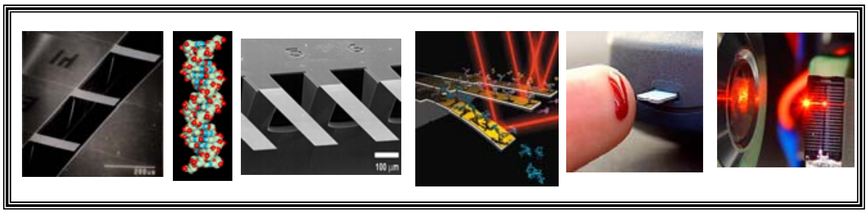
Sensores Nanomecánicos basados en tecnologías MEMS



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Esquema

- Introducción general: Biosensores, lab-on-a-chip, etc
- Sensores nanomecánicos
  - Definición
  - Mecanismos de funcionamiento
  - Sistemas de lectura
  - Fabricación y caracterización
  - Técnicas de inmovilización del receptor biológico
- Sistemas experimentales de medida
  - Sistemas de una única palanca
  - Sistemas en array
- Applicaciones
  - Aplicaciones en proteómica
  - Aplicaciones en genómica
- Integración en plataformas “lab-on-a-chip”
- Resumen y conclusiones generales

THE PROBLEM OF THE ACTUAL DIAGNOSTICS

One major unmet diagnostic need is to have a FAST, RELIABLE AND SENSITIVE **POINT OF CARE** DEVICE

The provision of a result (test) at the point in time at which the result will be used to make a **decision** and take appropriate **action** which will result in an improved health outcome

Glucose monitoring

i-STAT Portable Blood Clinical Analyzer

The future??

Tricorder Gene I

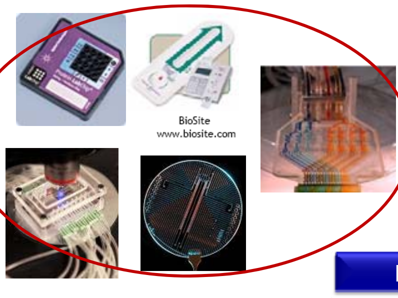
2015???

Tricorder Gene II

- **Instant Diagnostic**
- **In any place at any time**
- **Personalized care**
- **Inside the human body**

"Lab-on-a-chip": state-of-the art

Lab-on-a-chip devices can potentially perform diagnostic procedures on human blood, enabling cheap, fast, disposable tests to be performed at the POC.



Agilent Technologies, Evotec Technologies, Caliper Life Sciences, Hitachi, Philips Fluidigm Technology…

BUT…..

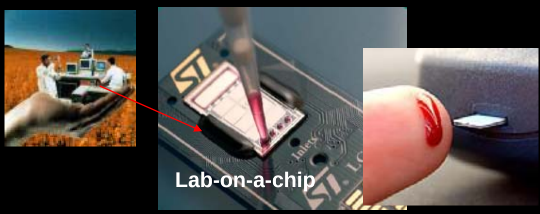
Detection → **Off-chip** / **On-chip**

90% of the detection methods are off-chip

Mainly using fluorescent labels

- Large equipment
- Few of them can be used outside laboratory

"Lab-on-a-chip" Biosensor based on MEMS technology



- MEMS biosensors
- Microfluidics
- Electronics
- Excitation and detectors

"POCT" point-of care testing

- precision
- fast
- sensitivity
- stability
- selectivity
- no pre-treatment

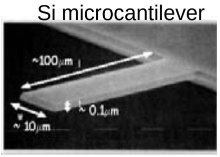
- Instant Diagnostic
- In any place at any time
- Personalized care
- Can operate inside the human body

MEMS-based biosensors

- Total Analysis System: integration of sources, sensors, detectors, flow system, electronics and data processing on a compact device.
- Silicon Microelectronics Technology: Mass Production. Low-cost fabrication.
- Sensor packed arrays
- CMOS integration

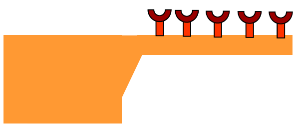
What is a microcantilever sensor?

Si microcantilever

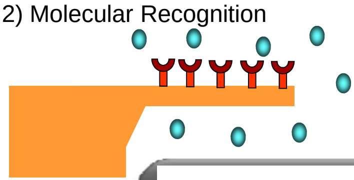


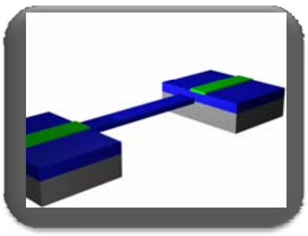
A new class of highly sensitive, label-free and direct biosensor which transduces the molecular recognition of biomolecules into a nanomechanical motion (SNPs-DNA and femtomolar-proteins)

1) Microcantilever functionalization

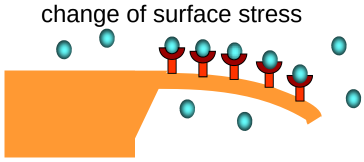


2) Molecular Recognition





3) Bending of the cantilever due to change of surface stress



Surface stress arises from intermolecular interactions (steric, electrostatic, hydrophobic)

Material properties

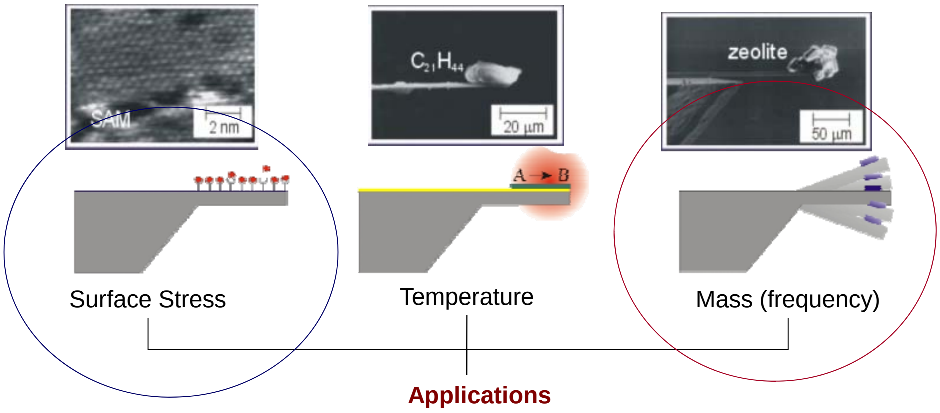
$$\Delta z \cong \frac{3(1-\nu)L^2}{Et^2}(\Delta\sigma_1 - \Delta\sigma_2)$$

length

thickness

Cantilever bending~nm

Nanomechanical Sensors : Introduction

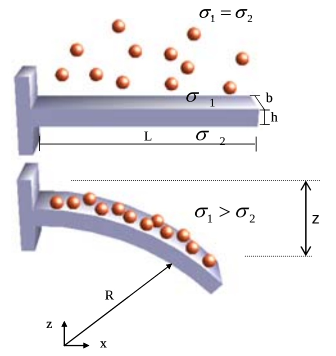


Applications

- Genomics
- Proteomics
- Environmental control

Nanomechanical Sensors : Introduction

Static response



$$z \approx 4\frac{1-\nu}{Et^2}L^2(\Delta\sigma_t - \Delta\sigma_b)$$

Surface stress detection ≤ 1mNm^{-1}

Adsorción sobre una única superficie
↓
Variación diferencial de tensión superficial
↓
Deformación elástica
↓
Radio de curvatura (deflexión)

Deflection is related to:

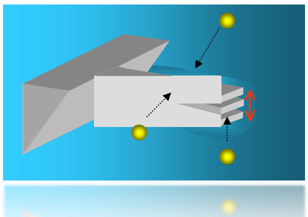
- Differential surface stress
- Length (L) and thickness (t) of the cantilever
- Material properties: Young´s Modulus (E), Poisson coefficient (ν).

- Wide application range: from chemical agents or explosives to antigens, macromolecules and microorganisms.
- Measurement of conformational changes of molecules and intermolecular forces.
- Direct measurement of the biomolecular interactions kinetics.
- Real time detection of specific analytes (pesticides, hormones,…), DNA hybridization (SNP) or viruses.

Resonant frecuency principle (DINAMIC MODE)

•The resonant properties depend on the cantilever mass, environment viscosity and surface stress. In liquid, Resonant frequency shifts to a lower frequency (increase of the effective mass).

•The sensitivity is proportional to the Quality factor (Q), wich is very low in fluid, in which biomolecular recognition takes place



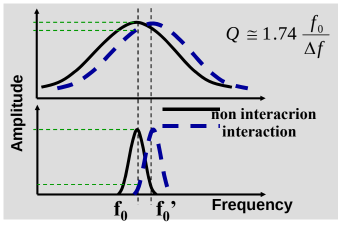
Quality factor: depends on the internal friction of the cantilever material and hydrodynamic damping between the cantilever and the environment

Air, Q≈ 100, Liquid, Q≈ 2

Frequency shift resolution given by

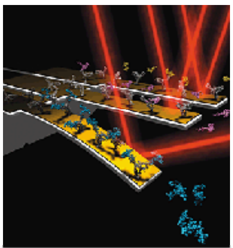
$$\Delta f \approx \frac{f_0}{Q}$$

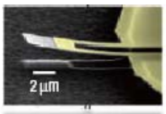
Low Q ⟹ Low resolution in Δf

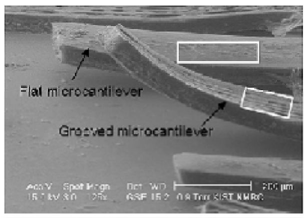


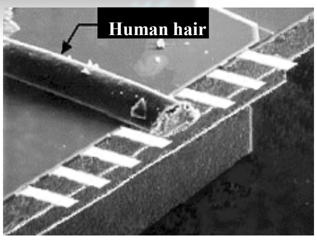
The low Q of the cantilever is amplified by implementing a positive feedback

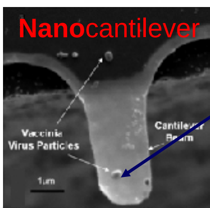
Microcantilever transducers







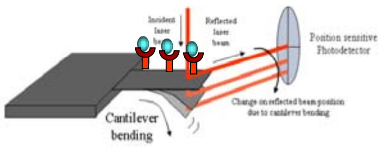


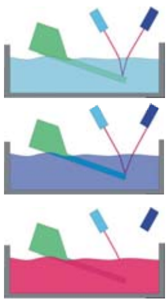


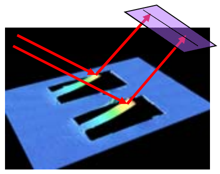
A single virus!!!

Nanomechanical sensors: read-out systems

OPTICAL METHOD







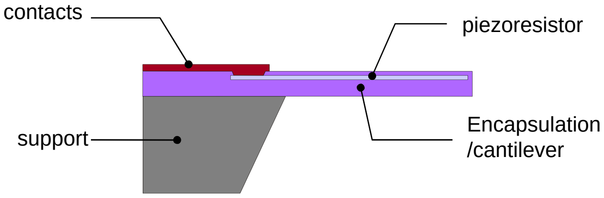
Advantages
- **High sensitivity**
- Sub-angstrom resolution

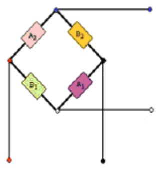
Disadvantages
- Complex integration (difficult alignment)
- Changes in the optical density: sensitive to refractive index of solution (new alignment required)
- Difficult for read-out of arrays
- Sequential switching of the laser due to overlapping of the reflection beams

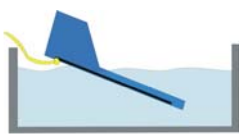
- **Interferometric** *(G.G. Yaralioglu et al., J. Appl. Phys. 83 (1998))*

Nanomechanical sensors: read-out systems

PIEZORESISTIVE METHOD







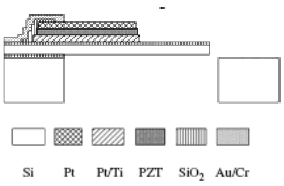
Advantages
- Easy integration, compact design and integrated electrical readout
- Insensitive to changes in the optical density of the sample (opaque samples)
- No optical alignment

Disadvantages
- **Lower sensitivity for surface stress**
- Poor resolution (high intrinsic electrical noise)
- Mechanical constraints (thick cantilevers)
- Isolation of electrical contacts from solution and thermal drift

	Poly Si	SOI	SU8
contacts	gold / aluminium	gold / aluminium	gold
piezoresistors	poly-crystalline Si	single crystalline Si	gold
Encapsulation/ cantilever	silicon nitride	silicon nitride	SU8
support	silicon	silicon	SU8

Nanomechanical sensors: read-out systems

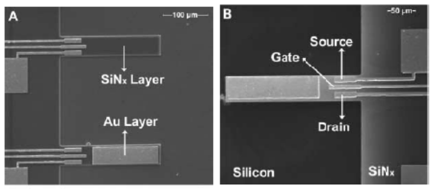
PIEZOELECTRIC METHOD



Advantages
- Easy integration, compact design and integrated
- Insensitive to changes in the optical density of the sample (opaque samples)
- No optical alignment
- Self-sensing/excited. **Higher quality factors**

Disadvantages
- **Lower sensitivity for surface stress**
- Mechanical constraints (thick cantilevers)
- Isolation of electrical contacts from solution

MOSFET Embedded microcantilevers (*G. Shekhawat, Science, 2006*)



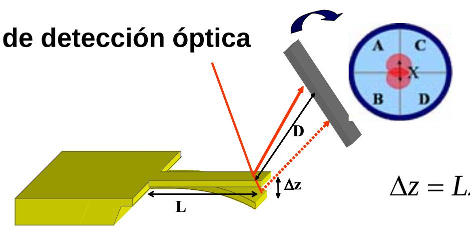
Advantages (over traditional piezoresistive)
- Similar sensitivity to optical read-out
- Small size, high sensitivity, and uncomplicated current measurement
- Compatibility with direct monolithic integration for application-specific integrated circuits
- Lower current noise than piezoresistive
- Provide more localized stress measurements

Disadvantages
- Large signal-to noise (S/N) ratios
- Mechanical constraints (thick cantilevers)

Técnicas de detección

	Ventajas	Limitaciones
Interferométrica	•amplio ancho de banda •medidas directas	•longitud onda láser •desplazamientos pequeños
Capacitiva	•medidas absolutas	•medios no electrolíticos •desplazamientos pequeños
Piezorresistiva y piezoeléctrica	•integrado	•aislamiento •fabricación •disipación de calor
Óptica	•simplicidad	•ancho banda fotodetector •opacidad del medio

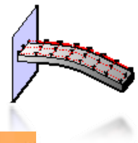
Sistema de detección óptica



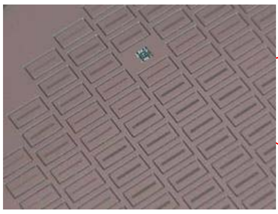
$$\Delta z = Lx / 2D$$

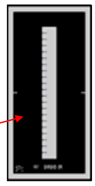
Microcantilever array: design and fabrication

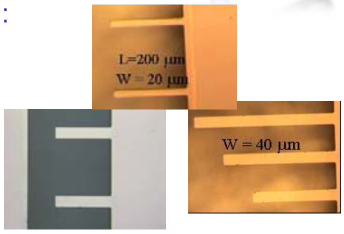
1. Design of microcantilevers for high sensitivity towards biomolecular interactions. FEM simulations (**Thickness, Length, Width, Frame effect, Material, biolayer requirements,..) (silicon nitride, polymer, polysilicon, crystalline Si,..)**



2. Crystalline Si cantilevers fabrication by using:
   - Bulk micromachining
   - BESOI wafers
   - DRIE etching

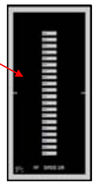


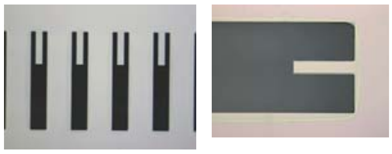




For common fluid cell

L = 100, 200, 500 µm
Thickness = 0.34 µm
Width = 20,40 µm
Pitch = 250 µm

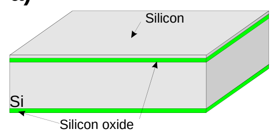




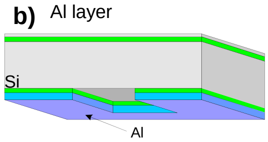
For discrete fluid cell

Microcantilever array: fabrication at Clean Room

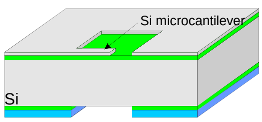
a) BESOI wafer



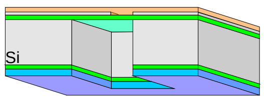
b) Al layer



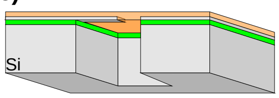
c) Definition of cantilevers: Dry etching of silicon after photolithography



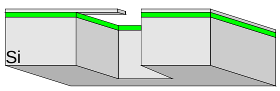
d) Cavities definition: DRIE etching of Si substrate



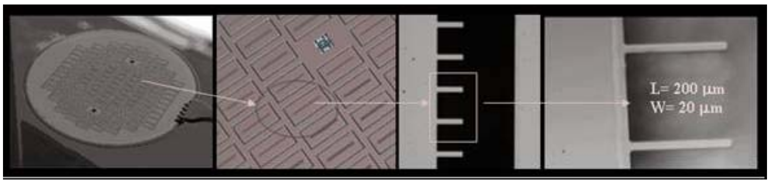
e) Removing of Silicon oxide



f) Removal of SiO_2 by wet etching (HF) and removing of photoresist. Cantilevers ready.



Microcantilever array: fabrication



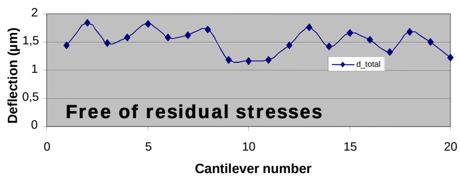
• **Avoid sticking**	**Bulk micromachining / No sticking**
• **Reduce area (20 cantilevers)**	**DRIE / 250 µm pitch for individual**
• **High Yield**	**100 %,** over 2560 cantilevers
• **High sensitivity**	**0.334 µm thick cantilevers**

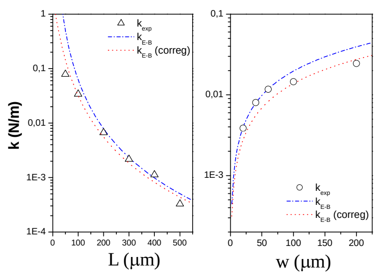
- **CMOS compatible, mass production at low cost**
- **Compatible with different surface coatings for biotesting**

Microcantilever array: characterisation

Experimental Mechanical Properties (Silicon)			
Length x width x thickness [µm]	**K (N/m)**	**Frequency [kHz]**	**Q**
500 x 20 x 0,3	$9{,}1\times10^{-3}$	1,5	1,8
200 x 20 x 0,3	$7{,}4\times10^{-3}$	9,9	6,3
100 x 20 x 0,3	$6{,}1\times10^{-2}$	34,9	13,6
50 x 40 x 0,3	$7{,}9\times10^{-2}$	86,5	20,7

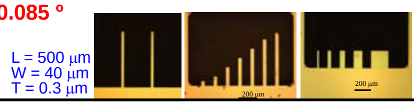
- Spring constant, resonant frequency, quality factor



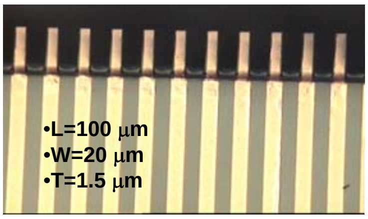


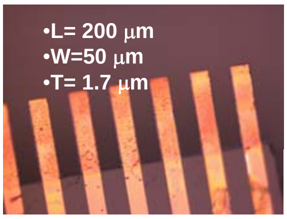
Off-set Deflection **< 2.0 µm/0.57º**

Dispersion **< 0.3 µm / 0.085 º**



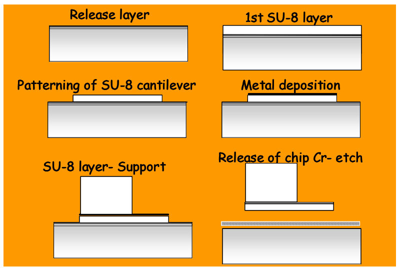
Microcantilever array: design and fabrication in polymer





Material: SU-8

- 40 times softer than Si: higher sensitivity to surface stress
- Chemically resistant, thermally stable and highly biocompatible
- Suitable for monolithic microfluidics integration:lab-on-chip
- Fast and easy processing and low requirements for lab-equipment



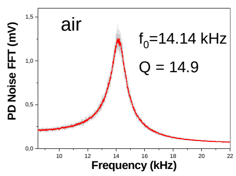
Characterisation of SU-8 cantilevers

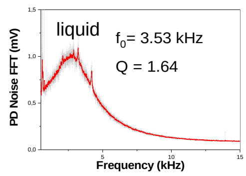
Experimental Mechanical Properties (SU-8)			
Length x width x thickness [µm]	k (N/m)	frequency [kHz]	Q
200 x 20 x 1.5	2.5×10^{-2}	17.0	15.0
200 x 50 x 1.7	6.4×10^{-2}	15.0	12.8
100 x 20 x 1.6	2.9×10^{-1}	41.0	15.6

Material properties, length, thickness

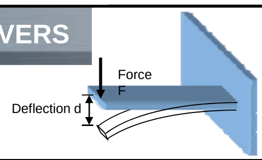
$$\Delta z \cong \frac{3(1-\nu)L^2}{Et^2}(\Delta\sigma_1 - \Delta\sigma_2)$$

Cantilever bending





COMPARISON WITH COMMERCIAL MICROCANTILEVERS



	Material properties E (10^{11} N/m^2)	ν	Thickness x width (μm)	k (N/m)	z (nm) For Δσ = 1 mN/m	Frequency* (kHz) air	liquid	Q Factor* air	liquid
commercial Veeco (SiN) (V-Shaped)	1.75	0.25	0.6 x 40	0.12	2.1	20.9	3.6	29.8	1.5
Commercial Olympus (SiN)	1.75	0.25	0.8 x 40	0.11	1.2	23.9	5.5	34.7	1.2
Silicon (CNM)	1.83	0.20	0.3 x 20	0.009	6.8	7.8	0.6	5.6	0.8
SU-8 polymer	0.05	0.25	1.5 x 20	0.024	9.3	17	3.4	15.6	1.4

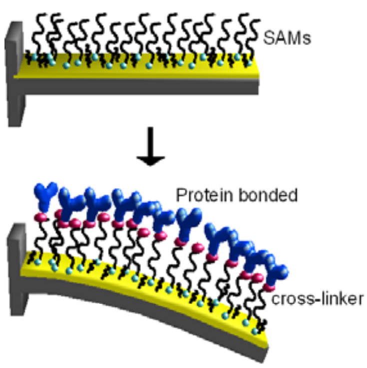
Z= deflexion
Δσ= surface stress change
E= Young´s modulus
ν= Poisson´s coefficient
h=thickness
L= length

Sensitivity

$$z \approx 4\frac{1-\nu}{Et^2}L^2(\Delta\sigma_t - \Delta\sigma_b) \leftarrow k = \frac{E}{4}\frac{wt^3}{L^3}$$

Nanomechanical sensors: Soft sensing layer

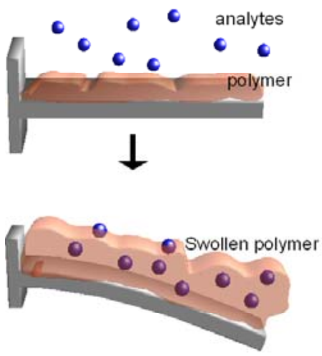
Self-assembled monolayers chemistry



Well controlled and robust technique

BIOSENSING

Polymers structures

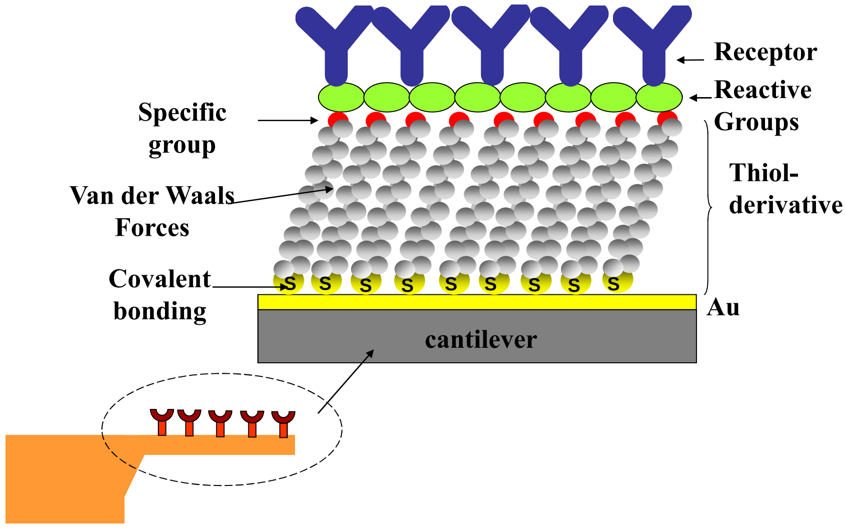


Higher surface stress change

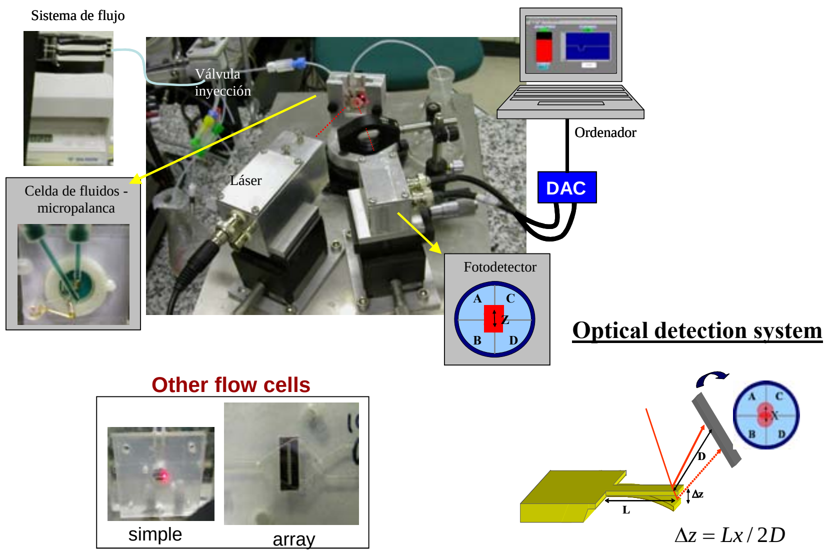
CHEMICAL SENSING

Microcantilever functionalization: Immobilisation procedures

SAMs: Self-Assembled Monolayer chemistry through thiol groups



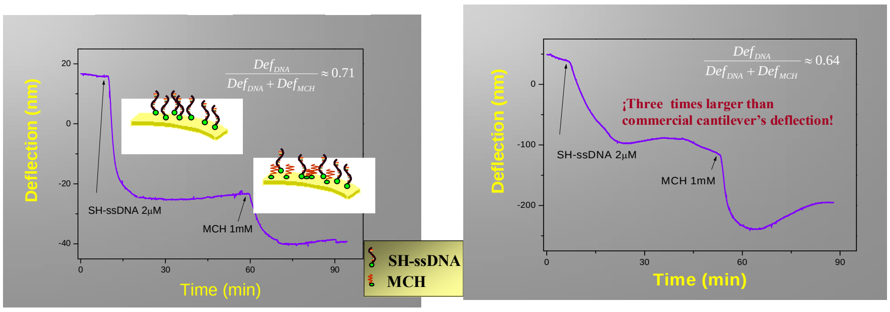
Experimental set-up for single-cantilever



Surface stress testing/ comparative

Commercial microcantilever

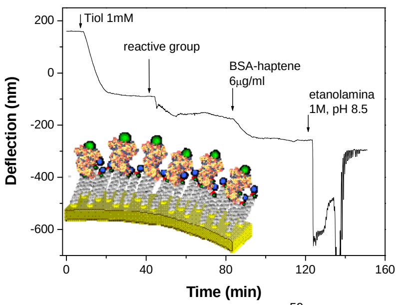
Silicon microcantilever

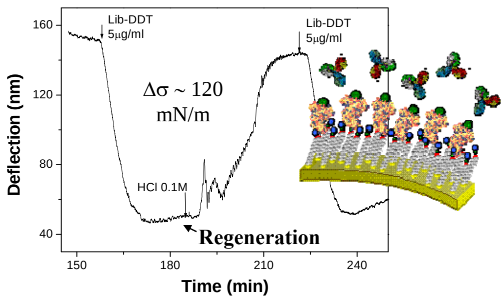


Single cantilever application: Pesticide detection

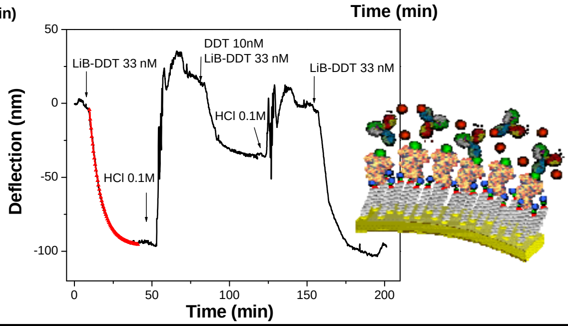
Surface modification

Immunoreaction



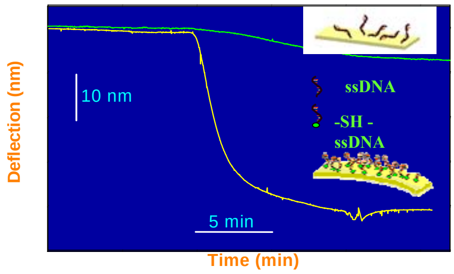


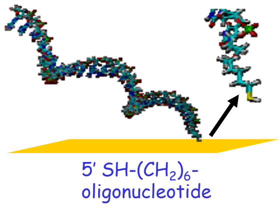
Competitive assay



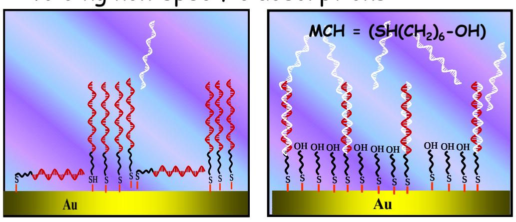
Single cantilever application: DNA detection

Effect of the oligonucleotide-thiol





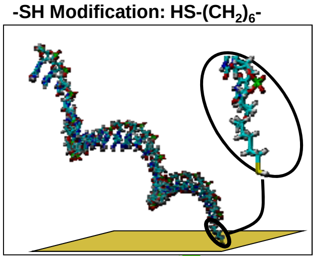
Avoiding non-specific adsorptions



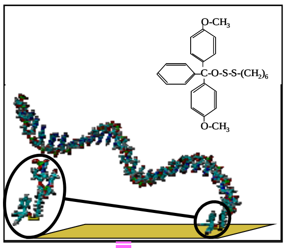
- Remove loosely bound DNA probes
- Enhance accessibility of DNA probe for hybridisation

IMPORTANCE OF THE SULFUR-GROUP ON THE SURFACE STRESS

-SH Modification: HS-(CH$_2$)$_6$-

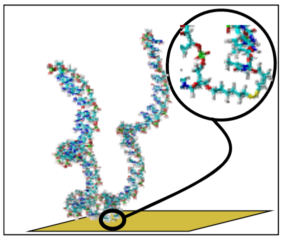


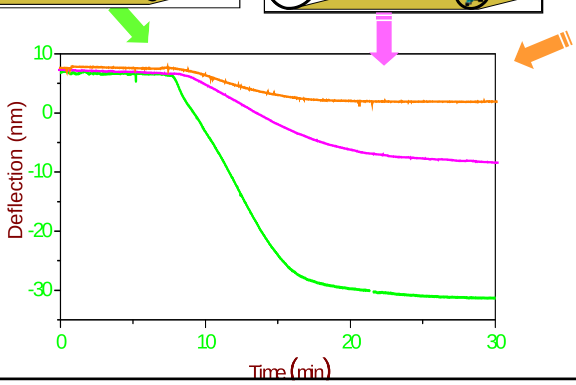
-SS-DMTO Modification



-SS-Modification:

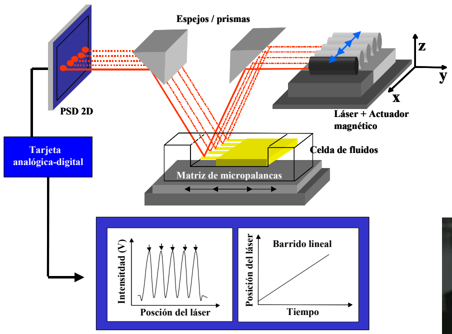
-(CH$_2$)$_6$-SS-(CH$_2$)$_6$-

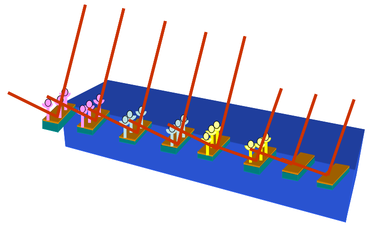




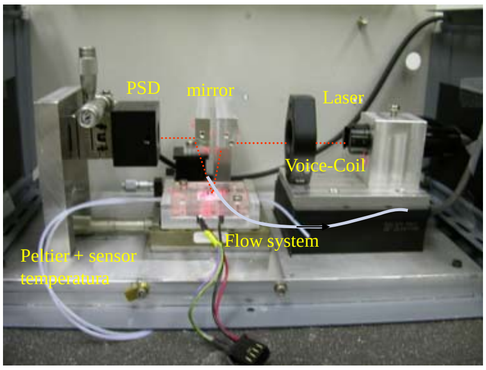
Microcantilever Arrays

Optical Sequential Readout of Microcantilever Arrays



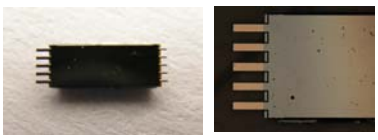


Scanning of the laser source is performed via voice-coil actuators:
6 mm range
100 nm resolution,
velocity ~10 mm/s

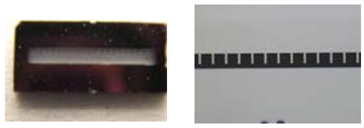


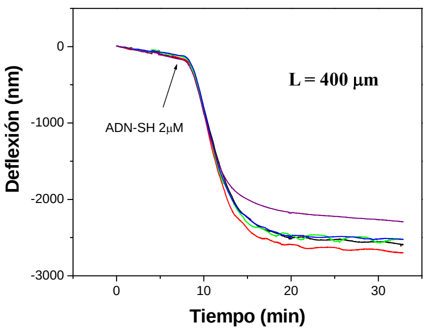
Optical Sequential Readout of Microcantilever Arrays

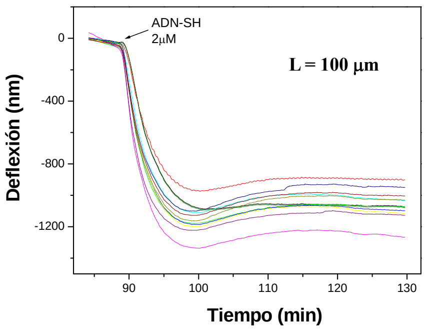
Comercial array



Home-made array

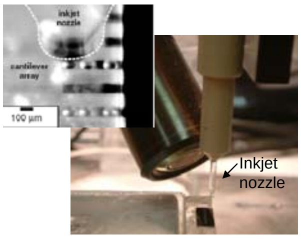




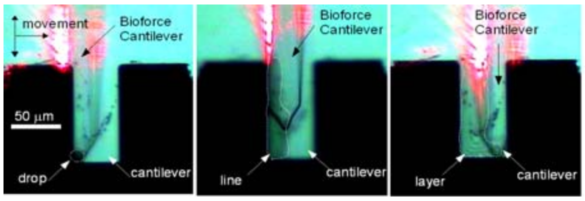


Microcantilever array functionalization

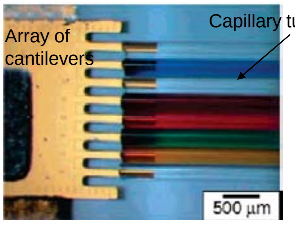
Ink-jet printing (Microdrop)



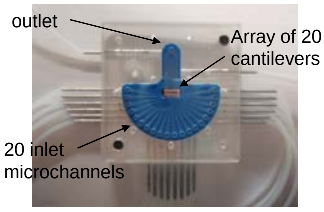
Contact printing (Nano eNabler)



Capillary

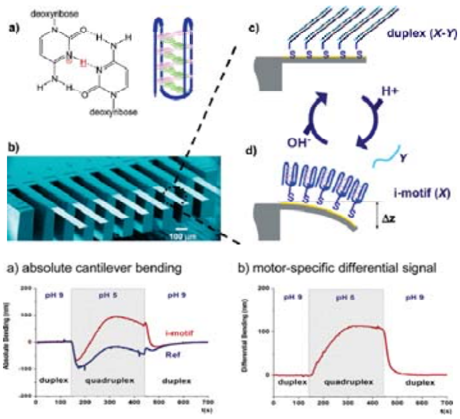


Microfluidics with individual microchannels



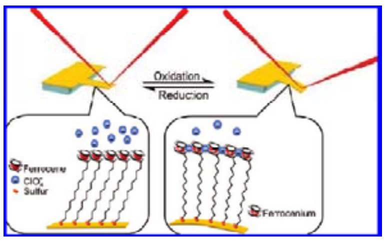
Examples of microcantilever biosensing in DC

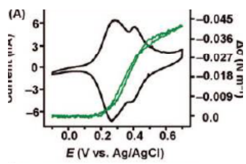
DNA Molecular Motor Driven Micromechanical Cantilever Arrays. Shu et al., JACS 127, 17054, 2005.



Conformational changes detection (molecular motors)

Redox Actuation of a Microcantilever Driven by a Self-Assembled Ferrocenylundecanethiolate Monolayer. Norman et al., JACS 131, 2328, 2009.

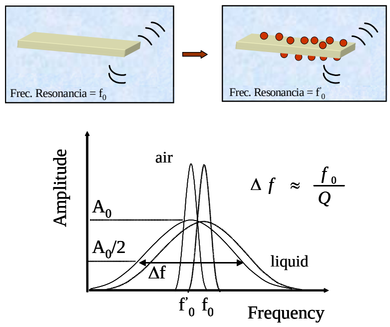




Electrochemically induced motion of free-standing microcantilevers (Red-ox reaction detection)

Examples of microcantilever biosensing in AC

Dynamic response



$$\Delta f \approx \frac{1}{2\pi}\sqrt{\frac{k+\Delta k}{m^* + \alpha \Delta m}}$$

Mass detection < fmg

Special interest for pathogens and cells detection:

- Measurement of physical properties of **single cells** (mass, size, volume, stiffness)
- Direct measurement of **adherent** cell mass (cell attached on surface)
- **Real time** measurements of cell mass change and conformational changes
- Detection of specific heat shock proteins in real time, …

Cantilever-cells dimensions:

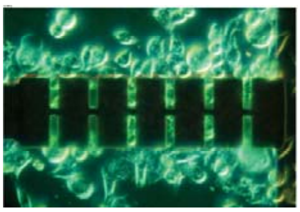
Cell mass ~ 5 ng (HeLa)

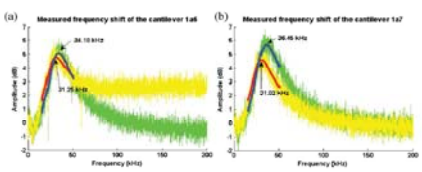
Cell diameter ~ 10 µm (Leukocytes)

Cantilever: 200 x 40 x 0.5 µm

Examples of microcantilever biosensing in AC

'Living cantilever arrays' for characterization of mass of single live cells in fluids. Park et al., Lab Chip, 8, 1034, 2008.

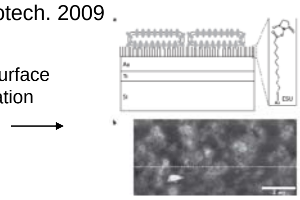


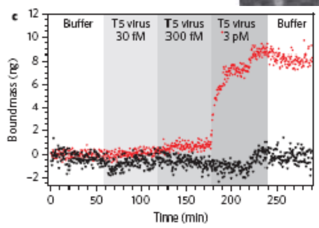


Characterization of a single adherent cell with a non invasive manner, under physiological conditions

Quantitative time-resolved measurement of membrane protein–ligand interactions using microcantilever array sensors. Braun et al., Nature Nanotech. 2009 .

Cantilever surface functionalization

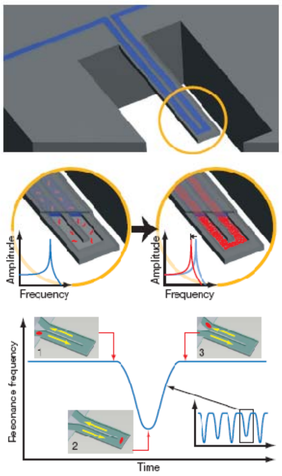


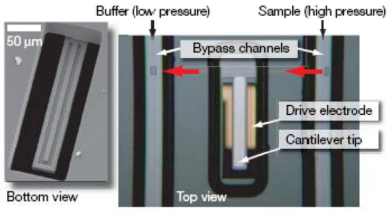


Measurements of the interactions between transmembrane protein receptors and their ligands

Examples of microcantilever biosensing in AC

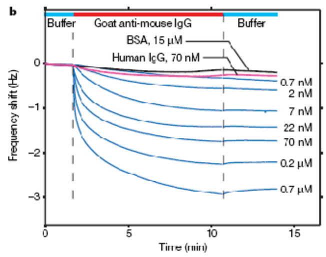
Weighing of biomolecules, single cells and single nanoparticles in fluid.
Burg et al., Nature, 446, 1066, 2007.



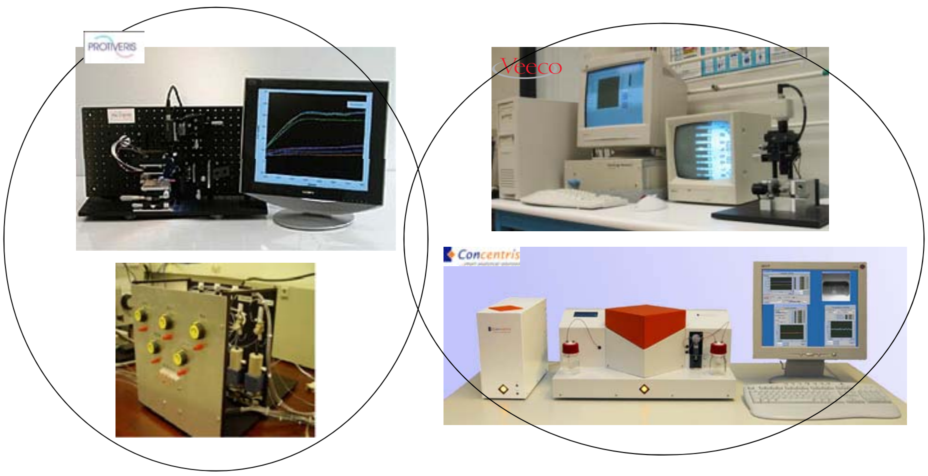


Microcantilevers with embedded microchannels.

High quality factors, high sensitivity



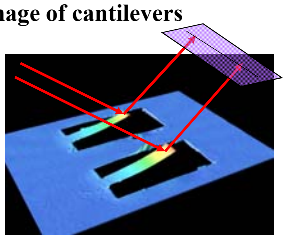
Commercial microcantilever sensors platforms

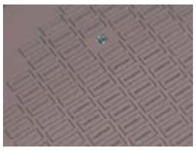


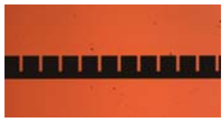
…….Ideally we must develop a portable biosensor microsystem (hand-held device) for decentralised clinical analysis

Integration in lab-on-a-chip: Integrated OptoNanomechanical Biosensor

Image of cantilevers





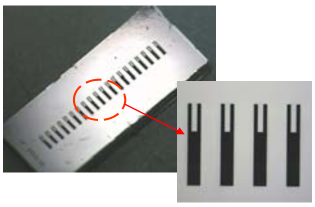


Array of 20 cantilevers

LAB-ON-A-CHIP DESIGN

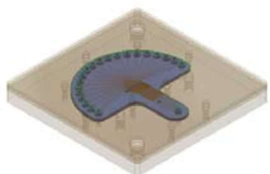
1. Microcantilever array
   Design, materials selection, fabrication and testing
2. Polymer microfluidic system
   common and discrete flow cell
3. Optical detection subsystem
   Lasers and photodetectors with signal processing CMOS circuits
5. Final integration and packaging

Microfluidic system: Discrete Fluid Path Header



Challenges

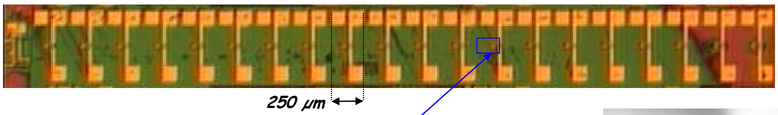
- -Fabrication of 20 channels of 250μm pitch
- -Low cost, disposable, biocompatible: PMMA
- -Optically transparent (infrared-850 nm)
- -Design to cause minimum bending or vibration
- -Gluing/sealing of cantilever chip to acrylic layer to ensure no leakage path between channels
- -Making multiple reversible fluid connections to header in a rapid and reliable way
- -Ensuring good optical path to and from cantilever surface



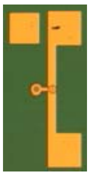
Optical detection system: lasers emitters

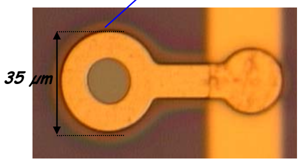
Growth by MOCVD

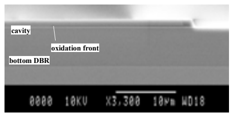
General view of a 20 VCSEL Array



View of a VCSEL







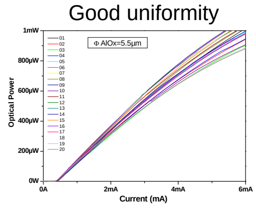
3.5 µm
(1.7 mA)

FEATURES

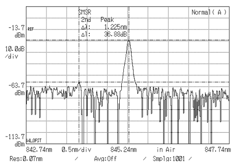
20 Addressable, Single Mode VCSELs
850nm wavelength
250 µm Pitch
Threshold I: 500 µA–2 mA
Threshold voltages: 1.5 - 3V
Beam divergence: 5°
Simple Electrical Interface

Source performances

Good uniformity



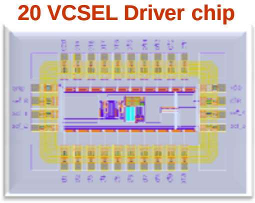
Single mode emission



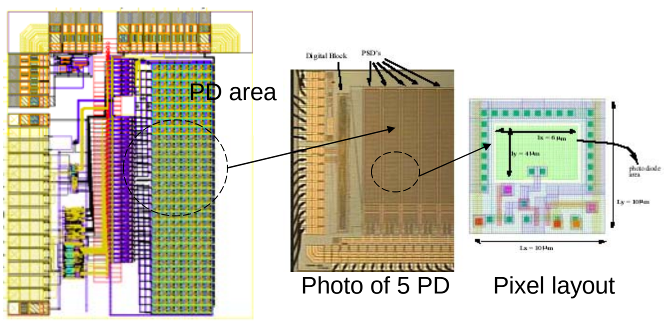
Optical detection system: drivers, PDs and CMOS circuits

1) Current Driver circuits for the VCSEL excitation
2) Electrical Model for VCSEL and PhotoDetectors (PD)
3) Photo- Detector circuits for Position Spot Detection (PSD)
4) Definition of the algorithm for PSD Signal Processing circuits for PSD
5) Definition of the measurement protocol and output signals.

20 VCSEL Driver chip

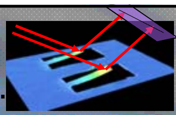


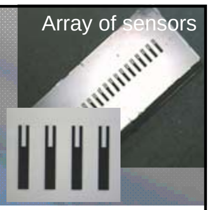
0.35 µm CMOS Chip: 2000x2500um2



Integration in Microsystems

For measuring arrays of microcantilevers.....





- **Advantages**
  - ✓ High sensitivity
  - ✓ Sub-angstrom resolution

- **Disadvantages**
  - ✓ Complex integration (difficult alignment): Difficult read-out of arrays
  - ✓ Sequential switching of the laser due to overlapping of the reflection beams
  - ✓ Complex detection by the PD chip
  - ✓ Microlens array onto laser chip
  - ✓ Immobilisation in each cantilever using the 20-flow cell

- Optics:20-VSCEL+Drivers
- Photodetector array. CMOS circuitry
- Microarrays of 20 microcantilevers
- Microfluidics:flow cell with 20 channels

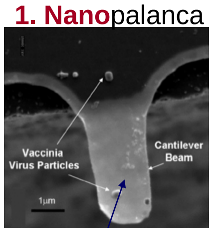
Flow cell-20-channels

Sensores nanomecánicos/MEMS/NEMS

MEMS → **NEMS**

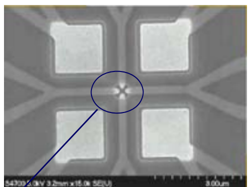
	MEMS	NEMS
Detección de masa:	pico-nano gramo	**femto-atto** gramo
Frecuencia de resonancia:	10-100 kHz	100 MHz -1 GHz
Área sensora:	1000 μm^2	**< 1 μm^2**
Límite de detección:	10^7 moléculas	**Moléculas y patógenos individuales**

1. Nanopalanca



Un sólo virus

2. Nanofluidica

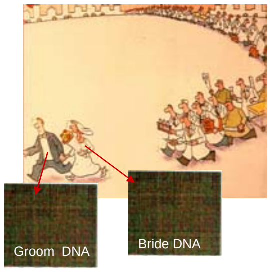


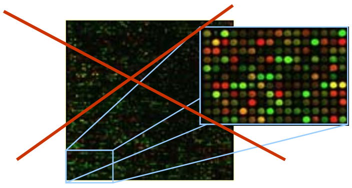
300 nm

Reducir tiempo de respuesta

Future opportunities

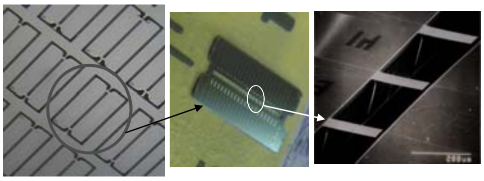
Actual DNA/Protein Biochips





- Need of high amounts of sample: (time consuming, expensive)
- Non-specific interactions
- Indirect read-out: fluorescent labeling. Labels restric assay types
- Low sensitivity for single mismatch detection
- Inconsistent activity of immobilised proteins

"Lab-on-a-chip" Nanobiosensors as an advanced platform



Array of microcantilevers

- Drastic reduction of sample amount
- **Direct read-out**: real time analysis
- High sensitivity
- Micro/nanotechnology: mass production with low cost

Conclusions

- Biosensors devices are a powerful technology allowing label-free, fast and real-time measurements
- The biological receptor layer is one of the crucial issues for making devices for field operation
- **Nanomechanical biosensors** is a emerging technology that allows:
  - Rapid detection of proteins, DNA and cells
  - It does not require labelling with fluorescent or radioactive molecules
  - The small sensor area allows analysis of minute amounts of sample (femtogram)
  - Arrays of microcantilevers can be fabricated with microelectronics technology: mass production and low cost
- The ideal situation is the development of a "lab-on-a-chip" microsystem through integration of sources, sensors, detectors, flow system, CMOS electronics and data processing on a compact device
- Biosensing is a highly multidisciplinary research area ranging from physics, chemistry, molecular biology, engineering,….

Nanobiosensors Group

- **Plasmonics**: SPR, Magneto-SPR and LSPR (nanostructures)
- **Integrated optics**: Nanophotonic biosensor (MZI)
- **Nanomechanical** biosensors (standard and optical)
- Biofuncionalization with biological receptors
- Microfluidics integration
- Lab-on-a-chip platforms (in-vitro and in-vivo)
- Applications: clinical diagnostics, environmental control

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