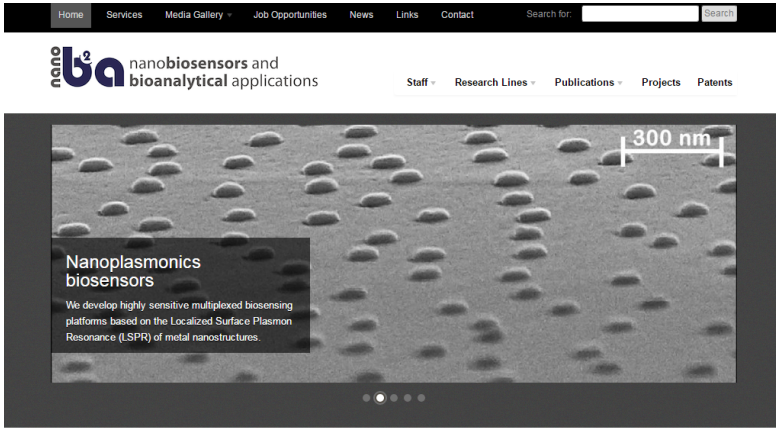
Biossensores plasmônicos

Cristina Kurachi

Nanobiosensors and Bioanalytical applications (NanoB2A) – ICN2

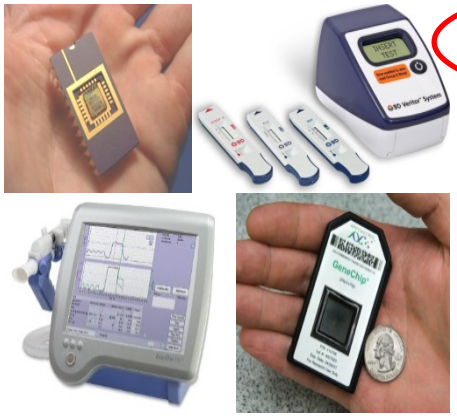


Institut Català de Nanociència y Nanotecnologia

Medical diagnostics

Clinical laboratory

- Limited and centralized laboratories
- Trained personnel and time-consuming analysis
- Expensive instrumentation



Point-of-Care diagnostics

- Fast, portable and user-friendly
- Decentralized analysis: diagnosis *in situ*
- Healthcare improvement in resource-constrained settings

Lateral-Flow Assay Test

Pregnancy Test

- Yes/No Response
- Low sensitivity
- Difficulties for multiplexed assays

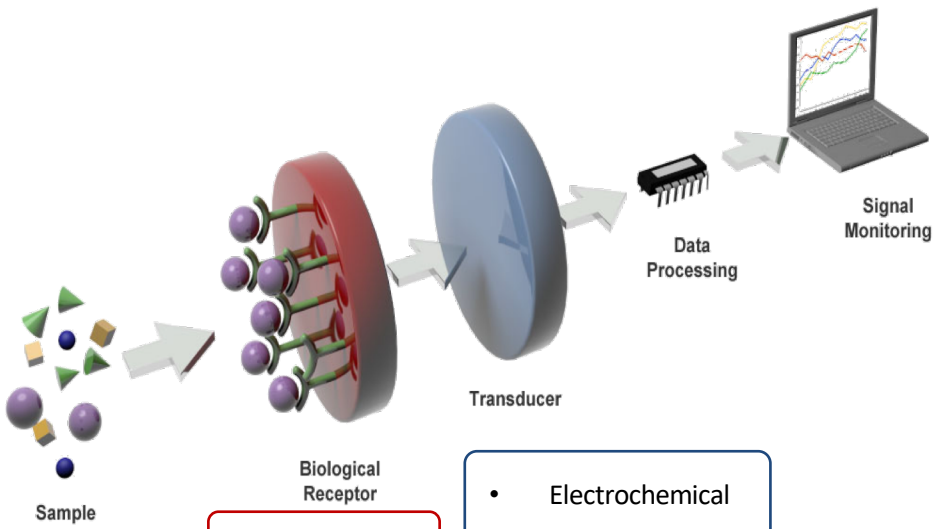
Biosensors

Glucose Biosensor

- Quantitative signal
- High sensitivity
- Integrated technology

Biosensors

"Self-contained integrated device that provides specific quantitative or semiquantitive analytical information using a biological or biomimetic recognition element which is in direct spatial contact with a transducer."
(IUPAC definition)



- Catalytic
- Affinity

- Electrochemical
- Mechanical
- **Optical**

Plasmonic Biosensors

Surface Plasmon Resonance

Main properties

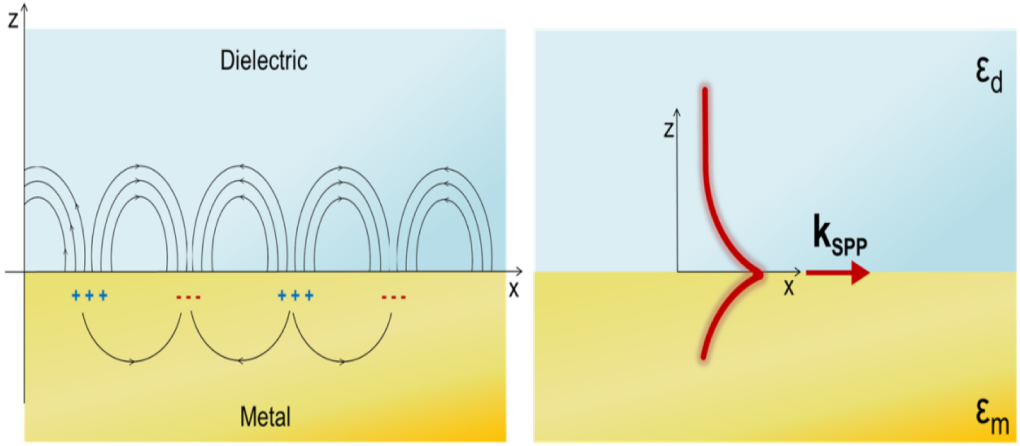
- Rapid and simple analysis
- Sensitive and selective
- Small devices
- Low-cost production

Biomedical Applications

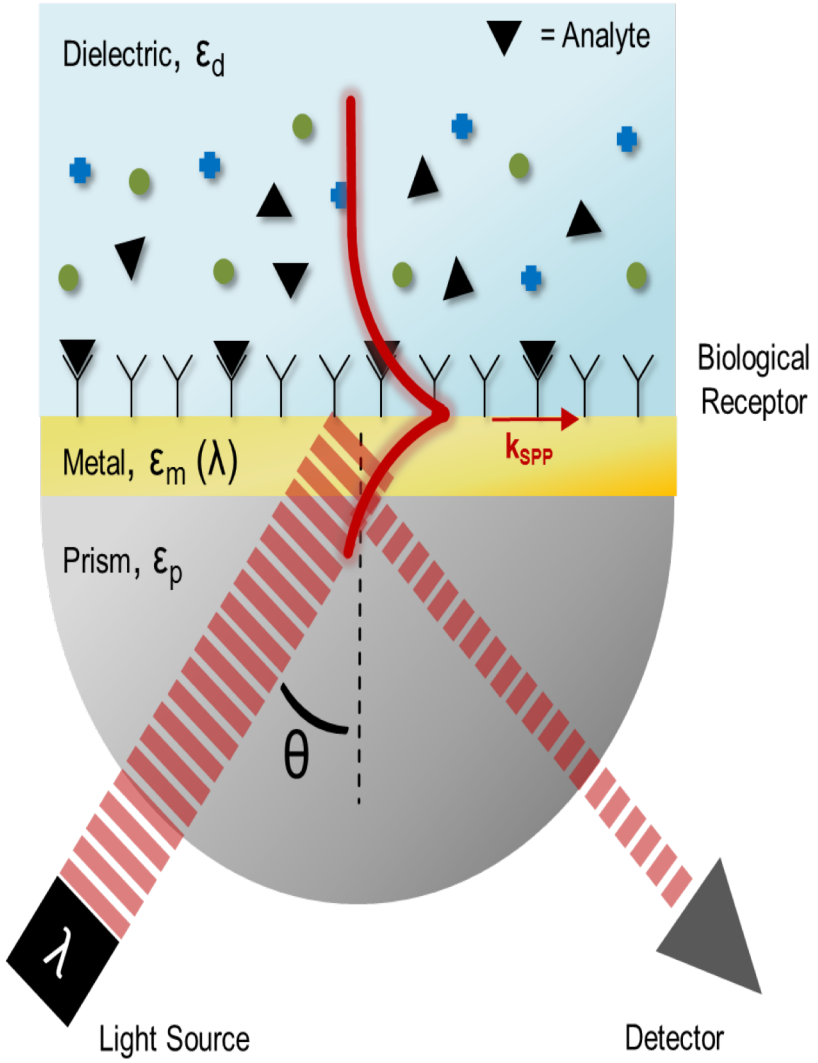
- Diagnosis and monitoring
- Biomarker identification
- Drug discovery

Surface Plasmon Resonance (SPR) Biosensor

Surface Plasmon Resonance: Collective oscillation of surface electrons at the interface of a metal (e.g. Au) and a dielectric that generates an evanescent wave sensitive to RI changes.

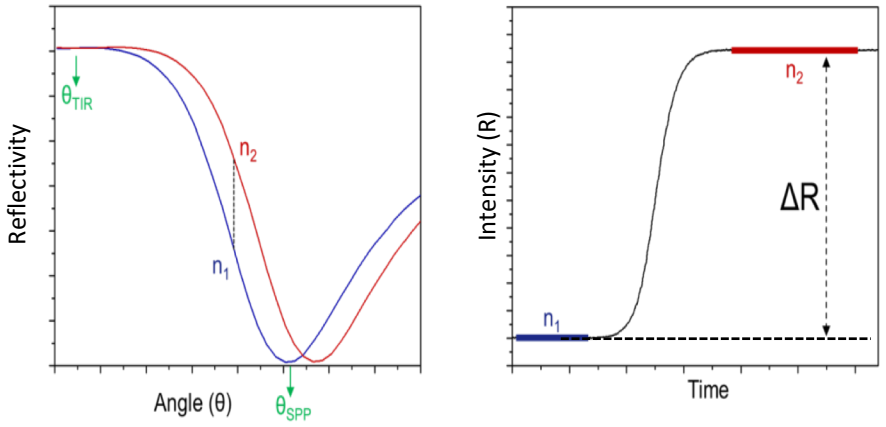


- **Evanescent Field Penetration:** 100 - 500 nm
- **Light Coupling:** Prism / Waveguide / Gratings

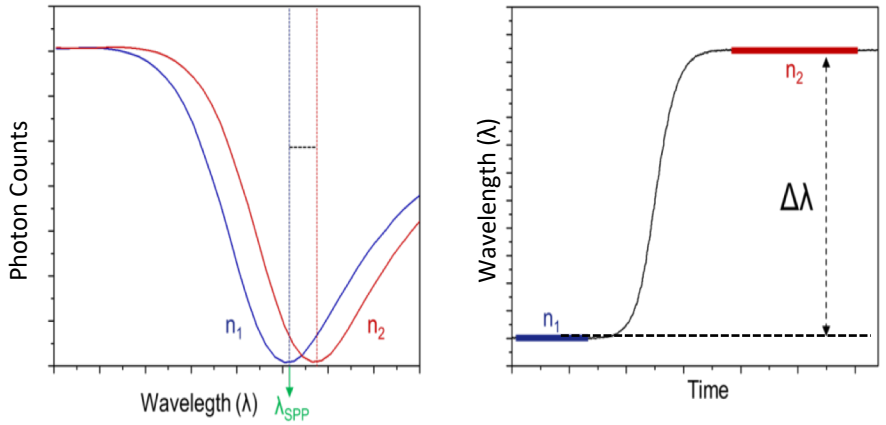


Surface Plasmon Resonance (SPR) Biosensor

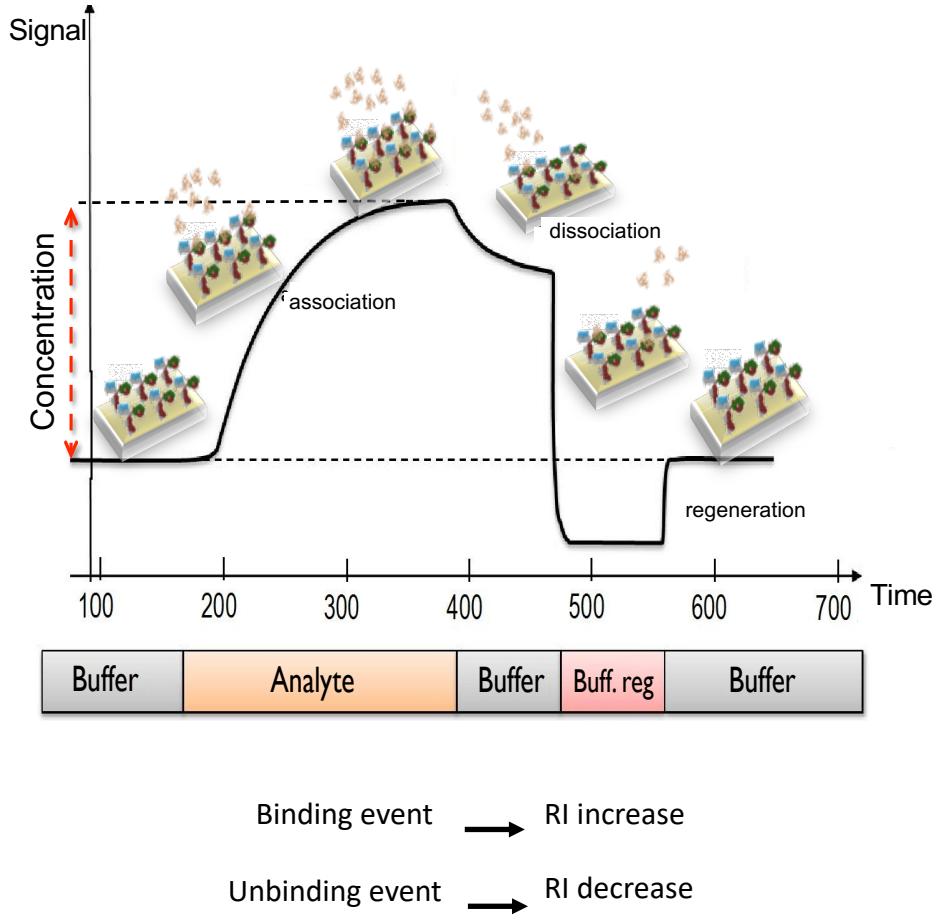
Intensity changes (ΔR)



Wavelength displacements (Δλ)



Real-time monitoring



Binding event → RI increase

Unbinding event → RI decrease

Surface Plasmon Resonance (SPR) Biosensor

- ✓ Versatility: analysis of any type of biochemical interaction
- ✓ Robustness and simplicity
- ✓ Sensitivity: LoD pM-nM (~10^{-5}–10^{-7} RIU)
- ✓ Affinity and kinetic studies
- ✓ Widespread technique and commercially available

Biacore (GE Healthcare)

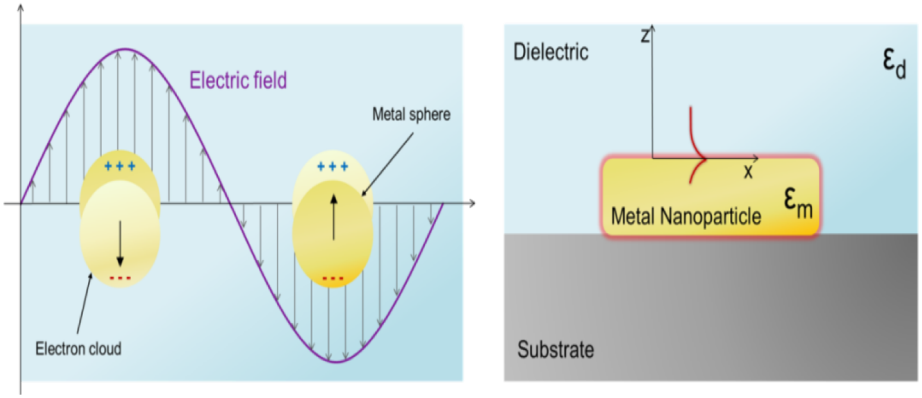
Sensia, S.L.

Nanoplasmonic Biosensors

Localized Surface Plasmon Resonance (LSPR)

Localized Surface Plasmon Resonance (LSPR) Biosensor

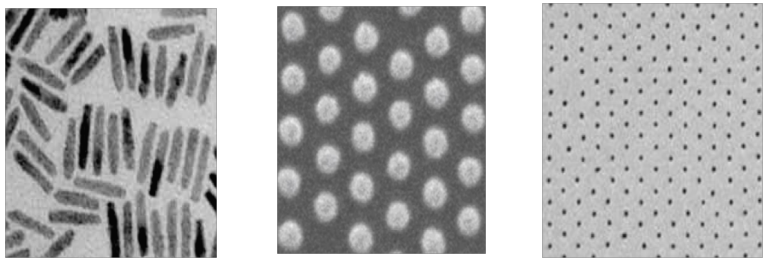
Localized Surface Plasmon Resonance: Oscillation of surface electrons of metal nanoparticles ($\varnothing < \lambda$), inducing a dipolar field that generates an evanescent wave sensitive to RI changes.



- **Evanescent Field Penetration:** 30 - 50 nm
- **Light Coupling:** not required
- **Size and shape tunability**

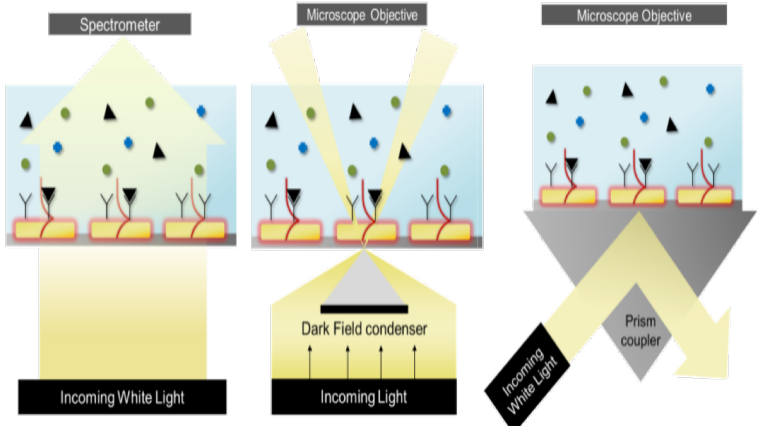
  Numerous sensor schemes and devices

Plasmonic Nanostructured Surfaces



Nanorods | Nanodisks | Nanoholes

LSPR Detection Systems



Extinction

High density of nanostructures

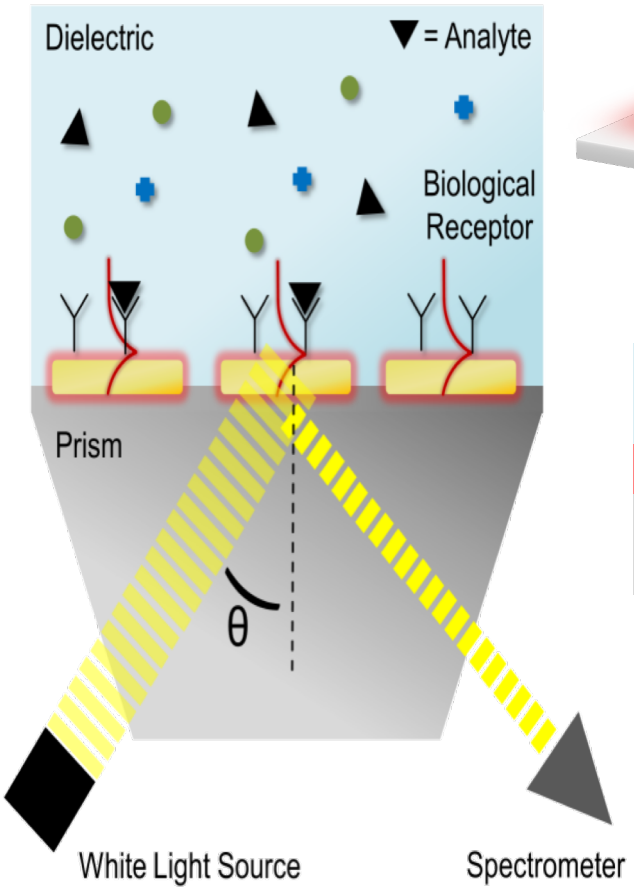
Scattering

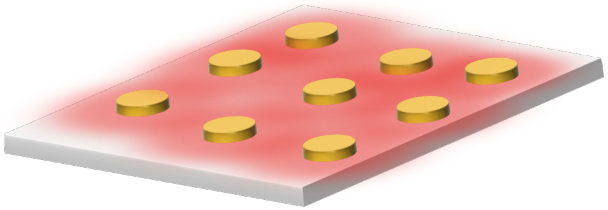
Low density of nanostructures

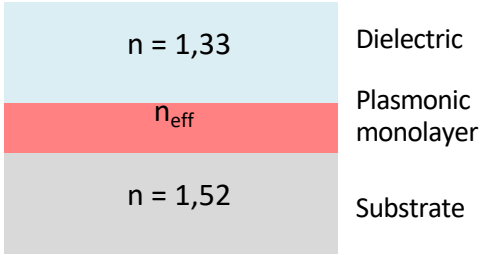
Localized Surface Plasmon Resonance (LSPR) Biosensor

LSPR-based Waveguide Biosensor:

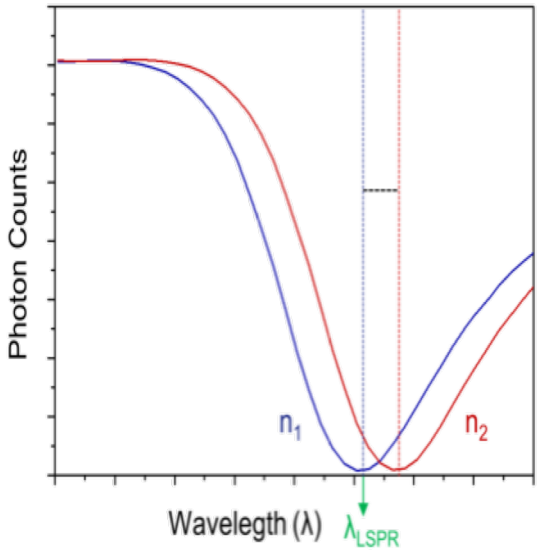
In-plane LSPR excitation enhances the nanoparticle polarizability, creating an effective RI sufficiently large to support a guided EM mode.

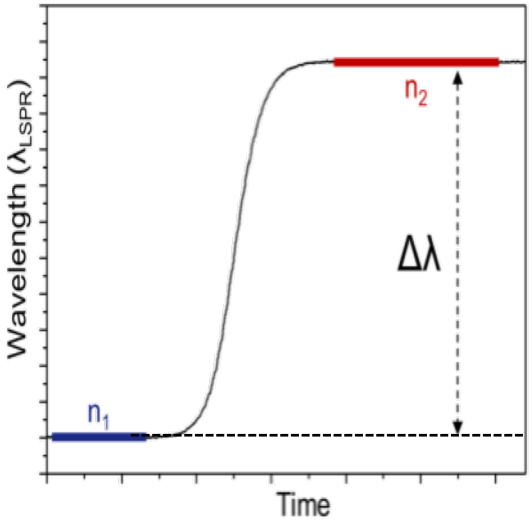






Improved RI sensitivity
Enhanced SNR





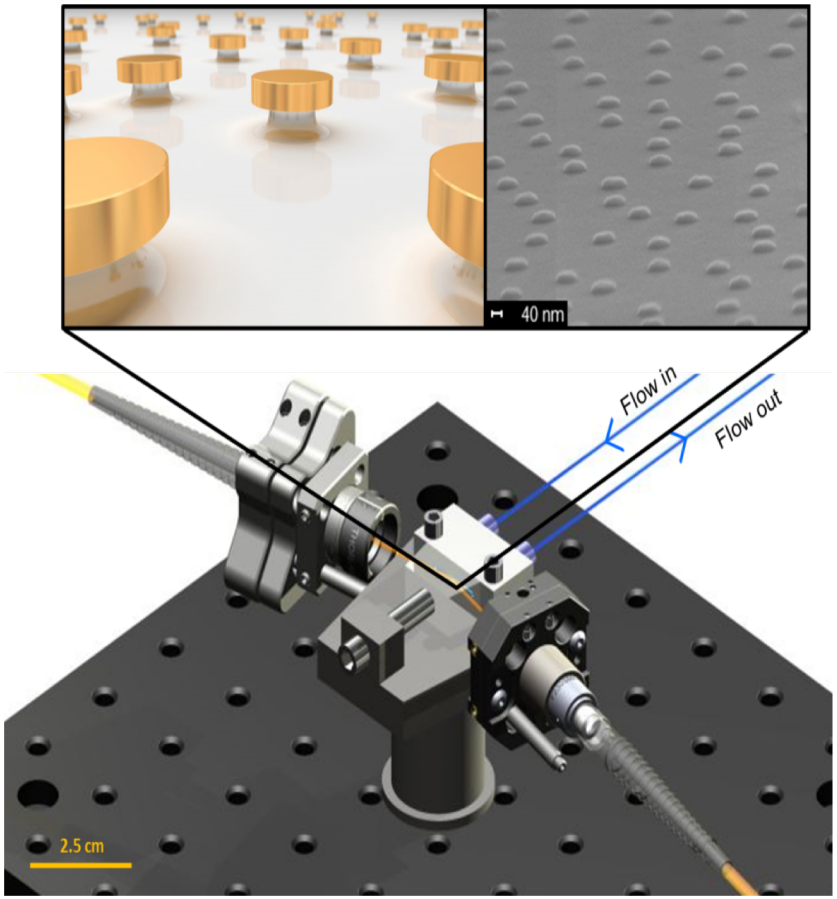
Otte, M.A. *et al.* **2011** ACS Nano 5 (11), 9179-9186

Localized Surface Plasmon Resonance (LSPR) Biosensor

Gold Nanodisks Structured Surfaces

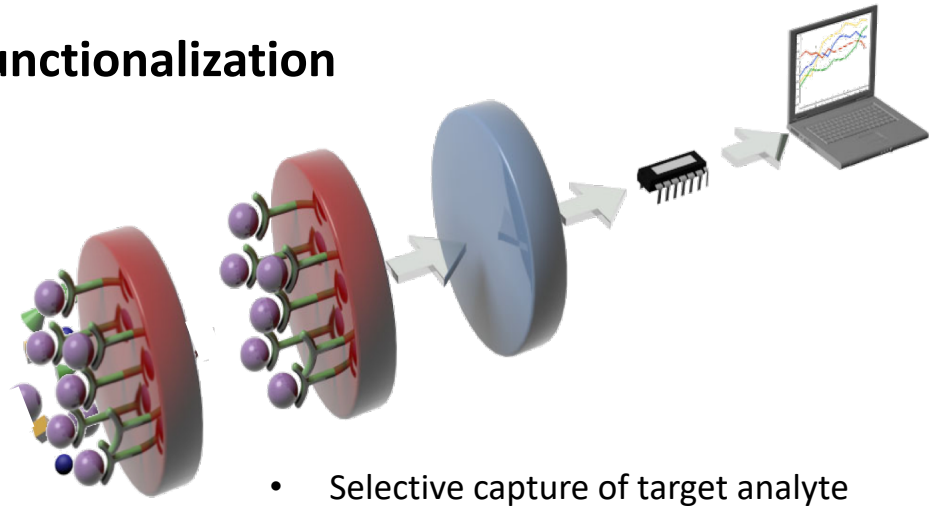
Hole-mask Colloidal Lithography:

Diameter = 100 nm / Height = 20 nm / Density = 6-7%



- ✓ User-friendly
- ✓ Real-time analysis
- ✓ Small (20 x 20 cm)
- ✓ Low-cost production
- ✘ Surface Functionalization

Surface Functionalization

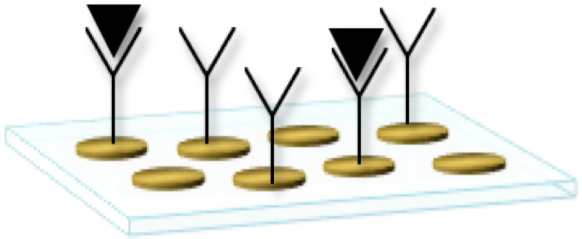


- Selective capture of target analyte
- Sensitive detection of small amounts
- Stability and reproducibility

Optimum analysis performance

Key factors

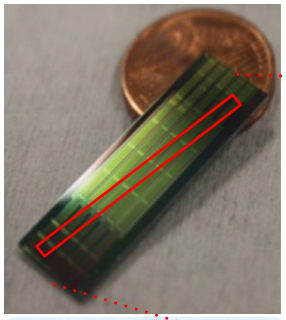
- Density of receptors
- Accessibility of the target
- Orientation
- Retaining of biological activity
- Stable linkage
- Anti-fouling properties



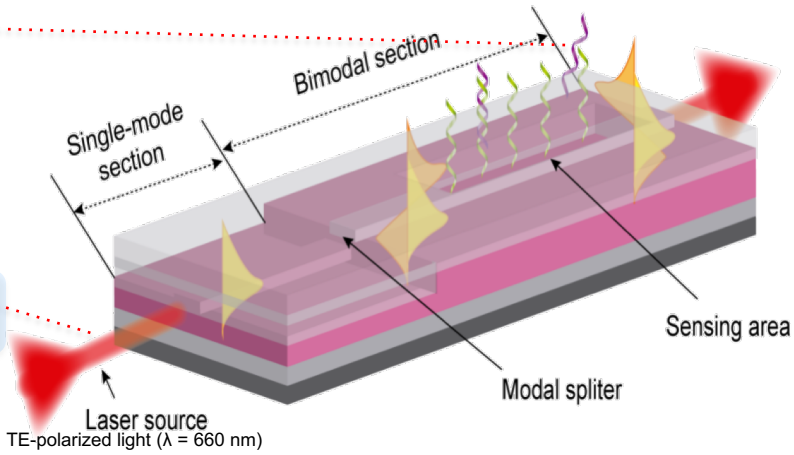
Nanostructured surface:

- Receptor immobilization solely on sensor spots (gold nanodisks)
- Anti-fouling coating of nonsensing areas (glass substrate)

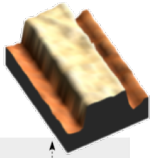
BiModal Waveguide sensor

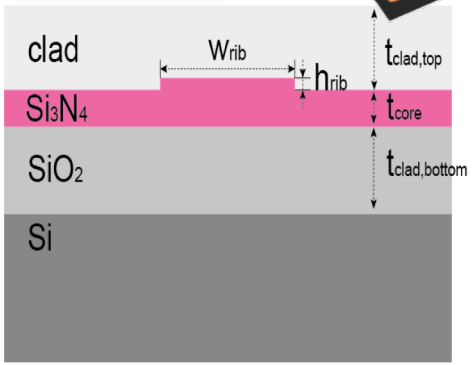


16 sensors in one chip

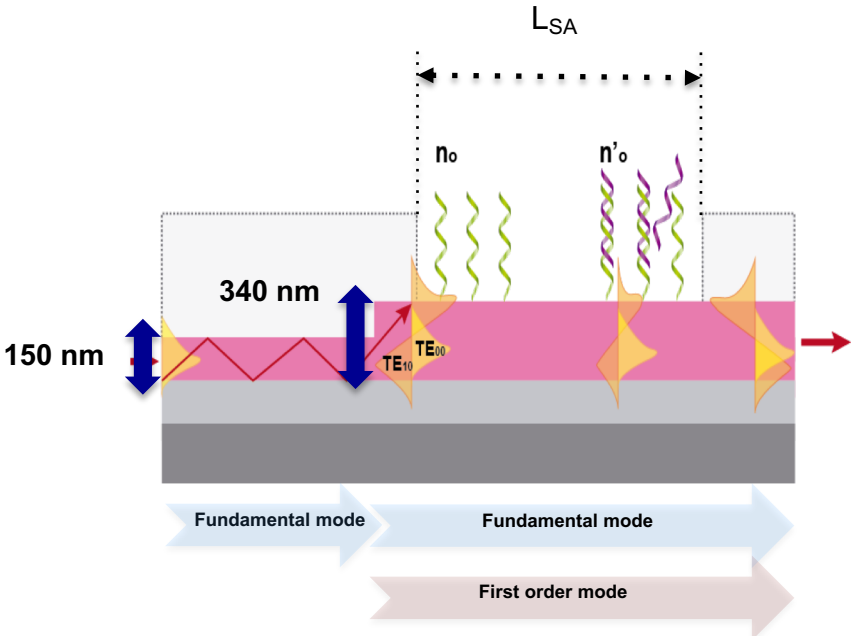


h_{rib} ≤ 3 nm

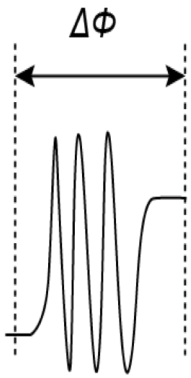




Propagation of light in guided modes

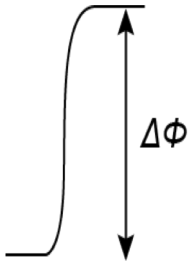


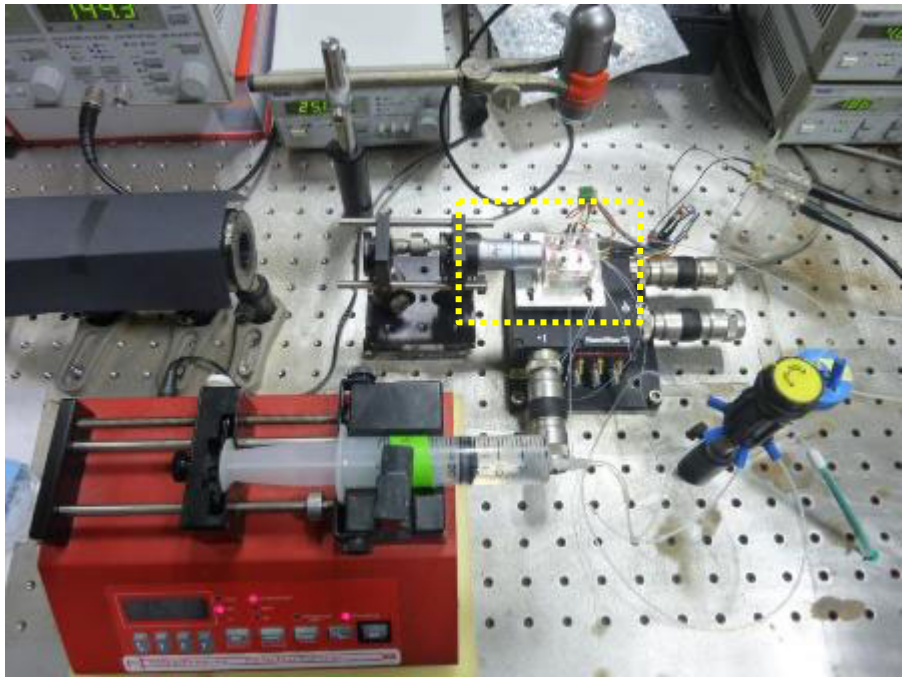
Interferometric readout

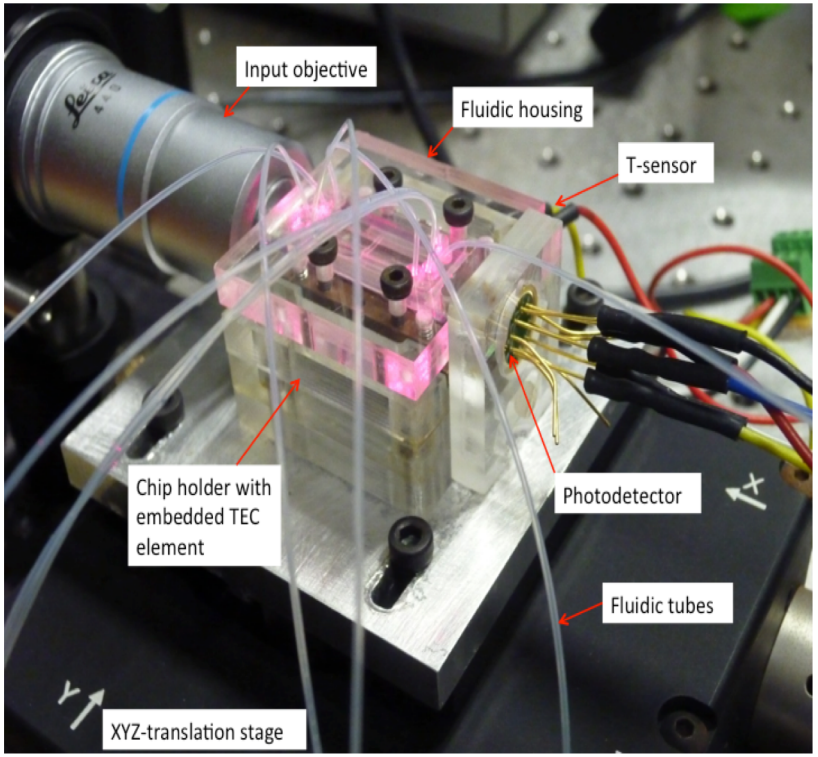


Linear phase readout

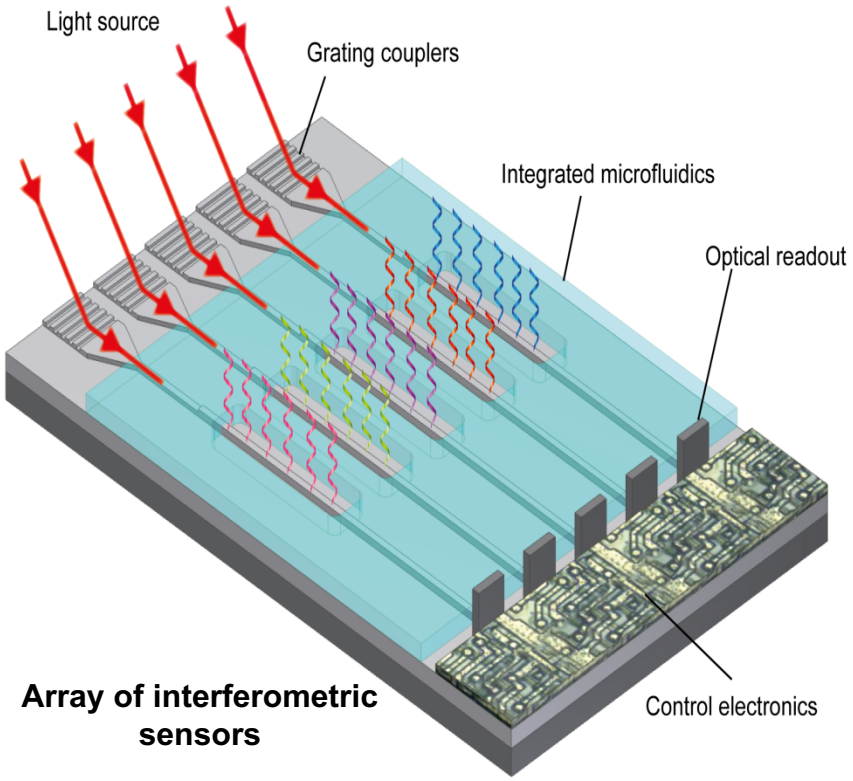
Phase modulation






Input objective
Fluidic housing
T-sensor
Chip holder with embedded TEC element
Photodetector
Fluidic tubes
XYZ-translation stage

BiModal Waveguide sensor



Array of interferometric sensors

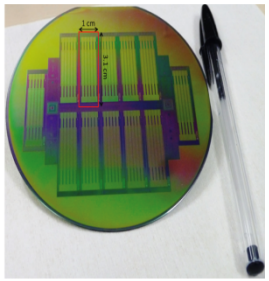
One of the most sensitive label-free existing biosensors

Standard Microelectronics technology

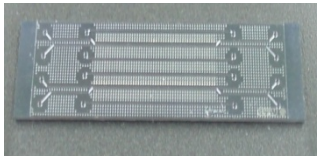
Miniaturization, integration & mass-production

Truly portable POC

Si technology



Polymer microfluidics

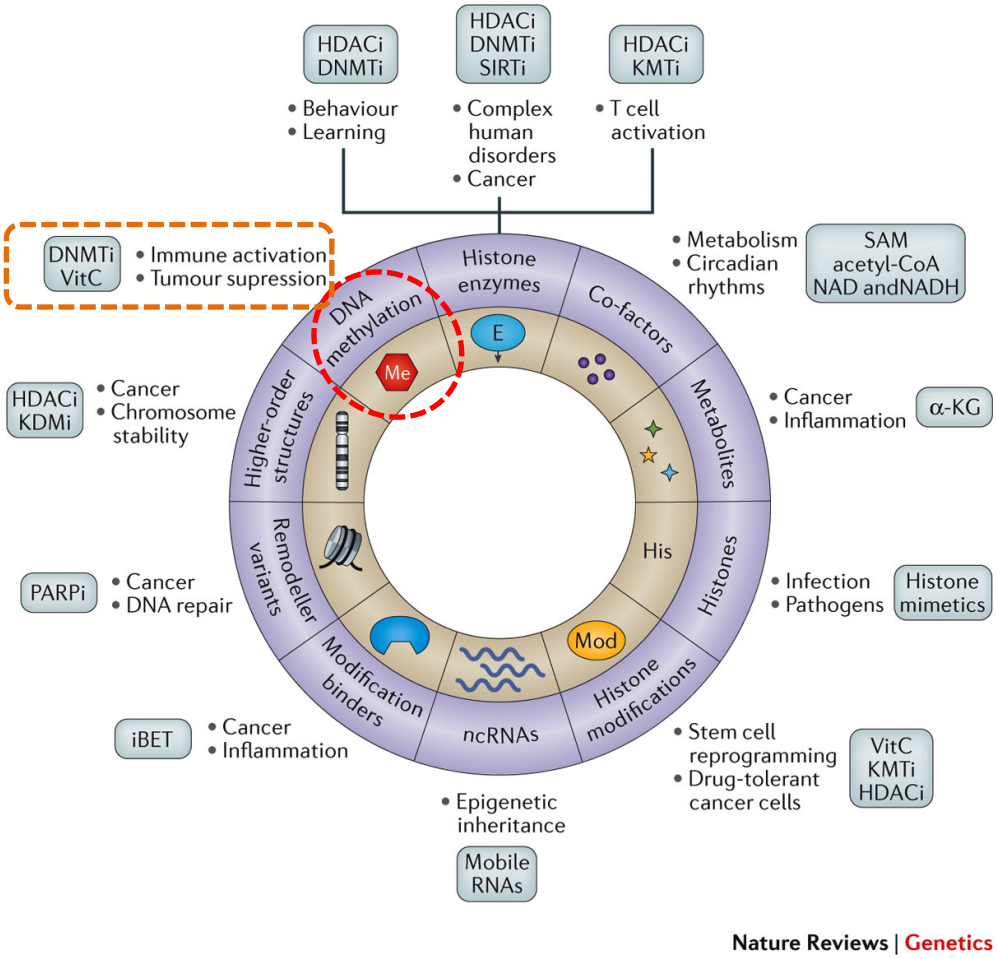


EP2278365 (**Granted 2014**), PCTES08070142 **(Granted 2013)**, CA2693423, CN102077124, US20110102777 (**Granted 2012**), JP2011519071. **TECHNOLOGY TRANSFER TO INDUSTRY IN APRIL 2014.**

DNA methylation analysis

César Sanchez
Albert Piqué
Cristina Kurachi
Laura Lechuga

Epigenetics mechanisms



Epigenetics

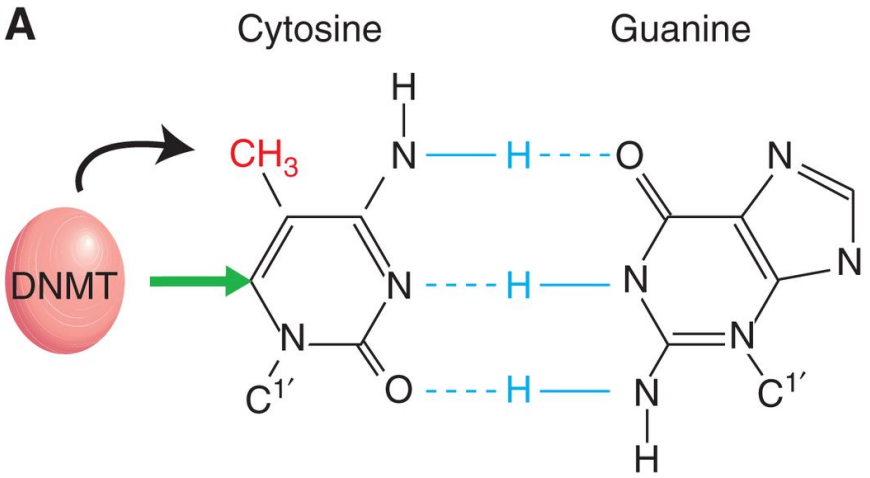
- changes in a chromosome that affect gene activity and expression;

- any heritable phenotypic change that doesn't derive from a modification of the genome;

- functionally relevant changes to the genome that do not involve a change in the nucleotide sequence.

CD Allis and T Jenuvein. Nat Reviews Genetics 17:487-500, 2016

DNA Methylation



Cytosine methylation in DNA. (A) Addition of a methyl group, CH3 (red), at the five position of the cytosine pyrimidine ring (black arrow) does not sterically interfere with GC base pairing (blue lines).

Li, and Yi Zhang, Cold Spring Harb Perspect Biol 2014;6:a019133

DNA Methylation - implications

- Embryonic development
- Cell differentiation
- Cell death (cDNA)
- Syndromes: Fragile X and Rett (learning disabilities and cognitive impairment)
- Cancer (cDNA)

K Warton and G Samimi, Front Mol Biosci 2:13, 2015
R Lehmann-Werman et al., PNAS E1826-34, 2016

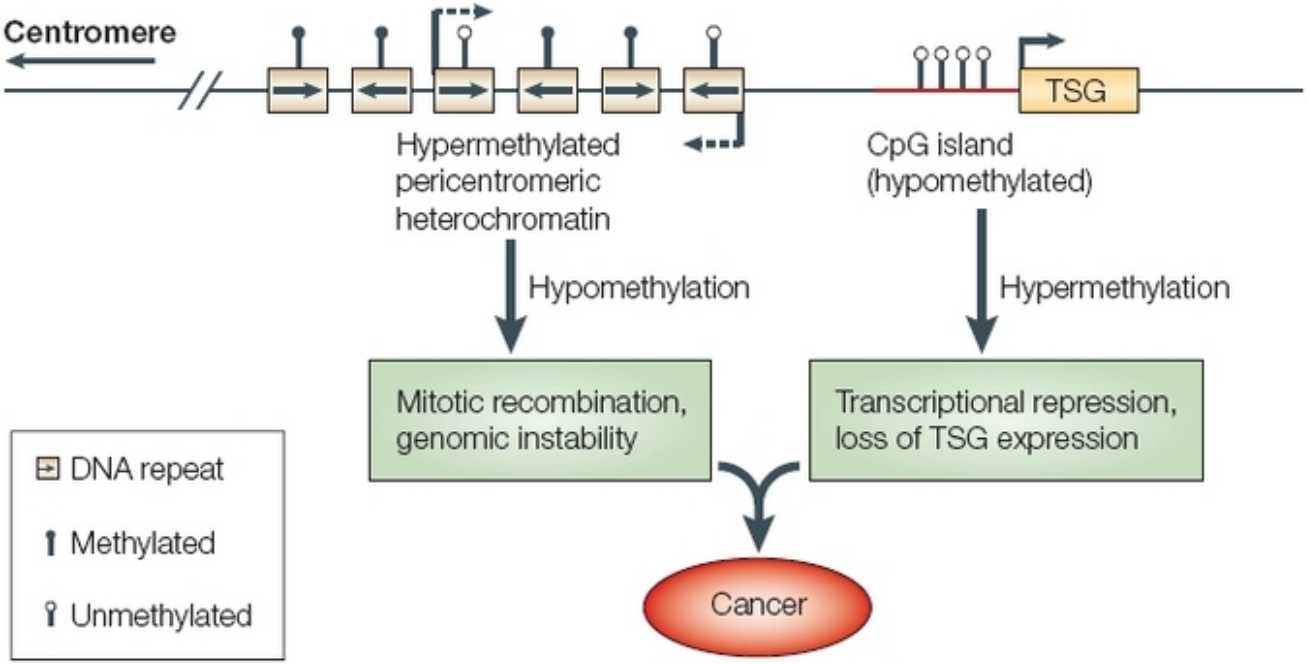


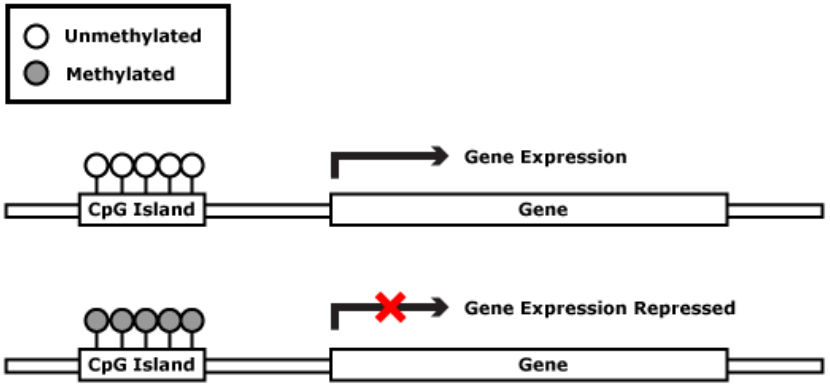
Figure 1: DNA methylation and cancer.
The diagram shows a representative region of genomic DNA in a normal cell. The region shown contains repeat-rich, hypermethylated pericentromeric heterochromatin and an actively transcribed tumour suppressor gene (TSG) associated with a hypomethylated CpG island (indicated in red). In tumour cells, repeat-rich heterochromatin becomes hypomethylated and this contributes to genomic instability, a hallmark of tumour cells, through increased mitotic recombination events. *De novo* methylation of CpG islands also occurs in cancer cells, and can result in the transcriptional silencing of growth-regulatory genes. These changes in methylation are early events in tumorigenesis.

cDNA methylation in cancer diagnosis

- Motivation: gene mutations can be spread over very large regions of its sequence

Complex development: blood tests based on DNA mutations

DNA methylation usually occurs in the CpG islands, it can be consistently measured.



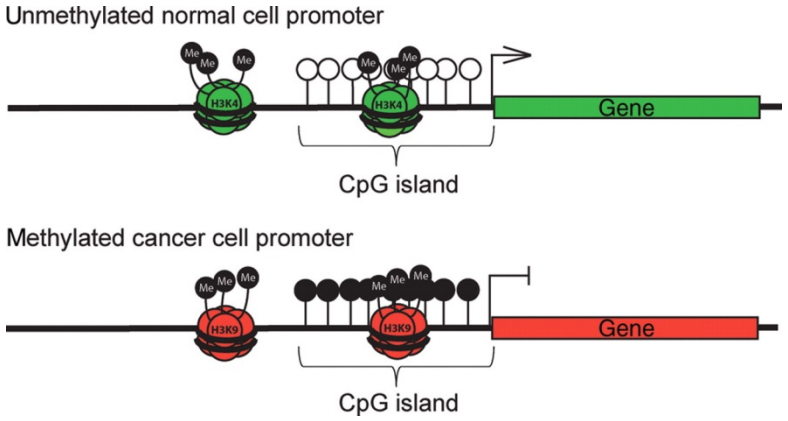


Table 1. Overview of various assays to assess global DNA methylation, depicting their biological relevance.

Assay	Biological relevance	Amount of DNA required (used in our assay)	Equipment needed
LINE1	~700,00 copies, which relates to ~17% in human genome§	500 pg –2 µg (250 ng)	Thermal cycler, Pyrosequencer
Alu	~1,100,00 copies, which relates to ~11% in human genome§	500 pg –2 µg (250 ng)	Thermal cycler, Pyrosequencer
LUMA	Proportion of CpGs located within HpaII sites (5'CCGG'3) in the human genome is 4.14% in transposable elements +3.90% in unique sequence (8.04% in total)#	100 –500 ng (100 ng)	Incubator, Pyrosequencer
HPLC-UV	Total 5mC in the human genome	1 –5µg (3 µg)	HPLC

§Information taken from [37] and [38]. # Information taken from [39].
doi:10.1371/journal.pone.0079044.t001

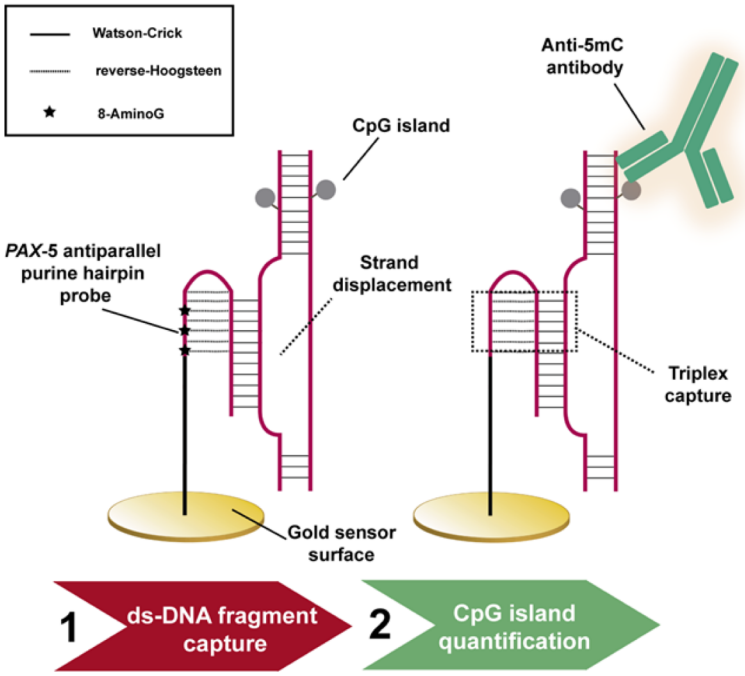
PCR

Gold standard

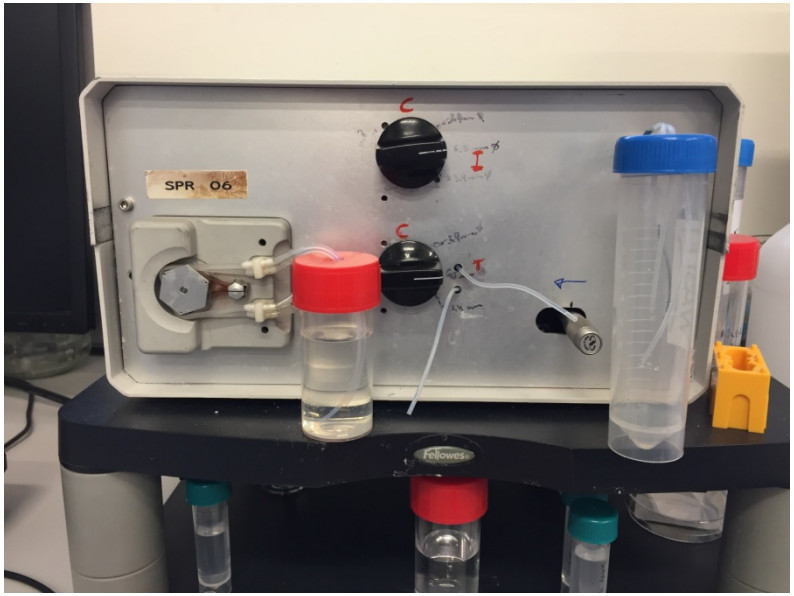
Lisanti S, Omar WAW, Tomaszewski B, De Prins S, Jacobs G, et al. (2013) Comparison of Methods for Quantification of Global DNA Methylation in Human Cells and Tissues. PLOS ONE 8(11): e79044. doi:10.1371/journal.pone.0079044
http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0079044

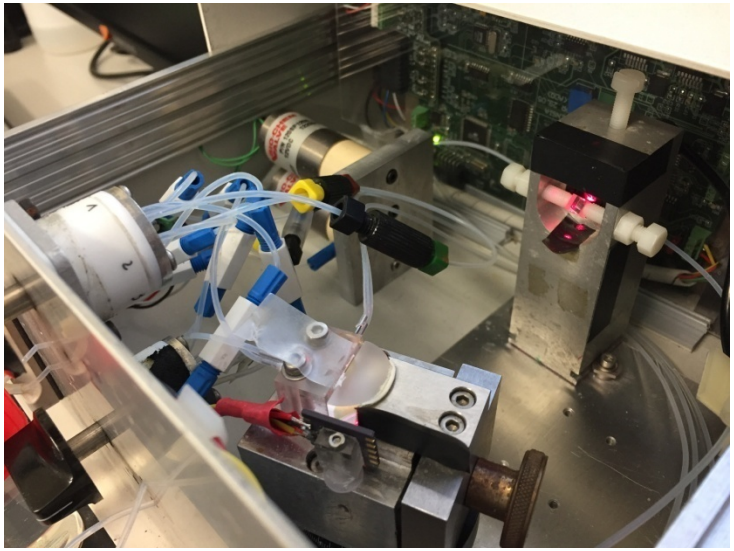
DNA sequences

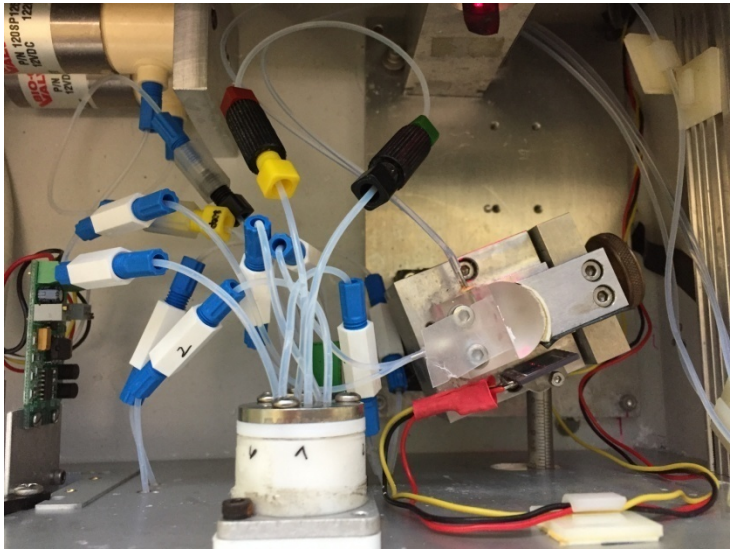
Duplex *PAX-5* gene probe	5′SH–(T)$_{15}$–GGAGGGAAGGAAGGCTTCAGCCTG 3′
***PAX-5* + strand 1x 5mCpG**	5′TCCCGTAGGTGC$_m$GCTGGCTAGCGCCCGGCGCAGGCTGAAGCCTTCCTTCCCTCCCCCCAACCCCTATAA AAGTCTGGGGCGGCG3′
***PAX-5* + strand 4x 5mCpG**	5′TCCC$_m$GTAGGTGC$_m$GCTGGCTAGCGCCC$_m$GGCGCAGGCTGAAGCCTTCCTTCCCTCCCCCCAACCCCTAT AAAAGTCTGGGGC$_m$GGCG3′
***PAX-5* – strand Non-methylated**	5′CGCCGCCCCAGACTTTTATAGGGGTTGGGGGGAGGGAAGGAAGGCTTCAGCCTGCGCCGGGCGCTAGCC AGCGCACCTACGGGA3′



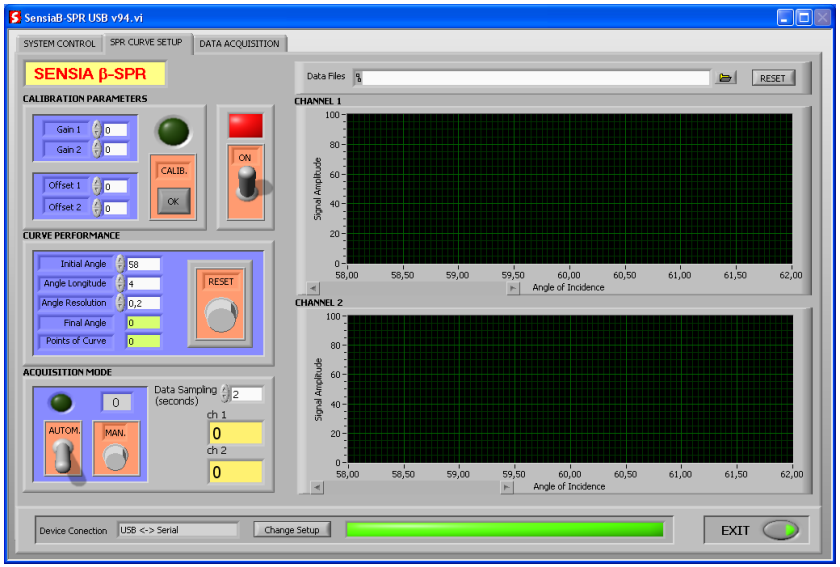
Anna Aviñó and Ramón Eritja
(IQAC)

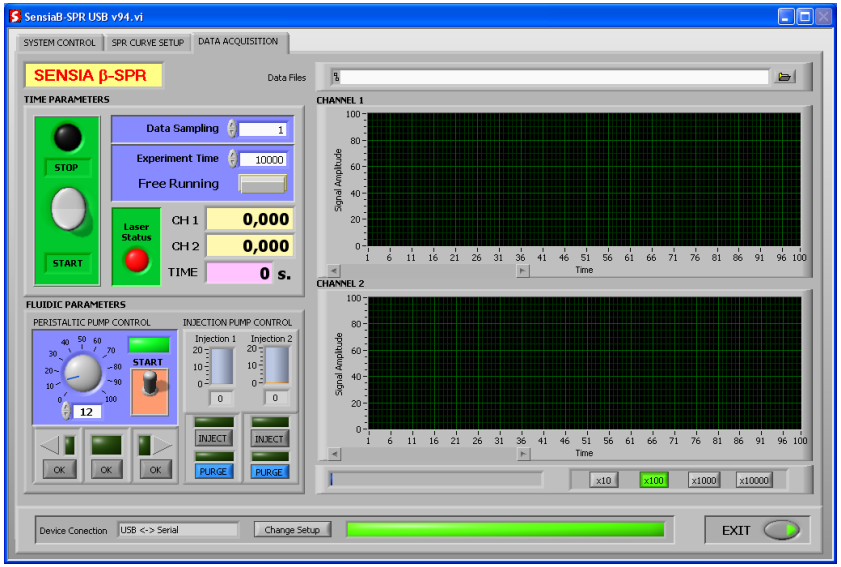

SPR 06
Fellowes

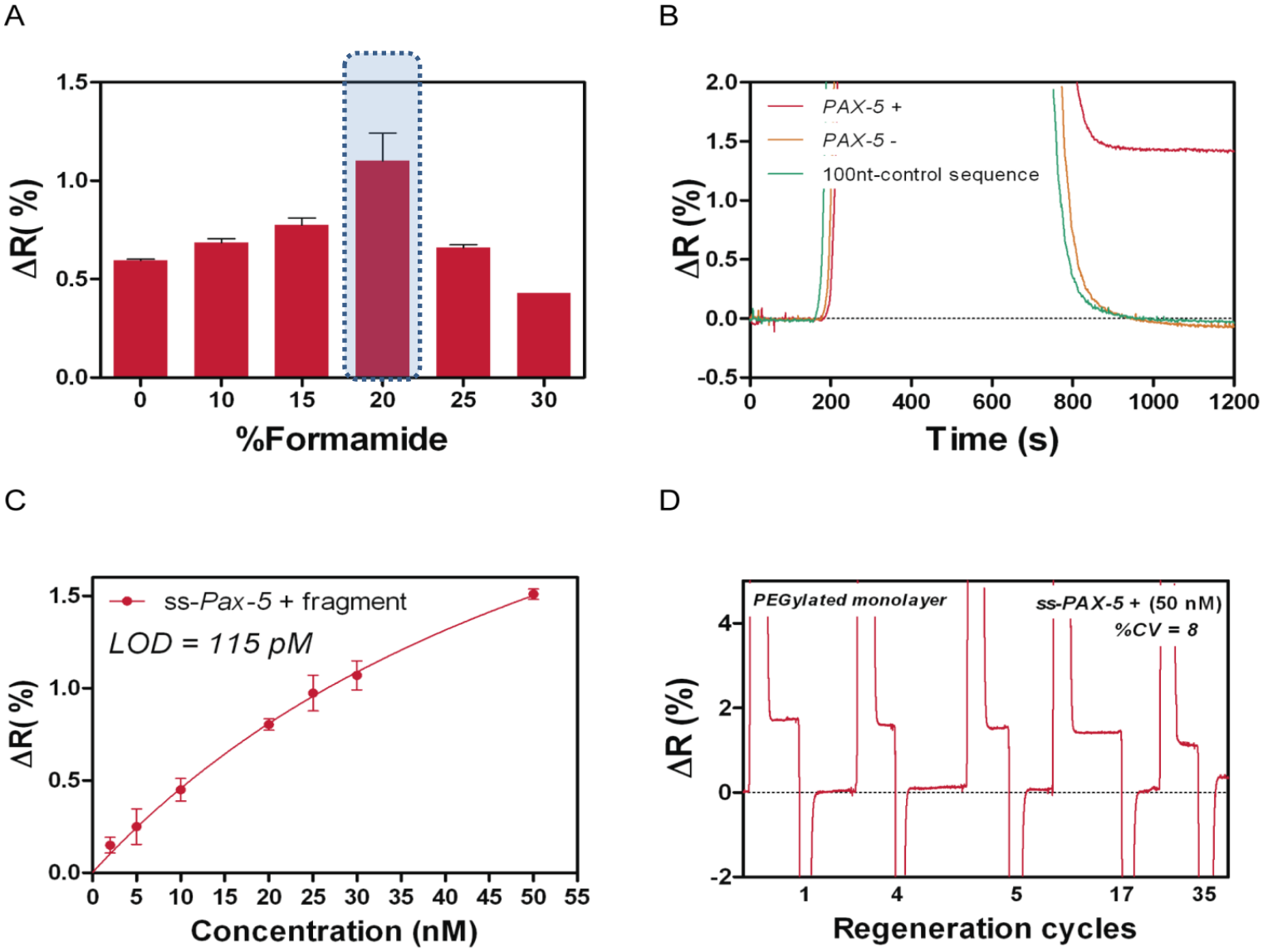


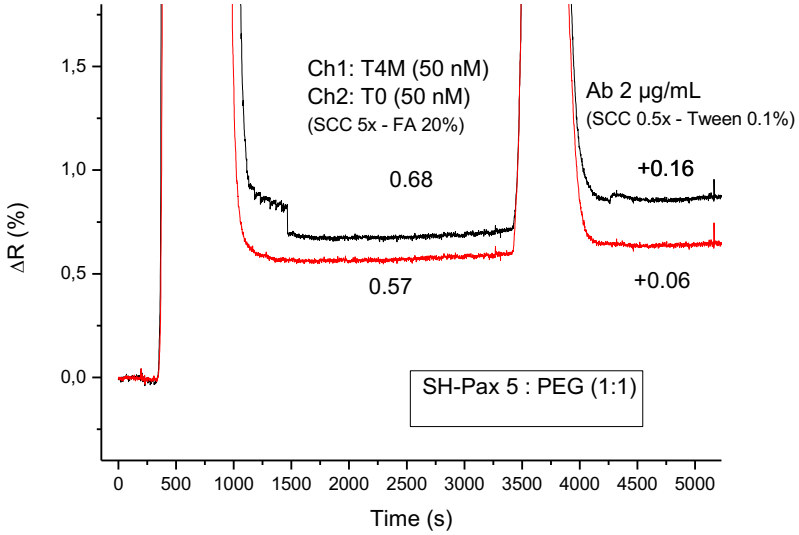


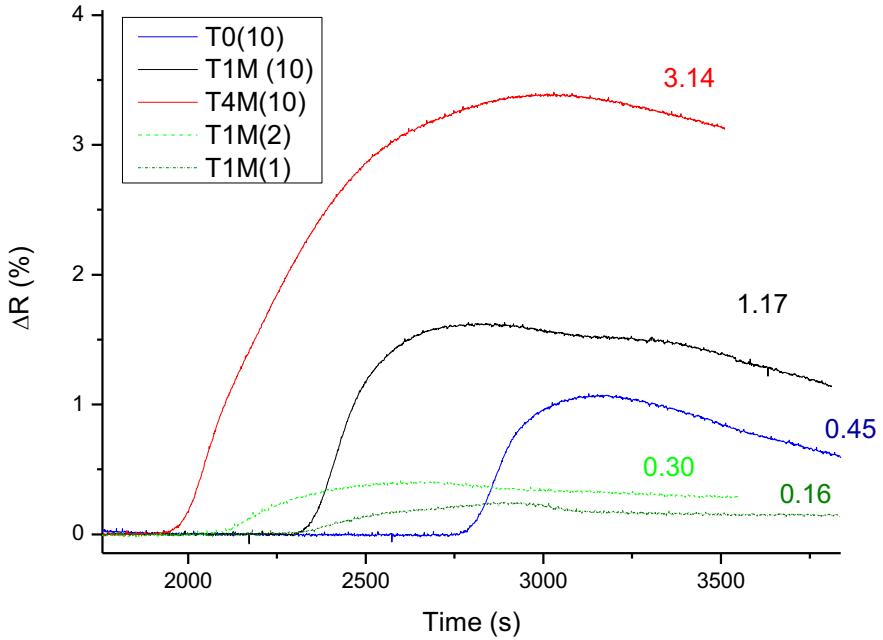

SensiaB-SPR USB v94.vi
SYSTEM CONTROL
SPR CURVE SETUP
DATA ACQUISITION
SENSIA β-SPR
Laser Control
ON
password
OK
Show Parameters
USB
Peristaltic Pump Control
Flow Control
START
STOP
Injection Pump 1
Sample 1
Injection Pump 2
Sample 2
Device Conection
USB <-> Serial
Change Setup
EXIT

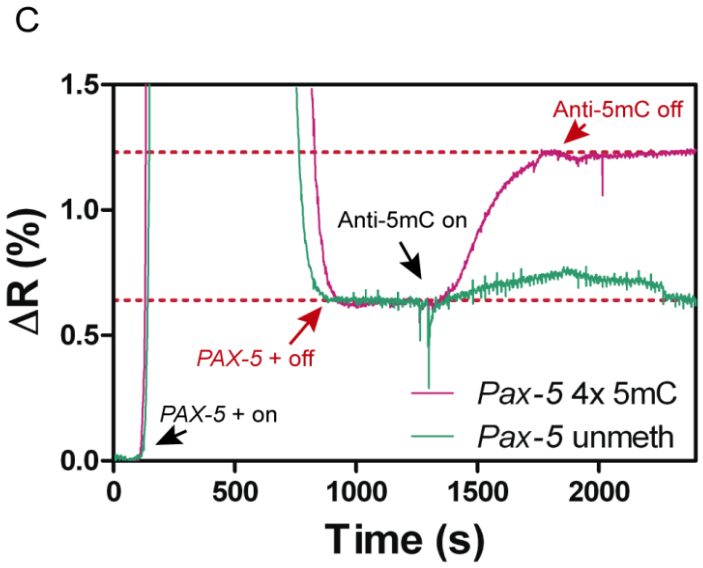

SensiaB-SPR USB v94.vi
SYSTEM CONTROL
SPR CURVE SETUP
DATA ACQUISITION
SENSIA β-SPR
Data Files
RESET
CALIBRATION PARAMETERS
Gain 1
Gain 2
Offset 1
Offset 2
CALIB.
OK
ON
CHANNEL 1
CHANNEL 2
Signal Amplitude
Angle of Incidence
CURVE PERFORMANCE
Initial Angle
Angle Longitude
Angle Resolution
Final Angle
Points of Curve
RESET
ACQUISITION MODE
AUTOM.
MAN.
Data Sampling (seconds)
ch 1
ch 2
Device Conection
USB <-> Serial
Change Setup
EXIT

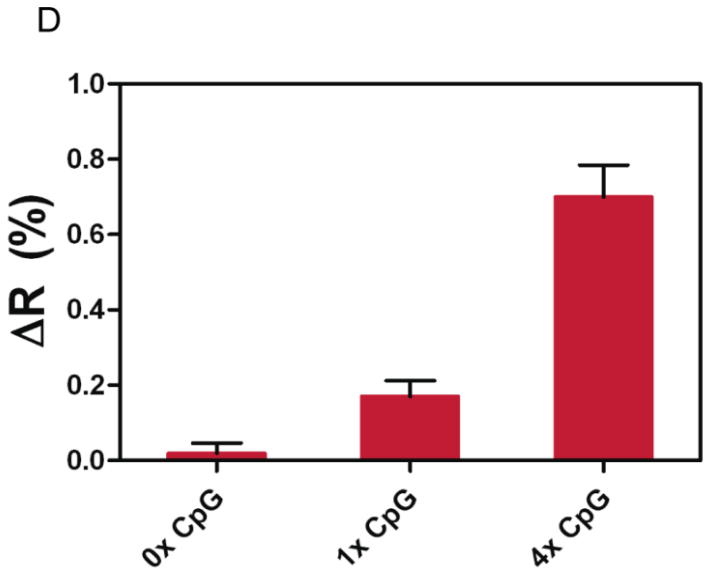

SensiaB-SPR USB v94.vi
SYSTEM CONTROL
SPR CURVE SETUP
DATA ACQUISITION
SENSIA β-SPR
Data Files
TIME PARAMETERS
STOP
START
Data Sampling
Experiment Time
Free Running
Laser Status
CH 1
CH 2
TIME
0,000
0,000
0 s.
CHANNEL 1
CHANNEL 2
Signal Amplitude
Time
FLUIDIC PARAMETERS
PERISTALTIC PUMP CONTROL
INJECTION PUMP CONTROL
Injection 1
Injection 2
INJECT
PURGE
x10
x100
x1000
x10000
Device Conection
USB <-> Serial
Change Setup
EXIT

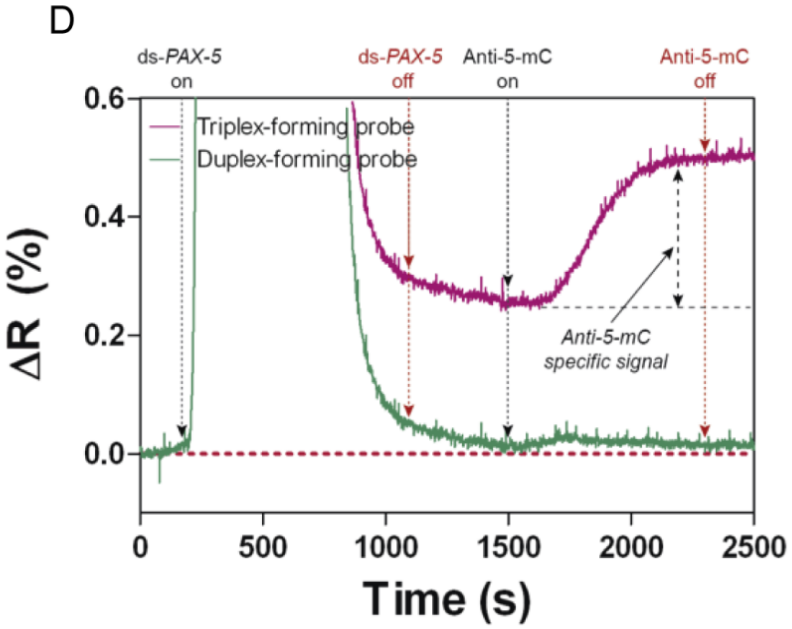

A
ΔR(%)
1.5
1.0
0.5
0.0
0
10
15
20
25
30
%Formamide
B
ΔR (%)
2.0
1.5
1.0
0.5
0.0
-0.5
PAX-5 +
PAX-5 -
100nt-control sequence
0
200
400
600
800
1000
1200
Time (s)
C
ΔR(%)
ss-Pax-5 + fragment
LOD = 115 pM
1.5
1.0
0.5
0.0
0
5
10
15
20
25
30
35
40
45
50
55
Concentration (nM)
D
ΔR (%)
PEGylated monolayer
ss-PAX-5 + (50 nM)
%CV = 8
4
2
0
-2
1
4
5
17
35
Regeneration cycles


Ch1: T4M (50 nM)
Ch2: T0 (50 nM)
(SCC 5x - FA 20%)
0.68
0.57
Ab 2 µg/mL
(SCC 0.5x - Tween 0.1%)
+0.16
+0.06
SH-Pax 5 : PEG (1:1)
ΔR (%)
Time (s)


T0(10)
T1M (10)
T4M(10)
T1M(2)
T1M(1)
3.14
1.17
0.45
0.30
0.16
ΔR (%)
Time (s)







$\Delta R_{triplex}$ is reduced by 50%
(compared to ΔR_{duplex})

(dsDNA) (ssDNA)

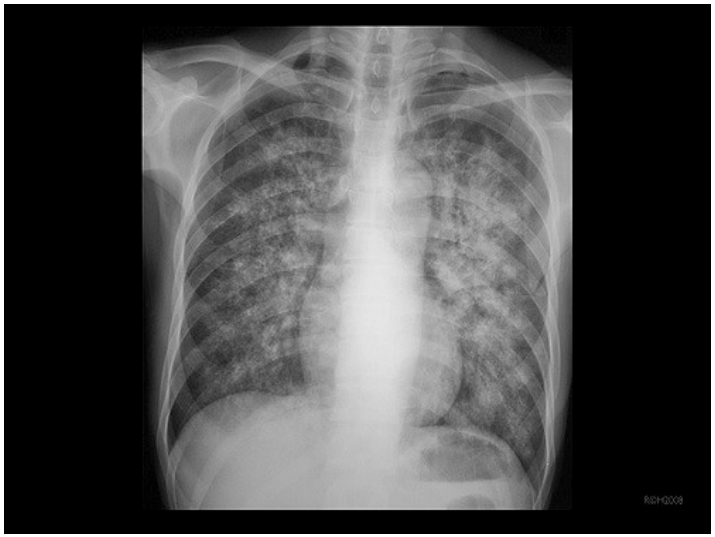
Pneumocystis jirovecii pneumonia (PcP) detection

César Sanchez
Cristina Kurachi
Anna Aviñó
Laura Lechuga

Pneumocystis jirovecii pneumonia

- Opportunistic infection caused by an ascomycete fungus
- Up to 20% of mortality rate

- AIDS patients
- Other immunocompromised conditions
  - Solid organ transplant
  - Hematological malignancies
  - Chemotherapy and/or steroidal therapy for prolonged duration
  - Auto-immune disorders
  - Chronic pulmonary disorders



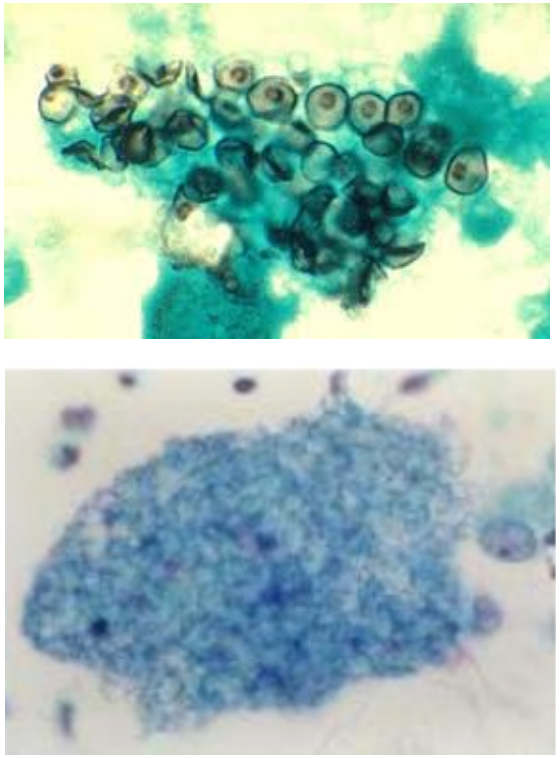
Y Singh et al., Eur J Clin Microbiol Infect Dis 2017

Laboratory diagnosis of PcP

•No *in vitro* system for routinely obtaining *Pneunomocystis* isolates

• Microscopic detection: bronchoalveolar lavage fluid (BALF), induced sputum samples or lung biopsy specimens *(costly, unpleasant to the patient, time consuming and specialized technicians in clinical settings are needed).* False negative: low fungal loads.

•Molecular detection: Conventional or real-time PCR assays based on the amplification of the large subunit of mitochondrial ribosomal RNA (mtLSUrRNA) *(sample preparation, instrumentation needed, and time consuming).* Overdetection: colonization x infection.



P. jirovecii at lung biopsy specimen (top) and Pap smear (bottom)

Silver Gram staining

Gold standard: visualization of *P jirovecii* cysts and/or trophozoites in biopsy lung specimens

F Robert-Gangneux et al. J Clin Microbiol 2014

Product Description

ingenetix

MycoReal *Pneumocystis*

Order No.: RTPM400, RTPM401, or RTPM403

Unit: 50 reactions

For research use only, not for diagnostic use

MycAssay Pneumocystis kit

(Myconostica, UK) – clinical test

- PCR technology
- Up to 4 hours
- > 2 mL sample (colected by BAL)
- 93% sensitivity (13 out of 14 pts)

PM Hauser et al., J Clin Microbiol 2011

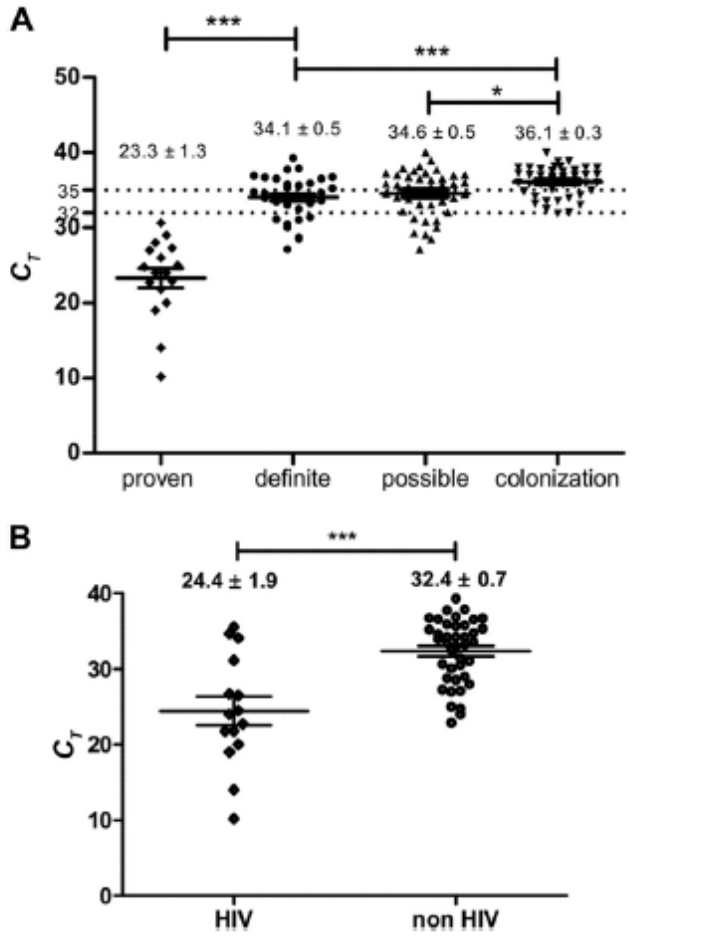


FIG 2 Results of qPCR (C_T of amplification) according to final diagnosis (A) or HIV status (B), expressed as the mean ± SEM. The values were compared using *t* tests. Statistically significant differences are marked as *, $P < 0.05$ or ***, $P < 0.001$.

Protocol:

- 1 mL BALF sample
- DNA extraction
- qPCR targeting mtLSU
- Amplification for 40 cycles

mitochondrial large-subunit of *Pneumocystis jirovecii* (mt LSU)

Quantification in copies/mL was not reliable

↓

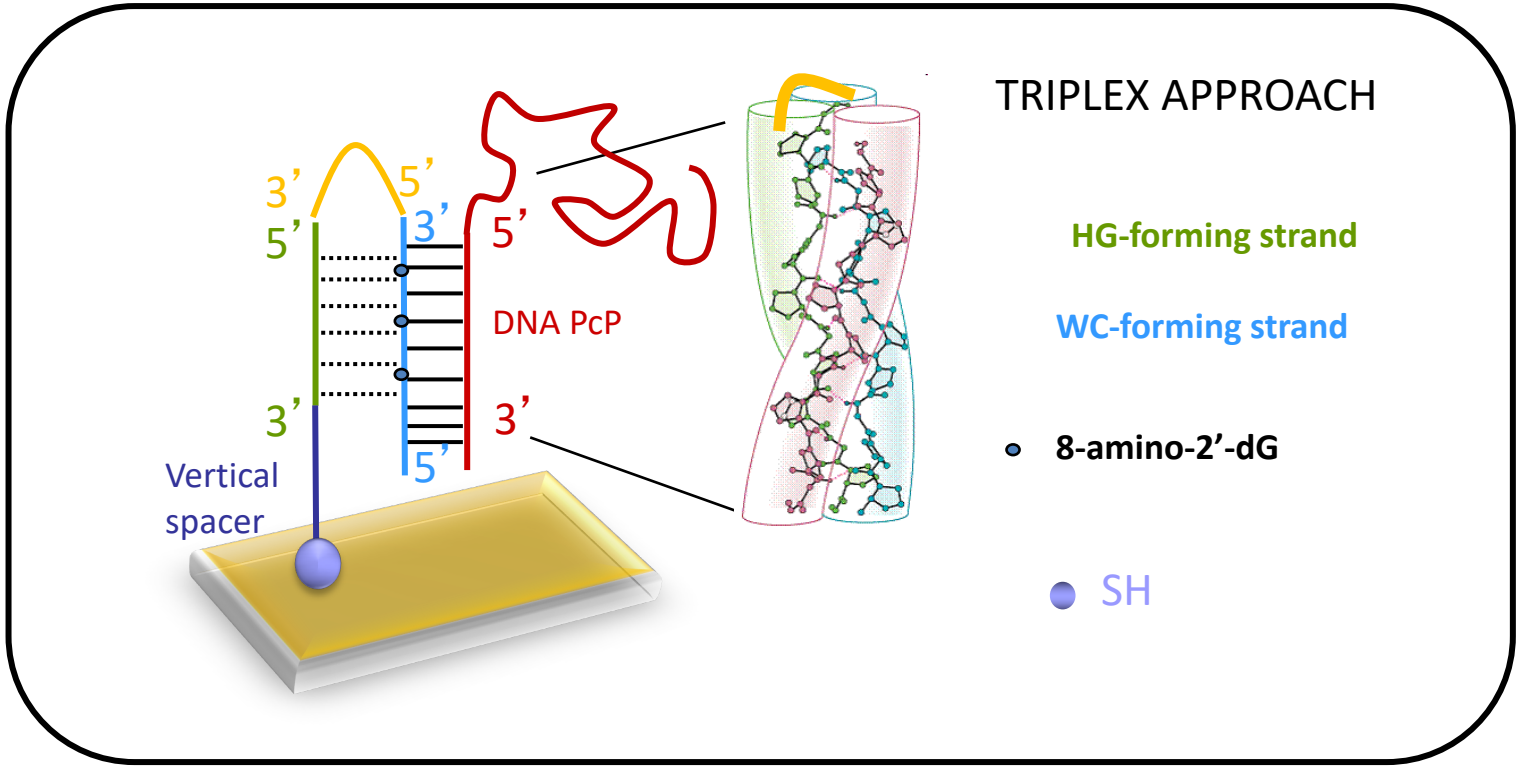
C_T: mean amplification cycle threshold

F Robert-Gangneux et al. J Clin Microbiol 2014

Triplex helix

Probe: DNA “tail-clamp”

Target: nucleic acid sequences of the *P jirovecii*



A Nadal, R Eritja *et al*. ChemBioChem 2005

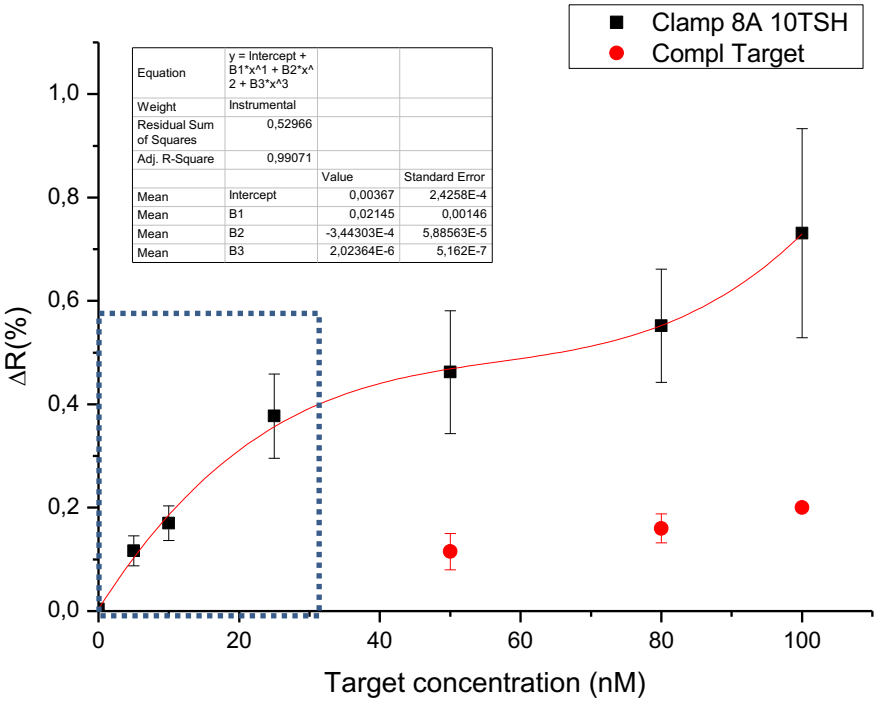
Targeting genes of *PcP* which are more suitable to be identified in the exhale of patients from oropharyngeal washes in pneumonia patients.

- **Selected the gene encoding the mitochondrial large-subunit of *Pneumocystis jirovecii* (mt LSU)**

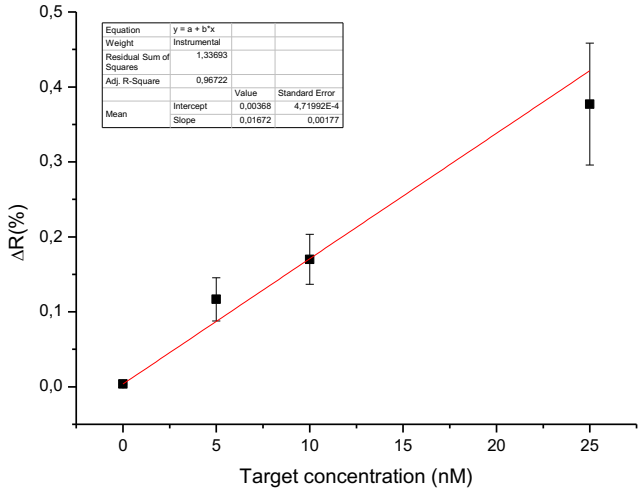
GTGTACGTTGCAAAGTACTCAGAAGAATTGTGGTAAGTAGTGAAATACAAATCGGAC
TAGGATATAGCTGGTTTTCTGCGAAAATTGTTTTGGCAAATTGTTTATTCCTCT**NAAA**
AATAGTAGGTATAGCACTGAATATCTCGAGGGAGTATGAAAATATTTATCTCA
GATATTTAATCTCAAAATAACTATTTCTTAAAATAAATAATCAGACTATGTGCG
ATAAGGTAGATAGTCGAAAGGGAAACAGCCCAGAACAGTAATTAAAGCTCCCY
AATTAATATTAAGTGAAATAAAAGTTGTTGGATATCTAAAACAGTTAAGAAGTGGGCTT
GGAAACAGCCAC

- Immobilization protocol
  - Pre-heating: 20 min @ 70^{o}C (DNA + TCEP)
  - 20 min @ room temperature
  - Overnight *ex situ* immobilization (1 DNA probe : 1 PEG)
- Target hybridization
  - SCC5x, SCC5x/FA20%
  - $MgCl_2$ (1.5, 2.5, 5 and 10 mM)

Sensor calibration



LOD = 1.2 nM



Muchas gracias!