3D nanostructures for Bio-Photonics and Neuro-Plasmonics

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IDEAS CO-GRANT n°616213
PhD and Post-Doc positions available

Nanostructures facility

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>1000 researchers
>30% of foreign people
Many departments in the same buildings
34 years old in average
500 m² clean room facilities
Electron/ion/optical lithographies
Evaporators, sputtering, ALD, RIE, etc...
Outline

- Superhydrophobic/oleophobic surfaces for biosensing
- Novel fabrication approach for multifunctional 3D nanostructures
- Neuro-plasmonics project (ERC Ideas-Consolidator)
- PROSEQO project (H2020, FET Open, 2016-2019)
What is a plasmon?

Surface Plasmon Polaritons (SPP) = surface electromagnetic waves
(solution of Maxwell equations)

Localized Surface Plasmon

\[ 2R \approx 60\text{nm} \]
\[ \lambda \approx 600\text{nm} \]

Polarizability:
\[ \alpha = R^3 \frac{\varepsilon_m - \varepsilon_d}{\varepsilon_m + 2\varepsilon_d} \]

Incoming wave

Field Enhancement or Quality Factor:
\[ Q = -\frac{\text{Re} \varepsilon_m}{\text{Im} \varepsilon_m} \approx 10 \]
**What is a plasmon?**

“plasma-oscillation”: density fluctuation of free electrons

Plasmons in the bulk oscillate at

$$\omega_{p}^{\text{drude}} = \sqrt{\frac{1}{4\pi\varepsilon_0}} \frac{4\pi ne^2}{m}$$

determined by the free electron density and effective mass

Plasmons confined to surfaces that can interact with light to form propagating “surface plasmon polaritons (SPP)”

Confinement effects result in resonant SPP modes in nanoparticles

A. Polman, “Nanophotonics lecture series”,
http://www.erbium.nl/nanophotonics/
Main features in Plasmonics:

They exist in noble metals: Au, Ag, Pt, Cu, ...

Skin depth: ≈ 20-30 nm (surface wave)

High electric field confinement, up to few nm in the visible range
(in contrast with Abbe diffraction limit ≈ $\lambda/2\approx 300$ nm)

High electric field enhancement: 10-1000 times the incident field amplitude.

Mean free path (e-)≈ 10-40 nm but SPP propagation length: 1-10 um

Very fast: plasmon response time $<< 1$ fs

What is a plasmon?

Plasmonics enables to manage the electromagnetic field at the nanoscale, but it requires Nanotechnology to be managed!!
Lotus effect & superhydrophobic surfaces

Natural systems

Artificial systems (by photolithography and RIE)

- Full controllable size
- High aspect ratio (up to 20 or more)
- Both rigid and flexible substrates

a, b, h ≈ 1-10 μm
Evaporation of 10 µl of water in few minutes

Miele et al. Controlling wetting and self assembly dynamics. *Advanced Materials* 2014

Beating the diffusion limit: proof of concept

F. De Angelis et al., Nature Photonics 2011

Breaking diffusion limits...

See also: E. Miele et al., Controlling wetting and self assembly dynamics....

Advanced Materials, 2014
....now looking for practical applications!

- Fluorescence based assay (immuno-assay!)
- MALDI
- IR spectroscopy, Raman
- Other spectroscopy
- Protein crystallization (@grenoble)

E. Miele et al.,
Controlling wetting and self assembly dynamics.... *Advanced Materials, 2014*
Combination of Focused Ion Beam (FIB) and ion-induced chemical modification on a proper polymer film that works as lithographic resist.

3D plasmonic hollow nanostructures for multifunctional plasmonics, F. De Angelis et al., Nano letters 13 (8), 3553-3558.
**Remark 1:** All devices are hollow and the channel passes through the whole structure up to the backside of the supporting membrane → Microfluidic & Optofluidic!

**Remark 2:** there is a uninterrupted metal layer that short-circuits the antennas → Optoelectronics, electrically driven optical properties, electrolytic cells, Photovoltaics, electro-photochemical catalysis.
Fabrication results

3D plasmonic hollow nanostructures for multifunctional plasmonics,
F. De Angelis et al., Nano letters 13 (8), 3553-3558.
3D plasmonic hollow nanostructures for multifunctional plasmonics, F. De Angelis et al., Nano letters 13 (8), 3553-3558.
Single antenna: planar vs out-of-plane (stronger lateral scattering)

Poynting vector stream lines for light impinges at 45°
3D: parallel to the surface
2D: normal to the surface
Field enhancement 140 vs 35

3D vertical nanostructures for enhanced infrared plasmonics.
M. Malerba et al., Scientific Report 2015
Optical properties 2: quality factors much above planar system

Array 5x5; height 0.85-3 um; pitches optimized to have best Q

Quality factor $Q = \frac{\omega}{\gamma} \geq 20-25$ in the whole mid-IR

Mid infrared field enhancement $\approx 200$ (in amplitude)

Commercial FTIR: NO collimated and polarized beam!!! 5x5 arrays!

3D vertical nanostructures for enhanced infrared plasmonics.
M. Malerba et al., Scientific Report 2015
3D Plasmonic meta-molecules

Tuning bond and antibonding modes without cross-talk (independently)

Hybridization in Three Dimensions: A Novel Route toward Plasmonic Metamolecules
Zilio et al., Nano Letters 2015.
FDTD investigations

Log \((E/E_0)^2\)

\(\lambda = 490\) nm

\(\lambda = 590\) nm

\(\lambda = 890\) nm

3D plasmonic hollow nanostructures for multifunctional plasmonics, F. De Angelis et al., Nano letters 13 (8), 3553-3558.
Practical applications in Biology??

Can we culture cells on the top?

Raman characterization

Height =1400 nm Radius=80 nm
Optimized for high field enhancement at 630 nm.

Inside the channel
\[ \frac{E}{E_0} = 15-20 \]
\[ I_{\text{raman}} = 10^5 \]
detection limit:
1–10 μM (liquid flow)
Real-label-free

outside
\[ \frac{E}{E_0} = 180 \]
\[ I_{\text{raman}} = 10^9 \]
Detection limit:
few molecules (dry conditions)

See also, E. Miele et al., Controlling wetting and self assembly dynamics.... Advanced Materials, 2014
Understanding the neuronal code, i.e. the rules which govern the way neuronal circuits process, store, and exchange information, is a major scientific and technological challenge that will revolutionize our capability of managing and exploiting neuronal circuits. Currently, progresses remain slow and face a dense multi-scale dynamics involving signaling at the molecular, cellular and large neuronal network levels.

**Neuro-Plasmonics**

Whereas the brain capabilities are most likely emerging from large networks of neuronal populations, available electrophysiological methods limit our access to single cells and typically provides only an averaged observation of neuronal signaling, fragmented to limited spatial and temporal scale. Moreover, this field suffers the lack of a method capable of accessing the molecular level.
State of the art: neuronal circuits investigations

**Basic principles: action potentials**

- **Patch-Clamp (in cell).** Single sharp electrode: detailed investigation of electric action potentials but just few cells, possible cell damage, difficulties in long term observations and automation.

- **Micro-Electrode-Arrays (MEA) (extra-cellular)**
  - Network investigation, long term, no damage, full automation, but poor electrical sensitivity (NO subthreshold!!) due to contact resistance between membrane and electrode.

**MEA + 3D micro-nano-electrodes.** Network investigation with low contact resistance!

**Challenges??**
- 3D nanofabrication electrode penetration
- Membrane damage
- Membrane reforming
- Chemical Signaling??
- Neurotransmitters??

**Investigations at molecular level by Plasmonics and Advanced Spectroscopies**
Three-dimensional nanoantennas are able to guide neuronal development along predefined patterns.

The guidance effect is not driven by material differences or by selective functionalization.

The nanoantennas present the same material and the same surface chemistry of the surrounding flat substrate.

Out-of-Plane Plasmonic Antennas for Raman Analysis in Living Cells.
La Rocca et al. Small 2015
3D vs 2D cell interface

- N2A cell line
- strong spontaneous adhesion
- Predictable adhesion sites

Out-of-Plane Plasmonic Antennas for Raman Analysis in Living Cells.
La Rocca et al. Small 2015
In-vitro Raman characterization of cell membrane
\( \lambda = 785 \text{ nm} \), acquisition time 10 seconds

Future perspective: membrane receptor investigations

How we can integrate them with electrical sensors??

Out-of-Plane Plasmonic Antennas for Raman Analysis in Living Cells.
R. La Rocca et al. Small 2015
Methodology: Integration with commercial hardware

3Brain commercial chipset **with 4096 recording electrodes** ([www.3brain.com](http://www.3brain.com))

- **Integration with confocal microscopes and spectroscopic tools** (including Fluorescence and Raman)
- **Rat-hippocampal or Human IPS cell culture** (induced pluripotent stem)
- **Our 3D plasmonic nanoelectrodes**
  - Large scale an fast processes (up to 40k structures/hour)
  - High plasmonic performances thanks to the 3D structures
  - Direct integration with commercial electronic chip
  - Direct access to the market ➔ **Strong medium/longterm impact on the the market and Neuroscience community.**

Direct integration with confocal microscopes and spectroscopic tools (including Fluorescence and Raman)

3D plasmonic nanoantennas integrated with MEA biosensors. **M. Dipalo et al. Nanoscale 2015**
Cultured neurons

- hippocampal neurons from rat, 10 days in vitro

Network level

extracellular contact!

Raman measurements

Electrical measurements

3D plasmonic nanoantennas integrated with MEA Biosensors. M. Dipalo et al. Nanoscale 2015

Out-of-Plane Plasmonic Antennas for Raman Analysis in Living Cells. La Rocca et al., Small 2015
Intracellular delivery by plasmonic nanotubes

- **Real time, quantitative, broad range of molecules delivered, cell selective...**
- **Fast (up to $10^{4-5}$ cell/s), compatible with low voltage electroporation.**

H2020 – FET OPEN
2016-2019
Single Protein Sequencing
Combination of advanced 3D fabrication, plasmonics, and FRET
Monitoring extracellular metabolites by Raman spectroscopy

Silver nano-islands separated from cell culture ==> reduced toxicity

<table>
<thead>
<tr>
<th>Peak (cm$^{-1}$)</th>
<th>Possible attribution</th>
<th>Refs.</th>
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<tbody>
<tr>
<td>534</td>
<td>Acetoacetate</td>
<td>36, 39</td>
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<tr>
<td>658</td>
<td>Histidine</td>
<td>32</td>
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<tr>
<td>685</td>
<td>Proteins</td>
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<tr>
<td>727</td>
<td>Methionine</td>
<td>32, 33</td>
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<tr>
<td>757</td>
<td>Cytocrome, Ring breath Tryptophan (Trp)</td>
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<tr>
<td>768</td>
<td>Fumarate</td>
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<tr>
<td>828</td>
<td>Tyrosine</td>
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<tr>
<td>894</td>
<td>Glycine</td>
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<td>1004</td>
<td>Phenylalanine (Phe)</td>
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<td>1121</td>
<td>Proteins; stretching CN</td>
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<td>Proteins; stretching CN; Carbohydrates: stretching C-O</td>
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<td>Nucleic Acids: Thymine (T)</td>
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<td>1244</td>
<td>Ammide III (β-Sheet)</td>
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<td>1291</td>
<td>Fumarate</td>
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<td>1316</td>
<td>Nucleic Acids: Guanine(G); Proteins: C=H; Lipids</td>
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<tr>
<td>1336</td>
<td>Proteins; twisting (CH2, CH3)</td>
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<td>1402</td>
<td>Deformation CH3 Asym; Stretching COO-</td>
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<td>1422</td>
<td>Nucleic Acids: Adenine (A), Guanine (G)</td>
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<td>1488</td>
<td>L-Histidine</td>
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<td>1521</td>
<td>Nucleic Acids: Citosine (C)</td>
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<td>Tryptophan</td>
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<td>Proteins: Ammide II</td>
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<td>Phe</td>
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<td>Ammide I; C=C Tyr, Trp, Lipids; stretching (C=C) olefinIC</td>
<td>43, 46, 48</td>
</tr>
</tbody>
</table>

By looking Raman signal variations a broad range of metabolites can be monitored in real time

Shalabaeva et al., Nanoscale, under review.
Micro-motors with asymmetric shape efficiently convert light into work by thermo-capillary effects.

Post-Doc positions
Available from March 2016

Thanks for your attention!!