Course on
Nano-Bio-Sensing and Bio/CMOS interfaces

1. Introduction
What’s about The Course?

Nano-Bio-Sensing and Bio/CMOS interfaces

Nano-Bio-Technology
What’s about Nano-Bio-Technology?

That means “techniques at the nano-metric scale to fabricate and/or modify structures related to biological processes”
What’s about Nano-Bio-Technology?

BioTechnology

NanoTechnology

NanoBioTecnology

The intersection zone between nanotech and biotech
What’s about Nano-Bio-Technology?

NanoTechnology

Atoms and molecules manipulation
Structures Fabrication at the nano-scale
Microscopy at atomic resolution
Spectroscopy on single atom/molecule

NanoBioTechnology

Proteins and DNA purification
DNA amplification (PCR)
Gens expression
ELISA Tests

BioTechnology

S.Carrara, EPFL - Lausanne (CH)
Nanobiotechnology, as part of nanotechnology, has gained increasing importance during the last 10 years. In particular in medicine and pharmacology, this area of research opens up new perspectives in analytics and therapy. Nanobiotechnology is an interdisciplinary field of research and is based on the cooperative work of chemists, physicists, biologists, medical doctors and engineers. At the interface between biotechnology and nanotechnology, nanobiotechnologists carry out research on the phenomena of self-assembly or self-organisation of biomolecules such as cell membranes or virus particles, in order to adapt these principles to the technical production of nanostructures.
Nanobiotechnology is a rapidly advancing area of scientific and technological opportunity that applies the tools and processes of nano/microfabrication to build devices for studying biosystems. Researchers learn from biology to create new micro-nanoscale devices to better understand life processes at the nanoscale. The Nanobiotechnology is characterized by its highly interdisciplinary nature and features a close collaboration between life scientists, physical scientists, and engineers.
How to do nano-things?

The Built structure

Top-Down Approach: parts removal

Chemical corrosion
Laser Ablation
Ionic Ablation
Mechanical Imprinting
How to do nano-things?

Bottom-up Approach:
Why build cars if we can simply grow them?
IEEE Transactions on NanoBioscience will address such themes as:

- Methods for fabricating nanostructured biomaterials.
- The biocompatibility of materials at the nanometer scale.
- Cell-cell interaction and cell mechanics at the nano scale.
- Nanotechnology as applied to biomolecules and cells.
- Tissue engineering at the nano scale, e.g., for wound repair and replacement of skin, cartilage, bone, nerve and other tissues.
- Bioinformatics, biocomputing and molecular computing.
- Measurement and sensing of single cells, cell systems and biomolecules by optical, chemical or physical methods (including molecular biosensors).
- Engineered surfaces using assembled molecular arrays to guide interactions with biomolecules and cells.
- Effects of electric and magnetic fields on biomolecules.
- Molecular electronics and nanoscale diagnostic devices.
Aims of Journals in NanoBioTecnology

Aims and Scope

*IEEE Proceedings on Nanobiotechnology* covers all aspects of research and emerging technologies related to the convergence of nanotechnology with biotechnology, especially those aspects relating to the interactions between biomolecules and biomolecular assemblies with electronic structures or materials.
Biological Objects @ the Nano-scale

Fig. 2  Hierarchical scales of biology
@ the Nano-Scale Means:

Table 2: Size and time scales in biology: nanometres to metres and nanoseconds to gigaseconds. The biological time constants at each scale are comparable diffusion time constants, $\tau_{\text{diffusion}}$, even though diffusive mixing is not acceptable for large systems.

<table>
<thead>
<tr>
<th>Length</th>
<th>Volume</th>
<th>Time</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 m</td>
<td>1000 L</td>
<td>$10^9$ s</td>
<td>Animal, bioreactor</td>
</tr>
<tr>
<td>10 cm</td>
<td>1 L</td>
<td>$10^2$ s</td>
<td>Organ, bioreactor</td>
</tr>
<tr>
<td>1 cm</td>
<td>1 mL</td>
<td>$10^8$ s = 1 day</td>
<td>Tissue, cell culture</td>
</tr>
<tr>
<td>1 mm</td>
<td>1 μL</td>
<td>$10^3$ s</td>
<td>Monovinon, well plate</td>
</tr>
<tr>
<td>100 μm</td>
<td>1 nL</td>
<td>10 s</td>
<td>Cell-cell signalling</td>
</tr>
<tr>
<td>10 μm</td>
<td>1 pL</td>
<td>0.1 s</td>
<td>Cell</td>
</tr>
<tr>
<td>1 μm</td>
<td>1 fL</td>
<td>1 ms</td>
<td>Subspace</td>
</tr>
<tr>
<td>100 nm</td>
<td>1 aL</td>
<td>10 μs</td>
<td>Organelle</td>
</tr>
<tr>
<td>10 nm</td>
<td>1 zL</td>
<td>100 ns</td>
<td>Protein</td>
</tr>
<tr>
<td>1 nm</td>
<td>1 pL</td>
<td>1 ns</td>
<td>Ion channel</td>
</tr>
</tbody>
</table>

Table 1: Time scales of systems biology (extended beyond [39])

<table>
<thead>
<tr>
<th>System or subsystem</th>
<th>Relaxation time, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aging</td>
<td>$10^8-3 \times 10^9$</td>
</tr>
<tr>
<td>Survival with chronic/congestive heart failure</td>
<td>$\sim 10^8$</td>
</tr>
<tr>
<td>Bone healing</td>
<td>$10^7$</td>
</tr>
<tr>
<td>Small wound healing</td>
<td>$10^6$</td>
</tr>
<tr>
<td>Atrial remodelling with AF</td>
<td>$10^5$</td>
</tr>
<tr>
<td>mRNA synthesis on operon</td>
<td>$10^3-10^4$</td>
</tr>
<tr>
<td>Cell proliferation, DNA replication</td>
<td>$10^2-10^4$</td>
</tr>
<tr>
<td>Translocation of substances into cells</td>
<td>$10^1-10^3$</td>
</tr>
<tr>
<td>Protein synthesis in polysomes</td>
<td>$10^1-10^2$</td>
</tr>
<tr>
<td>Heart with ventricular fibrillation</td>
<td>$10^1$</td>
</tr>
<tr>
<td>Allosteric control of enzyme synthesis, heartbeat</td>
<td>$10^9$</td>
</tr>
<tr>
<td>Glycolysis in the cytoplasm</td>
<td>$10^{-1-10^1}$</td>
</tr>
<tr>
<td>Oxidative phosphorylation in mitochondria</td>
<td>$10^{-2}$</td>
</tr>
<tr>
<td>Intracellular diffusion, enzymatic reactions and turnover</td>
<td>$10^{-6-10^{-3}}$</td>
</tr>
<tr>
<td>Receptor-ligand, enzyme-substrate reactions</td>
<td>$10^{-6}$</td>
</tr>
<tr>
<td>Ion channel gating</td>
<td>$10^{-7-10^{-9}}$</td>
</tr>
<tr>
<td>Electron transfer in photosynthesis</td>
<td>$10^{-11-10^{-13}}$</td>
</tr>
</tbody>
</table>
Examples in NanoBioTechnology

Fig. 3  Size of biological systems and the micro- and nanodevices being developed to study them. Adapted from [85]

Fig. 4  Cellular-scale sensors and actuators, combined with model-based computation and feedback, allow full exploration of dynamics of single cell

recording of multiple single-cell signatures. Externally controlled nano-actuators, needed to effect changes in the biochemical, mechanical and electrical environment both outside and inside the cell, will provide a major impetus for nanoscience.
Examples in NanoBioTechnology

Paramagnetic capture mode magnetophoretic microseparator for blood cells

From IEE Proceedings on Nanobiotechnology
Examples in NanoBioTechnology

Characterisation of pore structures in nanoporous materials for advanced bionanotechnology

Abstract: Porous materials are potential candidates for applications in various fields, such as bionanotechnology, gas separation, catalysts and micro-electronics. In particular, their applications in bionanotechnology include biosensors, biomedical implants and microdevices, biosupporters, bio-encapsulation, biomolecule separations and biomedical therapy. All these bionanotechnology

Fig. 1  Geometry of GIXS

Fig. 4  Pore radius and distribution determined from GIXS
Examples in NanoBioTechnology

Forming Microstructured Alkanethiol Self-Assembled Monolayers on Gold by Laser Ablation

Daniel Rhinow and Norbert A. Hampp

Fig. 1. Experimental setup. The beam of a pulsed frequency-doubled Nd:YVO₄ laser was controlled by a galvanometer scanner to form structures in the SAM mounted on the precision motorized XYZ stage. Nd:YVO₄ laser, 532-nm, 6-ns pulses, repetition rate 20 kHz. PBS: polarizing beam splitter; BE: beam expander; FTL: F-theta lens. IR radiation was blocked by an IR filter.

Two \(\omega\)-substituted alkanethiols were used to prepare SAMs on gold. Decanethiol (DT), HS(CH₂)₉CH₃ and 16-mercapto-hexadecanoic acid (MHDA), HS(CH₂)₁₅COOH (Fluka), were...
Examples in NanoBioTechnology

Multi-walled carbon nanotube exposure alters protein expression in human keratinocytes
Examples in NanoBioTechnology

Biotemplated Nanopatterning of Planar Surfaces with Molecular Motors

We report on the lattice of reconfigurable guiding nanotubes, enabling the generation of functionalized patterns of proteins on planar surfaces. In particular, we used the regular and anisotropic anisotropic alignment of microtubules to specifically bind and transfer kinesin-1 and non-claret disjunctional motor proteins. This enabled the generation of functionalized arrays of functional motor proteins, proved to be highly efficient for the transfer of motor proteins, and for the construction of nanostructures with functionalized guidance channels. Moreover, biotemplated nanopatterning is a promising tool for in vitro studies on the individual and cooperative action of motor proteins as well as for the reconstitution of complex subcellular machineries in synthetic environments.

**Figure 1.** Methods of biotemplated nanopatterning. (a) Biotemplated stamping: Kinesin molecules, which are bound in an oriented manner to the lattice of a template microtubule, are transferred onto the surface by a stamping process. After adsorption, the template microtubule is released when the deposited motor molecules propel the microtubule off the generated track in the presence of ATP. The same molecules will move and guide the transport microtubules. (b) Biotemplated binding: Motor proteins are bound to a template microtubule that was previously immobilized on the surface. Transport microtubules then move specifically on the motor track, thereby sliding along the template microtubule.

<table>
<thead>
<tr>
<th>Method</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Biotemplated Stamping</td>
<td>Kinesin molecules bound to a template microtubule are transferred onto the surface.</td>
</tr>
<tr>
<td>b) Biotemplated Binding</td>
<td>Motor proteins bound to a template microtubule move specifically on the motor track.</td>
</tr>
</tbody>
</table>
What’s about Nano-Bio-Sensing?

That means “techniques at the nano-metric scale to sense information related to biological processes”
## What to sense?

Bio-Markers may be simple molecules, proteins or genes.

<table>
<thead>
<tr>
<th>Simple Molecules</th>
<th>Glucose (Diabetes)</th>
<th>Thrombin (heart attack)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins</td>
<td>AFP (Hepato Carcinoma)</td>
<td>PSA (Prostate)</td>
</tr>
<tr>
<td>DNA sequences</td>
<td>PC-1 gene (prostate cancer)</td>
<td>p53 gene (Hepato Carcinoma)</td>
</tr>
</tbody>
</table>
The Measure of Bio-markers may be performed in a labeled manner or in label-free mode.
The Motivation

- 100,000 $ (machinery)
- 1,000 $ the single μ-array

Labeled

Label-Free

- 50 $ (machinery)
- 0.05 $ the single strip
How to measure Bio-markers in Label-Free Mode?

What change we can measure?

How?

• Mass
  - Resonant Quartz
  - Micro-Cantilevers
  - Mass Spectrometry

• Elasticity
  - AFM Microscopy

• Dielectric prop.
  - Surface Plasmon

• Charge
  - IS-FET
  - C-Measurements
How to measure Bio-markers in Label-Free Mode?

What change we can measure?

How?

- **Mass**
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  - Mass Spectrometry

- **Elasticity**
  - AFM Microscopy

- **Dielectric prop.**
  - Surface Plasmon

- **Charge**
  - IS-FET
  - C-Measurements
## Cancer Markers Detection by QCM

**S. Carrara et al. / Biosensors and Bioelectronics 24 (2009) 3425–3429**

<table>
<thead>
<tr>
<th>Functionalization</th>
<th>Quartz frequency [Hz]</th>
<th>Mass changing [ng]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bare chip</td>
<td>4993920 ± 1</td>
<td>–</td>
</tr>
<tr>
<td>E-G thiols film</td>
<td>4993900 ± 1</td>
<td>8.44 ± 0.42</td>
</tr>
<tr>
<td>NHS-EDC activation</td>
<td>4993897 ± 1</td>
<td>1.27 ± 0.42</td>
</tr>
<tr>
<td>Antibody</td>
<td>4993828 ± 1</td>
<td>29.10 ± 0.42</td>
</tr>
<tr>
<td>Antigen</td>
<td>4993616 ± 1</td>
<td>89.42 ± 0.42</td>
</tr>
</tbody>
</table>

**The Cancer Marker SCCA detected in Patients’ serum**

S. Carrara, EPFL - Lausanne (CH)
Bio-Chip Development

Development toward fully integrated chip for sensing cancer markers from the Project SmartHEALTH
How to measure Bio-markers in Label-Free Mode?

What change we can measure?

How?

- **Mass**
  - Resonant Quartz
  - Micro-Cantilevers
  - Mass Spectrometry

- **Elasticity**
  - AFM Microscopy

- **Dielectric prop.**
  - Surface Plasmon

- **Charge**
  - IS-FET
  - C-Measurements
Molecular Detection based on cantilevers deflection or changes in their resonance frequencies

From http://www.icmm.csic.es/

Displacements in nm scale!

90-135 nm

S. Carrara, EPFL - Lausanne (CH)
Future development toward fully integrated chip for sensing cancer markers
How to measure Bio-markers in Label-Free Mode?

What change we can measure?

- Mass
- Elasticity
- Dielectric prop.
- Charge

How?

- Resonant Quartz Micro-Cantilevers
- Mass Spectrometry
- AFM Microscopy
- Surface Plasmon
- IS-FET C-Measurements
MS Spectroscopy Principle

Ions Source | Mass Analyzer | Detector

Electric Field

M1

M2

I

M1

M2

m/z

t

S.Carrara, EPFL - Lausanne (CH)
Large Cooperative European projects is now running to develop Chromatography and MS on a single chip.
How to measure Bio-markers in Label-Free Mode?

What change we can measure?

- Mass
  - Resonant Quartz
  - Micro-Cantilevers
  - Mass Spectrometry

- Elasticity
  - AFM Microscopy

- Dielectric prop.
  - Surface Plasmon

- Charge
  - IS-FET
  - C-Measurements
Atomic Force Microscopy (AFM)

The AFM microscopy is based on a tip scan over a surface. The acquired signal is related to Tip-surface interactions.

S. Carrara, EPFL - Lausanne (CH)
Nanomechanical-Based Evaluation and Diagnostics of Human Patient Samples:

Nanomechanical-Based Analysis of Clinical Effusions

✓ Young’s elastic modulus, $E$: (a) All tumor and normal data: 7 different clinical samples (patients)
Nanomechanical-Based Analysis of Clinical Effusions

✓ Young’s elastic modulus, $E$:

(a) All tumor and normal data:
7 different clinical samples (patients)

(b) Gaussian fit for all "tumor" data:
$\langle E \rangle = 0.53 \pm 0.10$ kPa
(mean ± s.d.)

$P = 8.72 \times 10^{-22}$

(c) Log-normal fit for all "normal" data:
$\langle E \rangle = 1.97 \pm 0.70$ kPa
(mean ± s.d.)

Nanomechanics Can Distinguish Cells Despite Apparent Morphology

Benign or Tumor?

(i) E “tumor” cells:
\[
<\text{E}> = 0.54 \pm 0.08 \text{ kPa} \\
(\text{mean} \pm \text{s.d.; } n=8)
\]

(ii) E “normal” cells:
\[
<\text{E}> = 0.54 \pm 0.12 \text{ kPa} \\
(\text{mean} \pm \text{s.d.; } n=8)
\]

Both are tumor cells!
How to measure Bio-markers in Label-Free Mode?

What change we can measure?

How?

• Mass
  Resonant Quartz
  Micro-Cantilevers
  Mass Spectrometry

• Elasticity
  AFM Microscopy

• Dielectric prop.
  Surface Plasmon

• Charge
  IS-FET
  C-Measurements
Surface Plasmon Resonance

Kretschmann configuration (1971)
Molecules smaller than 200 Da or bigger than 400 nm could not be correctly detected. Within the range, it is possible to reach a sensitivity of ng.
SPR Chip Integration

Si PC μ-cavity, Noda, 2006

Si microdisk, Adibi, 2007

Silica μ-toroid, Vahala, 2007

Sol μ-ring, De Vos, 2007

Courtesy of Peter Seitz, talk on Nanophotonics for Lab-on-Chip
How to measure Bio-markers in Label-Free Mode?

What change we can measure?

- Mass
  
  - Resonant Quartz Micro-Cantilevers
  - Mass Spectrometry

- Elasticity
  
  - AFM Microscopy

- Dielectric prop.
  
  - Surface Plasmon

- Charge
  
  - IS-FET
  - C-Measurements
How to measure the Antibody-Antigen binding if they do not provide us a current?

The detection may be provided by the ions charge displacements and, thus, by means of a Capacitance Measurement.
System for Bio-sensing of DNA and Proteins

Capacitance variation during DNA hybridization

S.Carrara, EPFL - Lausanne (CH)
Gene Detection with Capacitance

Capacitance variation in DNA detection

al. et S.Carrara / IEEE SENSORS JOURNAL, VOL. 7, NO. 4, APRIL 2007

S.Carrara, EPFL - Lausanne (CH)
How to measure Bio-markers in Label-Free Mode?

What change we can measure?

How?

• Mass
  Resonant Quartz
  Micro-Cantilevers
  Mass Spectrometry

• Elasticity
  AFM Microscopy

• Dielectric prop.
  Surface Plasmon

• Charge
  IS-FET
  C-Measurements
IS-FET Detection

IonS-Field Effect Transistor (IS-FET) working Principle
Cancer Markers Detection


PSA detection by means of FET array

S. Carrara, EPFL - Lausanne (CH)
Great Potentiality for Bio-Markers Nano-Sensing

<table>
<thead>
<tr>
<th>TECHNOLOGY</th>
<th>Det. Limit</th>
<th>in Chip</th>
<th>Marker</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>QCM</td>
<td>0.61 nM</td>
<td>Yes</td>
<td>PSA</td>
<td>Y.Ukudag / Biosens. and Bioel. (submitted)</td>
</tr>
<tr>
<td>MS</td>
<td>14 zM</td>
<td>Yes</td>
<td>Biomol. Ions</td>
<td>pubs.acs.org/doi/abs/10.1021/ac048202r</td>
</tr>
<tr>
<td>IS-FET</td>
<td>27.3 zM</td>
<td>Yes</td>
<td>PSA</td>
<td>G.Zheng / NATURE Biotech 10 (2005) 1294-1301</td>
</tr>
</tbody>
</table>
The borders of Nano-Bio-Sensing

So, Nano-Bio-Sensing has not well defined borders with other well defined fundamental disciplines.
Nano-Bio-Sensing is a highly multidisciplinary field

Relations with other fields

- Biology
- Bioinformatics
- Electronics
- Informatics
- Mechanics
- Bio-Physics
- Bio-Chemistry
- Chemistry
- Bioinformatics
- Bio-Chemistry
- Chemistry
- Bio-Physics
- Electronics
- Informatics
- Mechanics
The interface between the CMOS circuit and the bio-sample needs to be deeply investigated and characterized.

Bio/CMOS Co-Design!
Course Lectures:

1. Introduction
2. Detection principle (Probe-Target interactions)
3. Nano-Technology for Probes immobilization
4. Nano-Technology for Sensing enhancement
5. CMOS building blocks
6. CMOS architectures for Sensing
The End
Lecture #1. Introduction

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