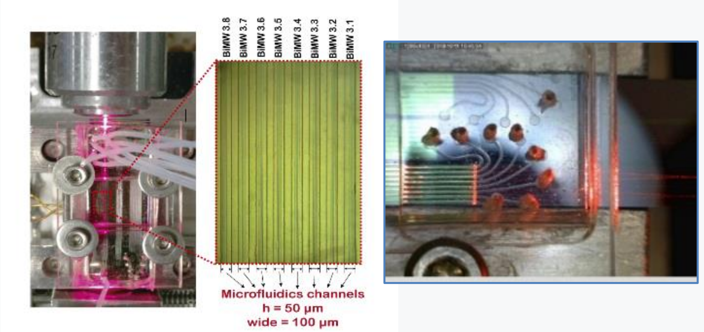
Engineering of the BiMW POC biosensor

Sensor chip

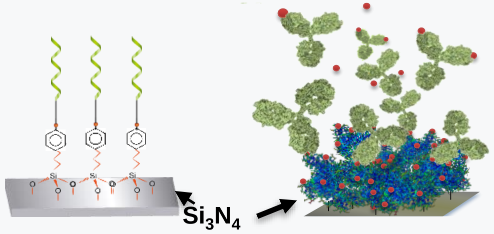


Fabrication
@Clean Room

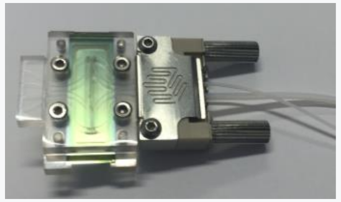
Polymer microfluidics



Surface Biofunctionalization

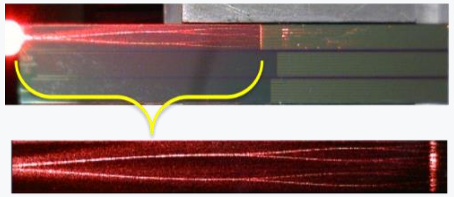


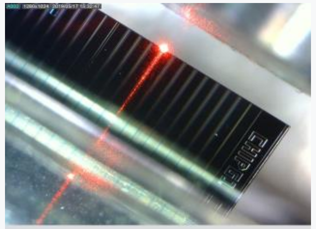
Cartridge development



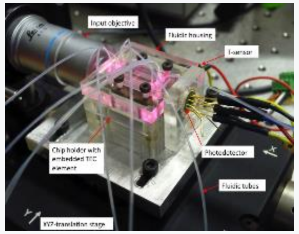
Packaging & Storage

Light incoupling & all- optical Modulation



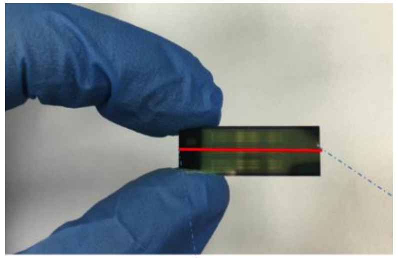


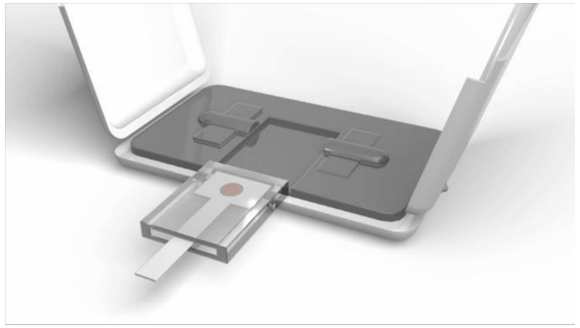
Optical readout & Signal processing



Portable POC Biosensor platform

Disposable Biochip





J Light Tech 40 (1), 237-244 (2023)
Laser & Photonics Reviews 9 (2), 248-255 (2015)
Journal of Physics: Photonics 1 (2), 025002 (2019)

BIOFUNCTIONALIZATION

Surface biofunctionalization

Chemical Surface activation (1st step)

- Introduction of functional groups to bind to the bioreceptor

Surface biofunctionalization (2nd step)

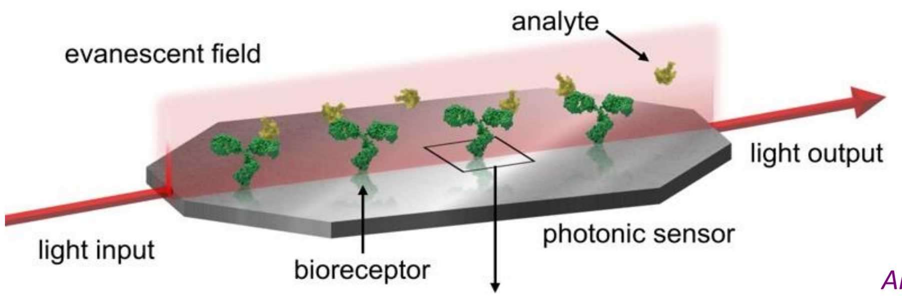
Maintaining structure and functional properties

- **Stable** linkage between the biomolecule an
- Optimized **density** of functional group
- Favorable **orientation**
- Good **accessibility** to the target d vertical spacers)

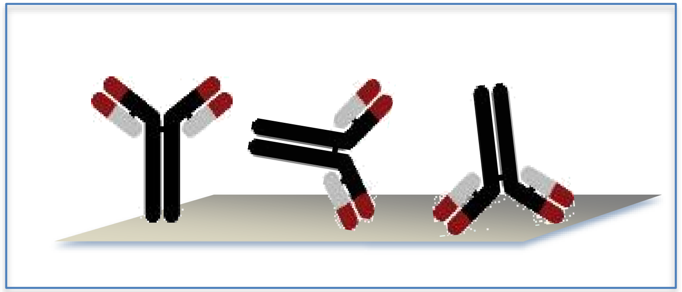
KEY STEPS

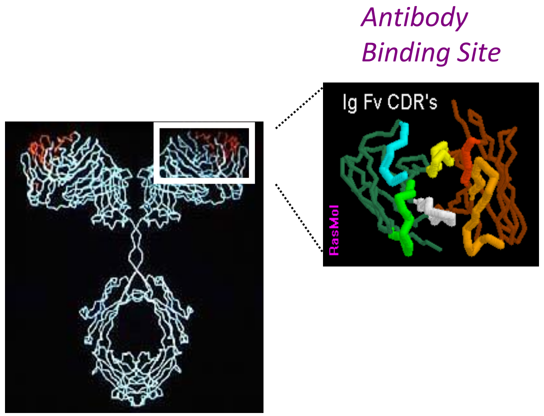
Antifouling surfaces (3rd step)

- Prevention of non-specific adsorptions from real samples

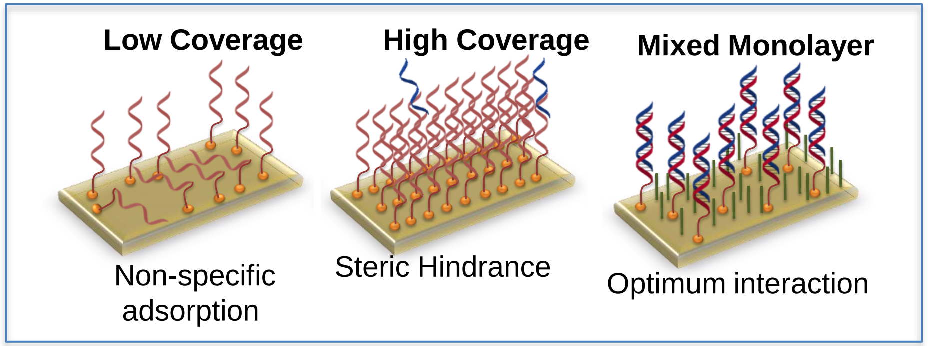


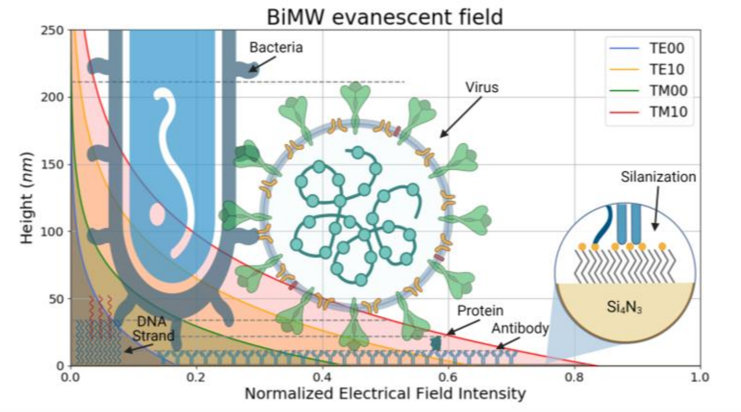
Orientation





Density

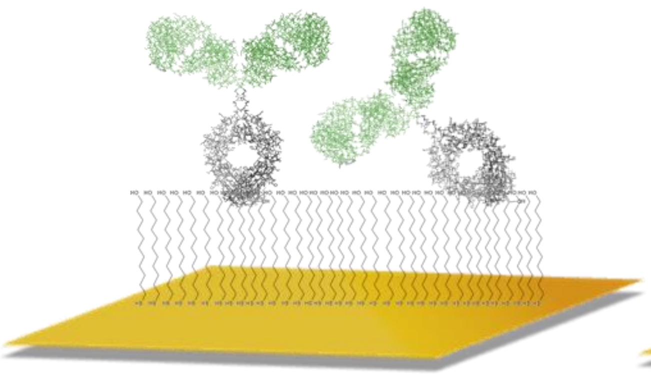




Anal Bioanal Chem 1-15 (2021)

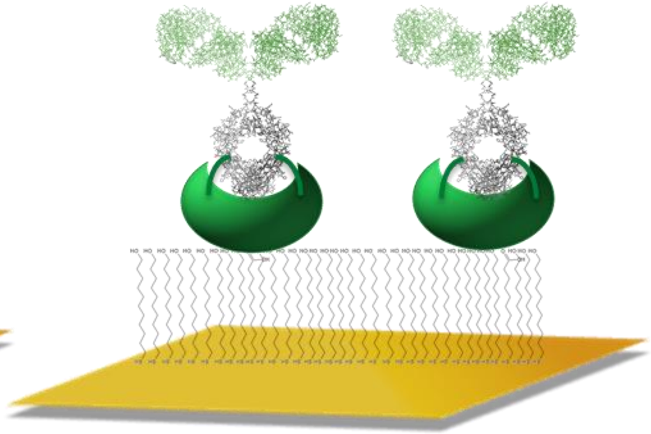
Inmovilización de Bioreceptores a la nanoescala

Covalent Strategy



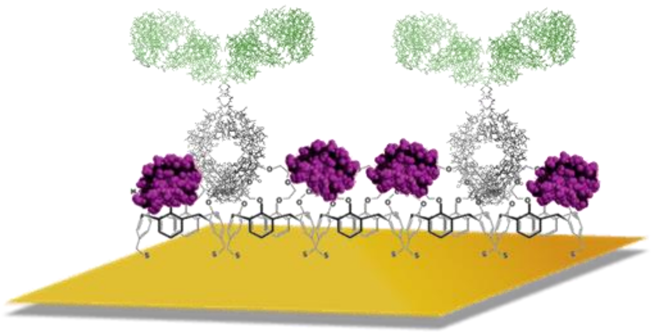
1) Alkanethiol SAM (SH-ROH:SH-RCO_2H)
2) Antibody covalent binding

Protein G Strategy



1) Alkanethiol SAM (SH-ROH:SH-RCO_2H)
2) Protein G covalent binding
3) Antibody (Fc region) affinity capture
4) Crosslinking ProteinG-mAb (BS^3)

Calixarene Strategy



1) Prolinker™
2) Antibody affinity capture
3) BSA blocking

Parameters to be optimized: surface chemistry, pH, ionic strength, receptor and Ab concentration, regeneration solution,…

Antifouling Strategies for real samples evaluation

- Modifying medium composition: surfactants, additives
- Modifying surface behaviour: hydrophylic blocking agents (as PEG)

Real Samples

Blood/Serum

Urine

Tears

- One-step assay
- Label-free & Real-time
- Crude samples or minimum treatment/dilution

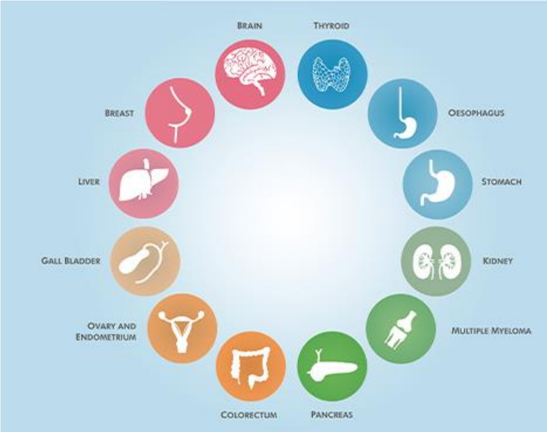
Analyst *138 (7) 2023 (2013);* ***Sensors*** *14(2) 2239 (2014),* ***Anal. Chem.*** *92 (18), 12596 (2020)*

EXAMPLES OF PHOTONIC BIOSENSORS FOR REAL APPLICATIONS

CANCER

Cancer is a major global health problem

There were an estimated **18.1 million** cancer cases around the world in **2020**. Of these, 9.3 million cases were in men and 8.8 million in women

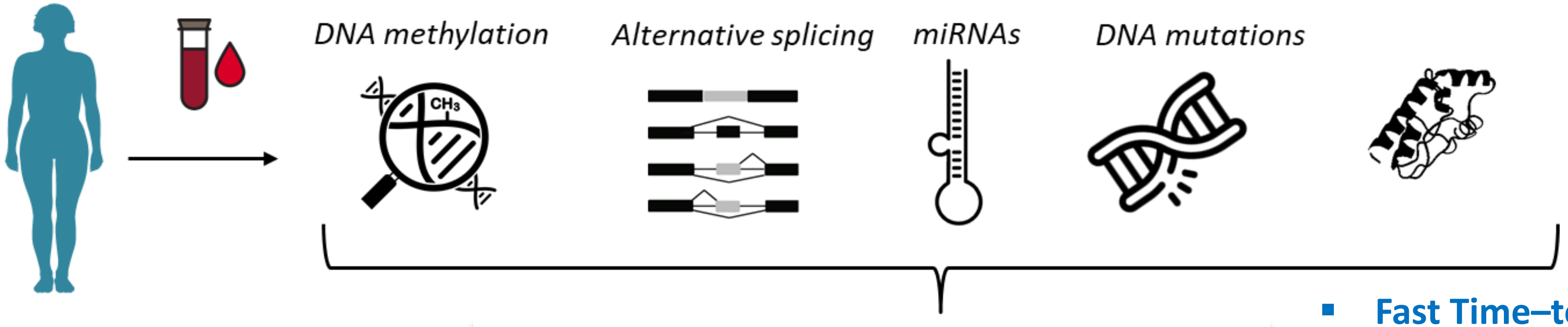


- 1 in 5 people develop cancer during their lifetime
- Prevention of cancer has become one of the most significant public health challenges
- At least 40% of all cancer cases could be prevented with effective primary prevention
- Further mortality can be reduced through early detection of tumours

We need new Diagnostic and Therapeutic tools that can significantly improve the survival rate

Genetics & Epigenetics | **Proteins**

BIOMARKERS DETECTION



POINT-OF-CARE BIOSENSORS FOR
EARLY CANCER DIAGNOSIS

- **Fast Time–to-result**
- **Minimum sample**
- **Label-free**
- **No amplification**

POC biosensor for Early colon cancer diagnosis

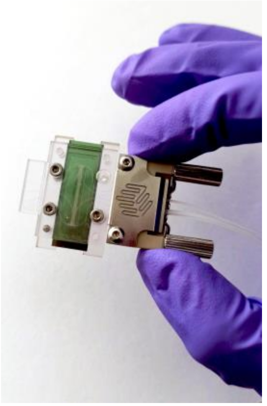
Colorectal Cancer Diagnosis:

- Colonoscopy/Sigmoidoscopy
- Faecal occult blood test (FOBT)

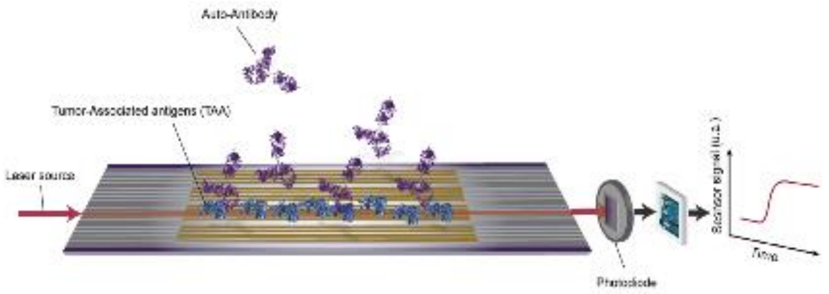
– Advanced stages – Low accuracy

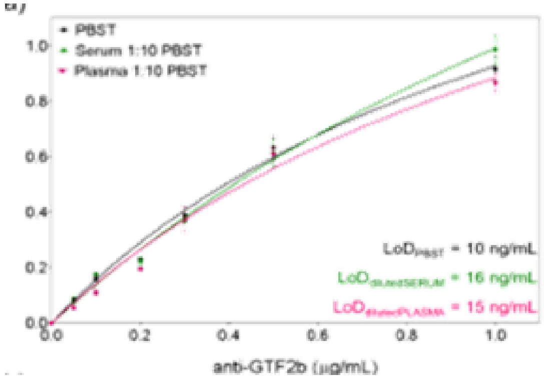
- **Direct and label-free detection of TAA autoantibodies**
- **Good sensitivity, selectivity and reproducibility**
- **Feasibility to quantify TAA autoantibodies in serum & plasma**
- **Correlation with clinical analysis results**

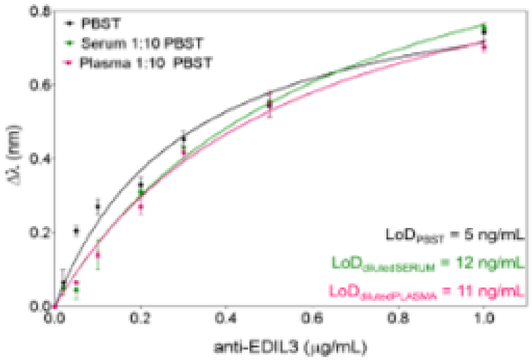
An easy POC biosensor test while avoiding colonoscopy

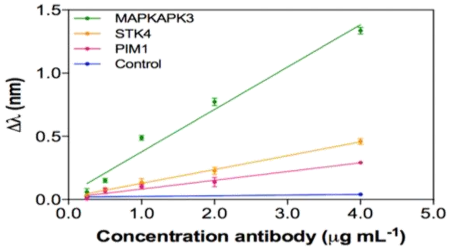


Biosensor for specific autoantibody panels



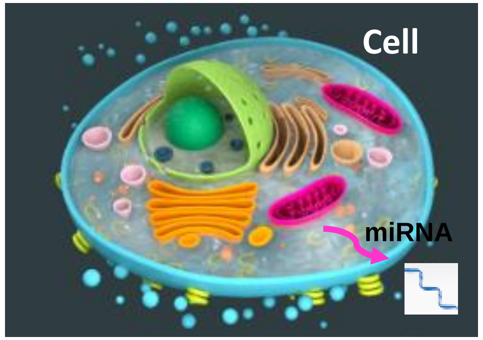






Analytica Chimica Acta, 930, 2016, 31-38

Early detection of bladder cancer

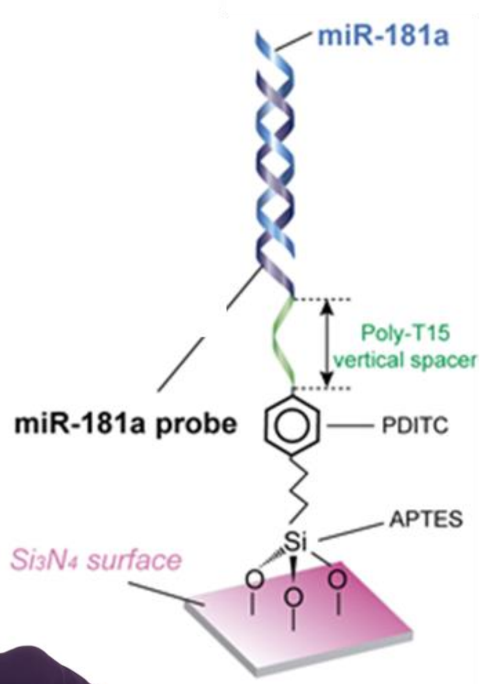


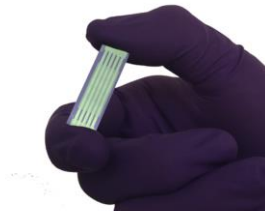
Micro-RNAs (miRNA) are **short RNAs (~ 20 nt)** implicated in many diseases as: **Cancer**, **Neurodegenerative disorders, Diabetes.** They are present in **biofluids** as blood, urine, saliva.

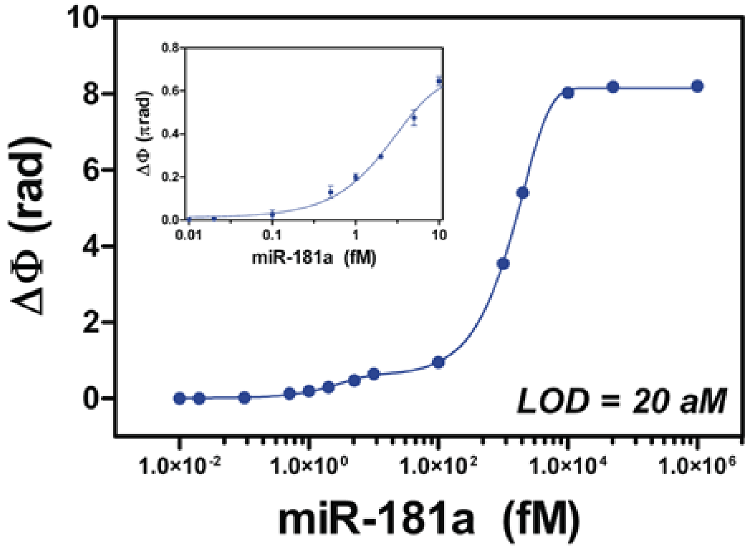
Detection drawbacks

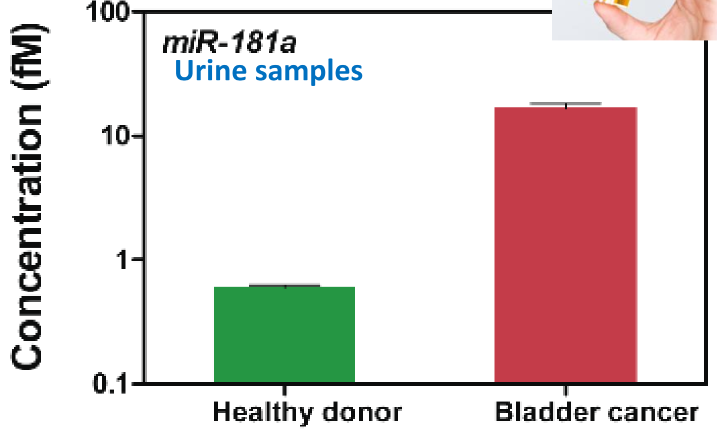
- ✖ Very low concentration levels in biofluids (pM-aM range)
- ✖ Difficult to detect due to the presence of homologous miRNAa

Development of a biosensor strategy to determine bladder cancer stage in urine using MicroRNA 181a as biomarker









- **Ultra-low LOD of only 20 aM**
  - miR-181a concentrations:10 aM to 10 pM
  - LOQ: 100 aM without amplification steps
  - Full selectivity as compared to miR homologous

- **Stratification of real patients**

ACS Sensors 1,748-756 (2016)

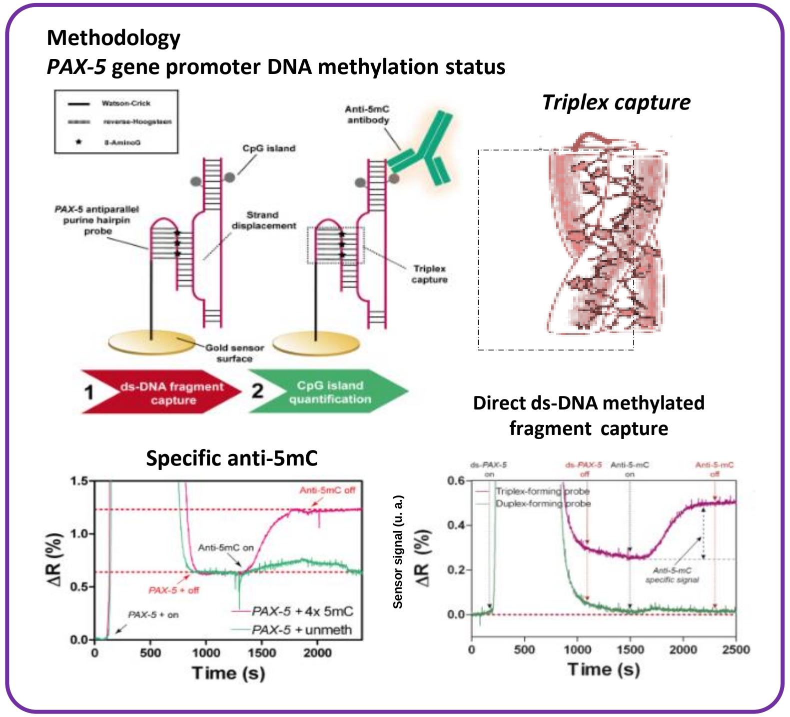
NnaoBiosensors for Cancer Epigenetics diagnostics

Early Cancer Diagnostics

- **Cancer protein biomarkers**
- **Cancer Epigenetics**

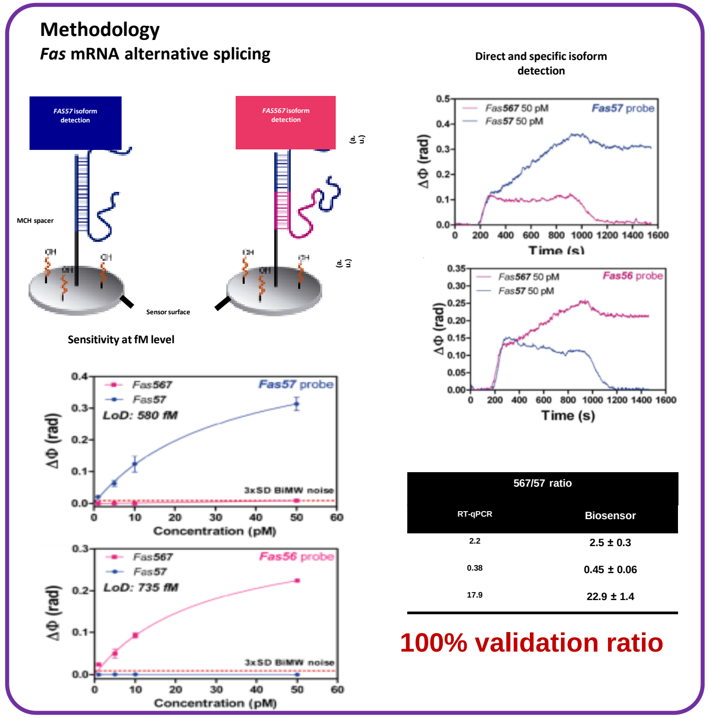
- Lung cancer
- Ovarian cancer

DNA methylation profiling



Detection in cell extracts

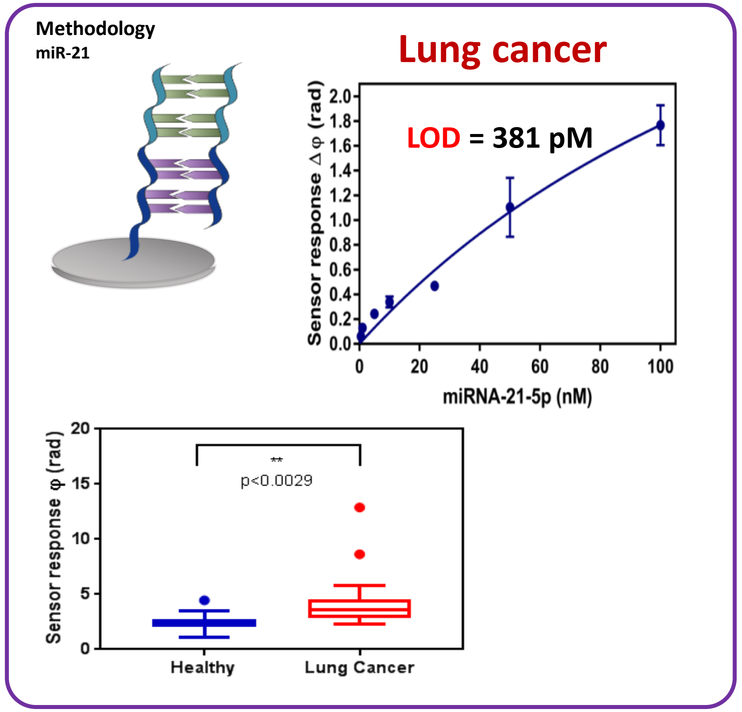
Alternative Splicing analysis



567/57 ratio	
RT-qPCR	Biosensor
2.2	2.5 ± 0.3
0.38	0.45 ± 0.06
17.9	22.9 ± 1.4

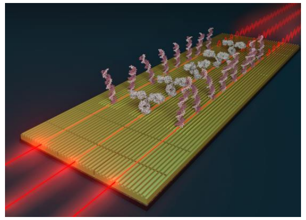
Detection in cell extracts

Micro-RNA detection



Detection in plasma

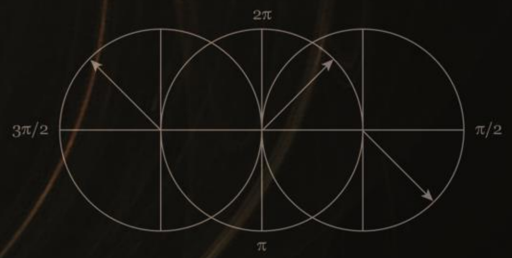
MULTIPLEX DETECTION



Frontiers in chemistry *7, 724 (2019)*
Analytical chemistry *91 (23), 15138-15146 (2019)*
Anal. Chem *(2022) in press*

Biosens & Bioelec. *78, 118–125 (2016)*
Anal. Bioanal. Chem. *(ABC) 408, 885-893 (2016)*
ACS Sensors *1, 748–756 (2016)*
Scientific Reports *7, 41368 (2017)*
Anal. Chim. Acta *930, 31-38, (2016)*

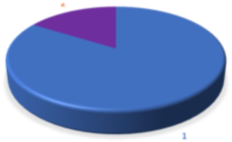
Diagnosis of Infectious Diseases



Diagnosis of infections: The Problem

Sepsis and Antimicrobial Resistance Infections (AMR) are a major concern

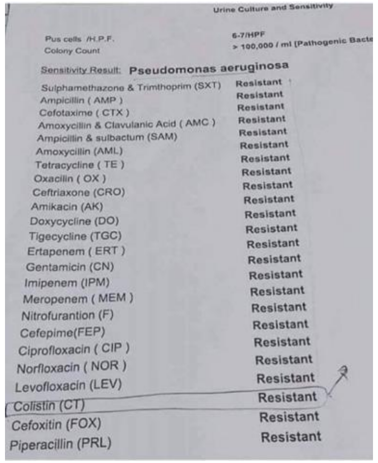
Sepsis



11 M deaths/2017

49 M cases/2017

<u>Mortality increasing every year</u>

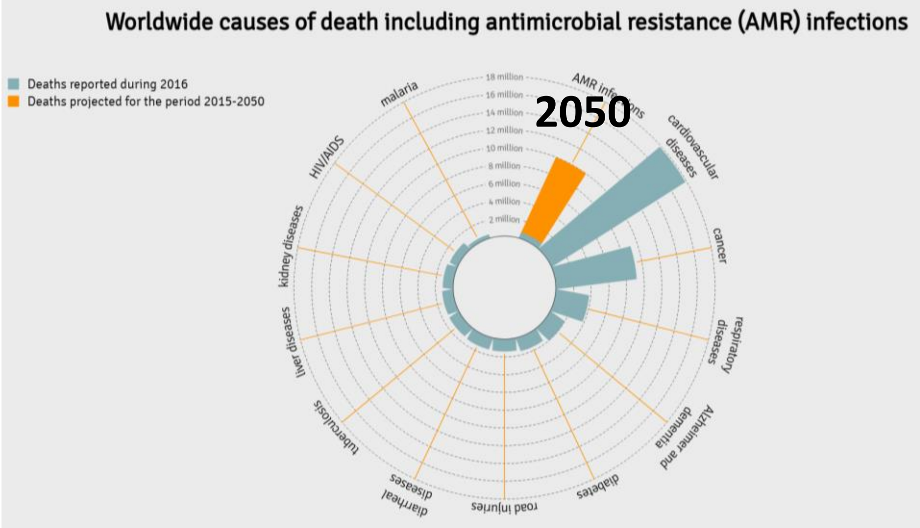


Urine Culture and Sensitivity

Pus cells /H.P.F.	6-7/HPF
Colony Count	> 100,000 / ml [Pathogenic Bact

Sensitivity Result: **Pseudomonas aeruginosa**

Sulphamethazone & Trimthoprim (SXT)	Resistant
Ampicillin (AMP)	Resistant
Cefotaxime (CTX)	Resistant
Amoxycillin & Clavulanic Acid (AMC)	Resistant
Ampicillin & sulbactum (SAM)	Resistant
Amoxycillin (AML)	Resistant
Tetracycline (TE)	Resistant
Oxacilin (OX)	Resistant
Ceftriaxone (CRO)	Resistant
Amikacin (AK)	Resistant
Doxycycline (DO)	Resistant
Tigecycline (TGC)	Resistant
Ertapenem (ERT)	Resistant
Gentamicin (CN)	Resistant
Imipenem (IPM)	Resistant
Meropenem (MEM)	Resistant
Nitrofurantion (F)	Resistant
Cefepime(FEP)	Resistant
Ciprofloxacin (CIP)	Resistant
Norfloxacin (NOR)	Resistant
Levofloxacin (LEV)	Resistant
Colistin (CT)	Resistant
Cefoxitin (FOX)	Resistant
Piperacillin (PRL)	Resistant

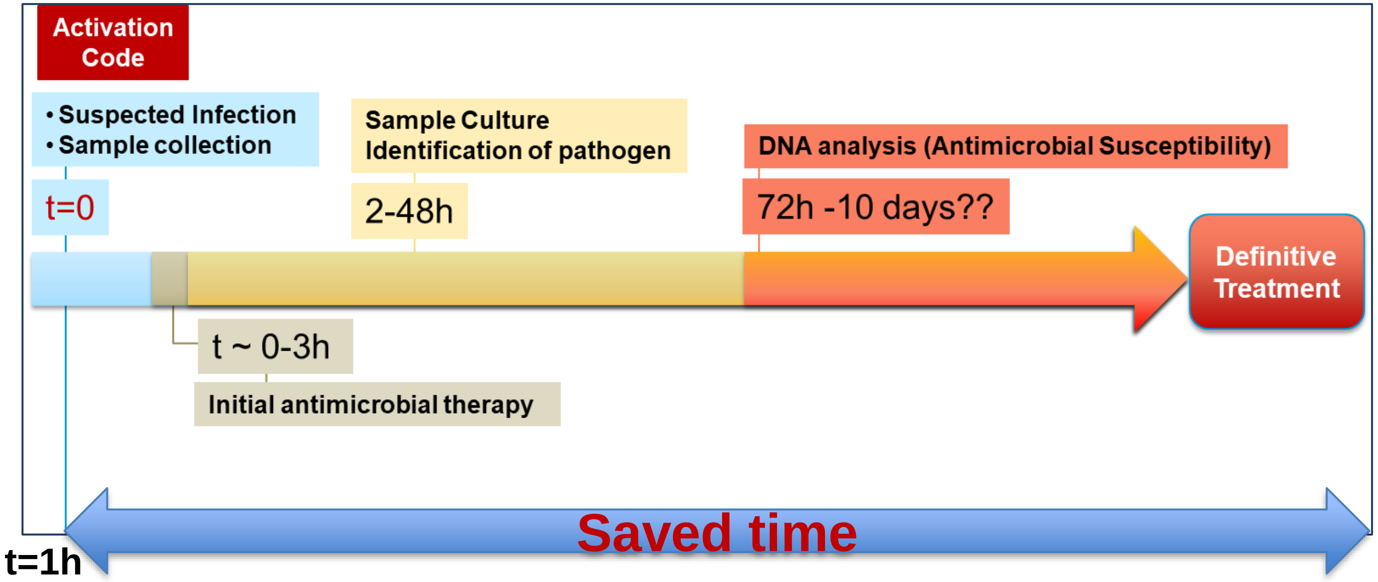


Rapid test for early sepsis & AMR diagnostics a need

Issues identified

- **TIME for triage**: death risk increases 8% by hour of delay
- Centralized labs, specialized equipment and personnel
- Slow and labor-intensive techniques

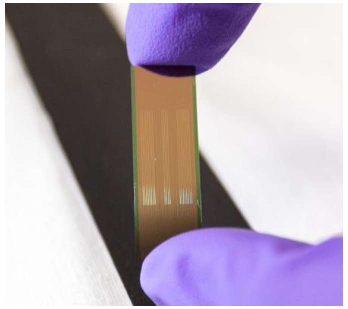
SOLVING DELAYED DIAGNOSIS AND INTERVENTION



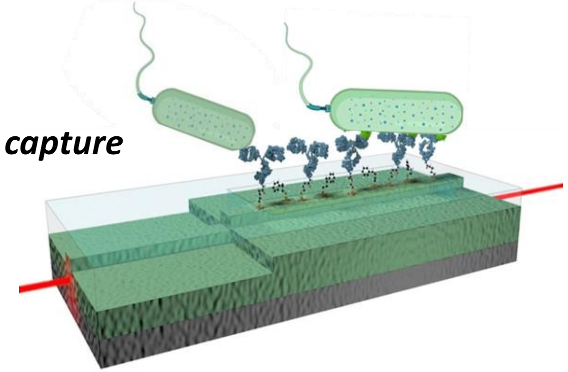
From days…. to less than 1 h

POC Biosensor for Early infection detection

Fast identification and quantification of bacteria

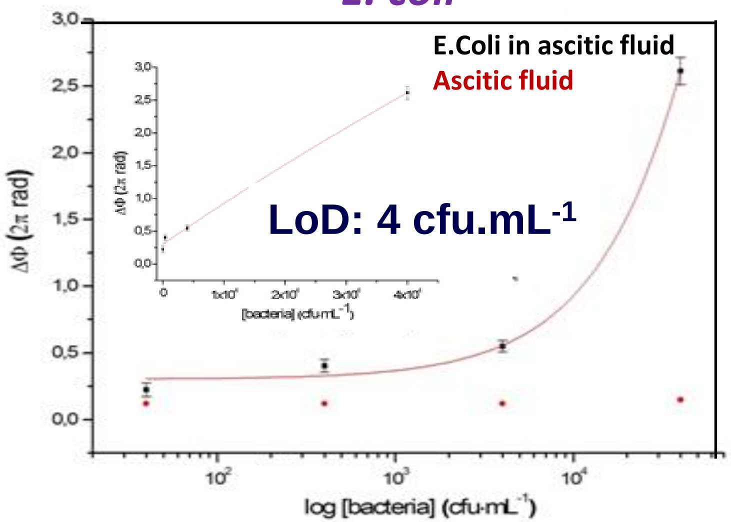


Bacteria capture



- Analysis time: 25 min
- Sample volume: 150-250 µL
- Direct detection (specific recognition)
- Custom biosurface for each bacteria
- Highly sensitive

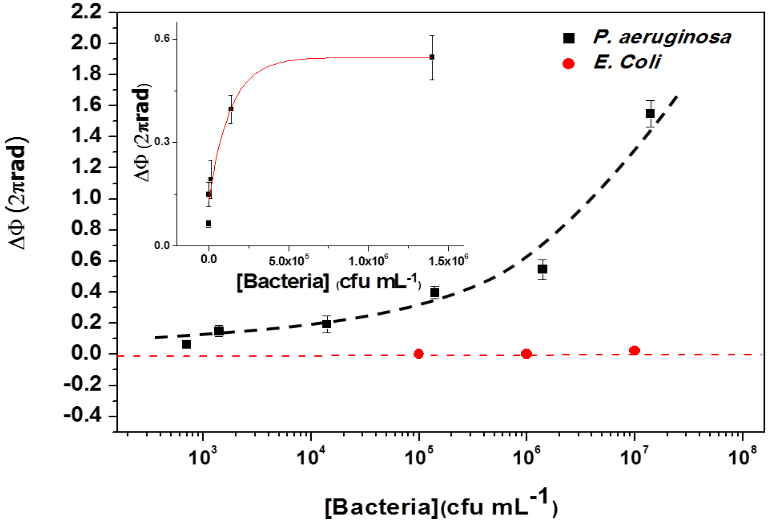
E. coli



- Identification of infections in cirrhotic patients
- Ascitic fluid
- LOQ= 40 cfu/mL

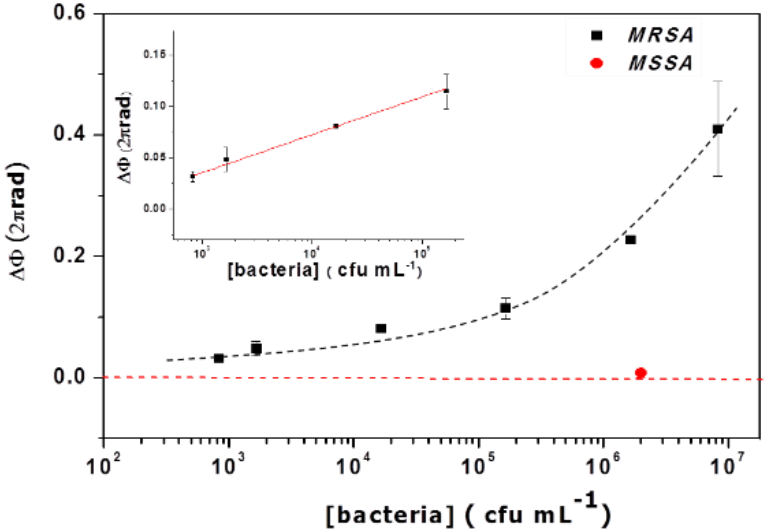
Vall d'Hebron Hospital

P. aeruginosa



- LOD= 50 cfu/mL

MRSA (vs MSSA)

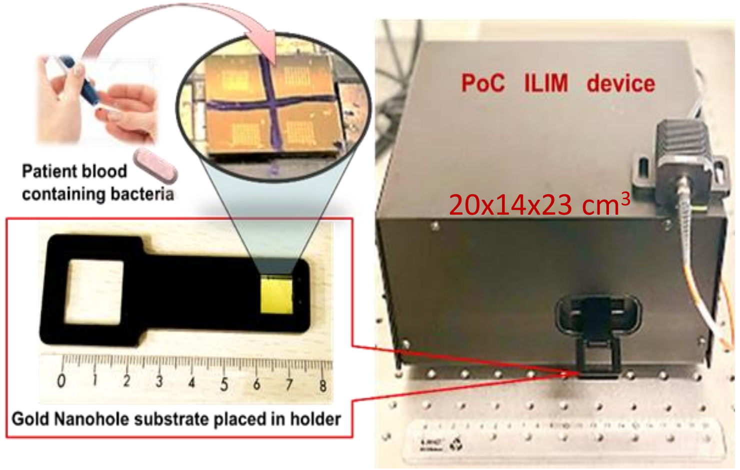


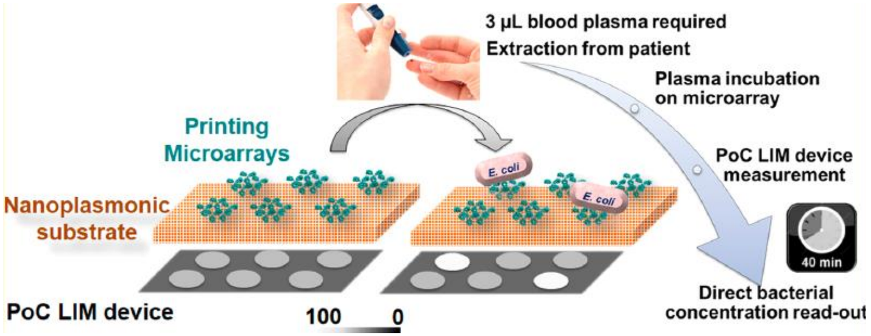
- Differentiation of the resistant strain (aptamer PBP2a)
- LOD= 30 cfu/mL

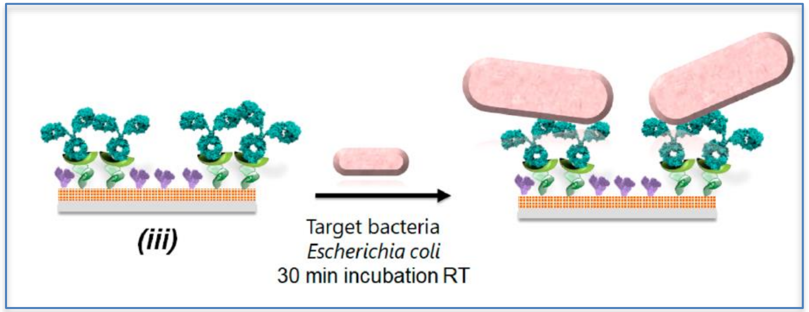
Biosens. Bioelectr., 85, 310-316 (2016)
Analyst, 145, 497-506 (2020)

POC Biosensor for Fast diagnostics of sepsis

PORTABLE OPTICAL PLATFORM

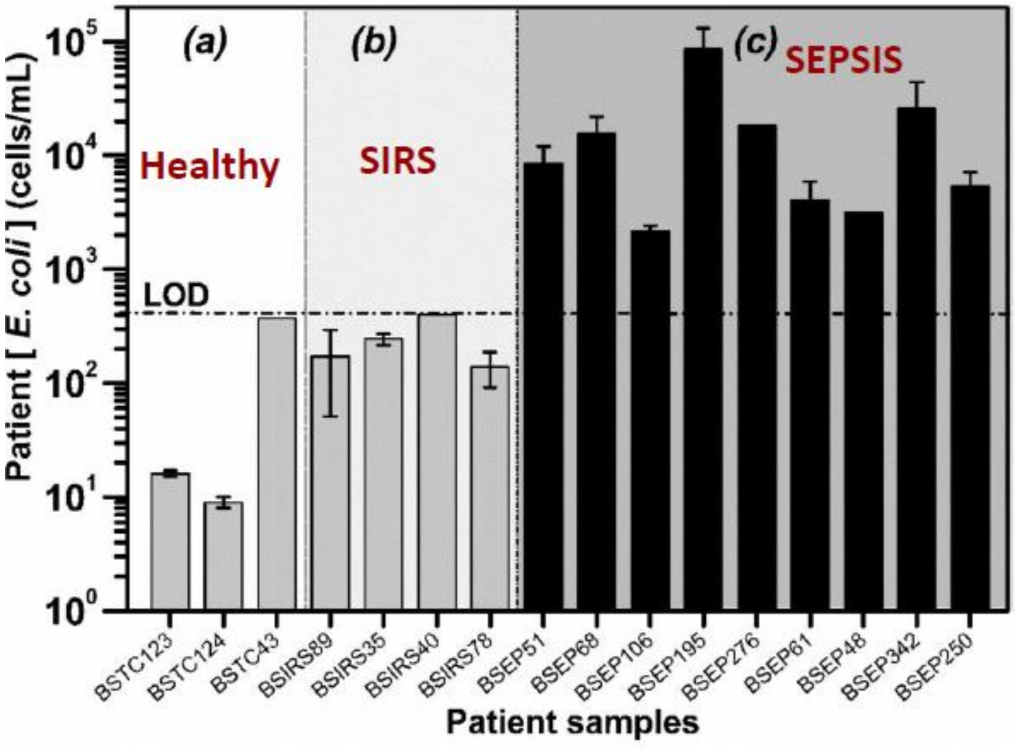






Vall d'Hebron Hospital

REAL SAMPLES VALIDATION (PLASMA)



- Accurate categorization of sepsis patients from healthy individuals and non-bacterial-infection (SIRS) patients
- **10 µL** sample volume
- Fast (40 min): one step on-site quantification
- POC deployed at the hospital
- Tested for the detection of sepsis protein biomarkers

RAIS FASTER THAN SEPSIS

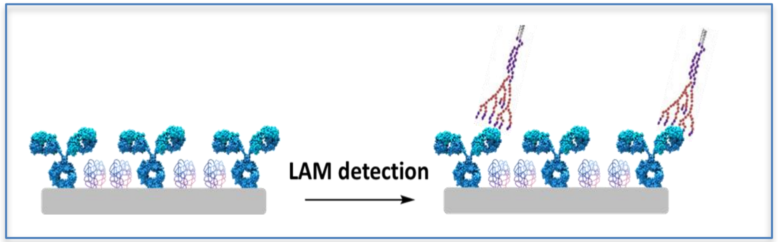
H2020 EU

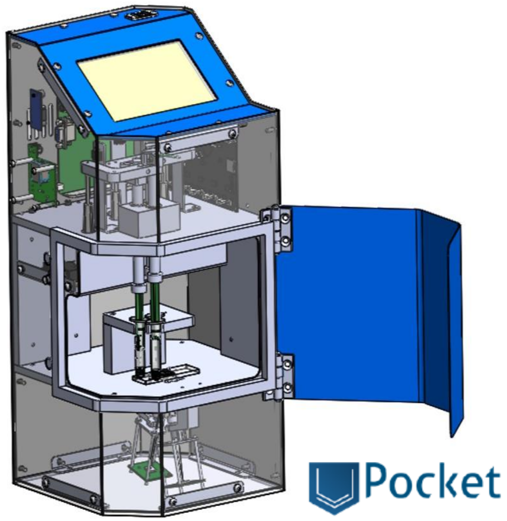
ACS Sensors, 4, 52-60 (2019)
Anal. Clinica Acta, 1077, 232-242 (2019)

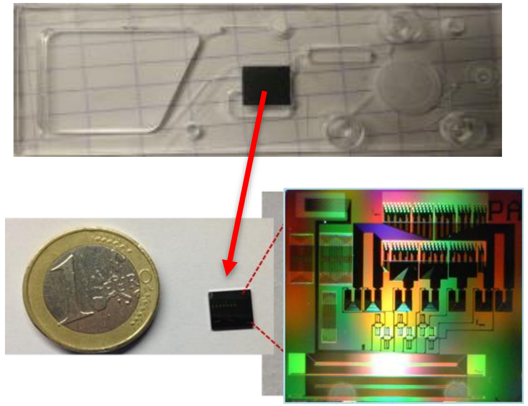
POC biosensor for Tuberculosis detection

Lipoarabinomannan (LAM)

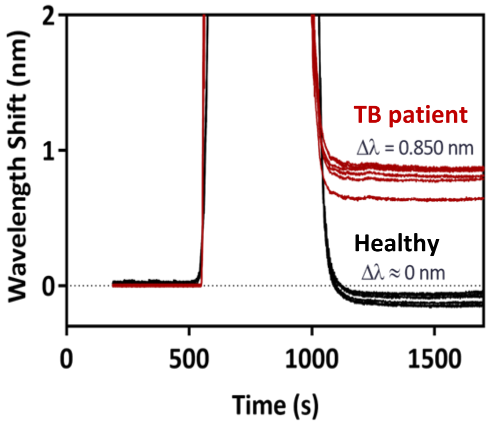
- Lipopolysaccharide found in **mycobacterial cell wall**
- Only present in **people with active TB**
- Confirmed presence in **urine**
- The only biomarker approved by WHO

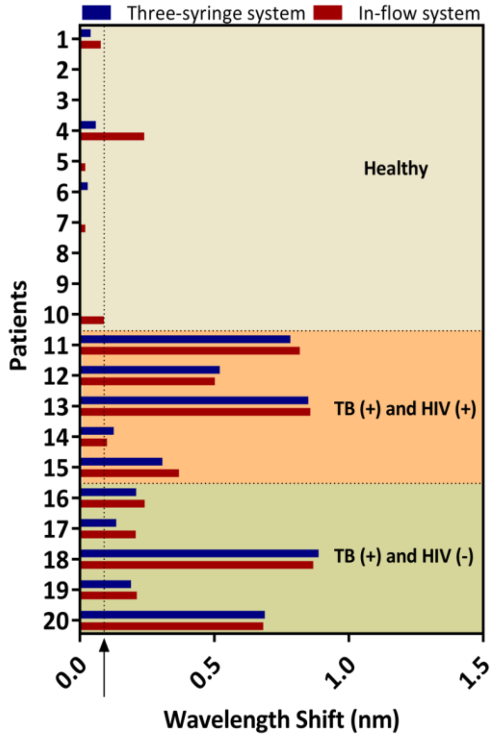






URINE SAMPLES





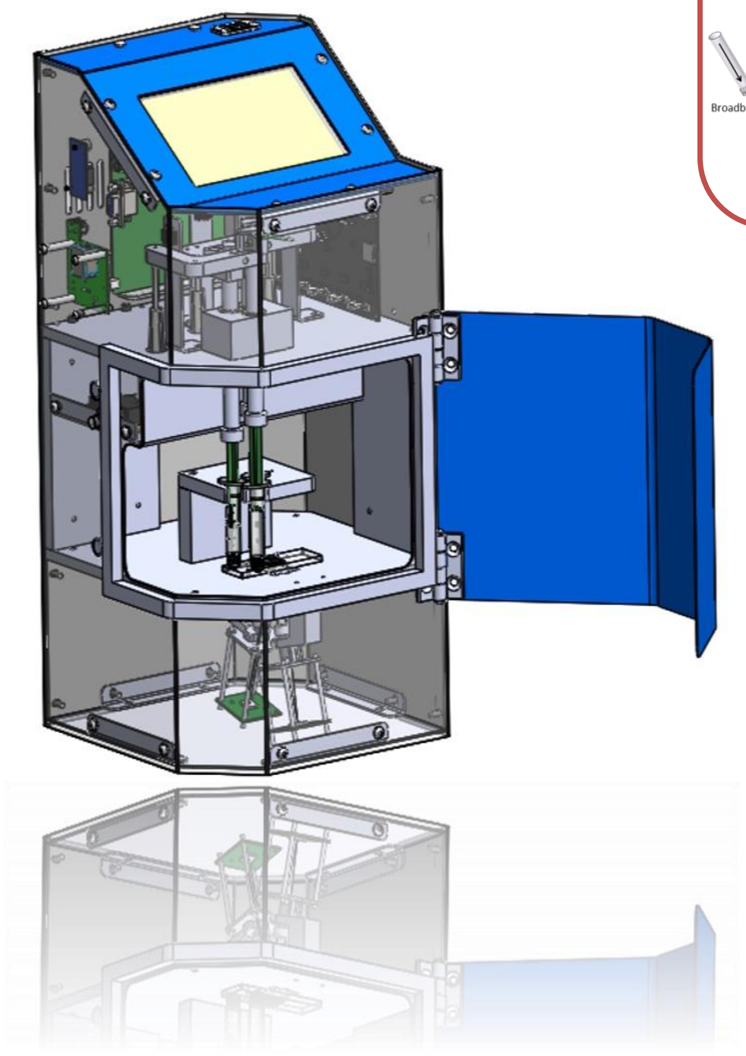
- **Sensitivity= 100 %**
- **Specificity = 95 %**
- **Directly in urine (150 µL)**
- **NO pre-treatment**
- **< 10 min**

ACS Sensors 3 (10) 2079-2086 (2018)
Anal. Methods 10, 3066-3073 (2018)

Low-cost Point-of-care for tuberculosis detection

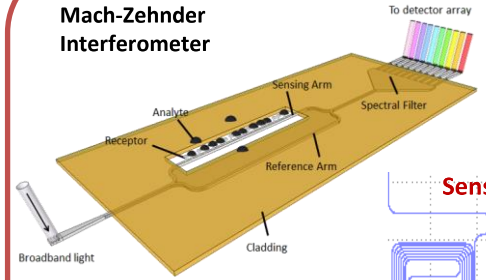
Pocket

POCKET EU PROJECT

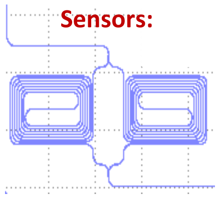


Mach-Zehnder Interferometer

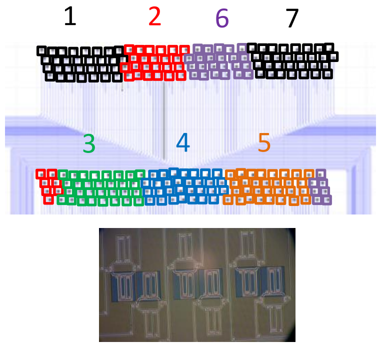
spectral output device



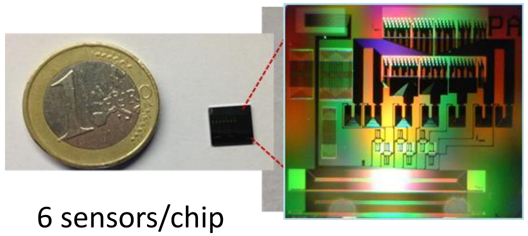
Sensors:



Output gratings



POCKET chips



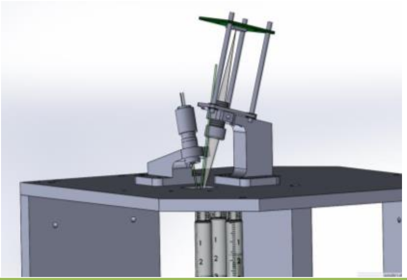
6 sensors/chip
1 reference/chip

7x7 mm^2

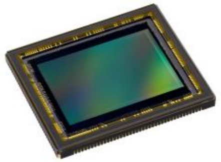
Light source

SLED
850 nm

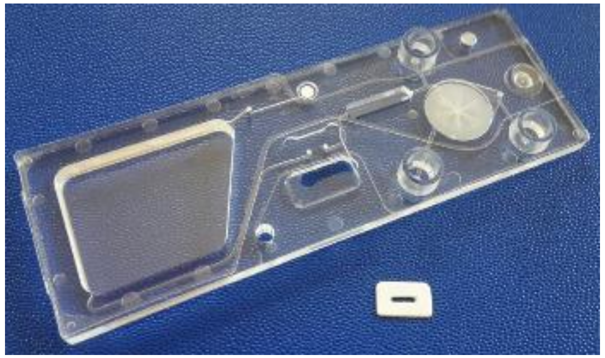
cheap and
allow **multiplexing**

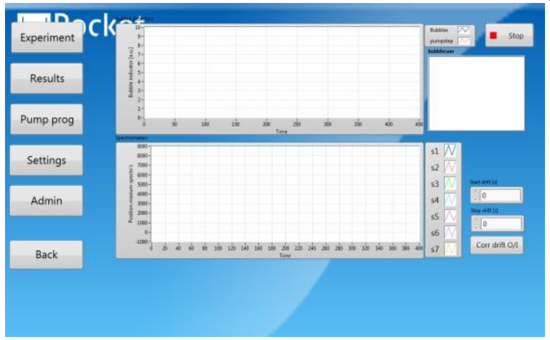


Optical readout



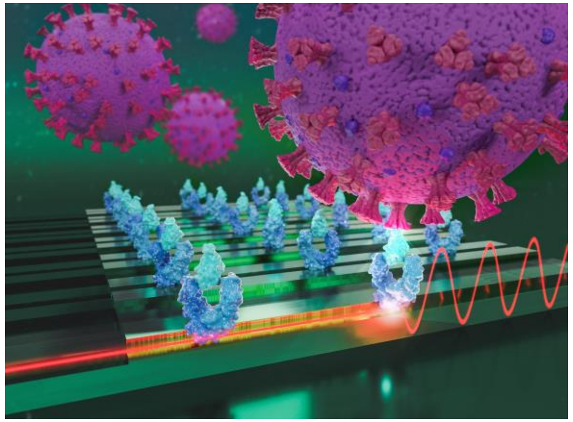
CMOS Camera





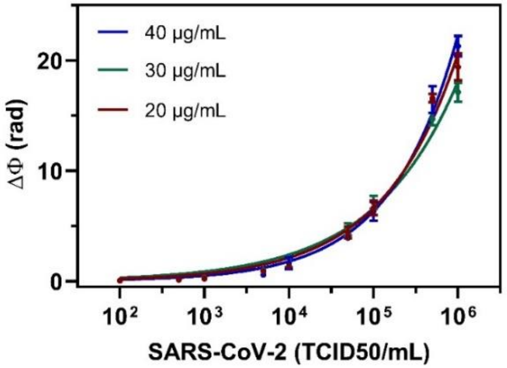
***Anal. Methods* 10**, *3066-3073 (2018)*

Biosensor for Direct SARS-CoV-2 detection



Sensitivity study

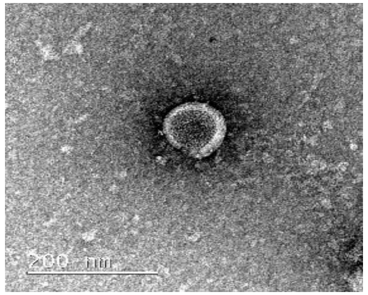
Fc-Nb concentration optimization

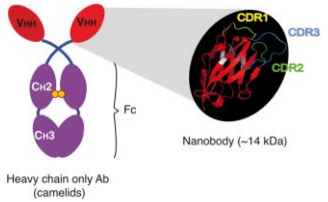


SARS-CoV-2 detection

	TCID50/mL			
µg/mL	LOD	LOQ	Linear range	%CV
20	100	544	$10^2 - 10^4$	15.79
30	69	386	$10^2 - 10^4$	11.12
40	643	1694	$10^2 - 10^4$	18.81

SARS-CoV-2
UV inactivated

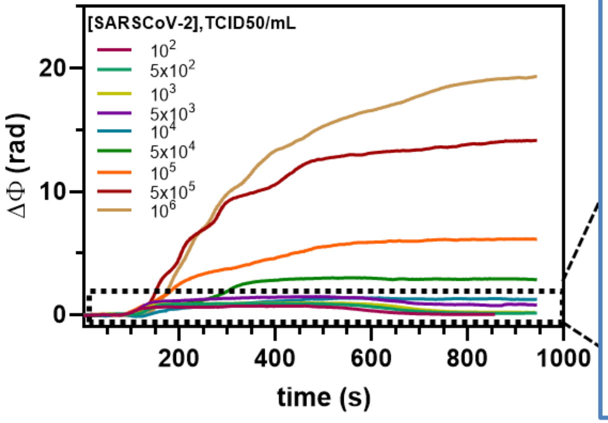




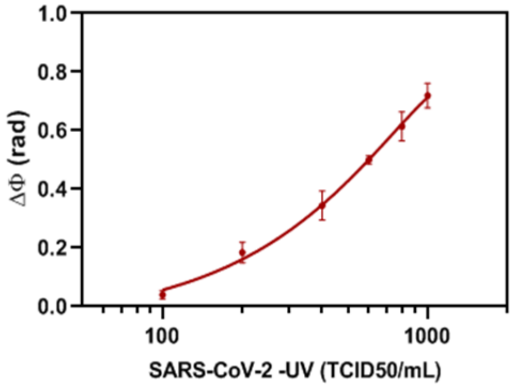
Specific Nanobodies as bioreceptors

In colaboration with Dr. Luis Ángel Fernández (CNB-CSIC)

Viral load detection



Direct detection and quantification



LOD ~ 100 TCID50/mL
LOQ ~ 400 TCID50/mL

Immobilization: Nb-Fc 1.26 (30 µg/mL) in MES pH 6
Buffer detection: 50 mM Hepes / 150 mM NaCl pH 7.1

EXCELLENT SENSITIVITY *(Patient´s levels: 10^3-10^5)*

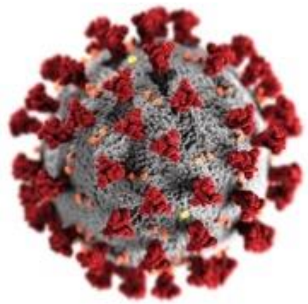
Adapted to SARS-CoV-2 variants of concern

Sensors & Diagnostics 2022

CONVAT

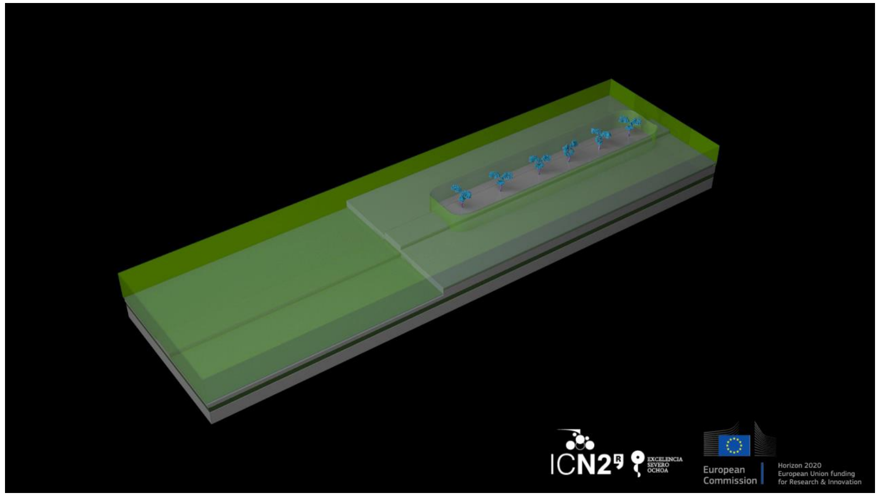
European Commission

Photonic Biosensor for virus detection

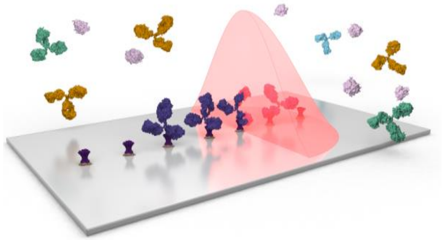


SARS-CoV-2

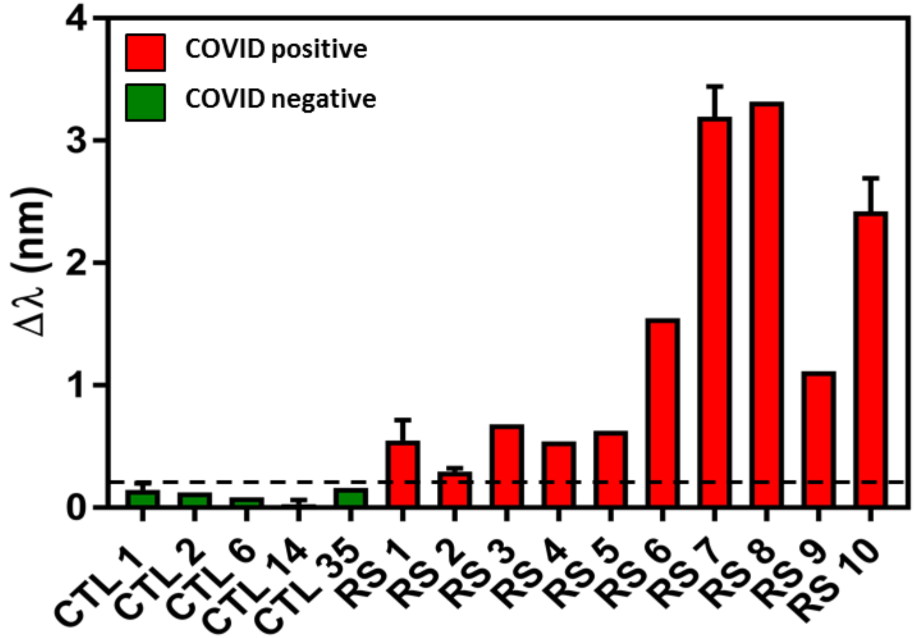
- YES/NO
- Intact virus
- **VIRAL LOAD**. From 100-10^7 virus/mL
- Time to result: : 15 min
- Clinical validation on-going



Photonic Biosensor for serological detection



- YES/NO
- **QUANTITATIVE**. Number of IgG
- Time to result: : 15 min
- Excellent Sensitivity
- Clinical validation-Tech Transfer initiated



Surveillance of the eco-evolution of coronaviruses in animals

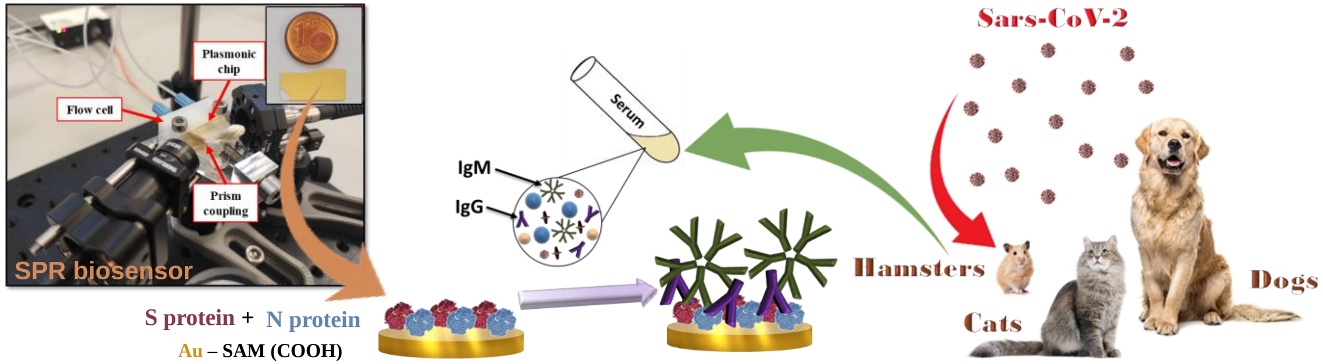
ICN2 EXCELENCIA SEVERO OCHOA

The main objective is an eco-evolutionary study of CoV in animals to improve their surveillance and the prediction of future health emergencies on a global scale.

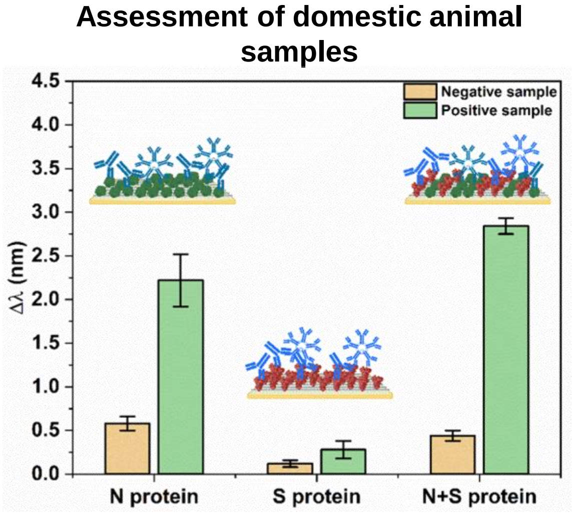
Biosensor for serological analysis of SARS-CoV-2 in domestic animals

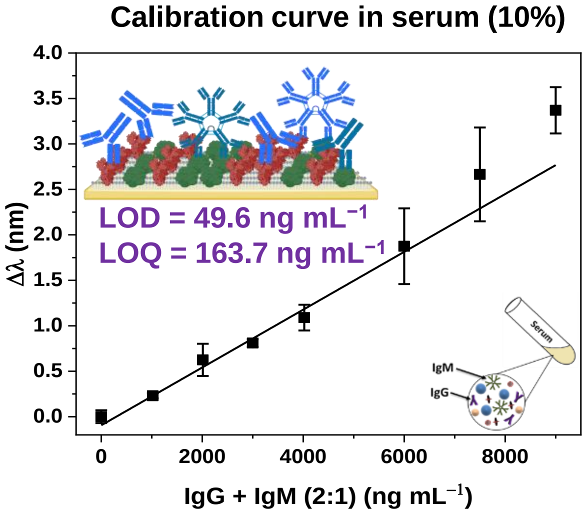
Biosensor for genomic analysis of CoV in domestic and wild animals

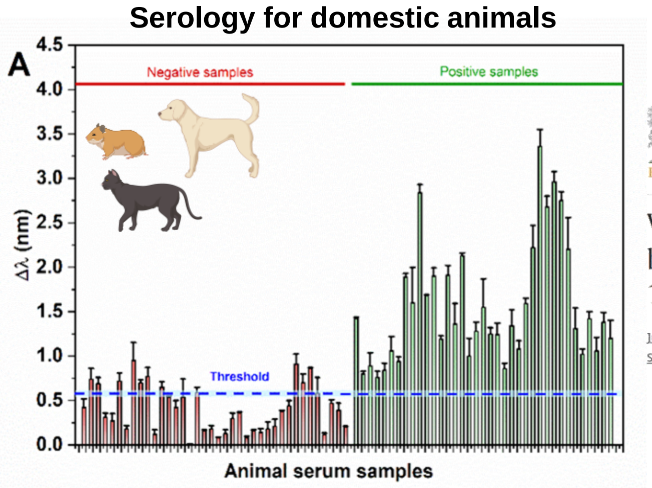
MUSECOV project (ERA-NET)



Circulating coronaviruses in European domestic animals (dogs, cats, and hamsters)







Summary of Biosensor Applications @NanoB2A Group

PROTEIN BIOMARKERS

Early Colorectal cancer (autoantibodies)

Gluten consumption

Hormone level alteration

Doping control

Tuberculosis diagnosis

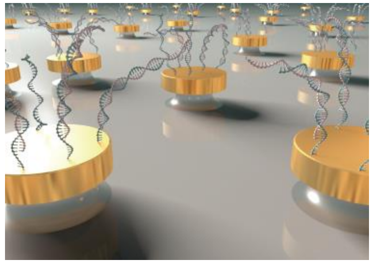
Allergy diagnosis (IgE)

Growth factors

Antibiotics

Urine, serum, plasma, tears

NUCLEID ACIDS

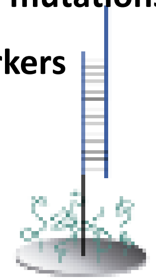


Single DNA cancer mutations

microRNAs biomarkers

Messenger RNA

DNA Epigenetics



Alternative splicing RNA

Antibiotic resistance markers

Urine, serum, plasma, tissue

SMALL ORGANIC MOLECULES

Environmental water pollutants
Pesticides, antibiotics
Organo-halogenated compounds, biocides

Food contaminants
Pesticides residues: canned food, oranges

Drugs
Antibiotics
Anticoagulants (Sintrom®)

Waste- sea-tap- river-water, food

INFECTIOUS PATHOGENS

Nosocomial infections

Chronic liver failure

Sepsis

Resistant bacteria

Water pathogens

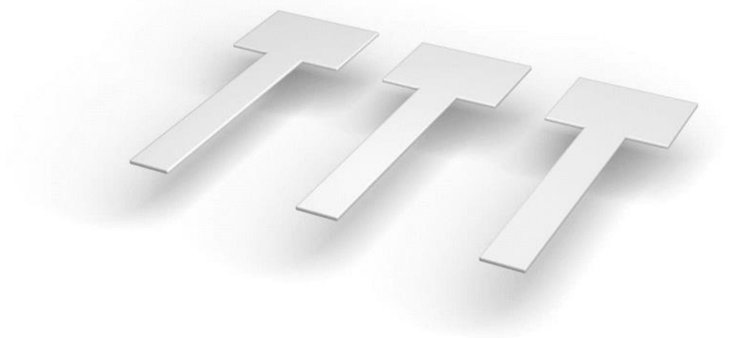
Respiratory virus

Urine, serum, plasma, ascetic fluid

Point-of-care photonic biosensors for decentralized analysis

Using light to make diagnostics devices accessible to everyone

- **Point-of-care biosensors** are required for fast, direct, label-free, high sensitivity, low sample volume and massive diagnostics for the **post-pandemic era.**
- Nanophotonics biosensors are one of the **most competitive technology**
- Surface chemistry **biofunctionalization** is the **key** for sensors specificity
- Biosensor platforms with **Multiplexing capabilities** will be required



2003

The Inventor: Out of Blood in Silicon Valley (2019) HBO

The story of Theranos, a multi-billion dollar tech company, and **its founder** Elizabeth Holmes

>$9 billones

"The Dropout"(Disney+)
"The Inventor: Out for Blood in Silicon Valley"(HBO)
"Bad Blood" (book by John Carreyrou)

THE nano b2a GROUP

GRACIAS!!!

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Multidisciplinary research

biology
engineering
chemistry
telecommunications
physics
mathematics
programming
biotechnology

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CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

EXCELENCIA SEVERO OCHOA

MINISTERIO DE CIENCIA E INNOVACIÓN

Financiado por la Unión Europea NextGenerationEU

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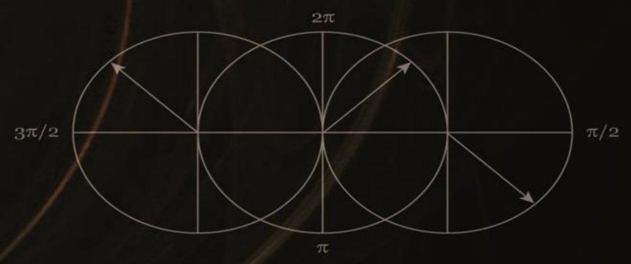
ciber-bbn
Centro Investigación Biomédica en Red Bioingeniería, Biomateriales y Nanomedicina

European Commission

HORIZON 2020

SILICON PHOTONICS BIOSENSORS

Basic concepts



Brief historical recap

Search of “integrated optics sensors” Google Scholar & Scopus:

1960-1969: Coupled Charge Devices for image acquisition

1970-1979: Integrated optics for gyroscopes

1980-1989: Chemical/gas sensors references start to appear

To my knowledge, W. Lukosz reported first as-we-know-today evanescent PIC sensor*:

*“When **these SiO_2-TiO_2 waveguides provided with surface relief gratings** were used for incoupling experiments with red HeNe laser light, the unexpected and surprising finding was that **the intensity of the light coupled into the waveguide at a constant angle of incidence was not constant in time as** is to be expected from the incoupling condition **but varied erratically***

*Soon we found that **the effect was caused by variations of the relative humidity in the environment** of the coupler grating on the waveguide. The effect can be **induced by the experimenter exhaling towards the waveguide or holding a finger near the waveguide”***

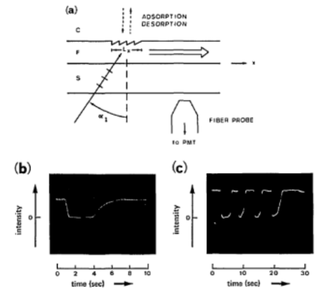


Fig. 1. (a) Schematic of switching or sensor experiment with an input grating coupler. S, substrate; F, waveguiding film; and C, air; with refractive indices n_S, n_F, and n_C, respectively. α_l, incoupling angle; L_x, length of grating; PMT, photomultiplier tube. (b), (c) Signal proportional to the intensity $I(t)$ of guided TE_0 mode ($\lambda = 514$ nm) versus time t for two sources of humidity at distance d from grating; (b) finger for a time of 2 sec, $d \simeq 1$–2 cm; (c) breathing experimenter $d \simeq 20$ cm.

Mach-Zehnder based PIC sensors are already mentioned by Lukosz, first as-we-know start to be reported at late 80’s and early 90’s, with a patent (1987) and paper by Heideman et al. (not fully integrated). **First integrated MZI biosensor was done at CNM, Barcelona (Spain) (1994)**

*W. Lukosz and K. Tiefenthaler, “Directional switching in planar waveguides effected by adsorption-desorption processes”, in Proc. 2nd Eur. Conf. Integrated Optics, Florence, 1983.
K. Tiefenthaler and W. Lukosz, "Integrated optical switches and gas sensors," Opt. Lett. 9, 137-139 (1984).
**Falk, Robert A., and Raymond W. Huggins. "Integrated optic field sensor consisting of an interferometer formed in substrate." U.S. Patent No. 4,899,042. 6 Feb. 1990. (Filed 1987)
R. Heideman, R. Kooyman, J. Greve, and B. Altenburg, "Simple interferometer for evanescent field refractive index sensing as a feasibility study for an immunosensor," Appl. Opt. 30, 1474-1479 (1991).
E.F. Schipper, A.M. Brugman, C. Domínguez, L.M. Lechuga, R. P.H. Kooyman and J. Greve. **The realization of an integrated Mach-Zehnder waveguide immunosensor in Silicon technology** Sensors and Actuators B 40, 147-153 (1997)

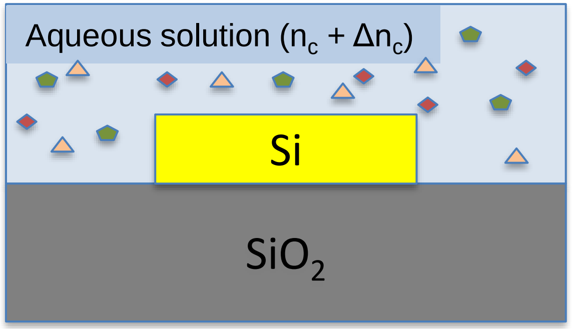
Courtesy of Dr. P. Muñoz (UPV)

PHOTONIC BIOSENSORS: Basic concepts

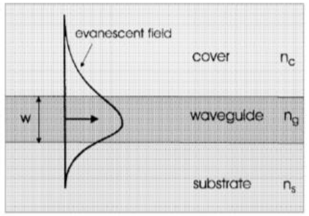
Evanescent Field Sensing: SENSITIVITY

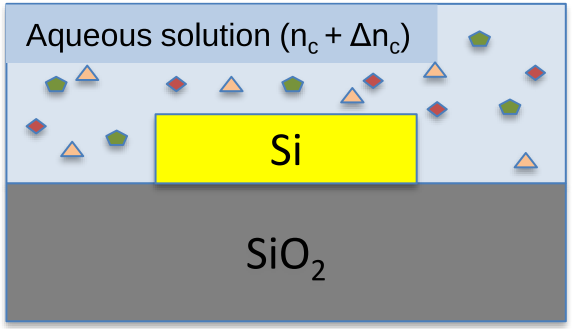
BULK SENSING

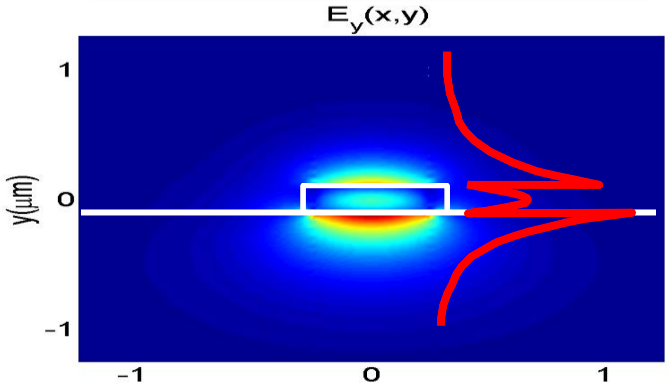
The analyte homogeneously distributed in the cover



$$S_b = \frac{\partial n_{eff}}{\partial n_c} \; (RIU/RIU)$$

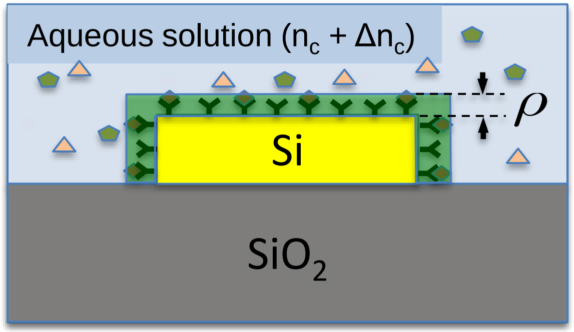




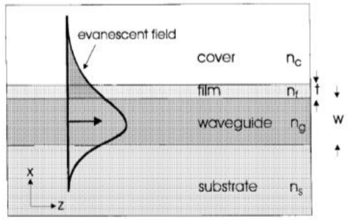


SURFACE SENSING

The analyte is a thin film at the core-cover interface



$$S_s = \frac{\partial n_{eff}}{\partial \rho} \; (RIU/nm)$$



$$\Delta n_{eff} = c \int \Delta n(x,y)^2 \, | E(x,y) |^2 \, dxdy \quad \longrightarrow \quad S_{Waveguide} = \frac{\partial n_{eff}}{\partial n_c}$$

TM and TE modes have different sensitivities

Metrics

REFRACTIVE INDEX UNITS (RIU)

- The amount of change in the analyte (cover) refractive index: Δn_c = 0.001 RIU
- Employed to relate the change of effective index n_{eff} with respect to cover index n_c (sensitivity)
- Independent of the sensor architecture
- Used to correlate the sensor readout to the analyte, e.g. a wavelength shift sensor has a given metric in, for instance, nm/RIU
- And a backward measurement can indicate a concentration, e.g. if cover is a liquid solution, a given shift in nm gives n_c, and from this the concentration is estimated (if composition known!)

LIMIT OF DETECTION (LoD)

- Smallest amount of analyte that produces a quantifiable output
- Can be specified in two different ways:
  - Bulk sensitivity expressed as refractive index units (RIU)
  - Surface sensitivity:
    - Typically in mass density pg/mm^2
    - Also as analyte concentration (ng/mL or molarity)
- LoD depends on the resolution of the redaout system
- LoD is strongly dependent on experimental noise
- LoD is typically used to compare different sensors

MASS DENSITY OR CONCENTRATION

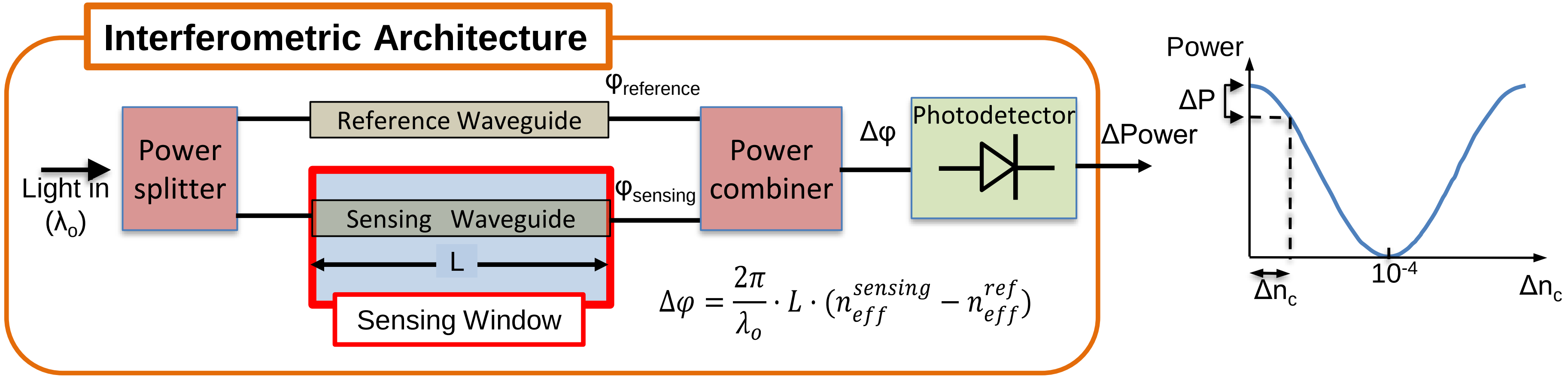
- Refers to the accumulation of mass on the sensor surface
- Independent of the sensor architecture
- Can be given in mass density units (pg/mm^2)
- Can be given as analyte concentration (ng/mL or molarity M, which is mole/L), related to the molecular weight of molecules

WARNING!

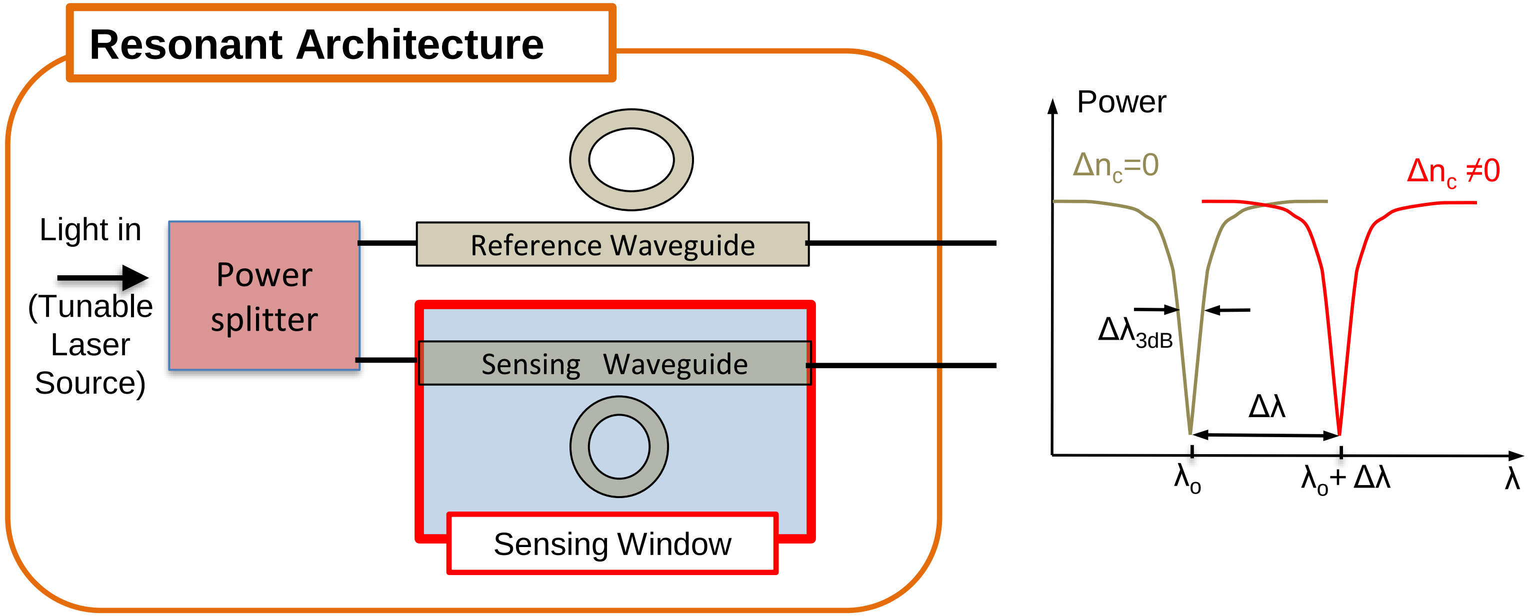
Which units to use for LoD when comparing sensors

- Either RIU or mass density (pg/mm^2)
- Molarity can be used if molecules have same MW!
- Otherwise comparison in ng/mL is not fair

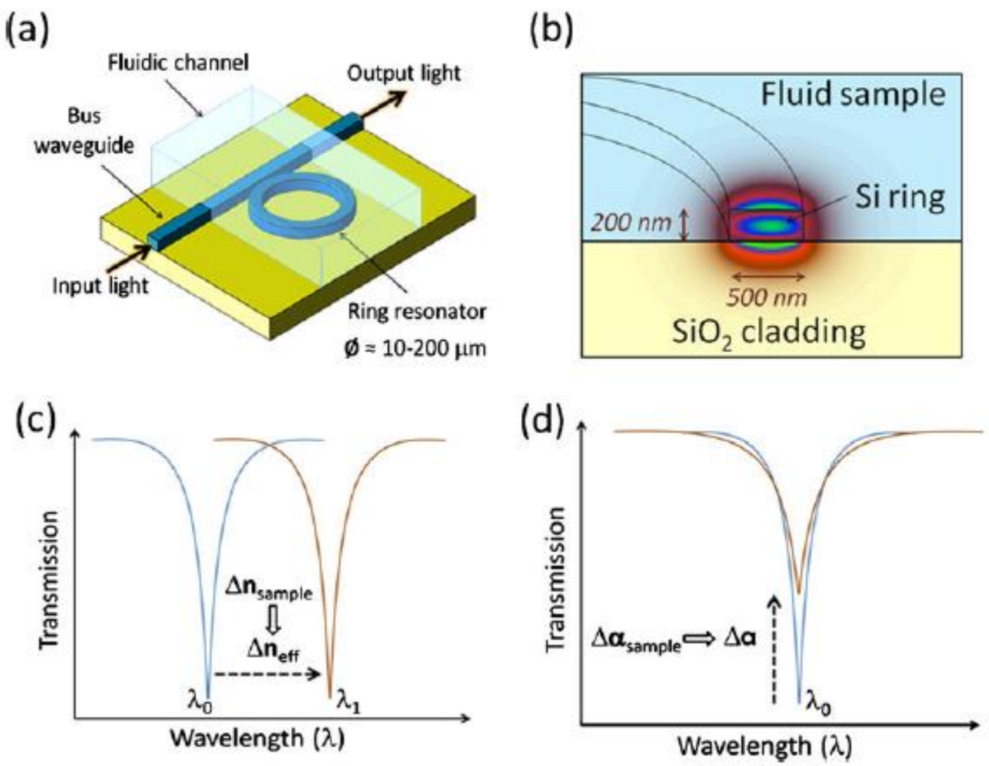
Sensing Architecture



$$\Delta\varphi = \frac{2\pi}{\lambda_o} \cdot L \cdot (n_{eff}^{sensing} - n_{eff}^{ref})$$



Ring Resonators based Biosensors



Monitoring spectral shift, gives quantitative information about the biointeraction

PRINCIPLE OF OPERATION

- **Light is coupled to the microring** by a straight (bus) waveguide on-chip.
- **At wavelengths that are factors of the ring circumference, photons** circulating in the ring **constructively interfere with those propagating in the bus waveguide**, leading to a resonant optical mode in the ring.
- The **sample** to be analyzed is **probed by** the **evanescent field**
- **The position and/or amplitude of the transmission minima**, i.e. resonance wavelengths, depend on the effective index of the mode, that is **modified by the refractive index of the analyte**

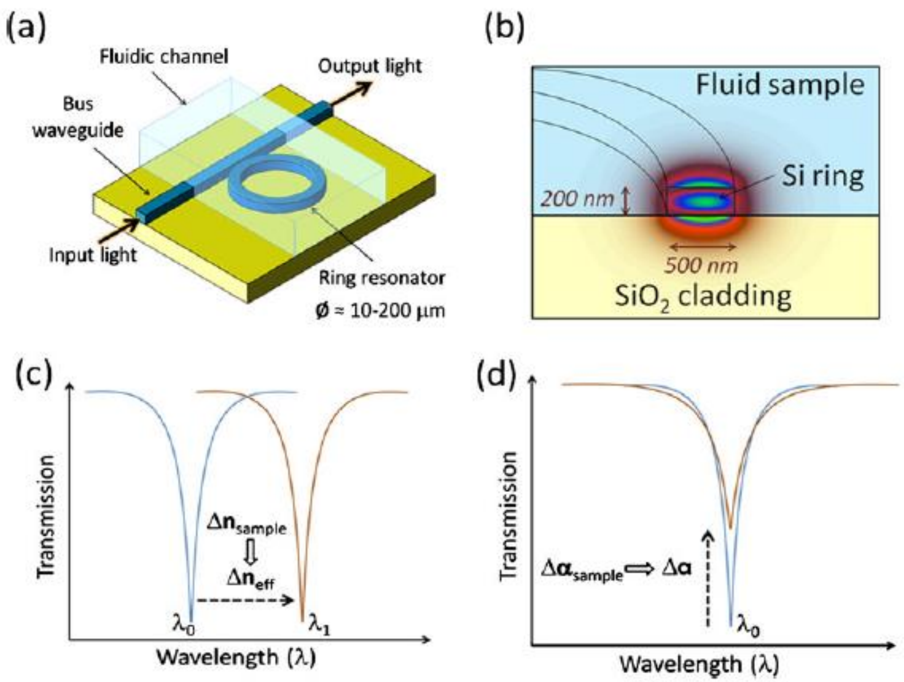
Sensitivity
Increases with increasing Q factor of the ring

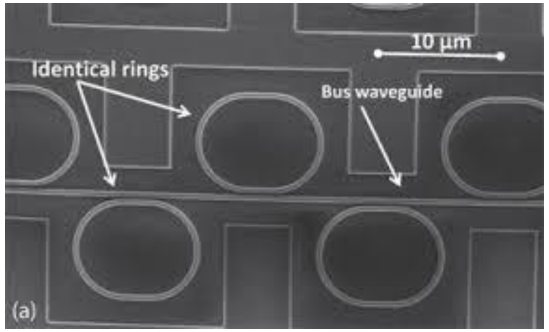
High Q factors: low losses/long photon lifetimes. Q factors: 10^6

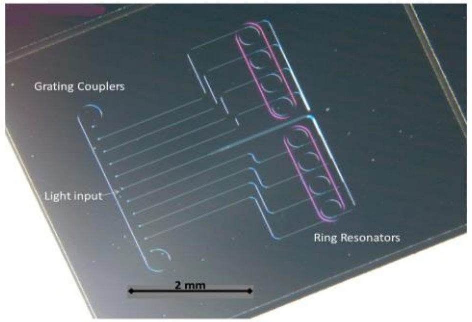
$$Q = \frac{\lambda_{resonance}}{\Delta\lambda_{3dB}}$$

Laser & Photonics Reviews 6, 463-487 (2012)
Sensors 16(3), 285 (2016)

Ring Resonators based Biosensors





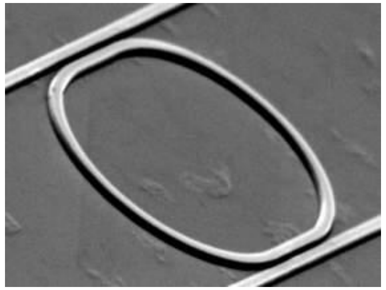


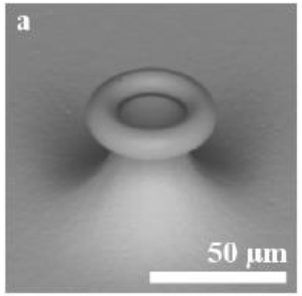
$$Leff = \frac{Q\lambda}{2\pi n} \qquad \lambda = \frac{2\pi r Neff}{m}$$

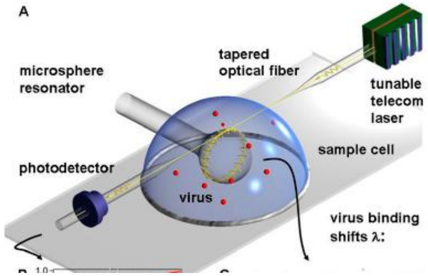
Monitoring spectral shift, gives quantitative information about the biointeraction

High Multiplexing
LOD: 8.10^{-7} RIU

Micro-rings | **Micro-toroids** | **Micro-spheres**







Advantages:
- Reduced footprint
- High multiplexing capabilities
- Good sensitivity (not scaling with lenght)
- CMOS compatible fabrication (low cost and lab-on-chip integration)

Drawbacks:
- 1550 nm design (strong water absorption)
- Expensive (tunable laser or optical spectrum analyzer)

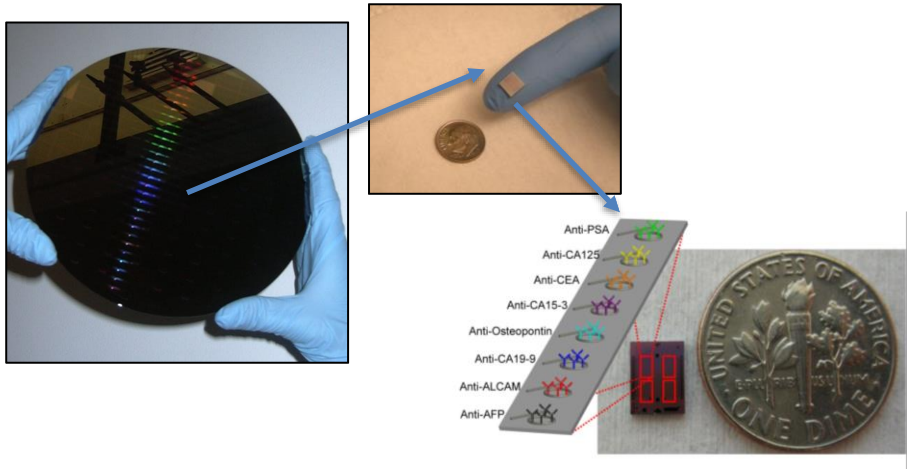
Laser & Photonics Reviews 6, 463-487 (2012)
Sensors 16(3), 285 (2016)

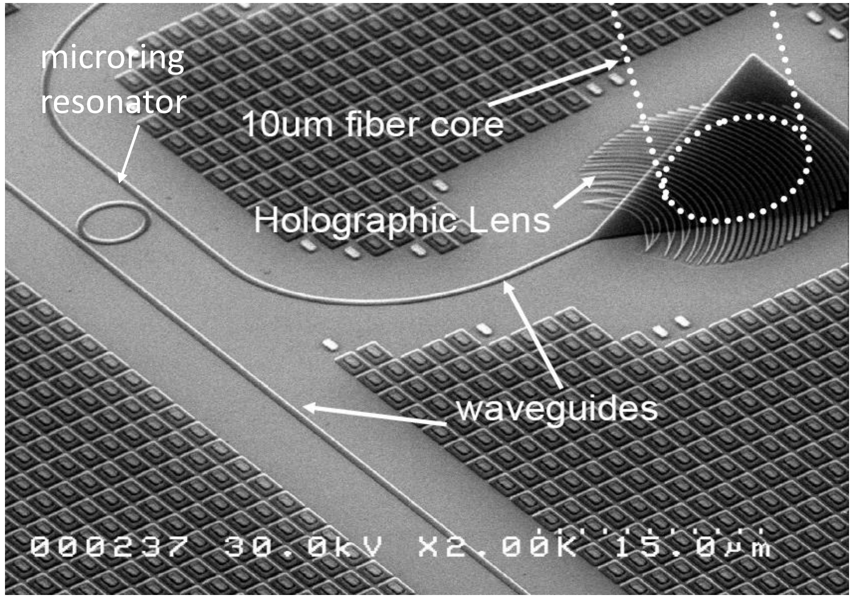
Microring resonator biosensor

Genalyte: up to 128 addressable rings (US)

- **Semiconductor processing**
  - All optical components are monolithically incorporated into the top layer Si.
  - Commercial fabrication on 8" SOI wafers via deep UV lithography
    - **600+ sensor chips/wafer**
    - **Low chip cost; disposable sensing platform**
  - **Sensors scale to over 10,000/cm^2**
    - Redundant measurement increases precision; on-chip referencing.
- **Si transparency** window at 1550 nm overlaps with telecom c-band
  - High speed and precision tunable lasers

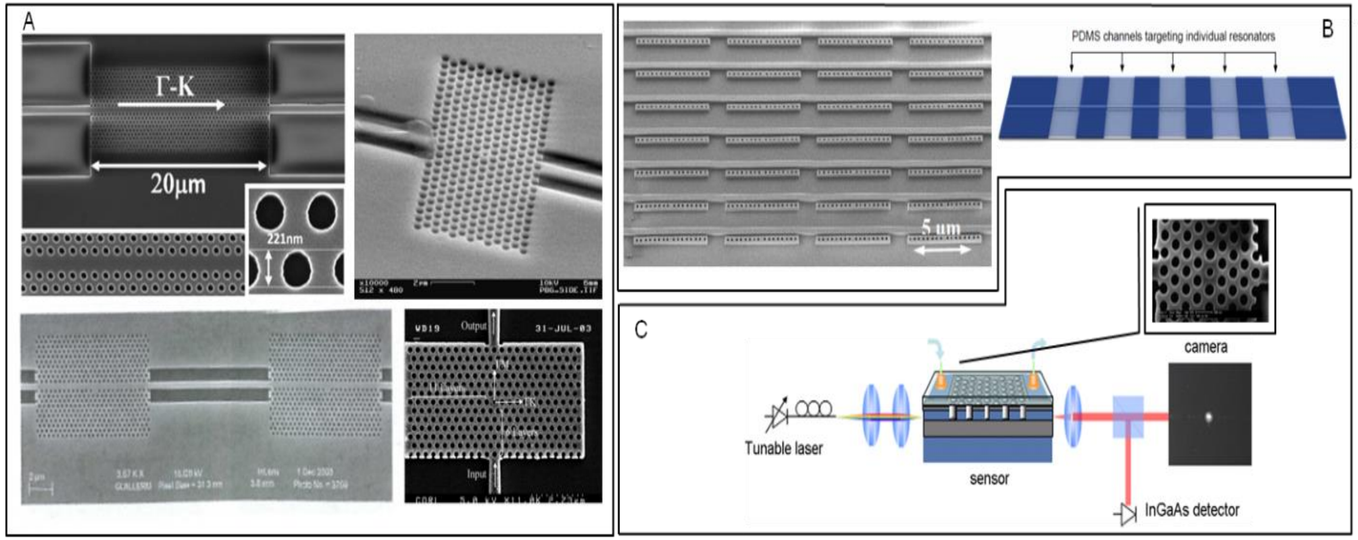
Silicon-on-insulator microrings offer incredible scalability and measurement convenience

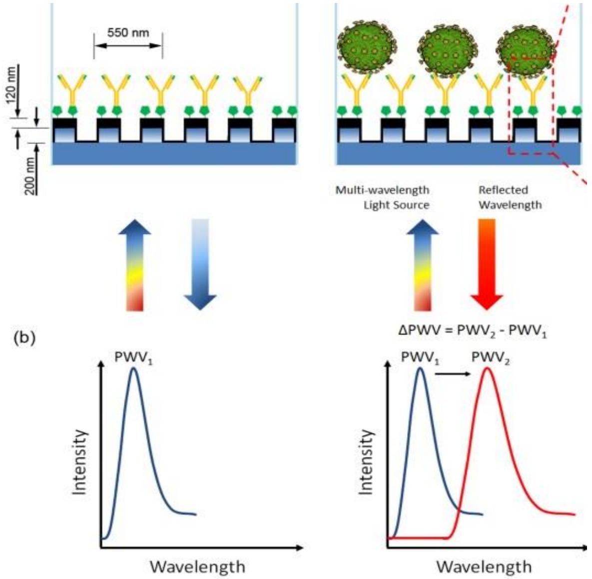




www.genalyte.com

Photonic crystals based biosensors



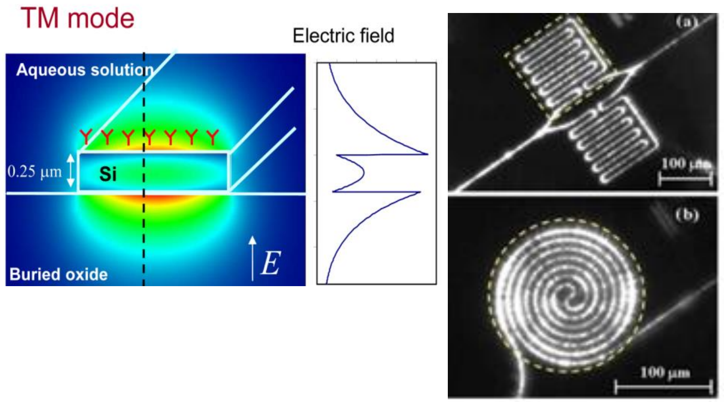


- Nanostructures with periodically (1D, 2D, 3D) repeated variations in the refractive index.
- Width and position of the **photonic bandgap** where light cannot propagate is highly dependent on the **n** change between the dielectric material and the periodicity of the structure.
- Strong confinement of the light in the periodic lattice.
- Attractive configuration due to their small dimensions: multiplexing

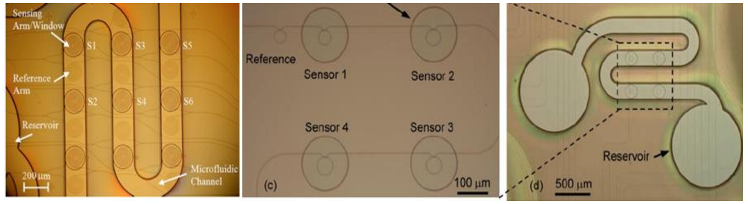
Mass sensitivity: 2 pg/mm^2
LOD: 3·10^{-5} RIU

Other waveguide configurations for sensing

Silicon wires as Sensing Waveguide

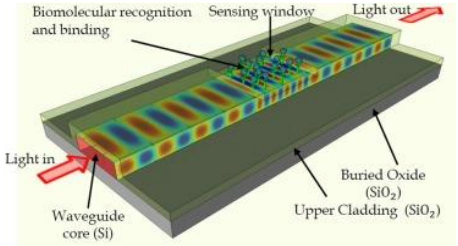


- Silicon photonic wires: submicron waveguides fabricated by ebeam lithography on SOI wafers
- High index contrast between the silicon core (n=3.5) and silica cladding (n=1.5): strong field confinement
- Sharp waveguide bends radii of few microns with low losses



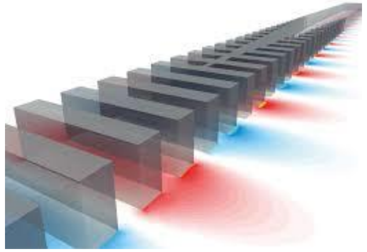
High Multiplexing
Mass sensitivity: 0.25 pg/mm^2
LOD: 2.10^{-6} RIU

Subwavelength Gratings as Sensing Waveguide (SWG)



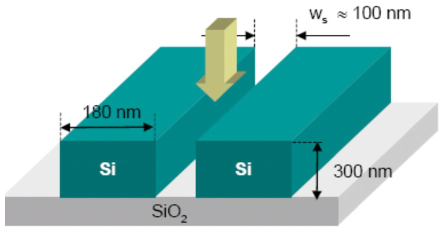
W= 600 nm; H= 220 nm; Λ= 200 nm

- **Periodic arrangement of silicon blocks**: pitch small enough to suppress diffraction effects and the structure behaves as a lossless waveguide
- Due to the segmentation, a significant delocalization of the electric field takes place and field enhancement at the sidewalls and between the silicon blocks: **high sensing capabilities**.



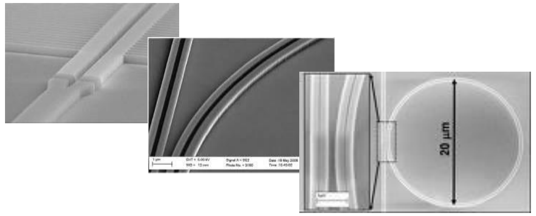
Few biosensing results

Slot waveguides as Sensing Waveguide



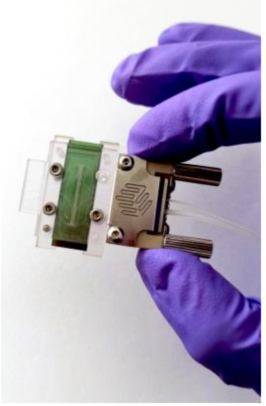
- **two sections of high refractive index** materials **separated by a nanometer low refractive index** slot region, surrounded by low n cladding. **Light is strongly confined in the slot region**
- **high sensing capabilities**. A non-trivial issue is **how to “fill-in” the slot with the analyte**

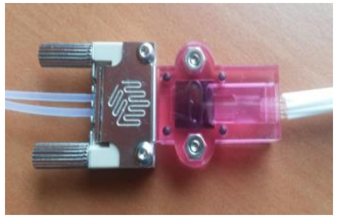
Slot-wg ring resonators



Take home messages

Point-of-care photonic biosensors for decentralized analysis





- Photonic chip technology is NOT the only factor defining the SENSITIVITY of a biosensor
- Benchmarking with SPR plasmonic biosensor technology
- Low-cost fabrication and integration technologies must be employed (disposable chips)
- Cartridge option (off-chip integration) is more suitable for real biosensing applications
- Surface chemistry and Biofunctionalization should not be UNDESTIMATED
- Working in the visible range highly recommended