3D nanostructures for Bio-Photonics and Neuro-Plasmonics

Michele Dipalo
Gabriele Messina
Valeria Caprettini
Laura Lovato
Francesco Tantussi
Andrea Toma
Pierfrancesco Zilio
Sara Perotto
Angelo Bozzola
....others
Francesco De Angelis

European Research Council
Established by the European Commission
Supporting top researchers from anywhere in the world

Neuro-Plasmonics

IDEAS CO-GRANT n°616213

PhD and Post-Doc positions available

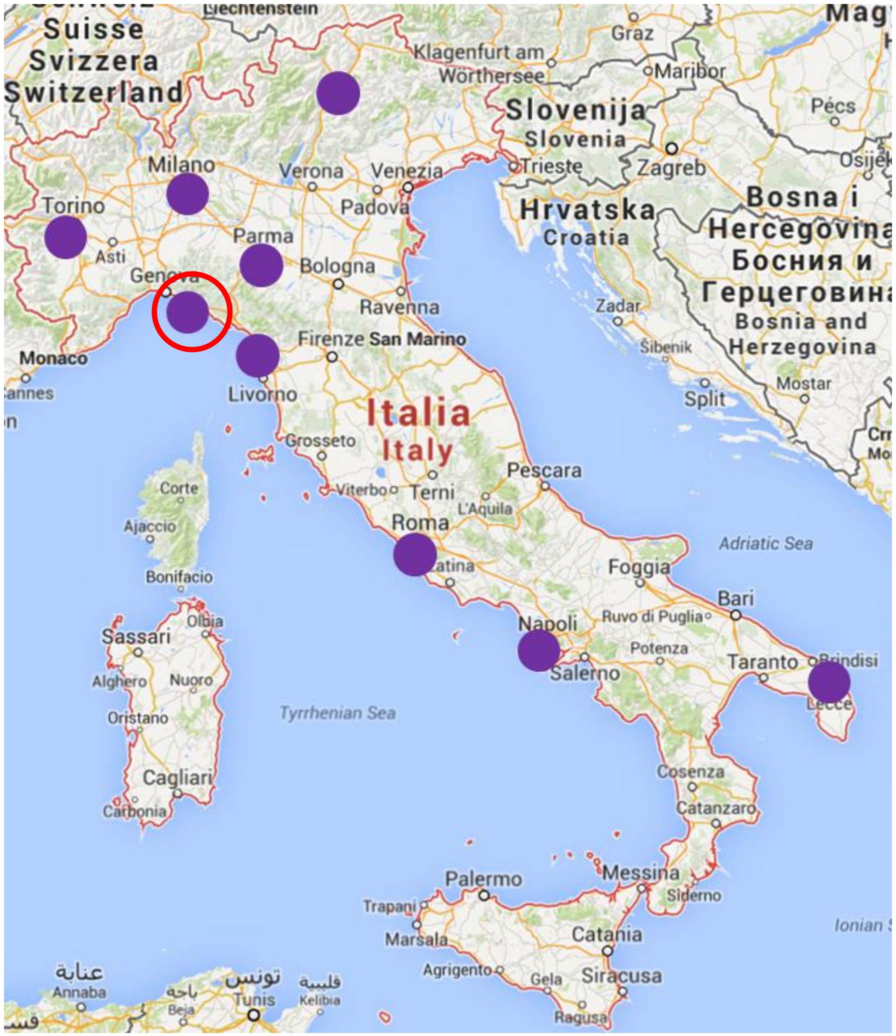
francesco.deangelis@iit.it www.iit.it

MALERBA FECIT

ISTITUTO ITALIANO DI TECNOLOGIA

>1000 researchers
>30% of foreign people
Many departments in the same buildings
34 years old in average

Genova
Pisa
Milano
Torino
Lecce
Roma
Napoli
Trento
Parma



Centers Overview

Click on any Center logo to visit the website

Center for Space Human Robotics
- Robotics for aerospace
- Sensors & MEMS

Center for Nano Science and Technology
- Artificial retina/eye
- Carbon nanocomposites
- Hybrid solar cells

Center for Genomic Science
- Robotized, imaging based, high-throughput Screening Unit for cell based screening
- Empowering genomics-proteomics platforms for analysis of biological samples
- Development of next generation sequence technologies with electrochemical detection systems

Center for Neuroscience and Cognitive System
- Active Vision
- Brain Stimulation

Brain Center for Motor and Social Cognition
- Neural mechanisms in cortical centers
- Mirror mechanism in physiological and pathological condition
- Dispositional moods and communicative actions

Center for Life Nano Science
- Neurosciences
- D4
- Smart Materials
- Computation

Center For Nanotechnology Innovation
- Lab on chip for ultrasensitive automated diagnostics
- Nanomedicine tools for diagnostics and therapeutics
- Nanoproducts and environmental safety assessment
- Energy storage/scavenging
- Modeling of biological/inorganic interfaces

Center for Micro-BioRobotics
- Microrobotics

Center for Advanced Biomaterials for Health Care
- Cell Instructive Materials
- Endothelial nanoshuttles
- 3D integrated platforms for high throughput and multiple detection

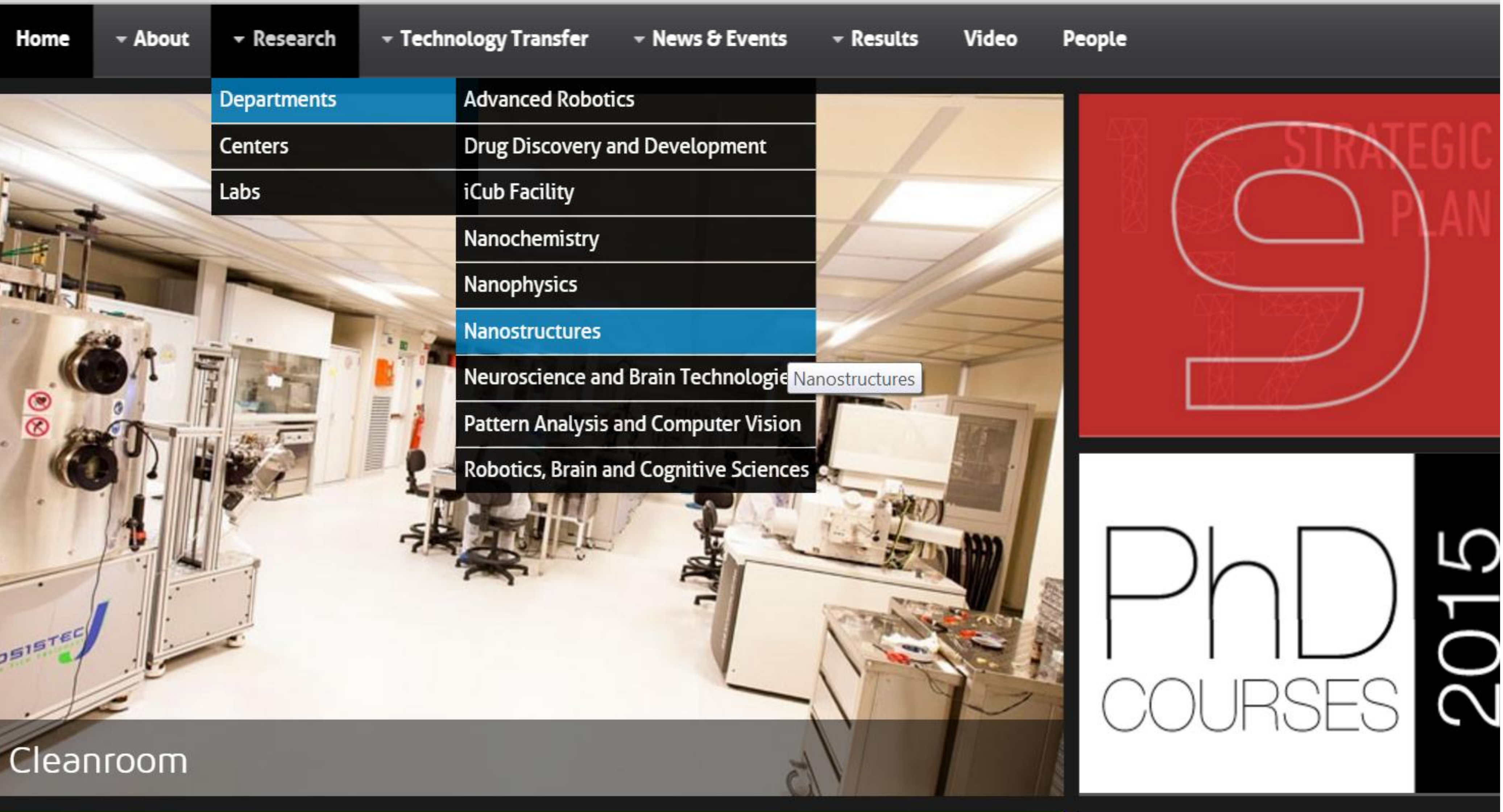
Center for Biomolecular Nanotechnologies
- MEMs/new sensors for robotics
- Solid state genomics
- Interaction of biological structures with nanomaterials
- Smart surfaces, Responsive composites
- Biocomposites
- Nanofibers
- Plastic solar cells, plastic lighting, Grezel cells
- Density functional software

iit ISTITUTO ITALIANO DI TECNOLOGIA

500 m^2 clean room facilites

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)

ISTITUTO ITALIANO DI TECNOLOGIA

Neuro-Plasmonics

THE FRAMEWORK PROGRAMME FOR RESEARCH AND INNOVATION
HORIZON 2020

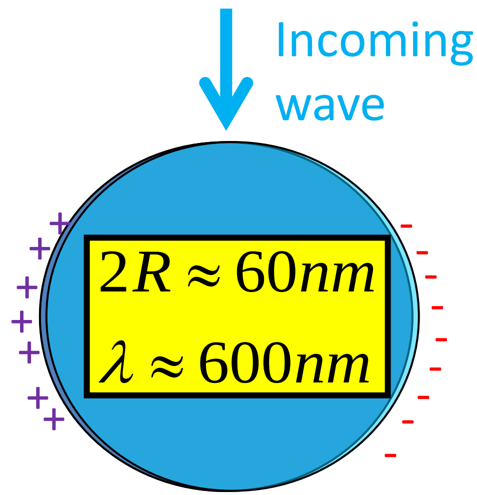
erc
European Research Council
Established by the European Commission
Supporting top researchers from anywhere in the world

What is a plasmon?

Surface Plasmon Polaritons (SPP) = surface electromagnetic waves

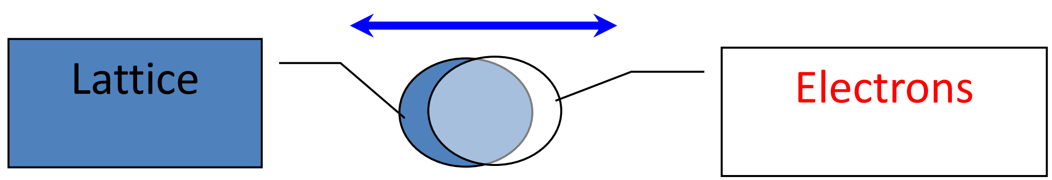
(solution of Maxwell equations)

Localized
Surface Plasmon



Polarizability:

$$\alpha = R^3 \frac{\varepsilon_m - \varepsilon_d}{\varepsilon_m + 2\varepsilon_d}$$



Strong & localized field

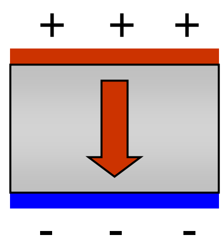
How much??

Field Enhancement or Quality Factor:

$$Q = \frac{-\mathrm{Re}\,\varepsilon_m}{\mathrm{Im}\,\varepsilon_m} \sim 10$$

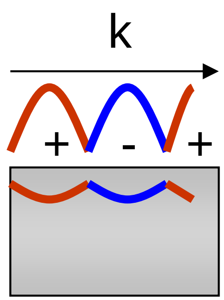
What is a plasmon?

“plasma-oscillation”: density fluctuation of free electrons

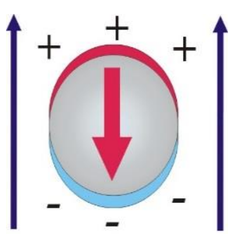


Plasmons in the bulk oscillate at $\omega_p^{drude} = \sqrt{\left[\frac{1}{4\pi\varepsilon_0}\right]\frac{4\pi n e^2}{m}}$

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/

What is a plasmon?

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 ≈ $\lambda/2$≈ 300 nm)

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

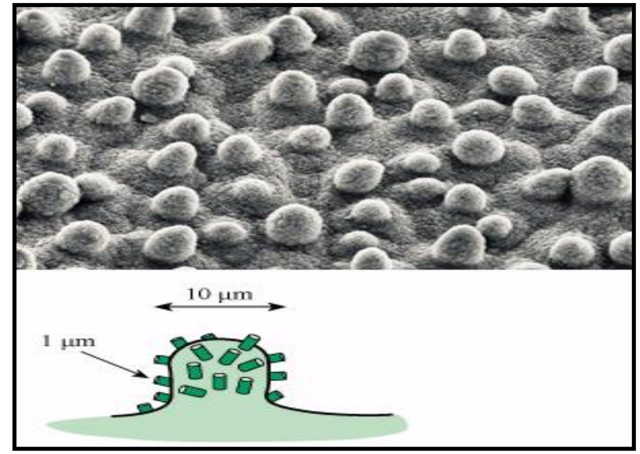
Mean free path (e-)≈ 10-40 nm but SPP propagation length: 1-10 um

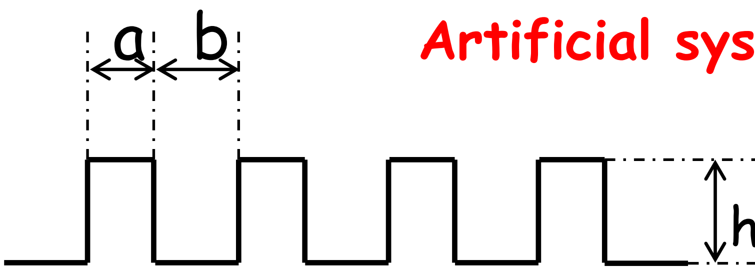
Very fast: plasmon response time << 1 fs

Plasmonics enables to manage the electromagnetic field at the nanoscale, but it requires Nanotechnology to be managed!!

Lotus effect & superhydrophobic surfaces

Natural systems

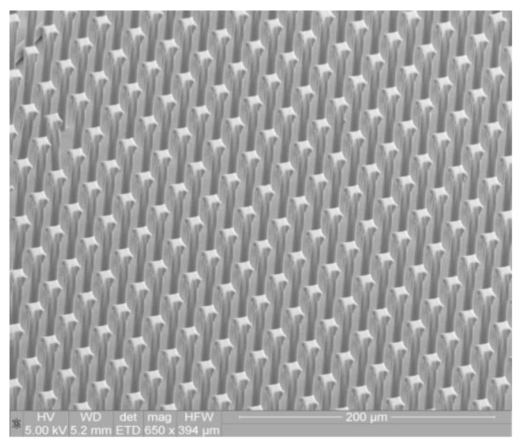


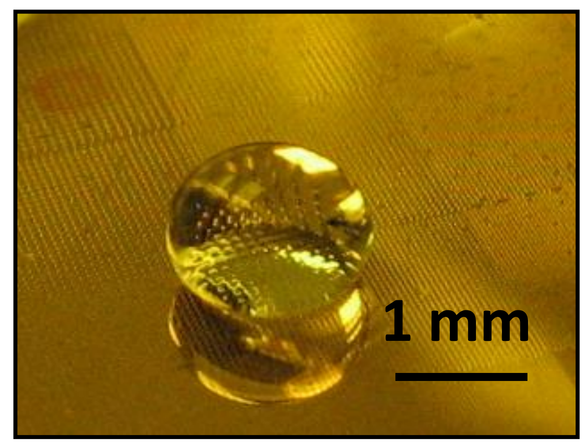


a, b, h ≈ 1-10 μm

Artificial systems (by photolithography and RIE)

Silicon micro-pillar
+ teflon layer



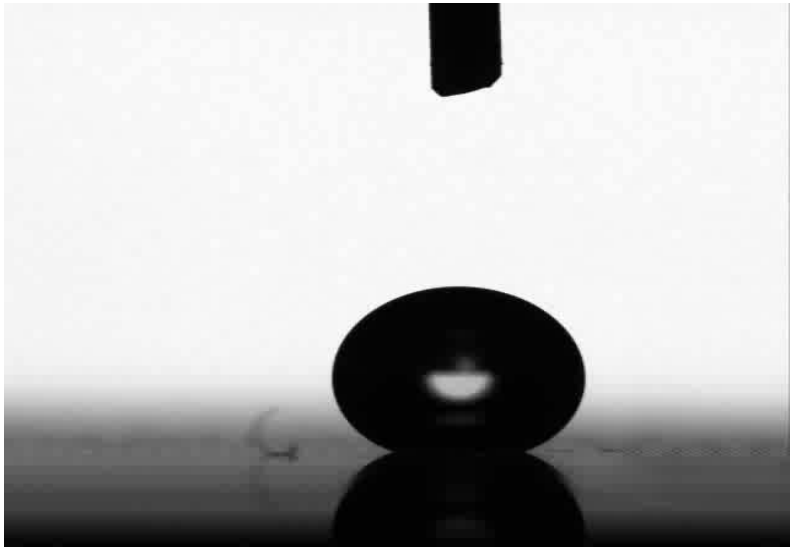


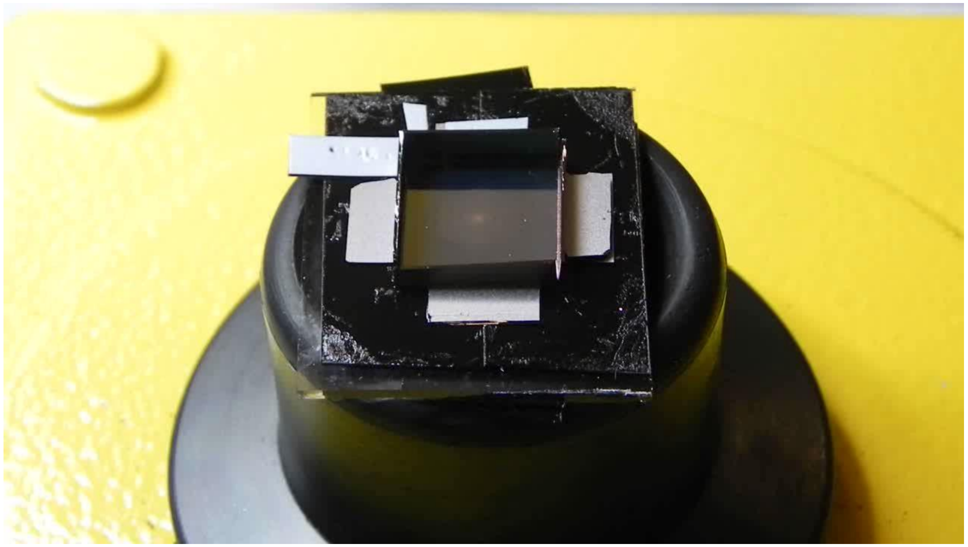
- Full controllable size
- High aspect ratio (up to 20 or more)
- Both rigid and flexible substrates

ISTITUTO ITALIANO
DI TECNOLOGIA

Beating the diffusion limit (patented)

Evaporation of 10 μl of water in few minutes

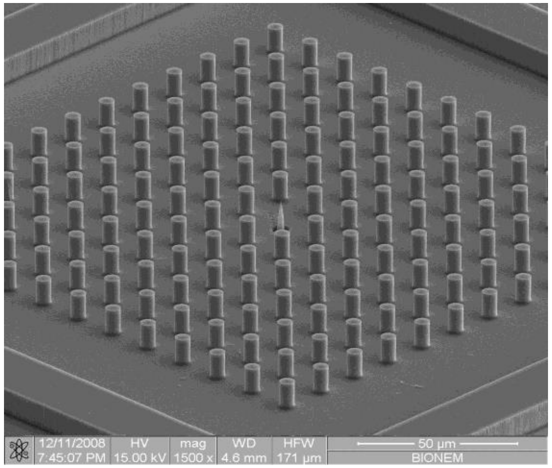


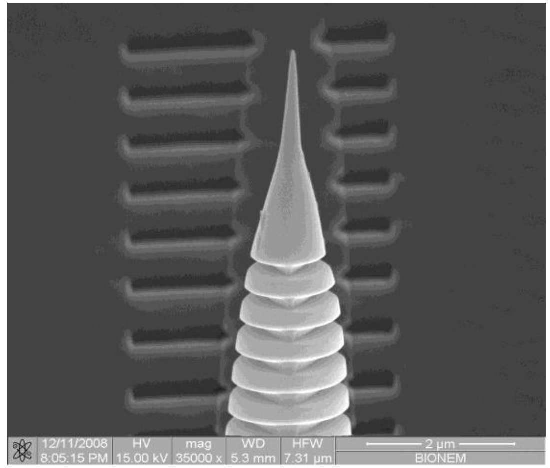


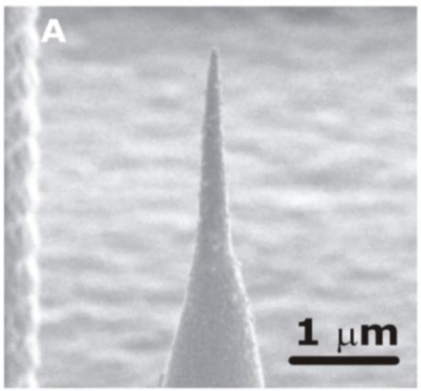
Miele et al. Controlling wetting and self assembly dynamics. ***Advanced Materials*** **2014**

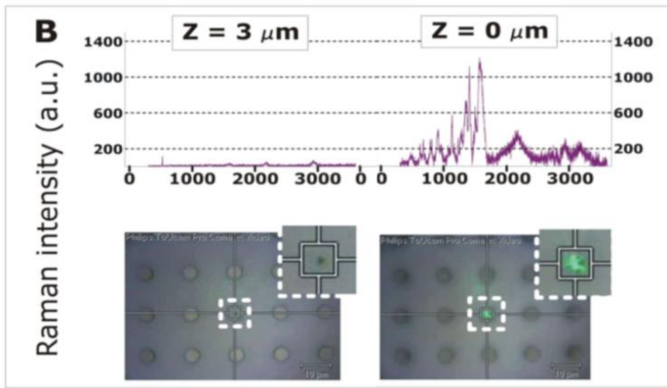
F. De Angelis et al., Breaking the diffusion limit. Nature Photonics, 5 (2011), 682-687.

Beating the diffusion limit: proof of concept







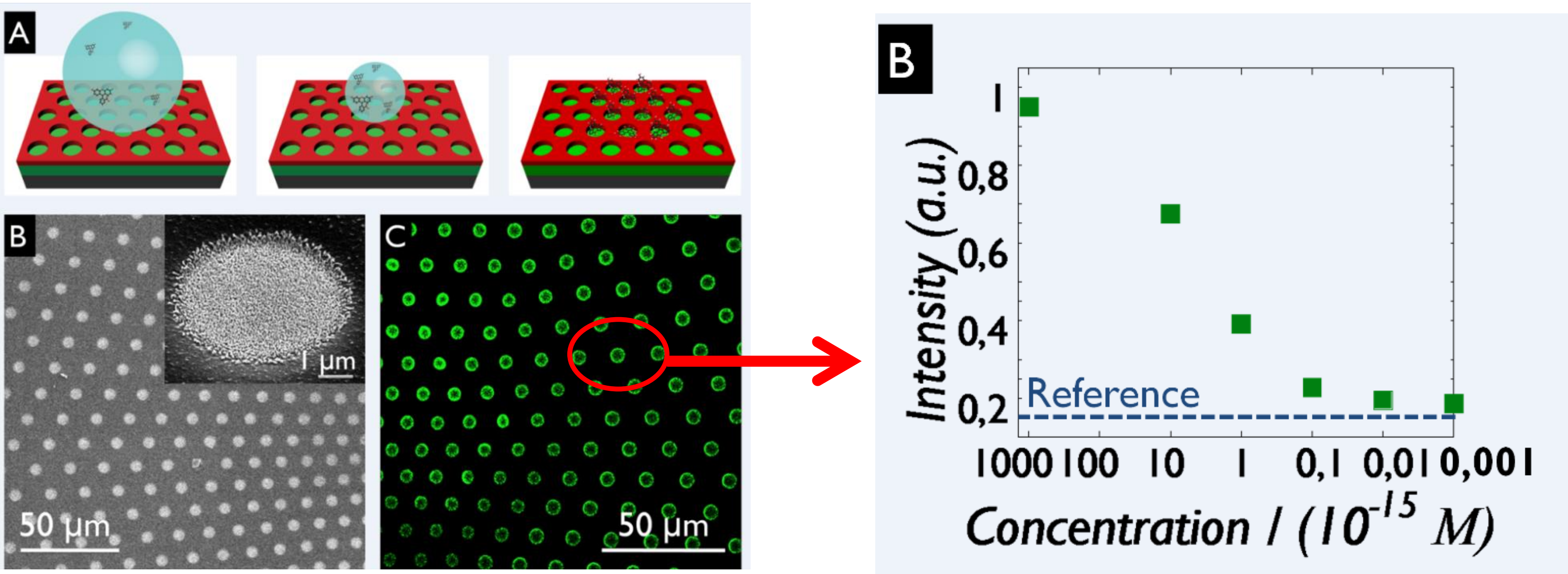


Breaking diffusion limits...
F. De Angelis et al., Nature Photonics 2011

See also: E. Miele et al., Controlling wetting and self assembly dynamics....
***Advanced Materials*, 2014**

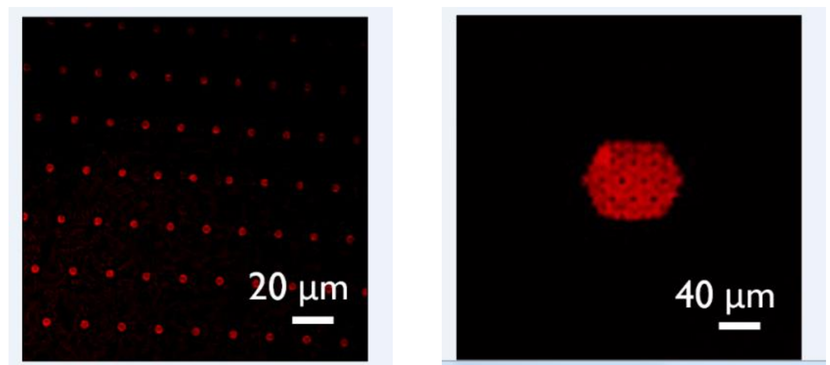
ISTITUTO ITALIANO
DI TECNOLOGIA

.....now looking for practical applications!



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

**Oleophobic devices:
Quantum dots delivery**



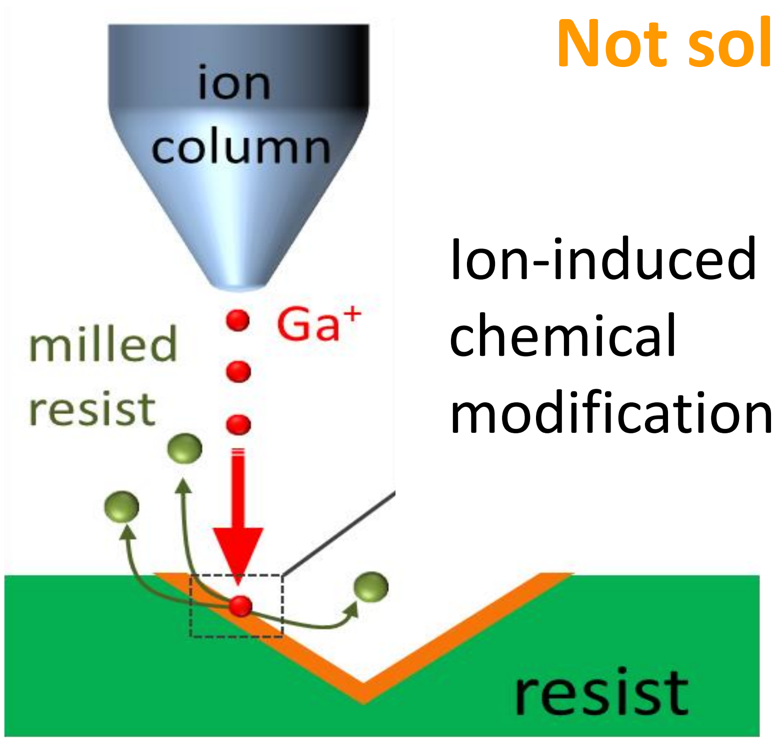
E. Miele et al.,
Controlling wetting and self assembly dynamics.... ***Advanced Materials*, 2014**

ISTITUTO ITALIANO
DI TECNOLOGIA

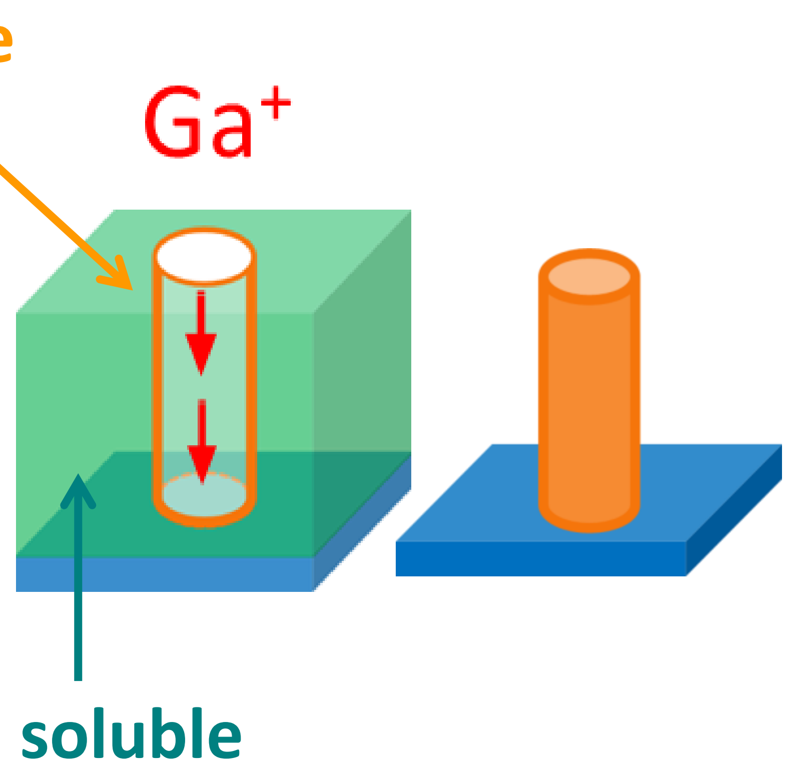
3D hollow plasmonic devices: fabrication method

European Research Council
Established by the European Commission
Supporting top researchers from anywhere in the world

Combination of Focused Ion Beam (FIB) and ion-induced chemical modification on a proper polymer film that works as lithographic resist.

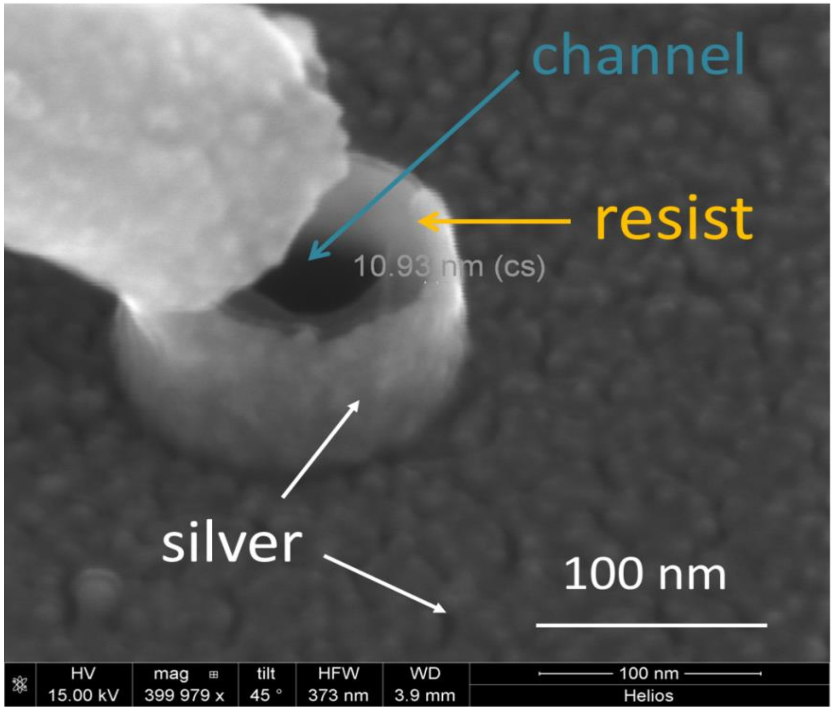


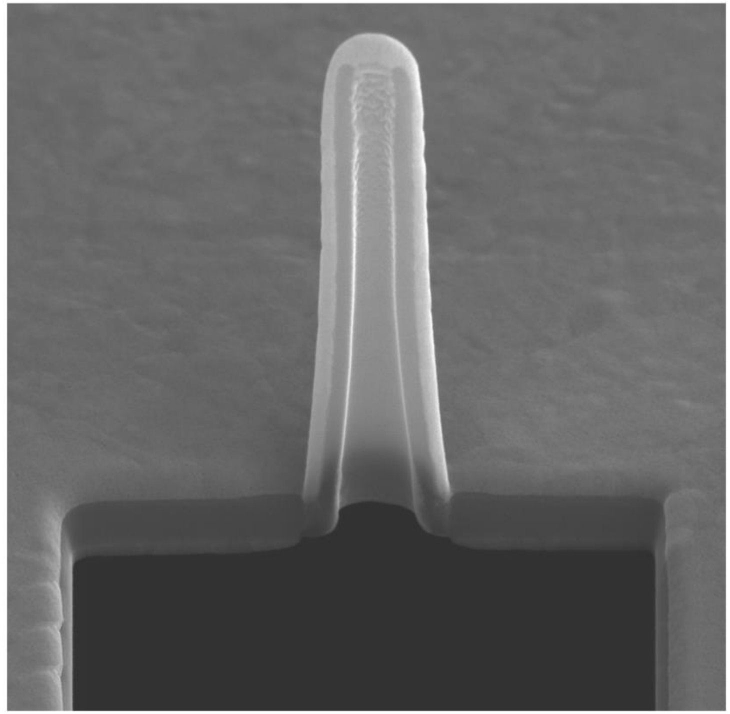
Ion-induced chemical modification

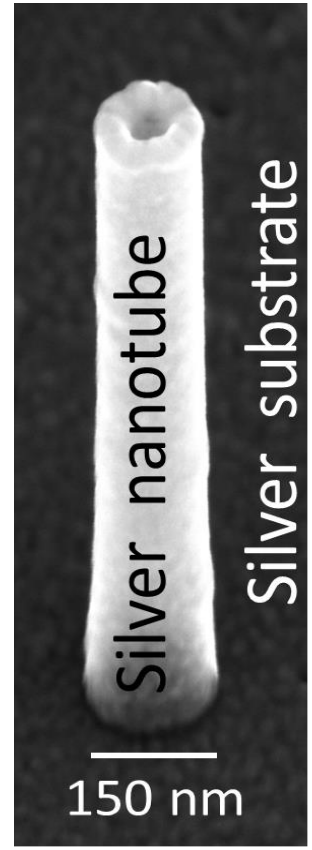


3D plasmonic hollow nanostructures for multifunctional plasmonics, F. De Angelis et al., Nano letters 13 (8), 3553-3558.

Fabrication results and capabilites



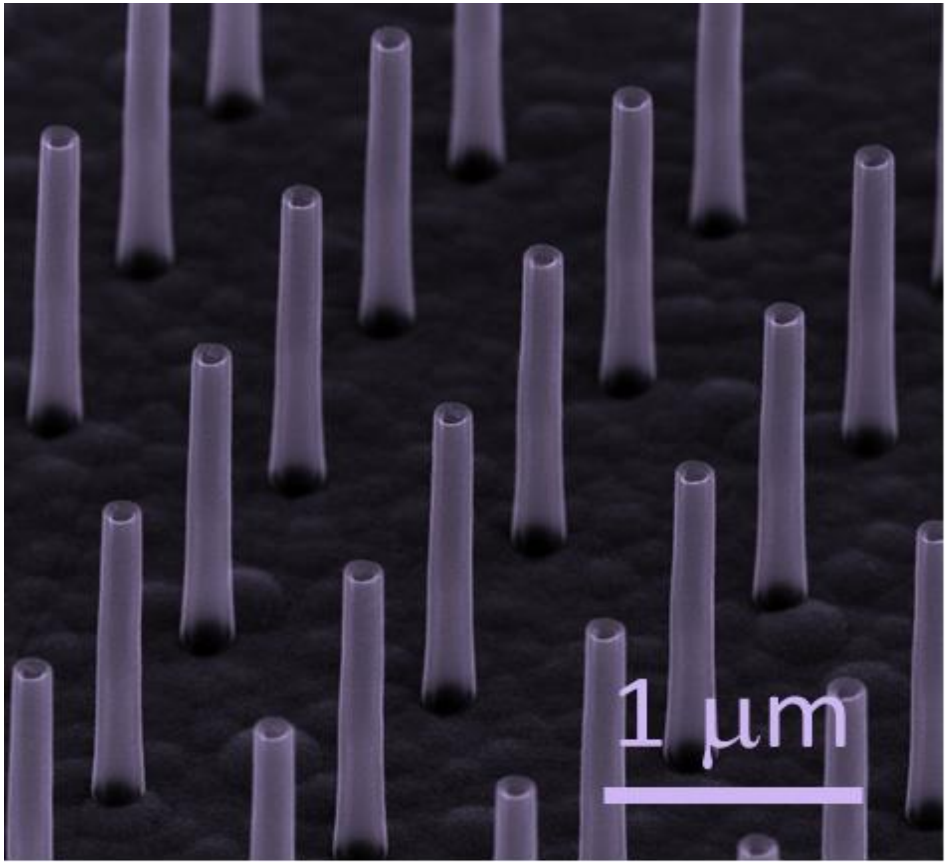


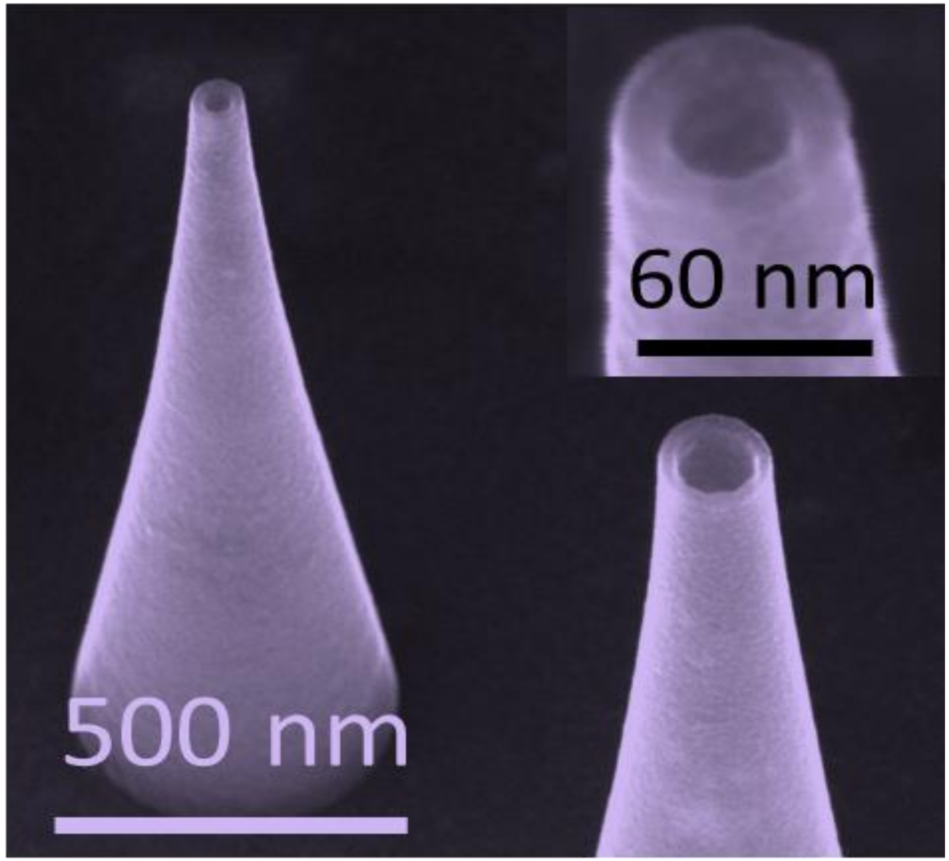


Remark 1: All devices are hollow and the channel passes through the whole structure up to the backside of the supporting membrane → Microfluidic & Optofluidic!

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

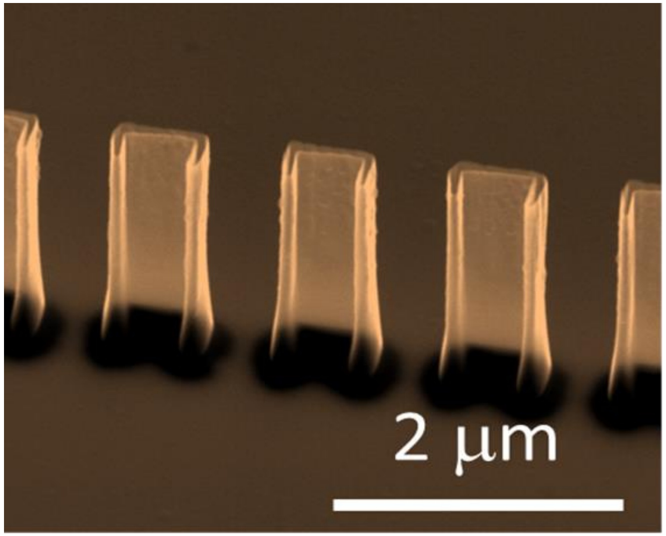
Fabrication results

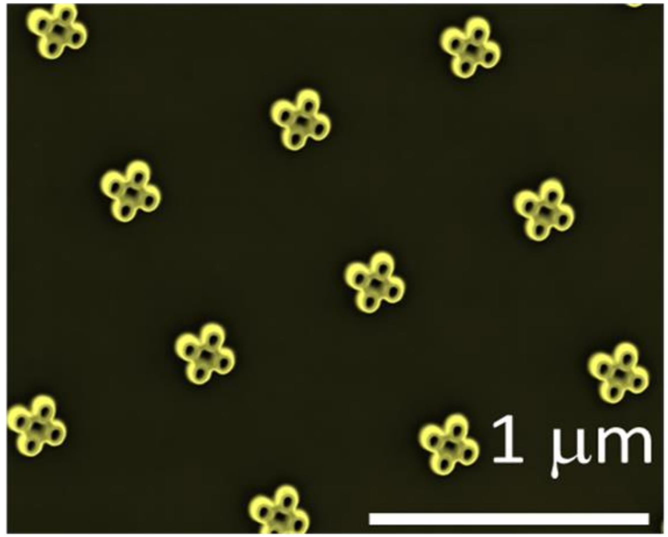


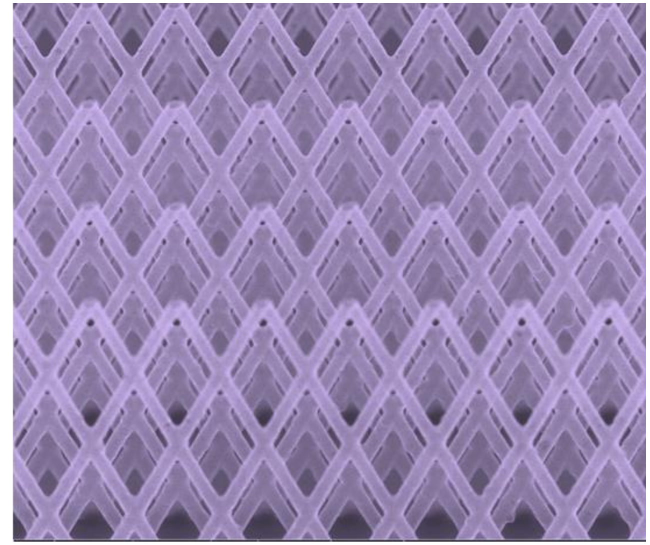


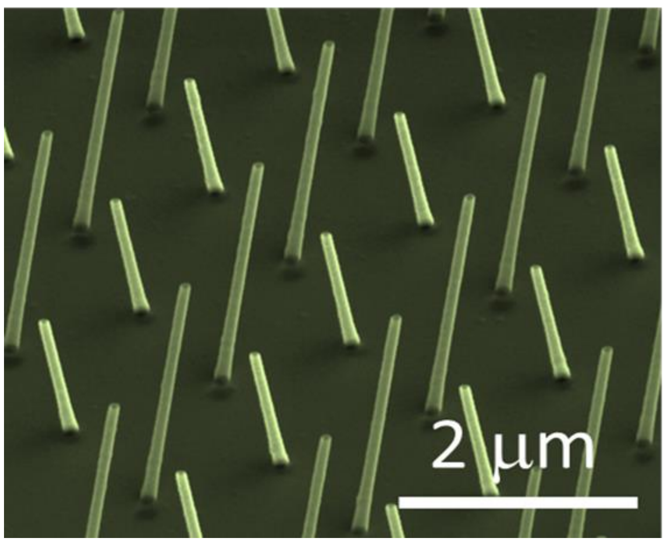
3D plasmonic hollow nanostructures for multifunctional plasmonics, F. De Angelis et al., Nano letters 13 (8), 3553-3558.

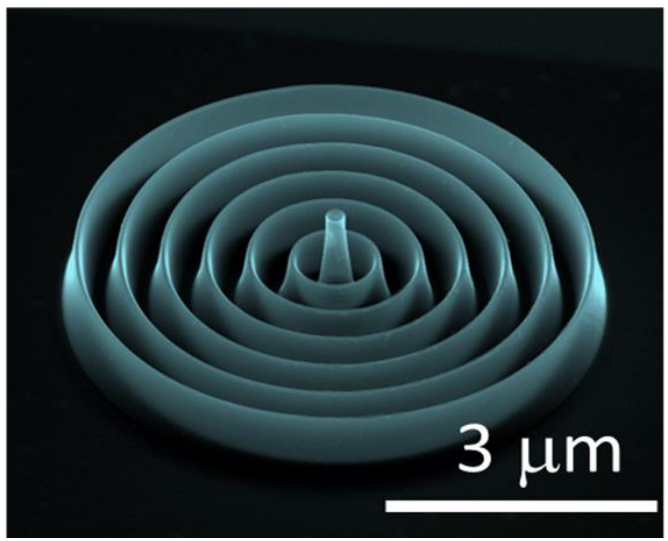
Fabrication results





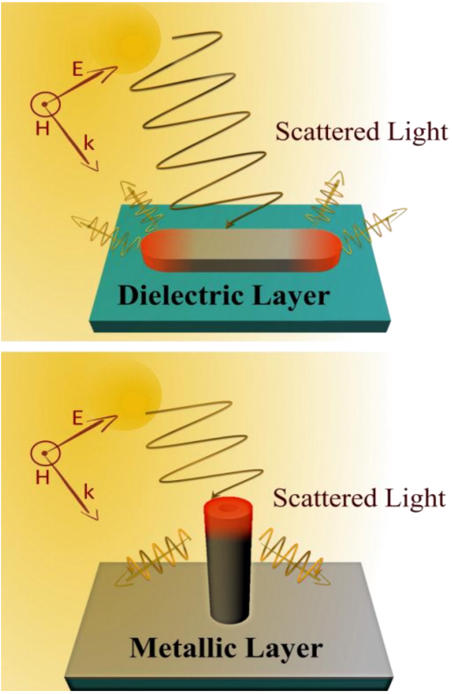


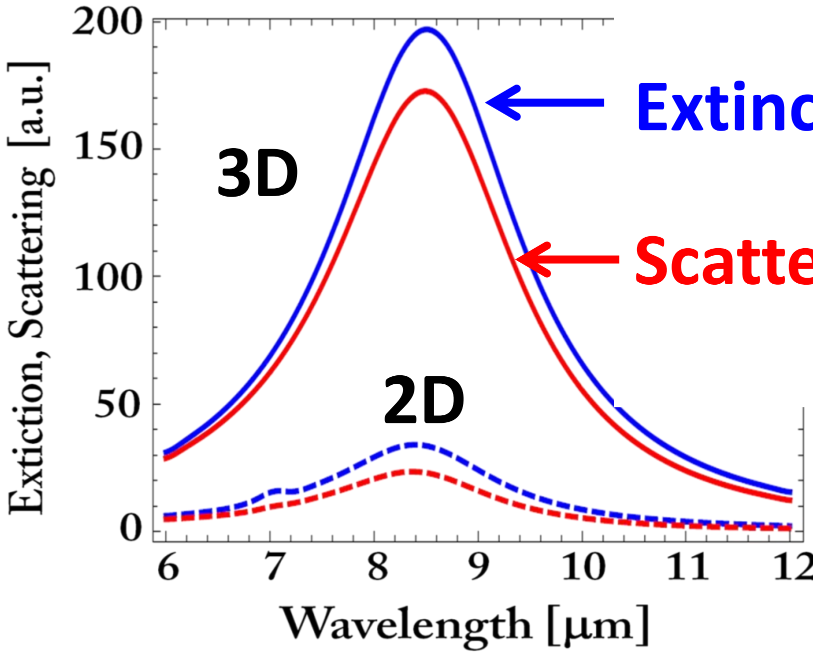




3D plasmonic hollow nanostructures for multifunctional plasmonics,
***F. De Angelis et al.,* Nano letters 13 (8), 3553-3558.**

Single antenna: planar vs out-of-plane (stronger lateral scattering)



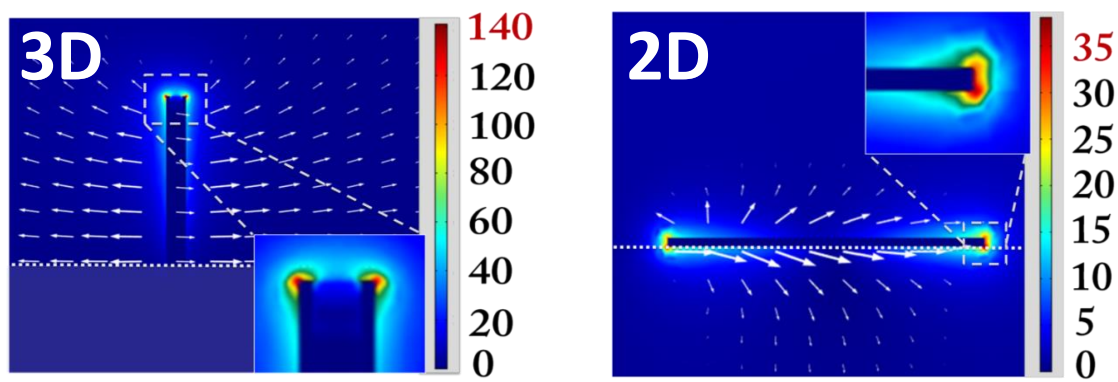


Poynting vector stream lines for light impinges at 45°

3D: parallel to the surface

2D: normal to the surface

Field enhancem. 140 vs 35

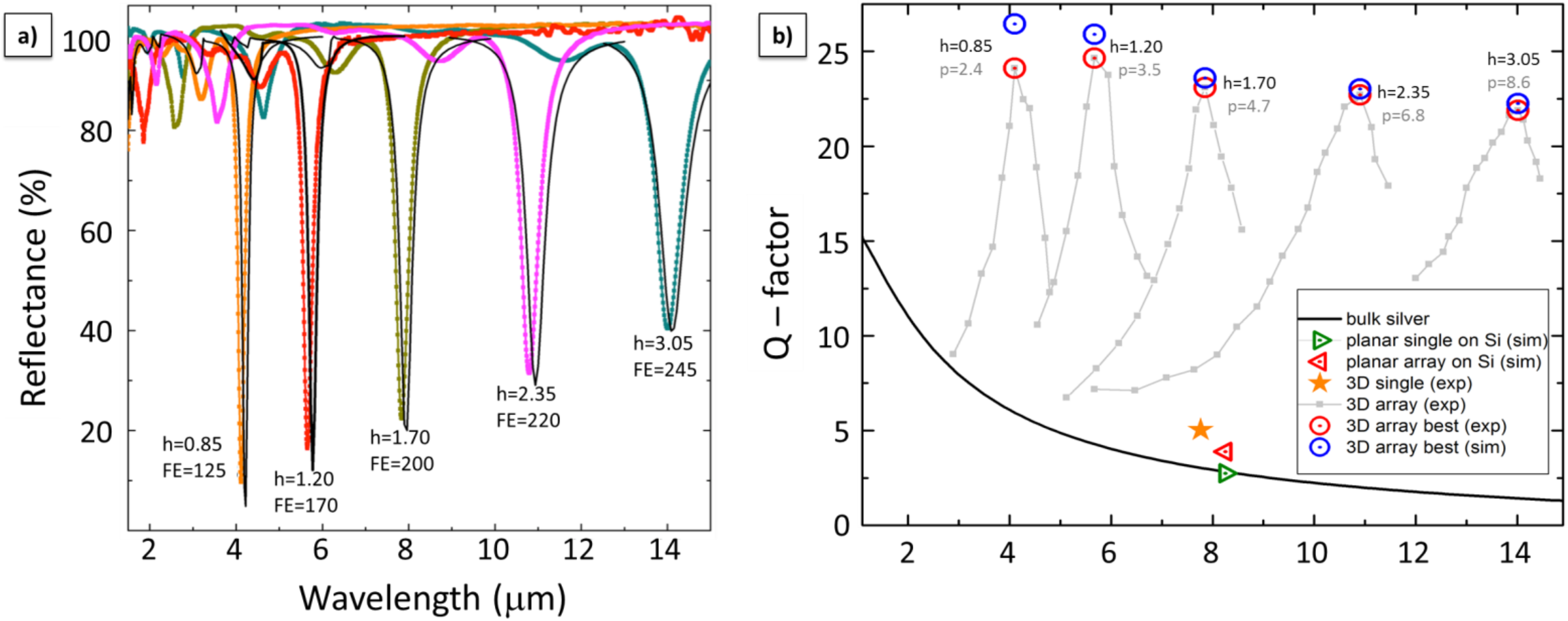


3D vertical nanostructures for enhanced infrared plasmonics.

M. Malerba et al., Scientific Report 2015

Optical properties 2: quality factors much above planar system

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



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

Mid infrared field enhancement ≈ 200 (in amplitude)

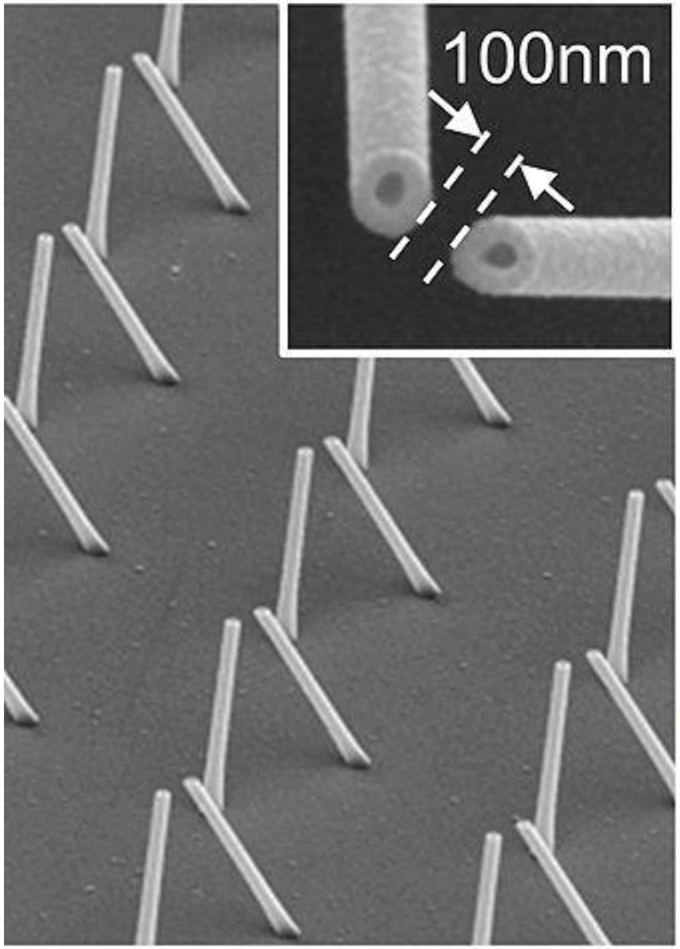
Commercial FTIR: NO collimated and polarized beam!!! **5x5 arrays!**

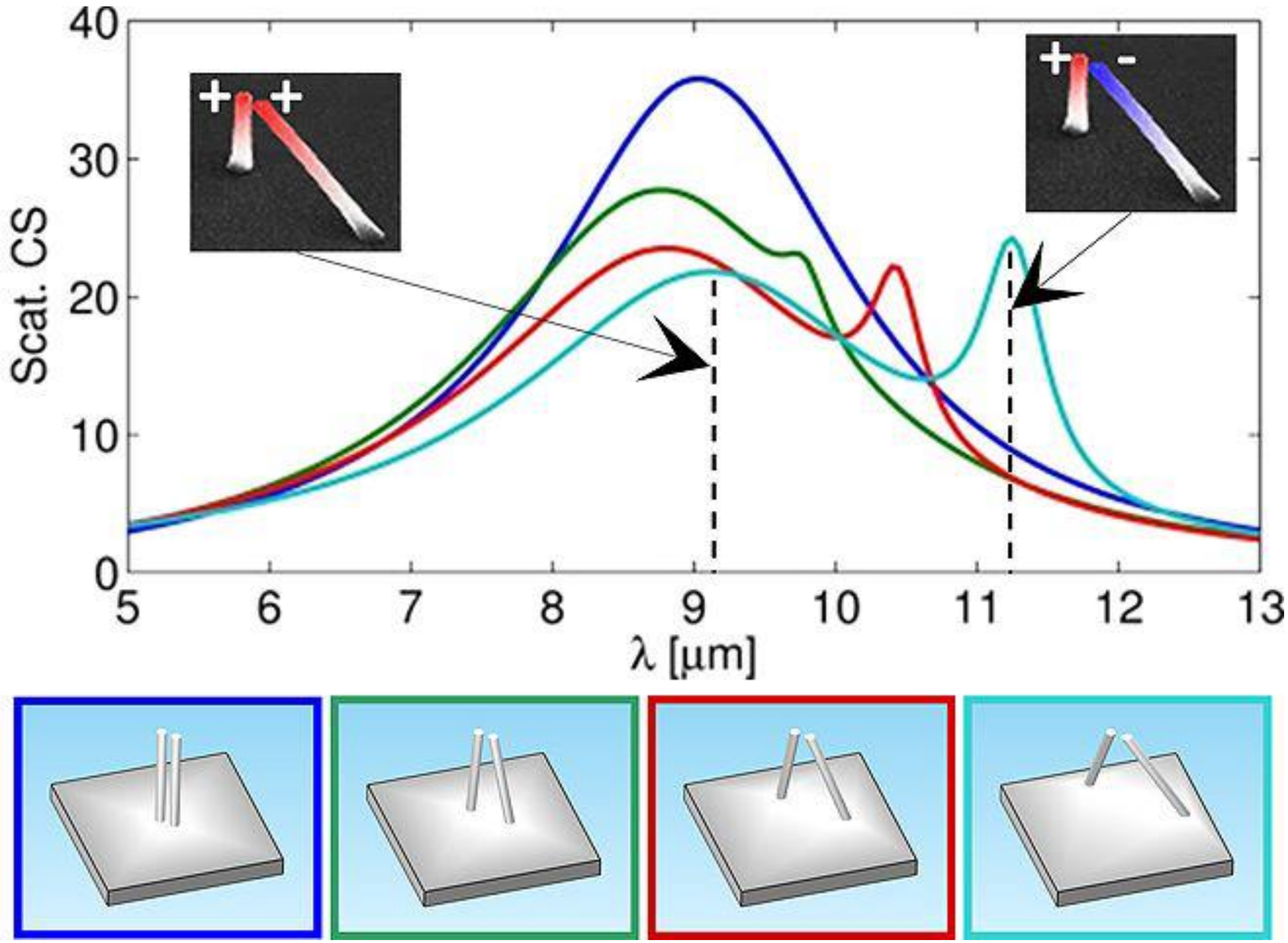
3D vertical nanostructures for enhanced infrared plasmonics.

M. Malerba et al., Scientific Report 2015

3D Plasmonic meta-molecules

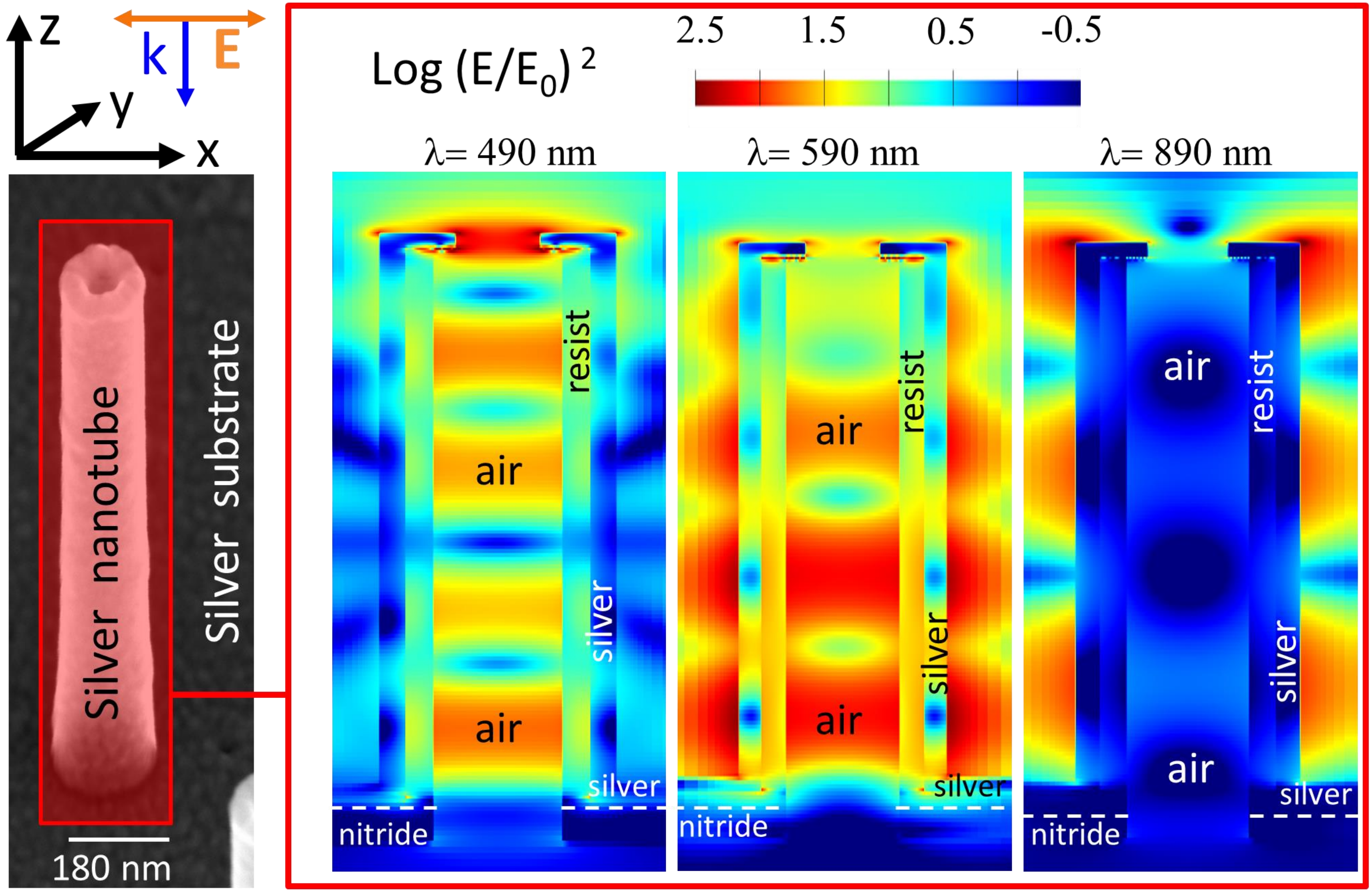
Tuning bond and antibonding modes without cross-talk (independently)





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

FDTD investigations

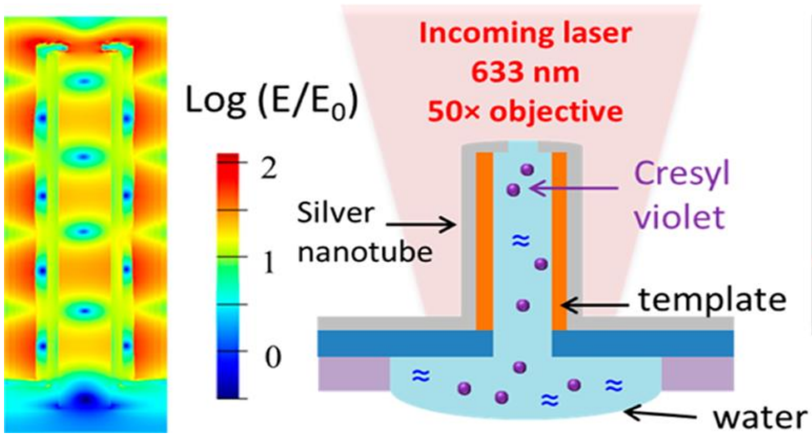


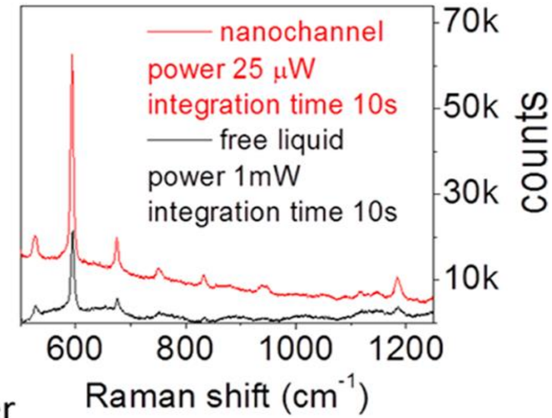
3D plasmonic hollow nanostructures for multifunctional plasmonics, F. De Angelis et al., **Nano letters 13 (8), 3553-3558.**

Raman characterization

Height =1400 nm Radius=80 nm

Optimized for high field enhancement at 630 nm.





Inside the channel

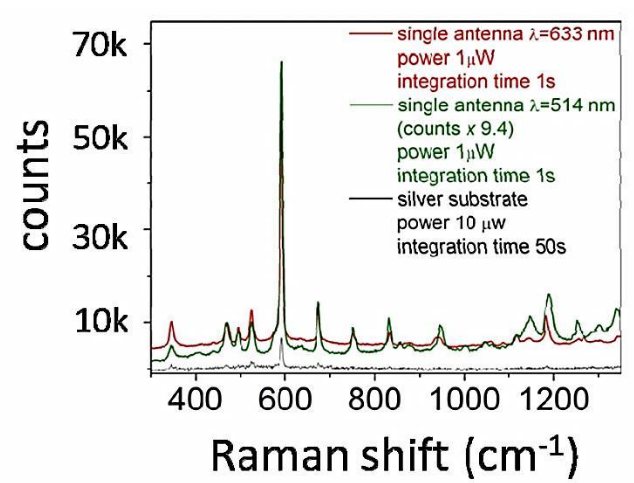
E/E_0 = 15-20

I_{raman} = 10^5

detection limit:

1–10 μM (liquid flow)

Real-label-free



outside

E/E_0 =180

I_{raman} = 10^9

Detection limit:

few molecules

(dry conditions)

Practical applications in Biology??

Can we culture cells on the top?

See also, E. Miele et al., Controlling wetting and self assembly dynamics.... ***Advanced Materials*, 2014**

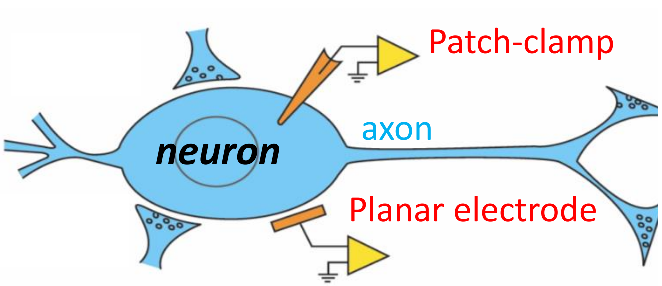
Understanding the neuronal code, i.e. the rules which govern the way neuronal circuits process, store, and exchange information, is a major scientific and technological challenge that will revolutionize our capability of managing and exploiting neuronal circuits.
Currently, progresses remain slow and face a dense multi-scale dynamics involving signaling at the molecular, cellular and large neuronal network levels.

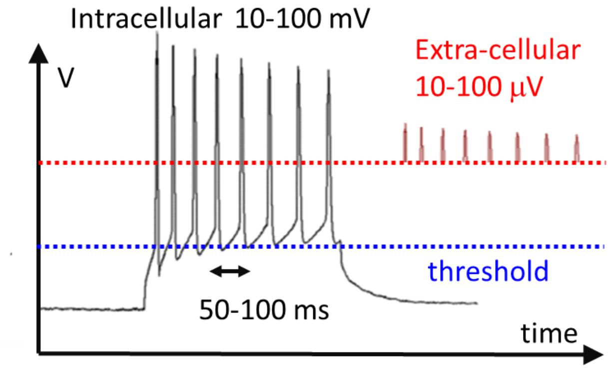
Neuro-Plasmonics

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

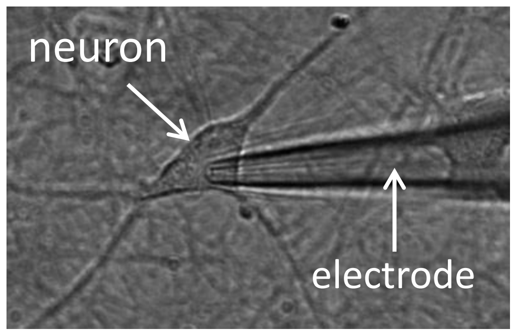
Basic principles: action potentials





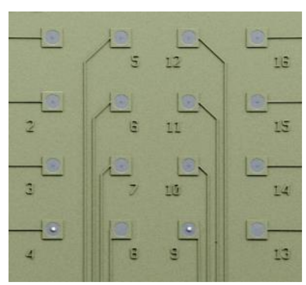
Patch Clamp (in cell).

Single sharp electrode: detailed investigation of electric action potentials but just few cells, possible cell damage, difficulties in long term observbations and automation.

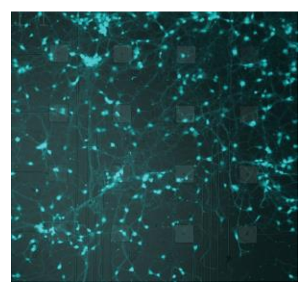


Micro-Electrode-Arrays (MEA) (extra-cellular)

Network investigation, long term, no damage, full automation, but poor eletrical sensitivity (NO subtreshold!!) due to contact resistance between membrane and electrode.



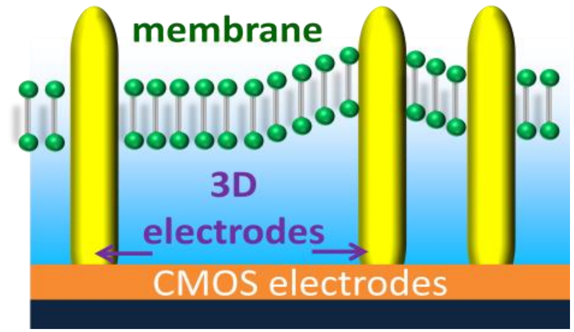
MEA



Cultured MEA

MEA + 3D micro-nano-electrodes.

Network investigation with low contact resistence!



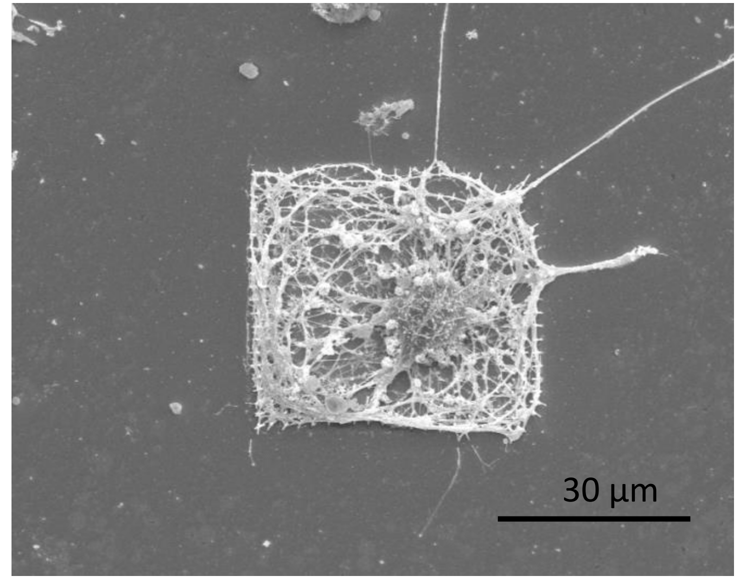
Challenges??

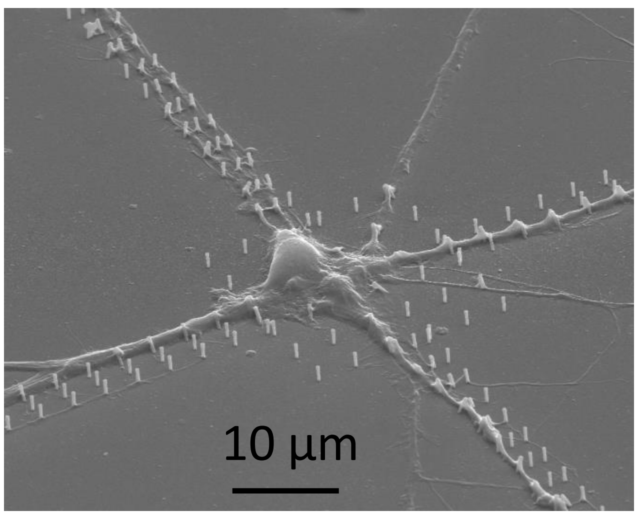
3D nanofabbrication
electrode penetration
membrane damage
membrane reforming
Chemical Signaling???
Neurotransmitters??

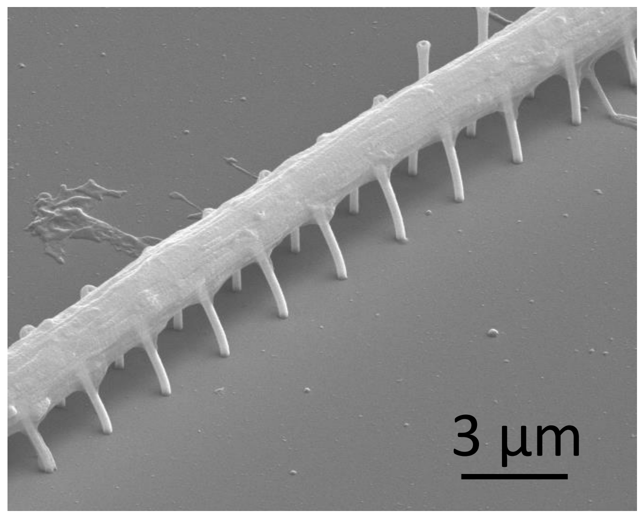
Investigations at molecular level by Plasmonics and Advanced Spectroscopies

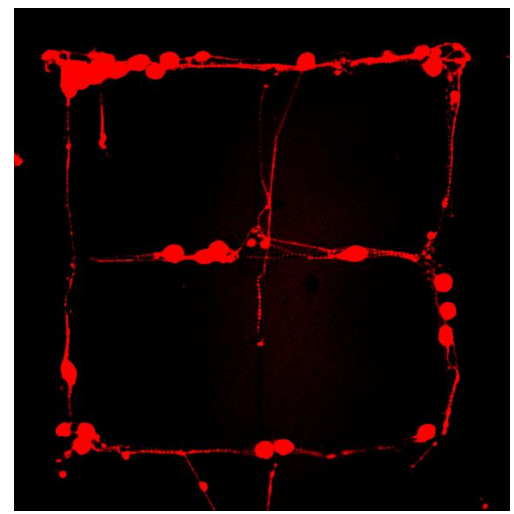
Neuronal guidance: place the cell in the right place

- 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.



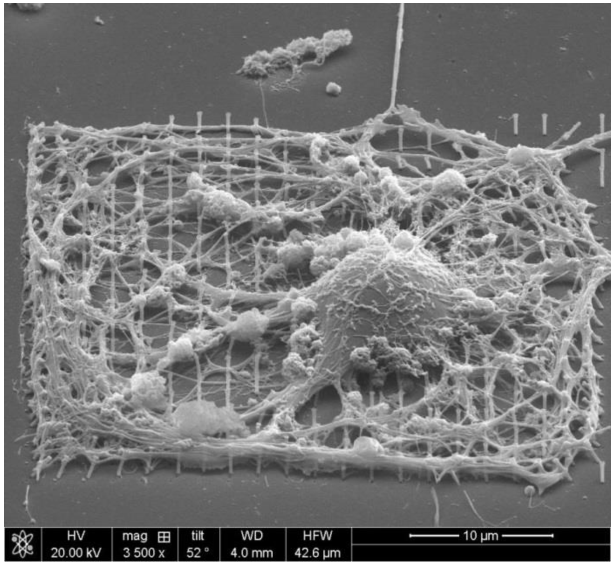


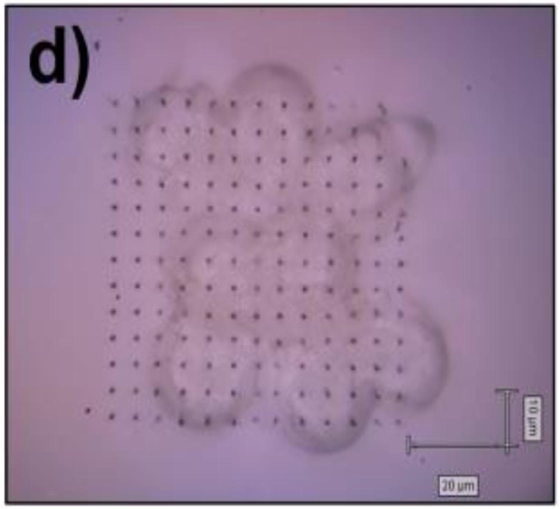




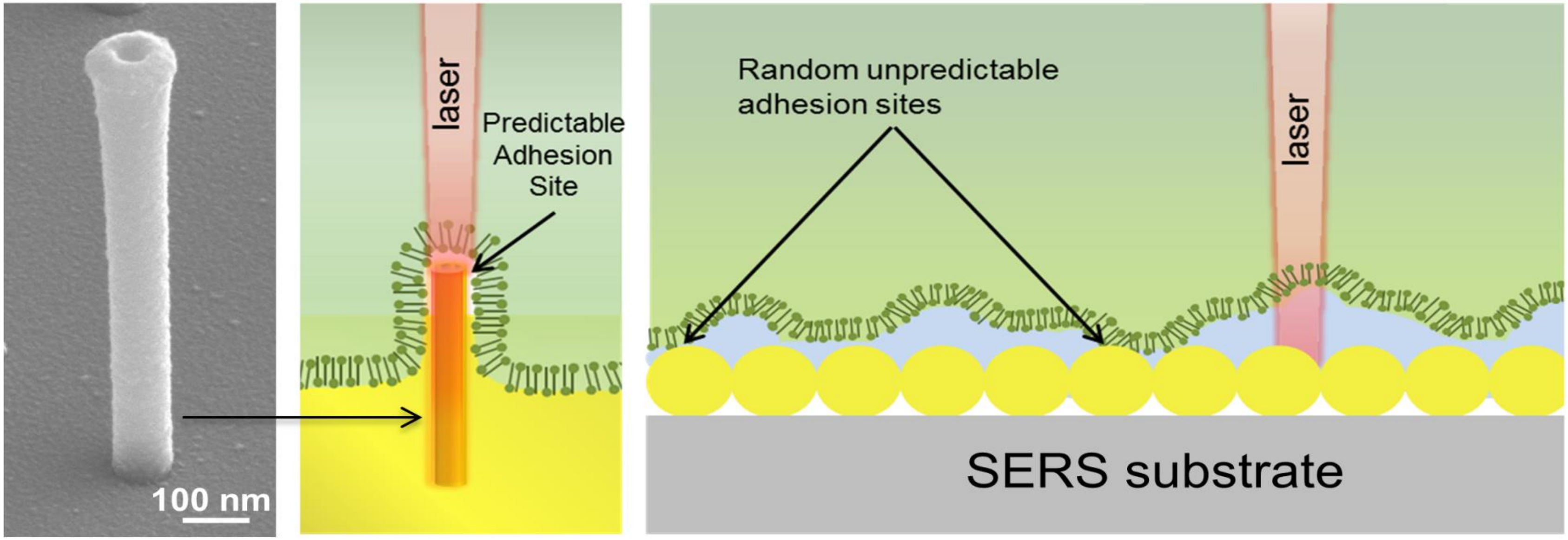
Out-of-Plane Plasmonic Antennas for Raman Analysis in Living Cells.
La Rocca et al. Small 2015

3D vs 2D cell interface



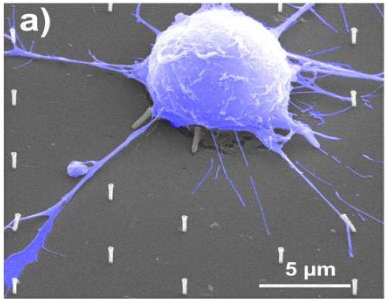


- N2A cell line
- strong spontaneous adhesion
- Predictable adhesion sites



Out-of-Plane Plasmonic Antennas for Raman Analysis in Living Cells.
La Rocca et al. Small 2015

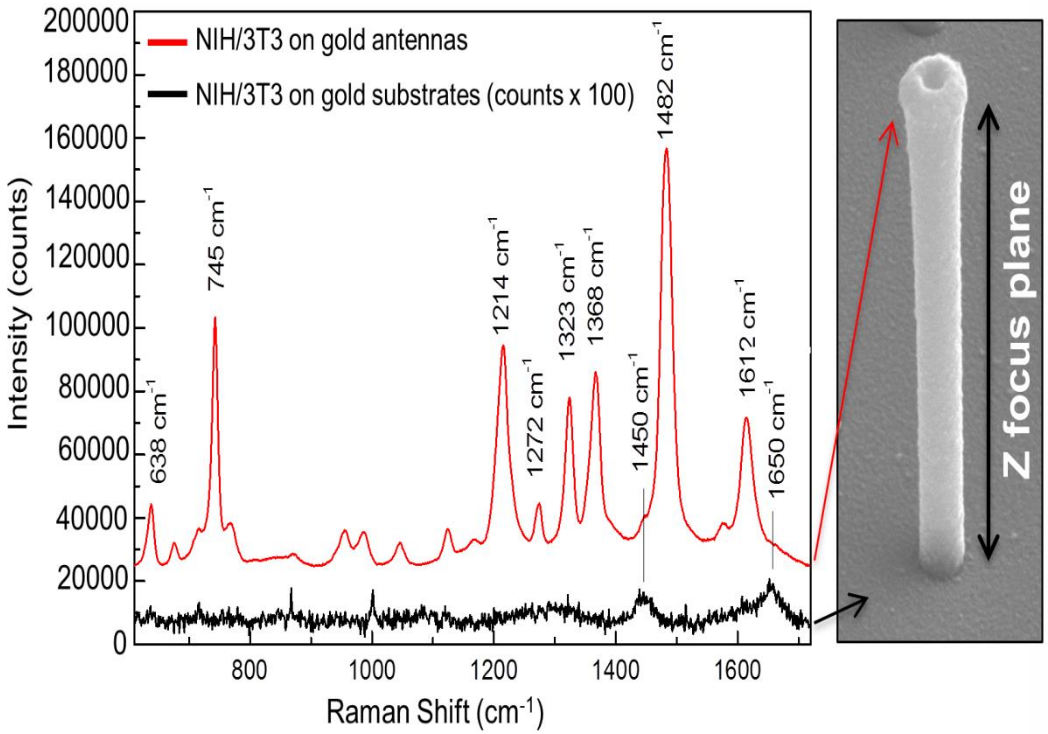
Raman characterization



In-vitro Raman characterization of cell membrane

λ=785 nm, acquisition time 10 seconds

Future perspective: membrane receptor investigations



Peak position (cm^{-1})	Possible attribution
638	C-C twist Phenylalanine, Tyrosine
677	
726	C-S protein, twist CH_2, rocking A (Adenine)
745	Ring Tryptophan
768	Ring Tryptophan
871	C-C-N sym. stretching of lipids, C-O-C carbohydrates
954	Hydroxyapatite, carotenoid, cholesterol
988	C-C BK stretching
1044	Phenylalanine
1123	O-P-O DNA backbone
1154	Tyrosine
1214	$C-C_6H_5$, Tyrosine, Tryptophan, Phenylalanine
1272	Saccharides, proteins
1323	Amide III alpha helix
1368	CH_3 symmetric stretching of lipids
1450	CH_2, CH_3 deformation, phospholipids
1482	Amide II
1572	G, A (Guanine, Adenine)
1612	C=C Tyrosine, Tryptophan
1650	Amide I

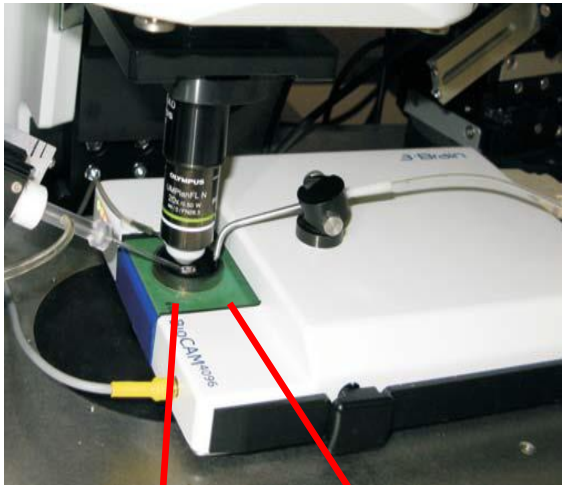
How we can integrate them with electrical sensors??

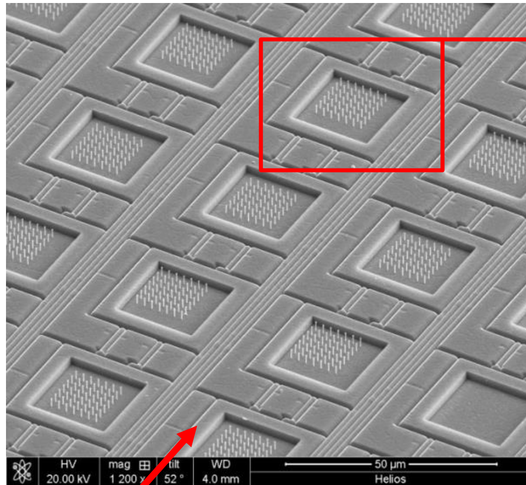
Out-of-Plane Plasmonic Antennas for Raman Analysis in Living Cells.
R. La Rocca et al. Small 2015

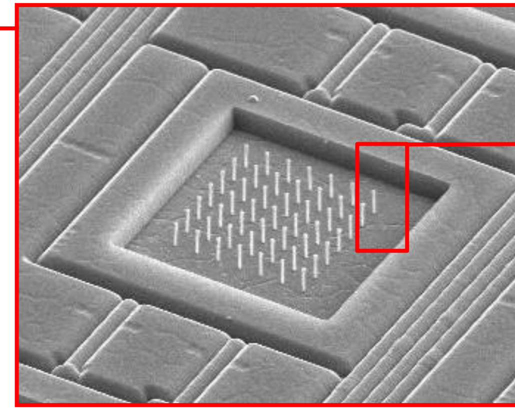
Methodology: Integration with commercial hardware

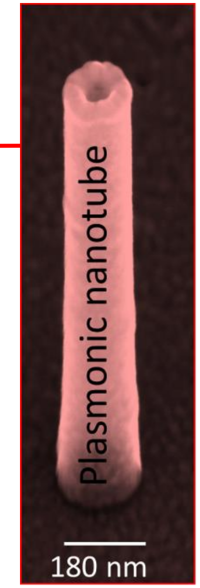
European Research Council
Established by the European Commission
Supporting top researchers from anywhere in the world

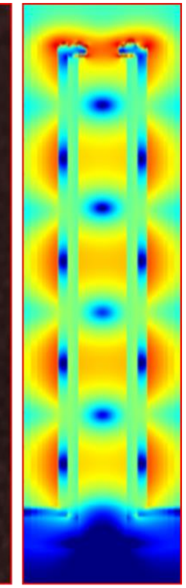
3Brain commercial chipset with 4096 recording electrodes (www.3brain.com)

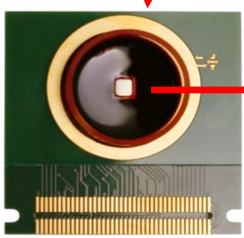




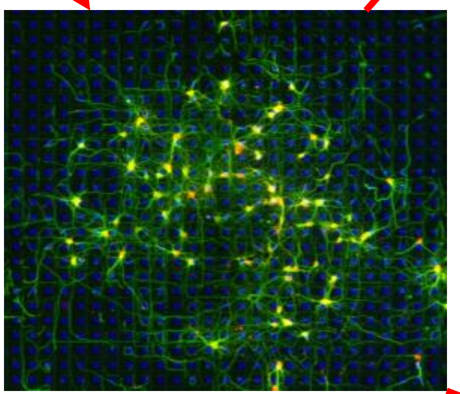








Plug&Play Commercial chip



Rat-hippocampal or
Human IPS cell culture
(induced pluripotent stem)

Our 3D plasmonic nanoeletrodes

- 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)

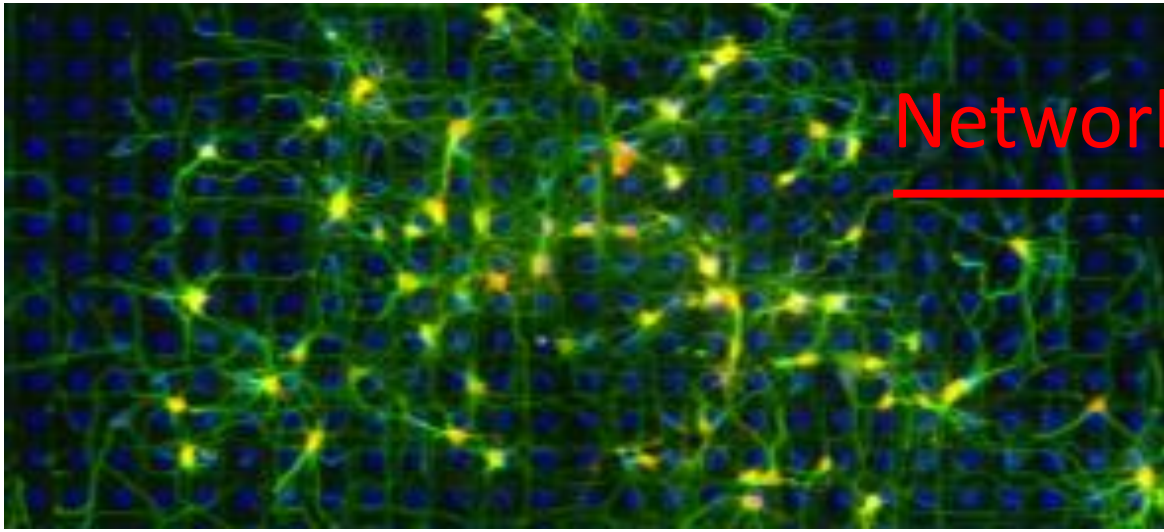
3D plasmonic nanoantennas integrated with MEA biosensors. **M. Dipalo et al. Nanoscale 2015**

Neuro-Plasmonics

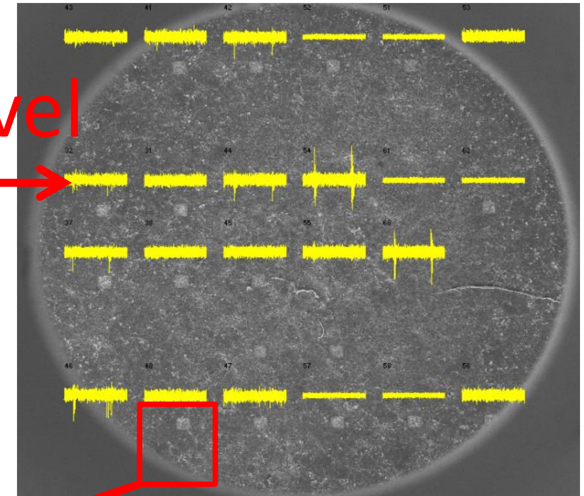
Cultured neurons

European Research Council
Established by the European Commission
Supporting top researchers from anywhere in the world

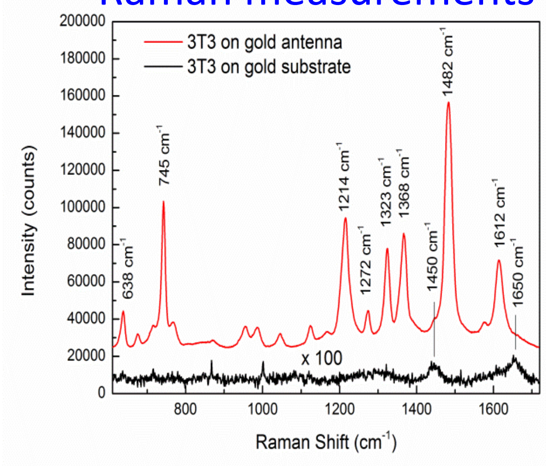
- hippocampal neurons from rat, 10 days in vitro

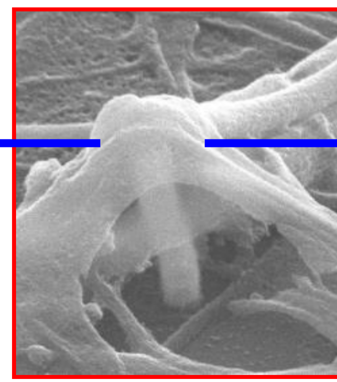


Network level

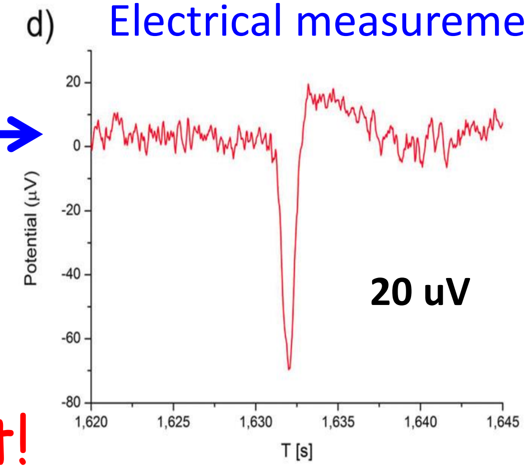


Raman measurements





Electrical measurements

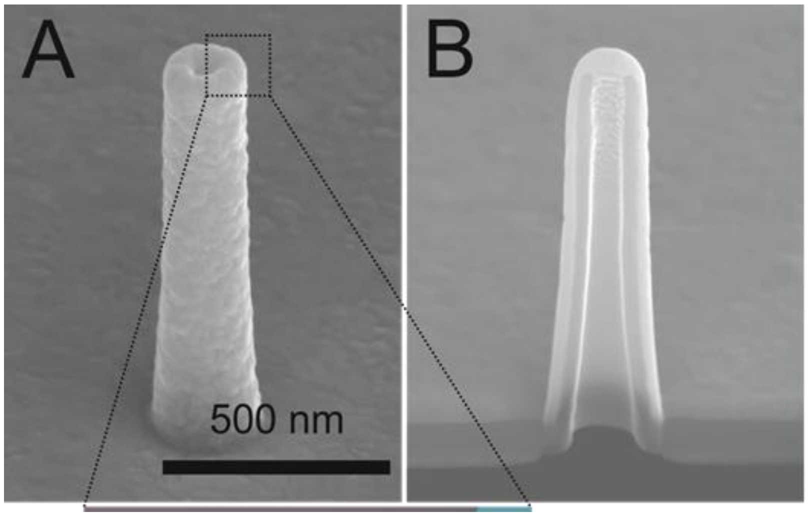


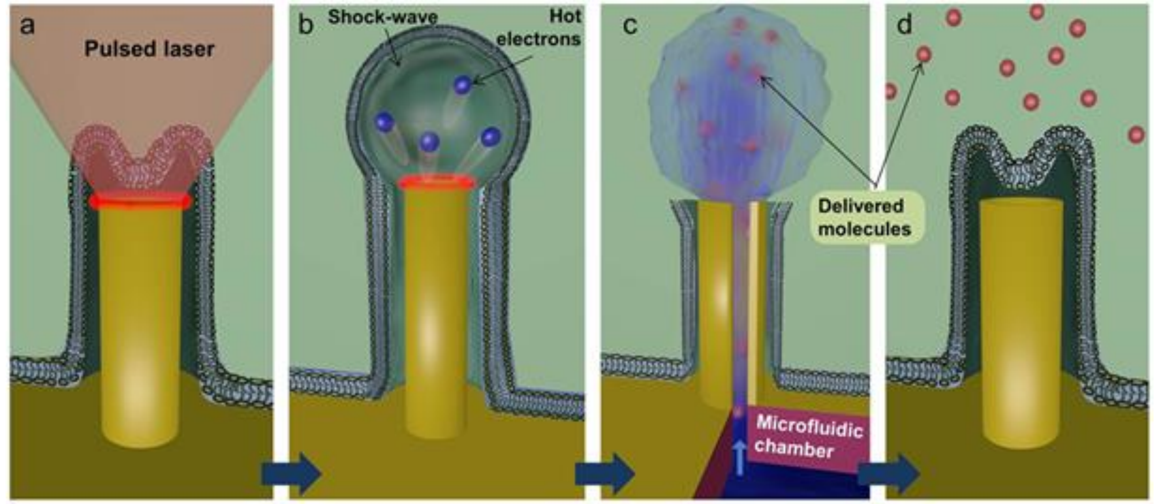
extracellular contact!

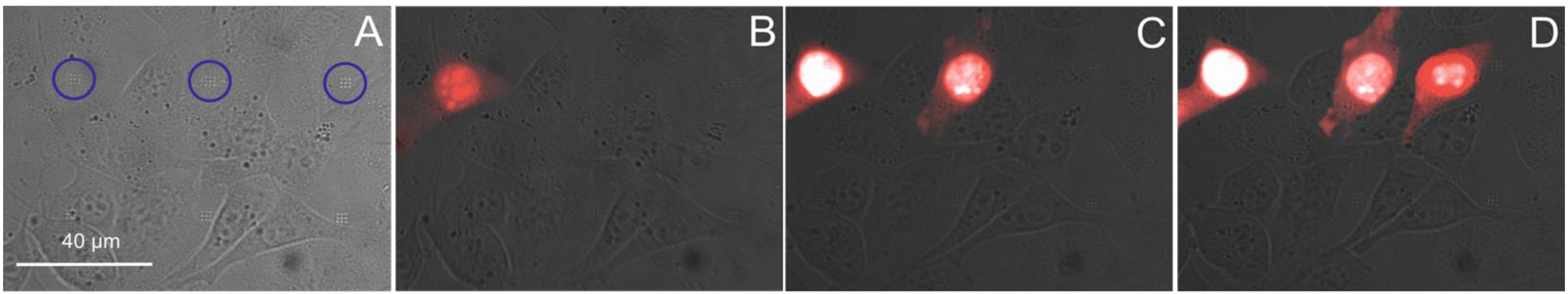
3D plasmonic nanoantennas integrated with MEA Biosensors. M. Dipalo et al. Nanoscale 2015

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

Intracellular delivery by plasmonic nanotubes

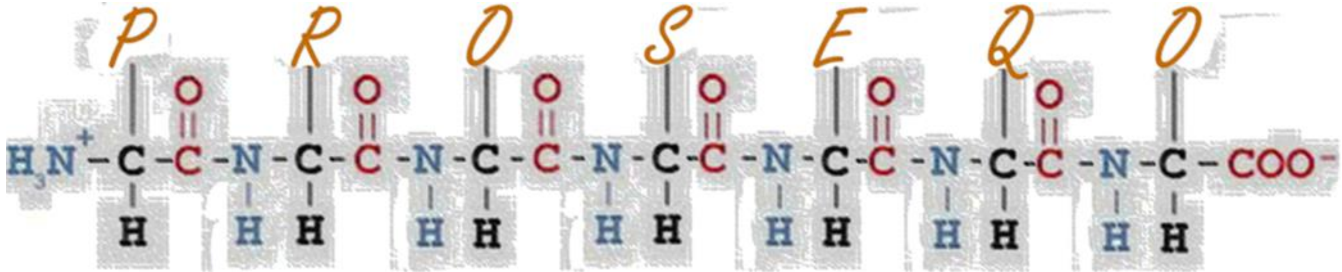






Real time, quantitative, broad range of molecules delivered, cell selective....
Fast (up to $10^{4\text{-}5}$ cell/s), compatible with low voltage electroporation.

Intracellular delivery with plasmonic nanotubes.
Messina et al, ***Advanced Materials 2015.***

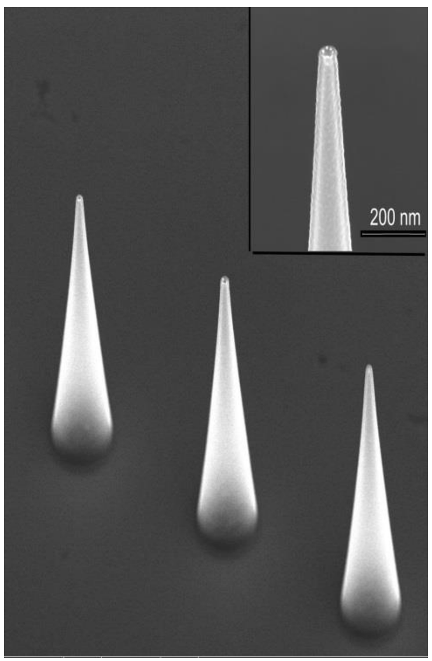


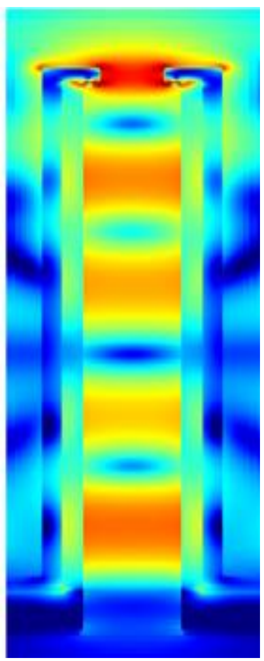
H2020 – FET OPEN

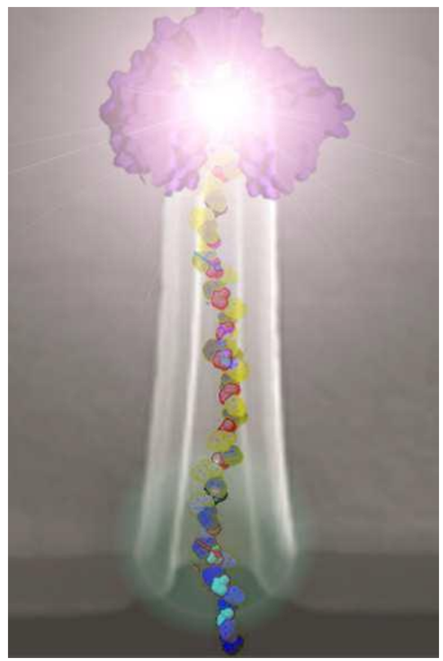
2016-2019

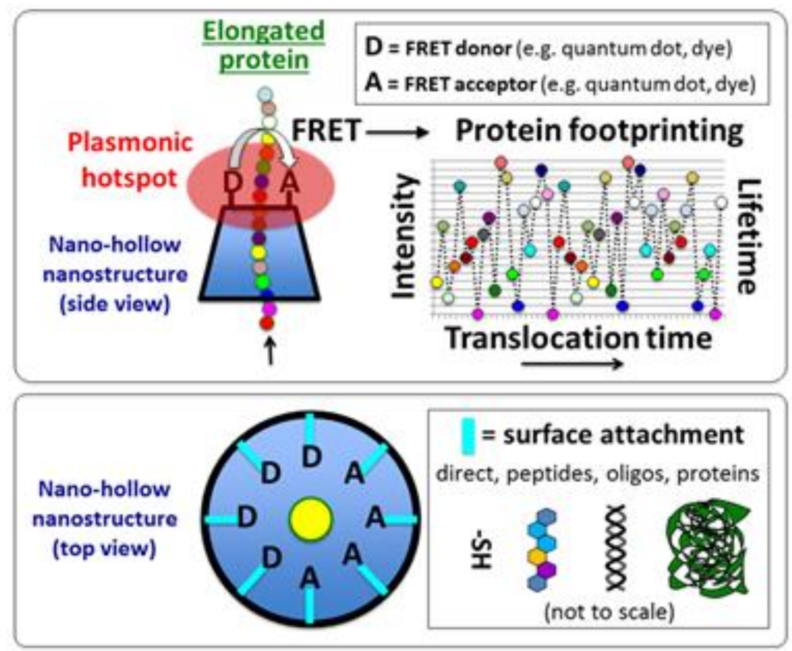
Single Protein Sequencing

Combination of advanced 3D fabrication, plasmonics, and FRET

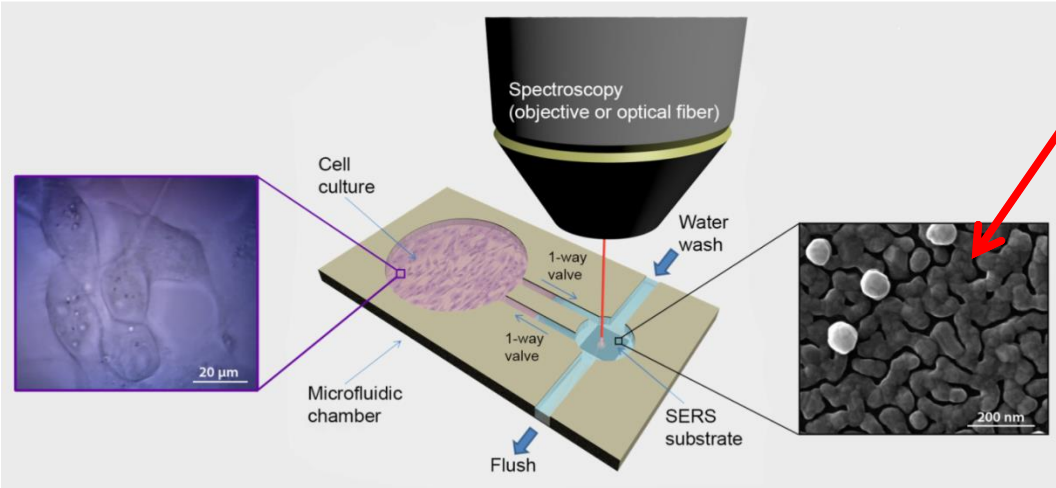






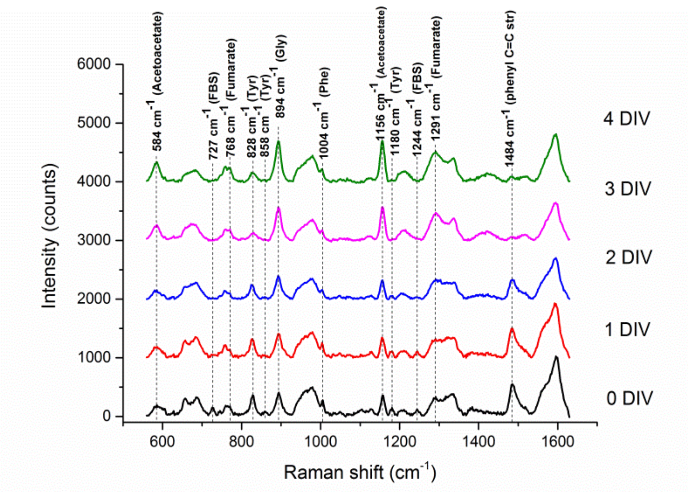


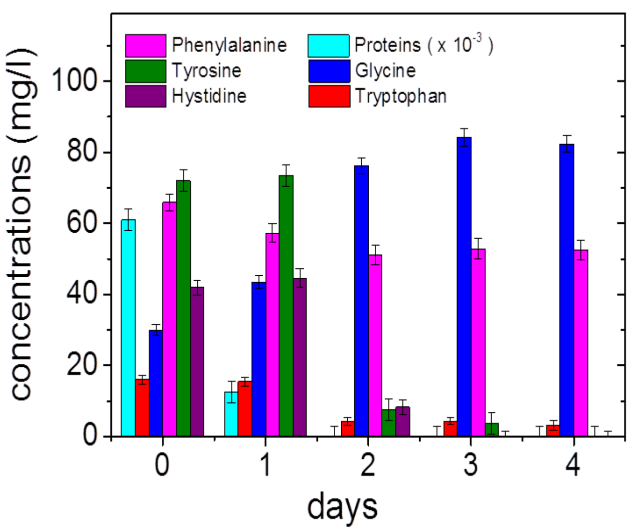
Monitoring extracellular metabolites by Raman spectroscopy



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

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



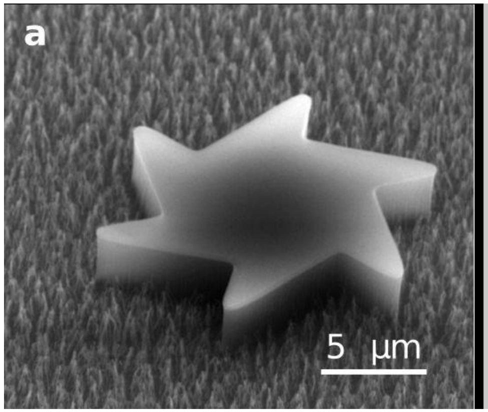


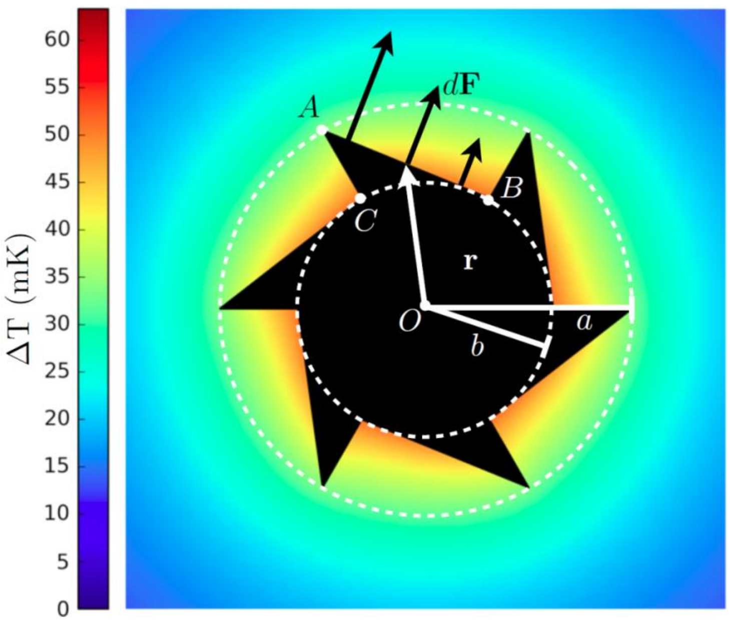
Peak (cm⁻¹)	Possible attribution	Refs.
584	Acetoacetate	36, 39
658	Histidine	32
685	Proteins	41
727	Methionine	32, 33
757	Cytocrome , Ring breath Tryptophan (Trp)	42,43
768	Fumarate	36, 40
828	Tyrosine	32-36
894	Glycine	32,33,36
1004	Phenylalanine (Phe)	32,-36
1121	Proteins: stretching CN	46, 44
1128	Proteins: stretching CN; Carboydrates: stretching C-O	43
1158	Acetoacetate	36, 39
1180	Tyrosine	32-36
1203	Phe	44
1210	Nucleic Acids: Thymine (T)	44
1244	Ammide III (β-Sheet)	34
1291	Fumarate	36, 40
1316	Nucleic Acids: Guanine (G); Proteins: C-H; Lipids	43, 45
1336	Proteins: twisting (CH2, CH3)	44
1402	Deformation CH3 Asym; Stretching COO-	44
1422	Nucleic Acids: Adenine (A), Guanine (G)	46, 47
1488	L-Histidine	34
1521	Nucleic Acids: Citosine (C)	46
1556	Tryptophan	34
1569	Proteins: Ammide II	46
1596	Phe	46
1620	Ammide I; C=C Tyr, Trp, Lipids, stretching (C=C) olefinic	43, 46, 48

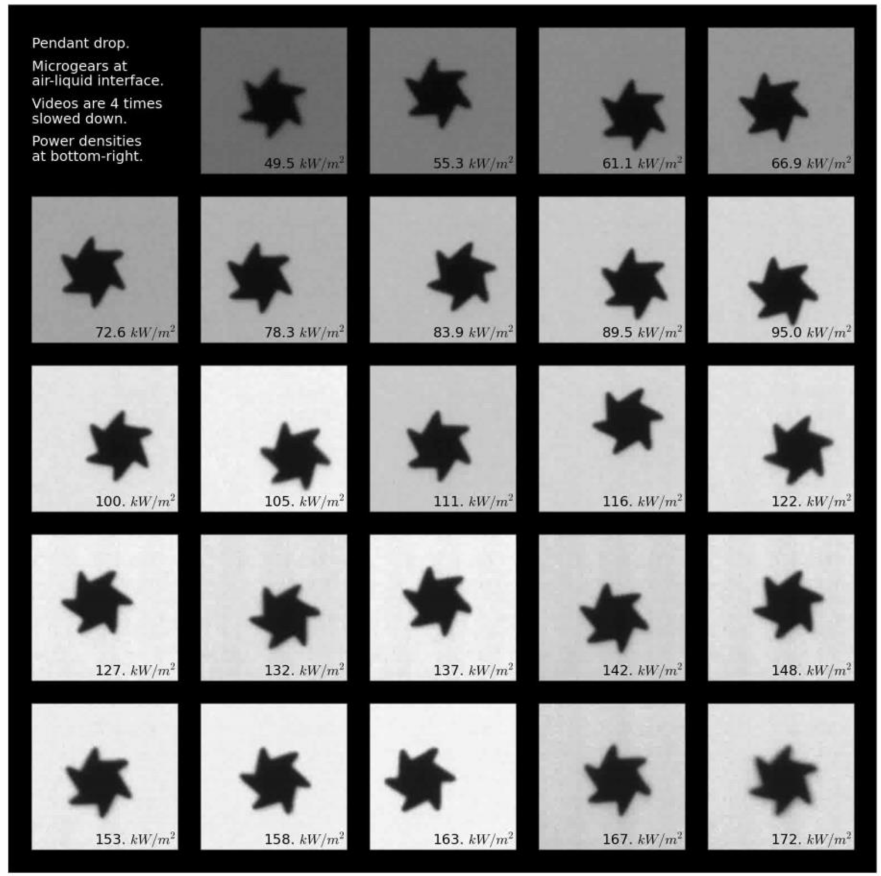
Shalabaeva et al., Nanoscale, under review.

Micro-motors with asymmetric shape efficiently convert light into work by thermo-capillary effects.

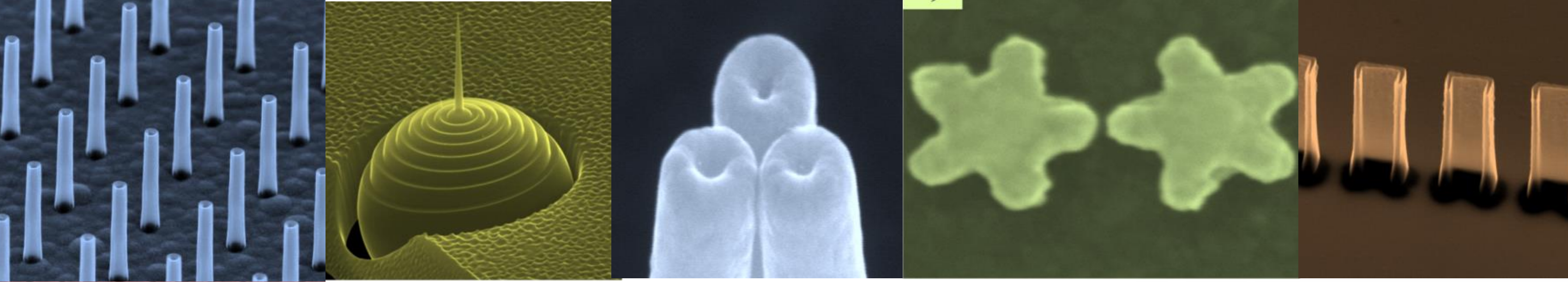
C. Maggi et al., Nature Communication (2015)

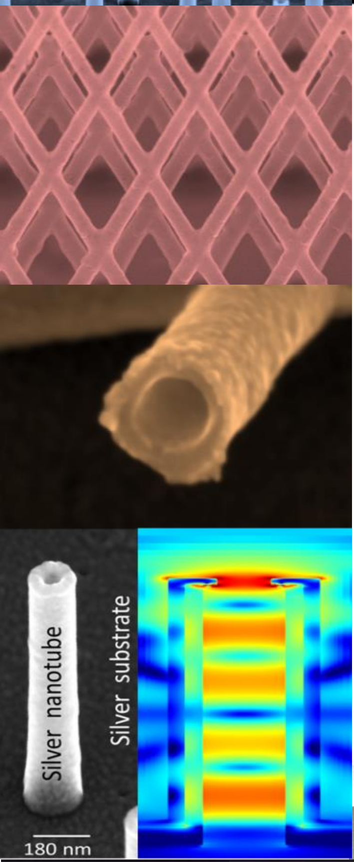






(in collaboration with R. DI Leonardo, Rome University)





Neuro-Plasmonics

European Research Council
Established by the European Commission
Supporting top researchers from anywhere in the world

Post-Doc positions
Available from march 2016

Thanks for your attention!!

ISTITUTO ITALIANO
DI TECNOLOGIA

francesco.deangelis@iit.it