PhD Maria Alessandra Cutolo

Engineering SERS-active substrates: design and characterization of advanced structures and innovative materials

Tutor: G. Breglio

co-Tutor: A. Cusano, M. Pisco

Cycle: XXXVI

Year: 2020-2023

Background information

- **M.Sc. in Electronic Engineering – 12th July 2019**
- *Thesis title:* "Lab-on-Fiber" thermo-plasmonic platforms for the localized release of drugs.
- *Tutors:* Prof. Giovanni Breglio, Prof. Andrea Cusano (University of Sannio, BN).
- **Research contract by Cerict (BN) – July 2019 to July 2021:**
  - Development of thermo-plasmonic platforms with a view to the localized release of
  - drugs through microgel.
  - Design of a needle as an ultrasound probe for selective and localized destruction of cancer cells.
  - Design and development of a barcode and QRcodes for a low-cost wireless sensor for
  - structural, medical and environmental monitoring.
  - Design of an innovative in-line control system for soft tissue and bone drilling.
- **Ph.D. started in Nov 2020 (XXXVI cycle):**
- *Title:* Engineering SERS-active substrates: design and characterization of advanced structures and innovative materials
- *Tutors:* Prof. Giovanni Breglio
- *Co-Tutor:* Prof. A. Cusano, Prof. M. Pisco

Summary of study activities

	Courses	Seminars	Research	Tutorship	Total
1st	23	5	35	0	63
2nd	18	3.8	45	0	66.8
3rd	0	0.4	60	0.6	61
Total	41	9.2	140	0.6	190.8
Expected	30 - 70	10 - 30	80 - 140	0 – 4.8	

Courses

- Imprenditorialità accademica
- Matrix Analysis for signal processing with matlab examples
- How to Boost your PhD
- Nanotechnologies for electrical Engineering
- Circuiti e sistemi elettronici per applicazioni biomedicali
- **CI-LAM Summer School III 2021**
- **CI-LAM Summer School III 2022**
- **IV Scuola Nazionale Biosensori Ottici e Biofotonica 2022**
- **SIE phd school 2022**

Conferences

1. **AIV XXV** *[**Oral** Presentation] 2022*
2. **ICOP** *[Poster Presentation] 2022*
3. **SIE** *[Poster Presentation] 2022 and 2023*

Research products

Journal contributions

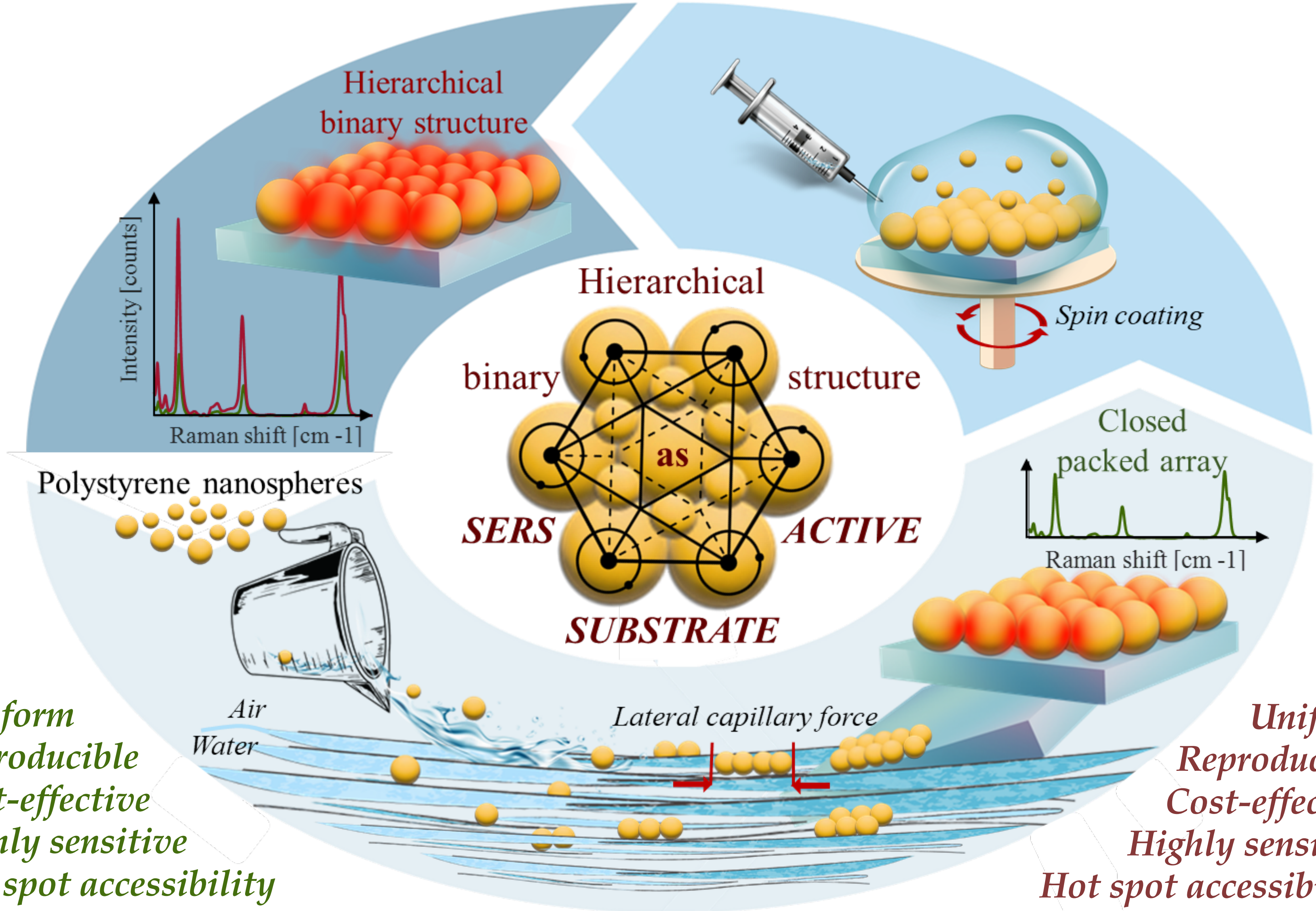
[P1]	M. A. Cutolo , F. Galeotti, S. Spaziani et al. **Self Assembled Hierarchical nanostructures: Towards Engineered SERS active platform.** ***Under submission.***
[P2]	B. Rossi, M. A. Cutolo, M. Giaquinto, Advanced Lab-on-Tip ultrasound detectors: A numerical analysis, Results in Optics, Volume 9, 2022,100312,ISSN 2666-9501, https://doi.org/10.1016/j.rio.2022.1003128.
[P3]	M.A. Cutolo, G. Breglio, Interferometric Fabry-Perot sensors for ultrasound detection on the tip of an optical fiber, Results in Optics, ISSN 2666-9501, https://doi.org/10.1016/j.rio.2021.100209.
[P4]	M. A. Cutolo, C. Cafiero, L. Califano et al., Feasibility analysis of an ultrasound on line diagnostic approach for oral and bone surgery. Sci Rep 12, 905 (2022). https://doi.org/10.1038/s41598-022-04857-0 .
[P5]	A. Cutolo, A. R. Carotenuto, M. A. Cutolo et al., Ultrasound waves in tumors via needle irradiation for precise medicine. Sci Rep 12, 6513 (2022). https://doi.org/10.1038/s41598-022-10407-5
[P6]	A. Minardo, R. Bernini, G. M. Berruti et al., Innovative photonic sensors for safety and security, Part I: Fundamentals, Infrastructural and ground transportations, Sensors. 2023; 23(5):2558. https://doi.org/10.3390/s23052558
[P7]	A. Cutolo, R. Bernini, G.M. Berruti et al., Innovative Photonic Sensors for Safety and Security, Part II: Aerospace and Submarine Applications, Sensors. 2023; 23(5):2417. https://doi.org/10.3390/s23052417 .
[P8]	G. Breglio, R. Bernini, G.M. Berruti et al., Innovative Photonic Sensors for Safety and Security, Part III: Environment, Agriculture and Soil Monitoring, Sensors. 2023; https://doi.org/10.3390/s23063187 .

Research products

Conference contributions

[C1]	**AIV XXV** Conference Materials, Interfaces, Processes in Industrial and Basic Research Applications 2022. Hierarchical binary structures as SERS-active substrates. Authors: M. A. Cutolo, G. Quero, V. Calcagno, S. Spaziani, F. Galeotti, M. Pisco, A. Irace, G. Breglio, A. Cusano. **[Oral Presentation]**
[C2]	M. A. Cutolo, G. Quero, V. Calcagno, S. Spaziani, F. Galeotti, M. Pisco, A. Irace, G. Breglio, A. Cusano. Hierarchical binary structures as SERS-active substrates. **ICOP** Italian Conference on Optics and Photonics 2022. **[Poster Presentation]**
[C3]	M. A. Cutolo, G. Quero, V. Calcagno, S. Spaziani, F. Galeotti, M. Pisco, A. Irace, G. Breglio, A. Cusano. Hierarchical binary structures as SERS-active substrates. **SIE** Riunione Annuale dell'Associazione Società Italiana di Elettronica SIE 2022 **[Poster Presentation]**
[C4]	M. A. Cutolo, G. Quero, V. Calcagno, S. Spaziani, F. Galeotti, M. Pisco, A. Irace, G. Breglio, A. Cusano. Advanced self-assembled SERS-active substrates based on nanosphere lithography. **SIE** Riunione Annuale dell'Associazione Società Italiana di Elettronica SIE 2023 . **[Poster Presentation]**

PhD thesis overview



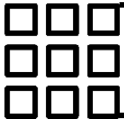
- ✓ *Uniform*
- ✓ *Reproducible*
- ✓ *Cost-effective*
- ○ *Highly sensitive*
- ○ *Hot spot accessibility*

Uniform ✓
Reproducible ✓
Cost-effective ✓
Highly sensitive ✓
Hot spot accessibility ✓

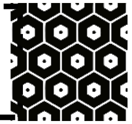
Closed packed array (CPA) → *Hierarchical binary structure (HSN)*

itee PhD information technology electrical engineering

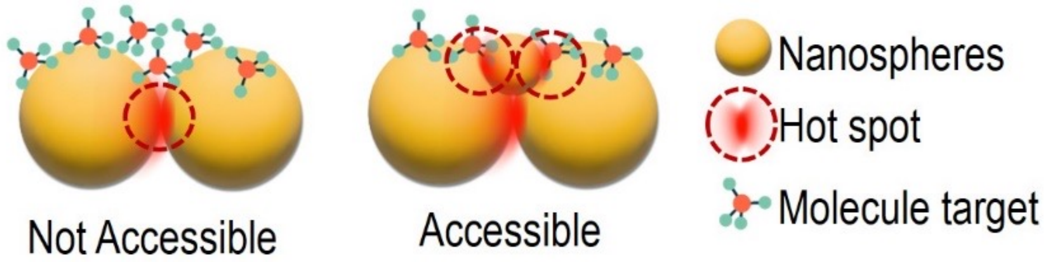
Objective



Development of SERS-active substrates

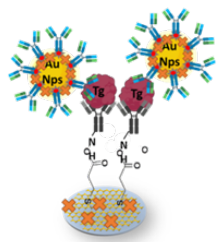


- ❑ *Highly sensitive*
- ❑ *Uniform*
- ❑ *Reproducible*
- ❑ *Cost-effective*
- ❑ *Hot spot accessibility*

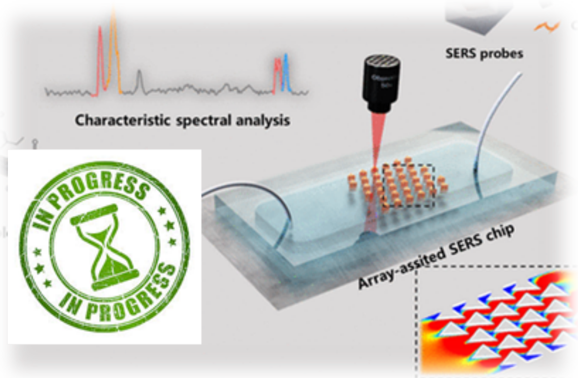


Motivations

Liquid biopsy in a smart needle



Point of Care (POC) diagnostic devices

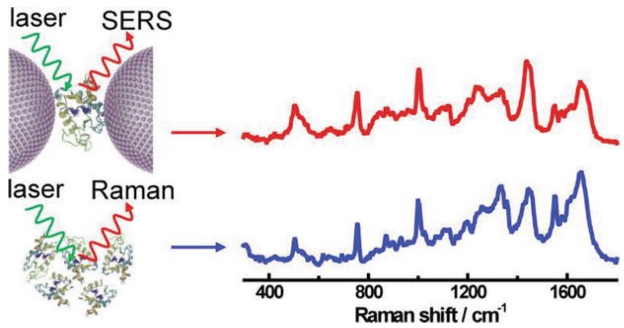


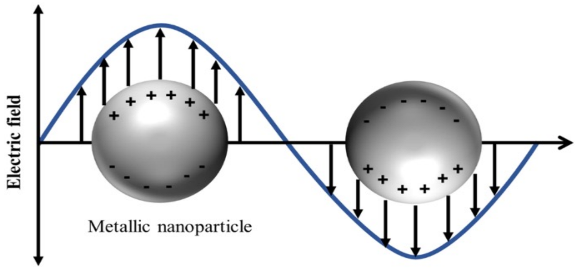
Surface Enhanced Raman spectroscopy (Sers)

Raman scattering is:

- Inelastic scattering
- 1% of the resulting overall scattering

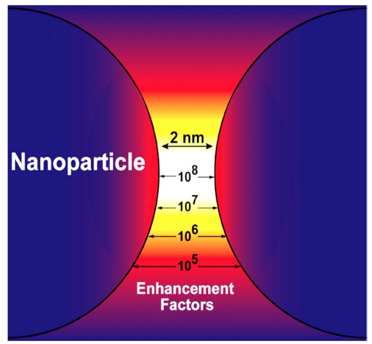
The "Raman spectrum" (***molecular fingerprint***) is unique and allows to provide structural information at the molecular level.





The "***sers***" effect is a phenomenon associated with the *amplification* of Raman signals of several orders of magnitude, achieve using plasmonic nanostructures, exhibiting a localized surface plasmon resonance (LSPR).

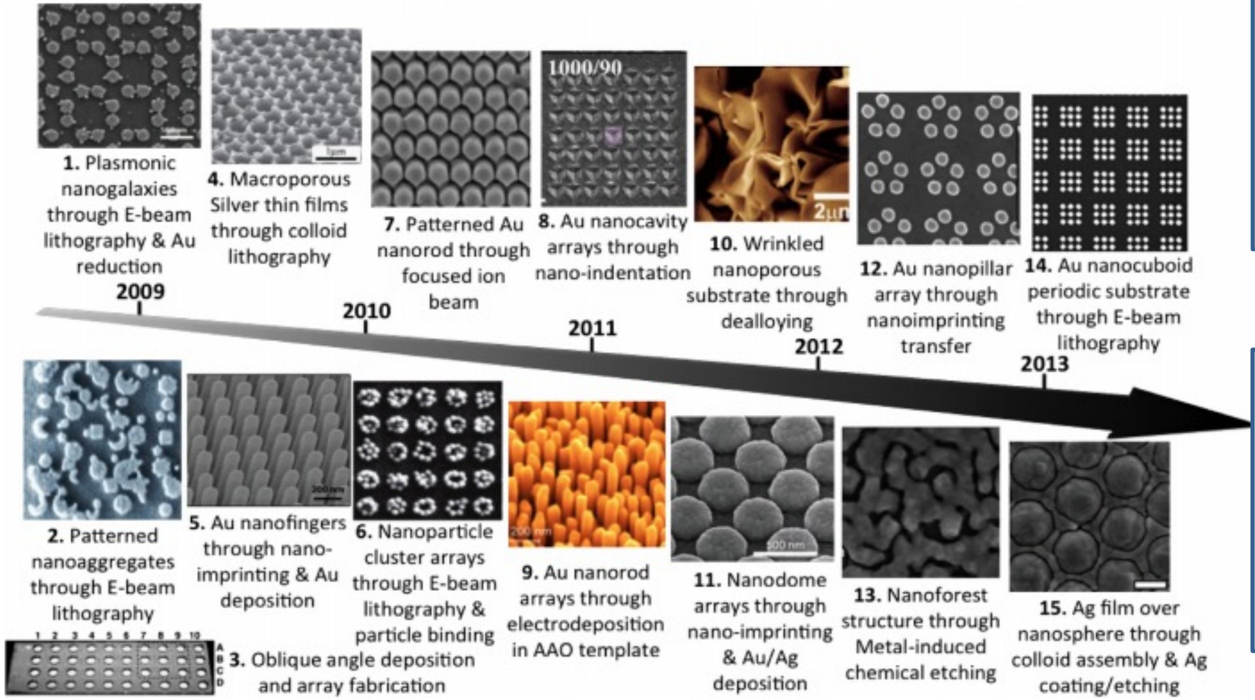
The local enhancement of the electromagnetic field is mainly responsible for the signal amplification and the spatial regions of highly intense local field enhancement are commonly called "*hot spots*".



$$EF \approx \left| \frac{E_{loc}(\omega_0, r_m)}{E_0(\omega_0, r_m)} \right|^4$$

To quantify the ability of the SERS surface to enhance the electromagnetic field nearby the target analyte, *"the enhancement factor (EF)"* is defined.

State of art



The fabrication techniques includes photolithography, nano-imprinting, electron-beam lithography, focused ion-beam milling, femtosecond laser ablation, and thin-film deposition.

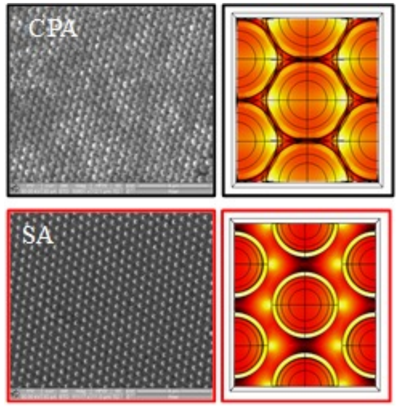
Self-assembly techniques is able to provide different periodic metallo-dielectric or metallic patterns exploiting a self-assembled monolayer of polystyrene nanospheres as building block

The molecular target size plays a major role in the intensity of the Raman spectrum.

	CPA	SA	SR
EF	10^6	6×10^5	7×10^5

Hot spot "accessibility"

An ideal SERS substrate features a high number of intense and accessible hot spots

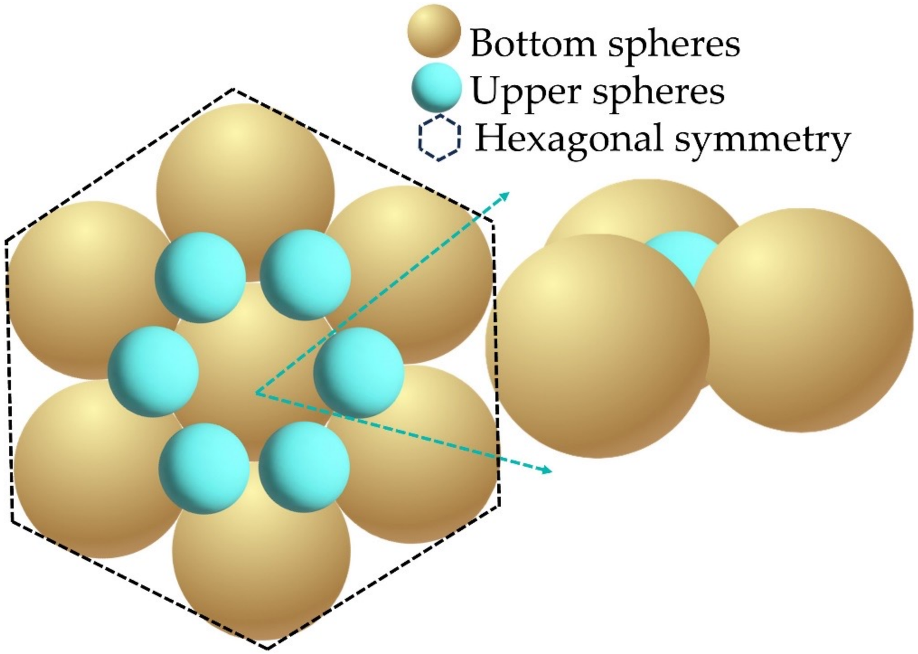


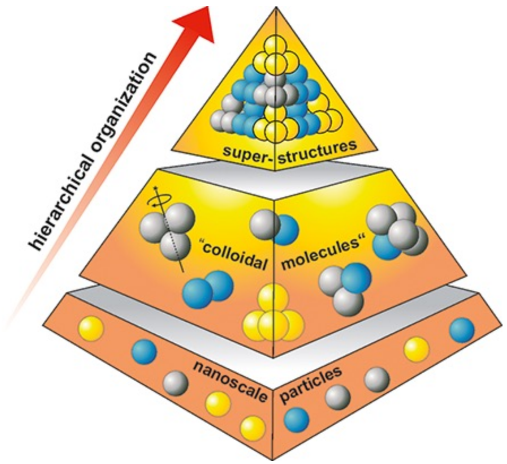
Stefano Managò, Giuseppe Quero, Gianluigi Zito, Gabriele Tullii, Francesco Galeotti, Marco Pisco, Anna Chiara De Luca, Andrea Cusano, Tailoring Lab-on-fiber SERS optrodes towards biological targets of different sizes, Sensors and Actuators B: Chemical, 2020, 129321, ISSN 0925-4005,

Proposed Substrate

✓ ***Hierarchical binary structure (HSN)*** ➡

- layer of big spheres periodically patterned on the surface of which there is a second layer of spheres, proportionally reduced in size (**Polystyrene**).
- Coated with 30nm of **gold** film .





✓ Fabrication method: ***Self-Assembly*** ➡

$$\delta = \frac{D_{\,upper\ spheres}}{D_{\,bottom\ spheres}}$$

❖ Co-deposition (for $\delta \leq 0.3$)

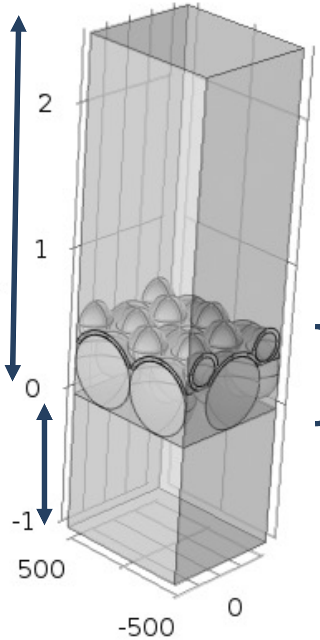
❖ Sequential deposition (for $\delta \geq 0.3$)

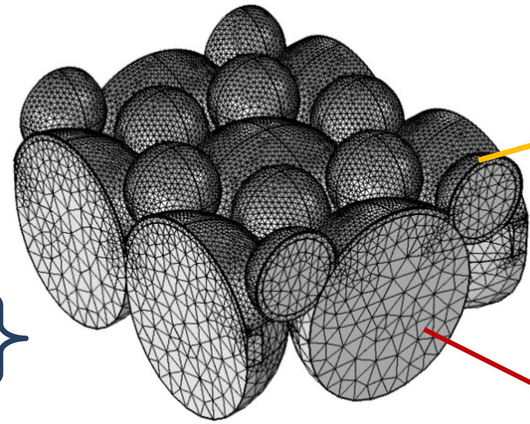
My work

Numerical method

Air:
- Height=10*r_1
- η =1

Glass:
- Height= 10*r_1
- η =1.45



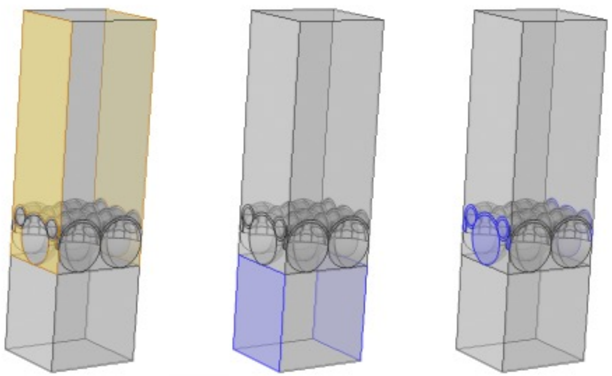


Gold
- Thickness=30nm

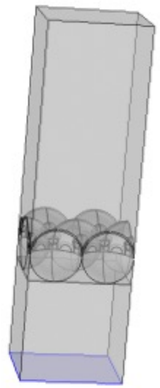
Resulting number of elements of the total mesh verge on 282554, with an average quality of 0.6

Polystyrene:
- η=1.6

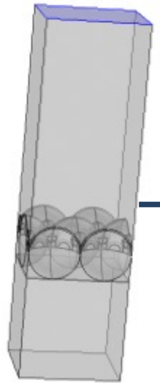
Floque's periodic condition



Port 2: *"receiver"*



Port 1: *"Source"*



Wavelength range:
- 600 to 1200 nm

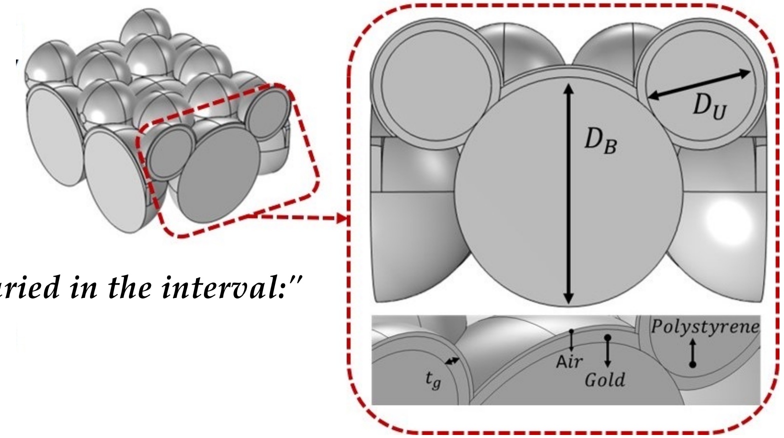
Initial condition:
- Power = *1W*
- Planar waves
- Polarization: *linear along x*

Numerical method

COMSOL MULTIPHYSICS

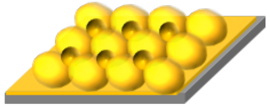
Three sets of simulations I performed:

1. Diameter of bottom spheres=D_B= 500nm
2. Diameter of bottom spheres =D_B= 750nm
3. Diameter of bottom spheres =D_B= 1000nm

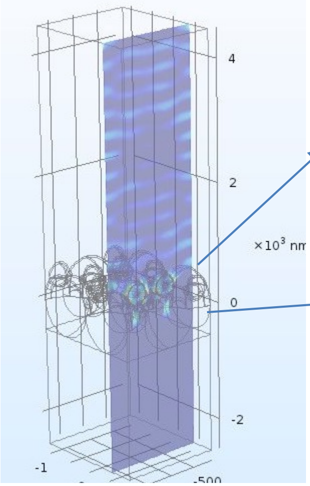


"Once the diameter of the bottom spheres is fixed, the upper diameter is varied in the interval:"

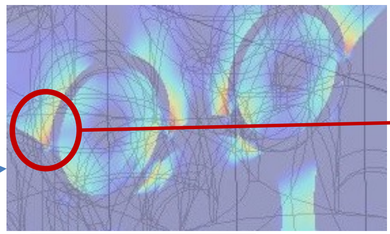
$$D_U/D_B = [0.24:0.56]$$



For each structure is taken the cut plan xy, in correspondence with the z coordinate where the hot spot is located:

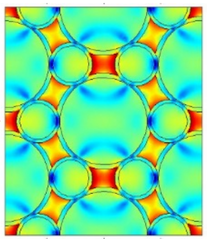


Plane zy



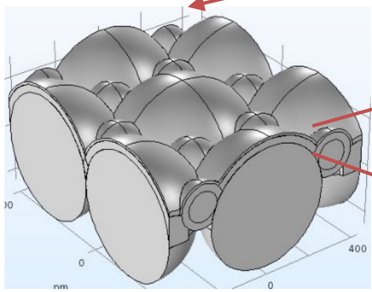
Choosen z

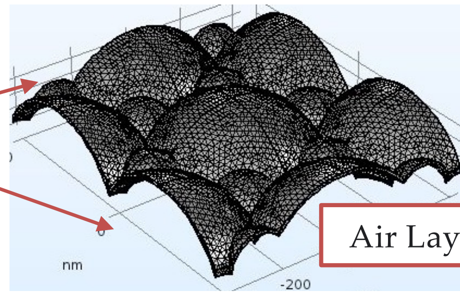
Plane xy



$$\log_{10}\left(\left|\frac{E_{loc}}{E_0}\right|^4\right)$$

The *conformal layer* is an air layer of 10 nm thickness that follow the anatomy of the gold layer.





Air Layer

On the conformal layer, to obtain a numerical estimation of the sers substrate performance, we evaluate:

$$AVI = mean \iiint \left|\frac{E_{tot}}{E_0}\right|^4 dV$$

itee PhD information technology electrical engineering

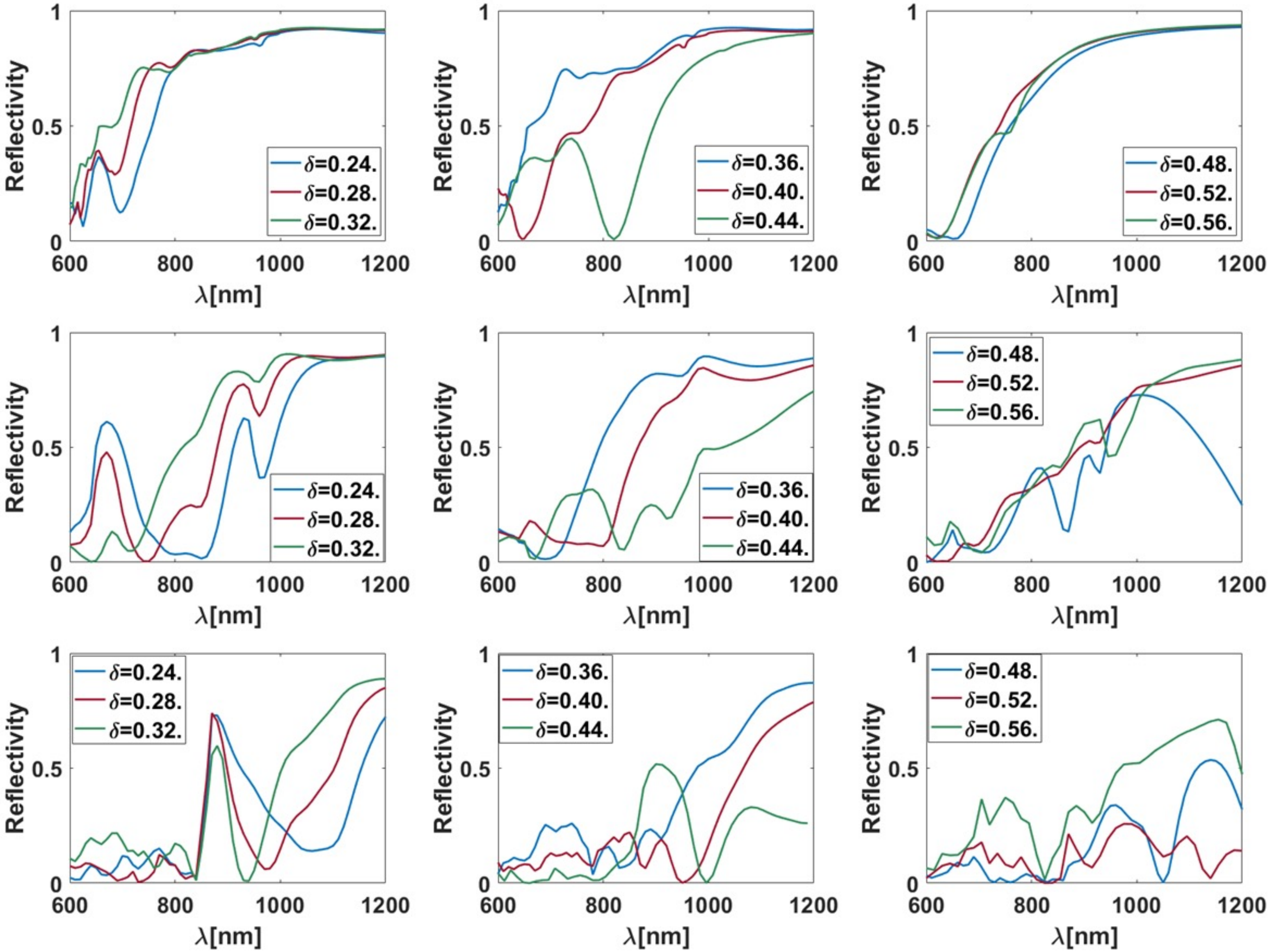
My work

Numerical Analysis

$D_U = 500$ nm

$D_U = 750$ nm

$D_U = 1000$ nm



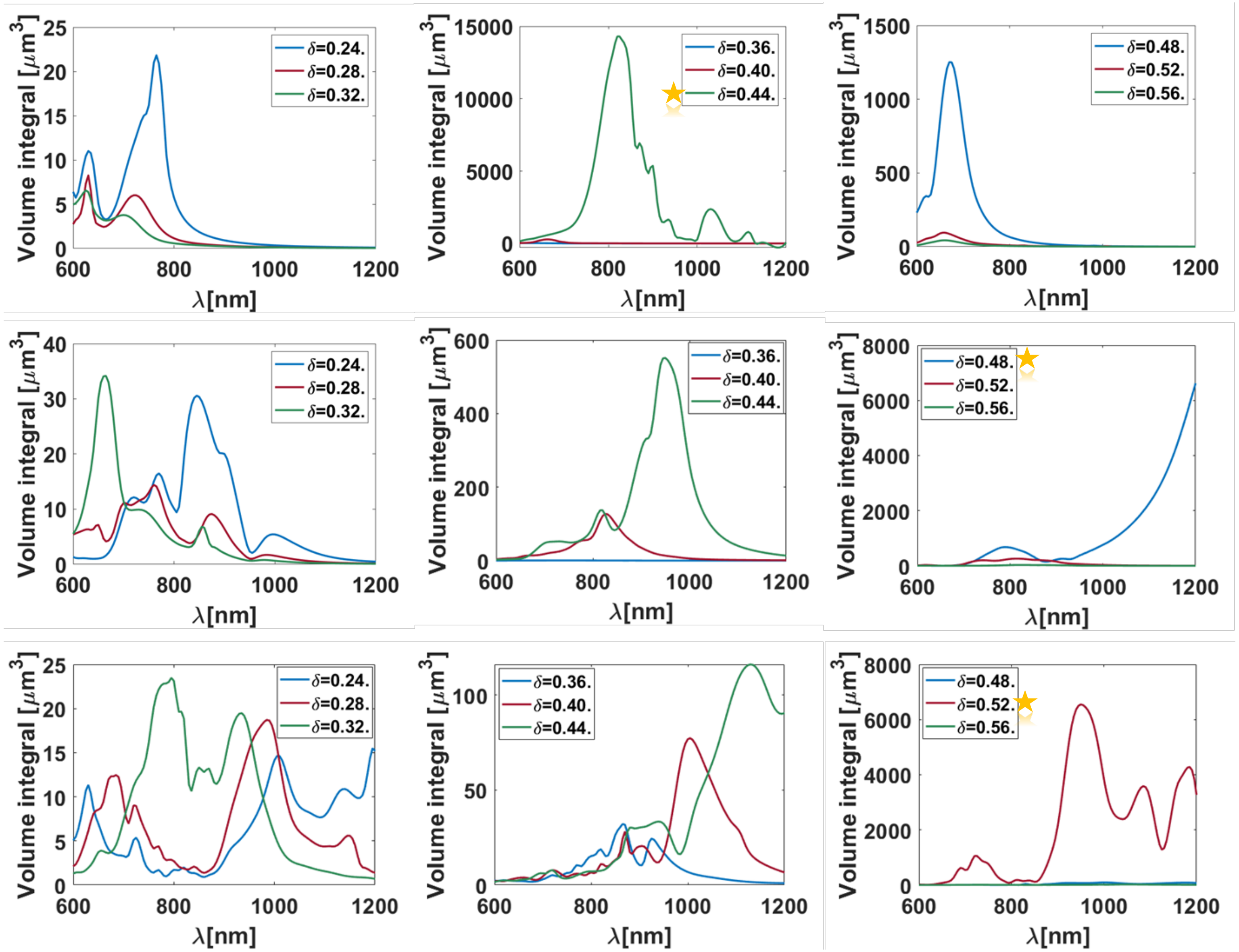
My work

Numerical Analysis

$D_U = 500$ nm

$D_U = 750$ nm

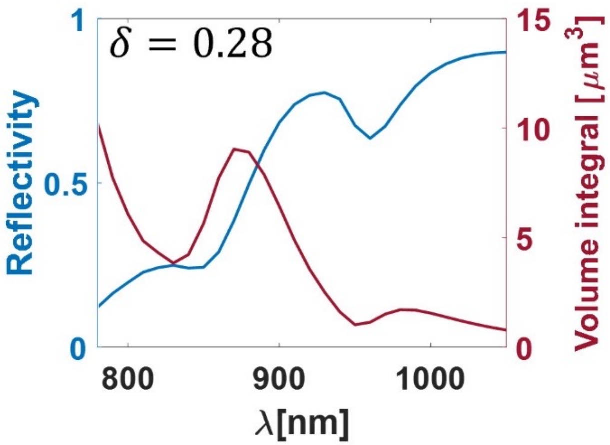
$D_U = 1000$ nm

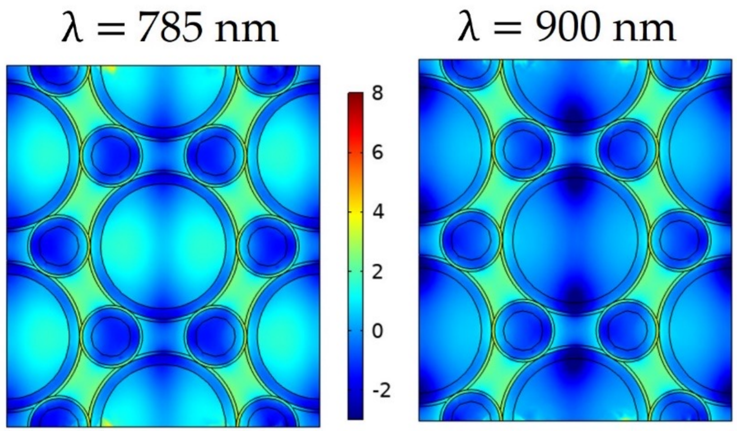


My work

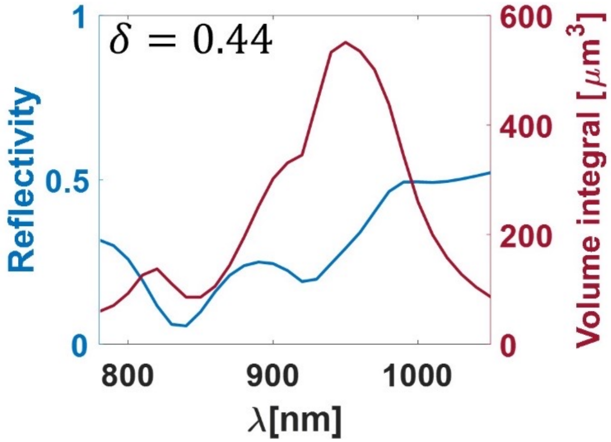
Numerical Analysis: outcomes

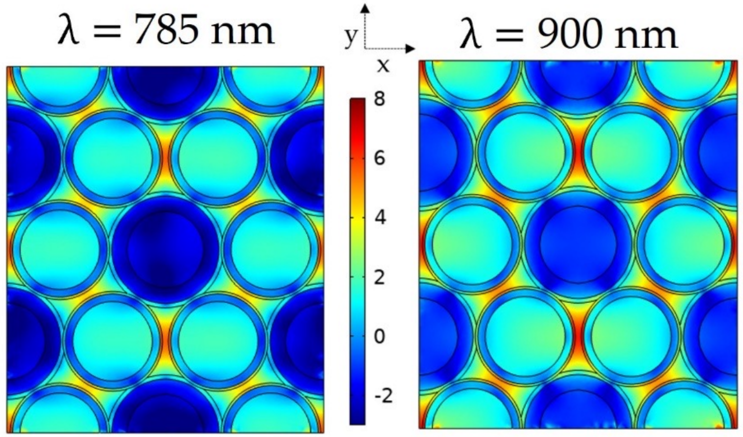
HSN= { D_B =750 nm, D_U =210 nm, δ =0.28 }





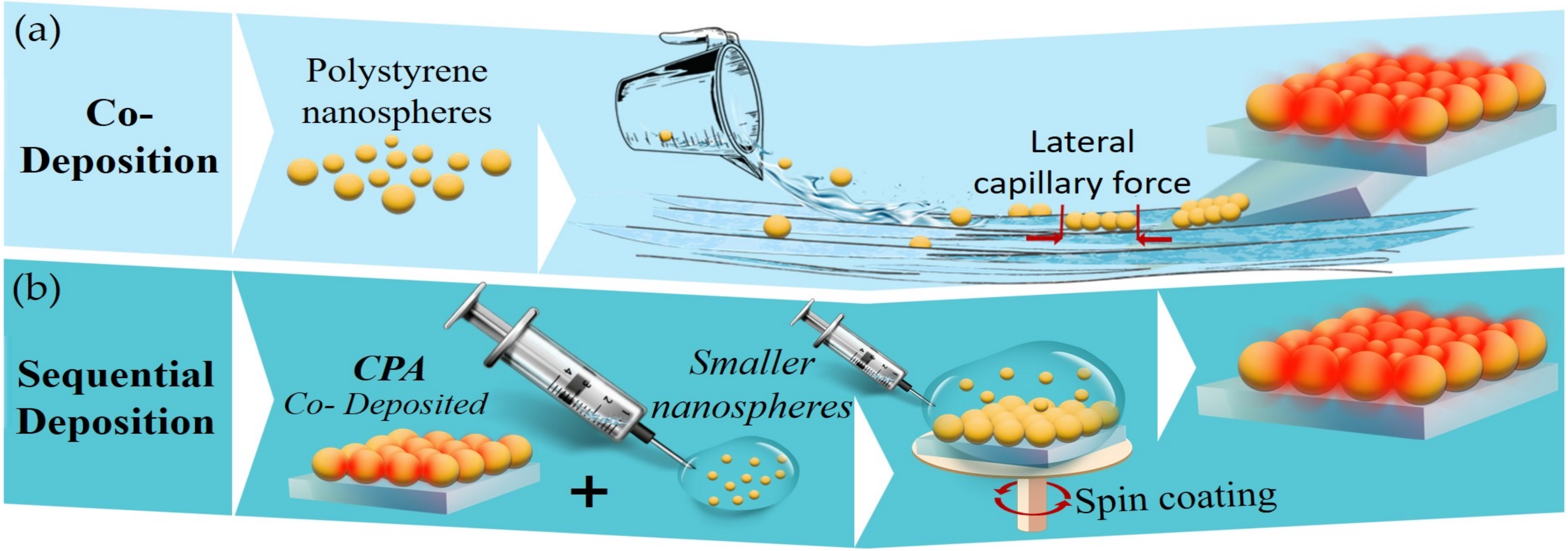
HSN= { D_B =750 nm, D_U =330 nm, δ =0.44 }





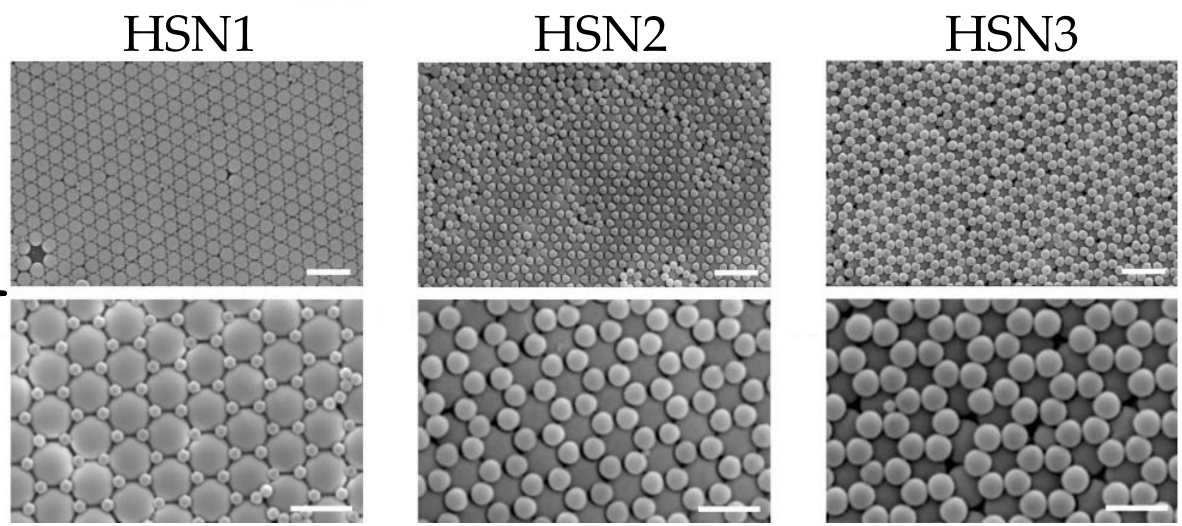
fingerprint region: 785-910 nm

Fabrication approaches:



Geometrical parameters of the fabricated substrates.

	D_B	D_U	δ
CPA	757 nm	none	-
HSN1	757 nm	196 nm	0.26
HSN2	757 nm	350 nm	0.46
HSN3	757 nm	425 nm	0.56

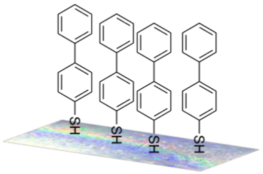


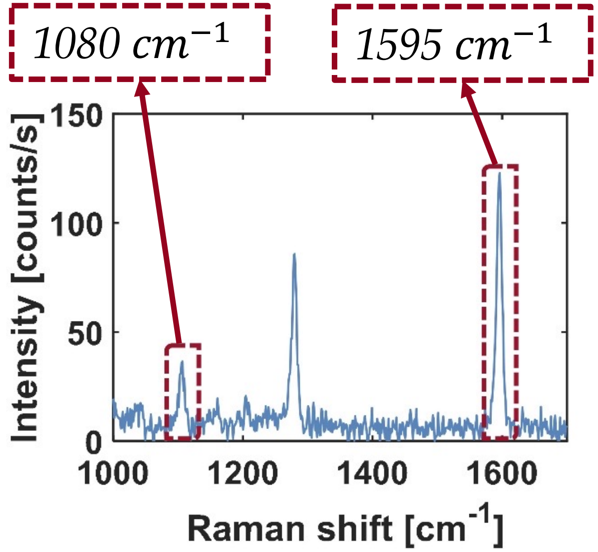
SERS characterization and performance assessment

To estimate the performance of the sers substrate, the BPT is used as benchmark:

- **Protocol for BPT :**
  - 1mM 4-biphenyl thiol (**BPT**) in EtOH V_f=1,5mL

    (Sigma-Aldrich cod. 752207 - Molecular Weight: 186.27)
  - EtOH Washing
  - Drying (5′ under the hood)





The selected parameters are the same both for Raman and Sers measurements:

- ❖ Objective: 100X (Air, NA=0.9)
- ❖ Wavelength: 785nm/633 nm
- ❖ Power laser: 3.2% ND Filter
- ❖ Pinhole: 400 μm
- ❖ Integration time: 1s
- ❖ Accumulations: 1acc
- ❖ Grating: 300 lines/mm
- ❖ XY map: Area 10x10 μm^2, step 0.5 μm, 400 points

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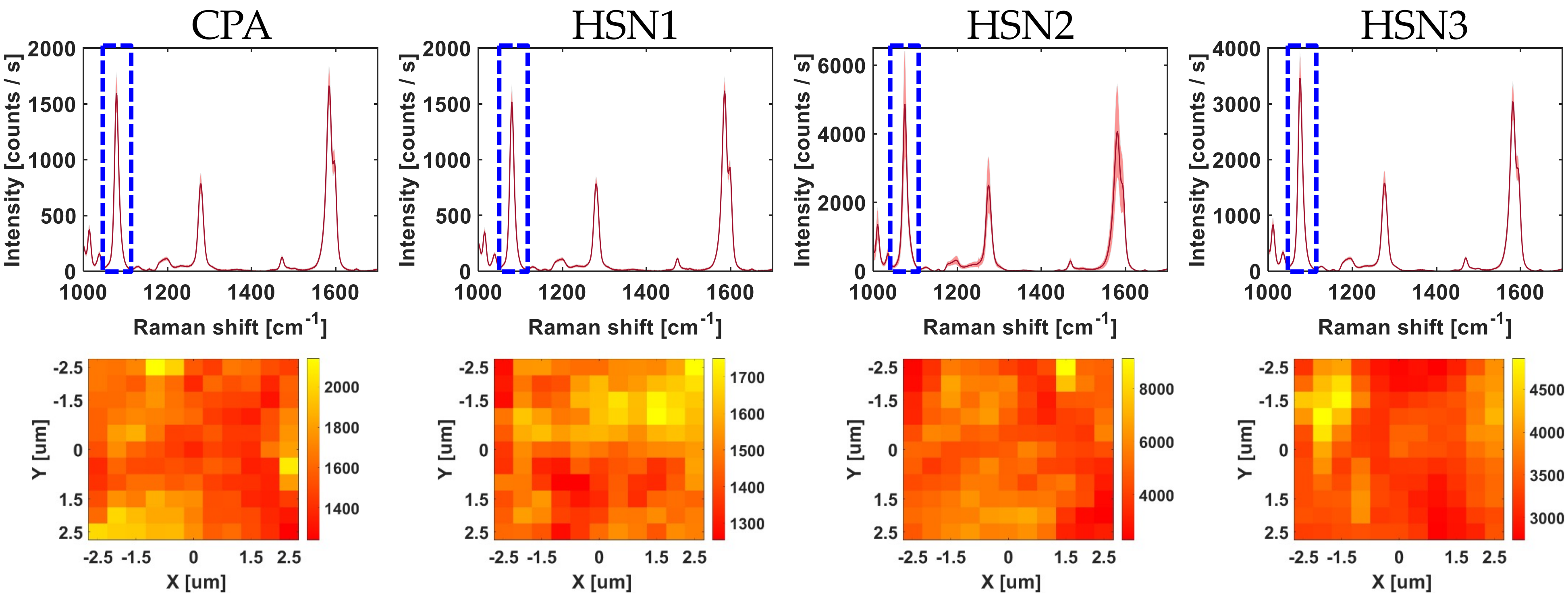
SERS characterization and performance assessment



	Peak Intensity (PI) [counts/s], μ±σ	σ/μ [%]	$\frac{PI_{HSN}}{PI_{CPA}}$	Average Volume Integral (AVI) [μm^3]	$\frac{AVI_{HSN}}{AVI_{CPA}}$	Enhancement Factor (EF) [10^5]	$\frac{EF_{HSN}}{EF_{CPA}}$
CPA	1578.11 ± 196.70	12.5	1.00	2.14	1.00	2.22	1.00
HSN1	1515.63 ± 106.20	7.0	0,96	0.96	0.45	2.29	1.03
HSN2	5094.35 ± 1117.40	21.9	3,23	11.61	5.40	6.74	3.03
HSN3	3701.24 ± 443.90	12.0	2,35	5.25	2.40	5.12	2.30

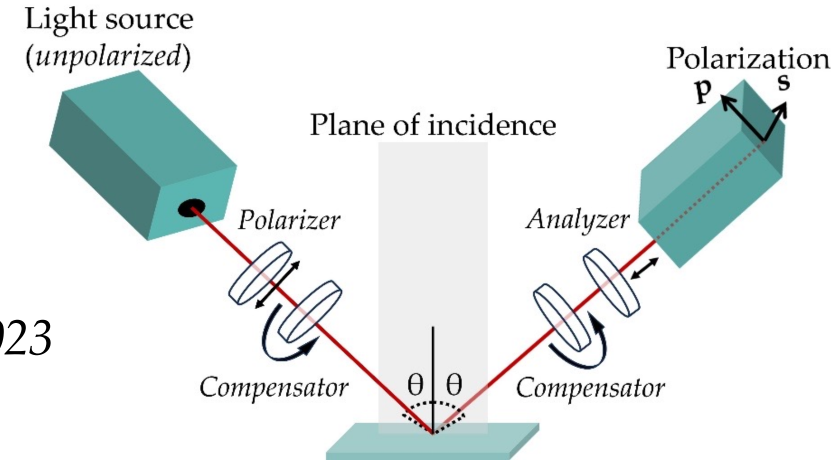
In the fingerprint region: 785-910 nm

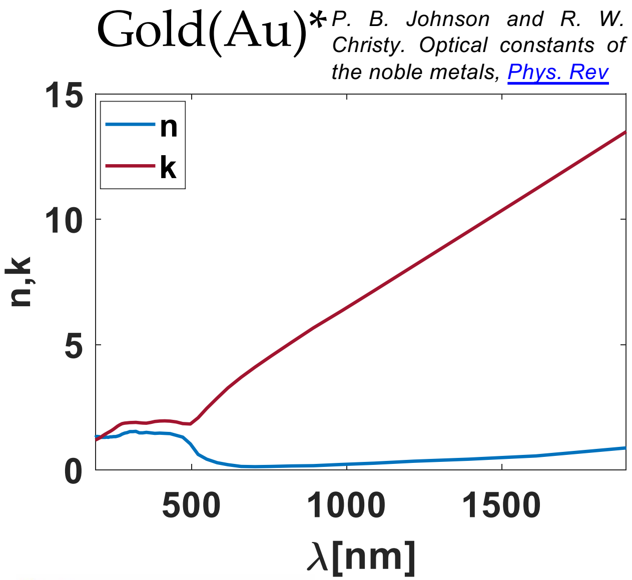
My work

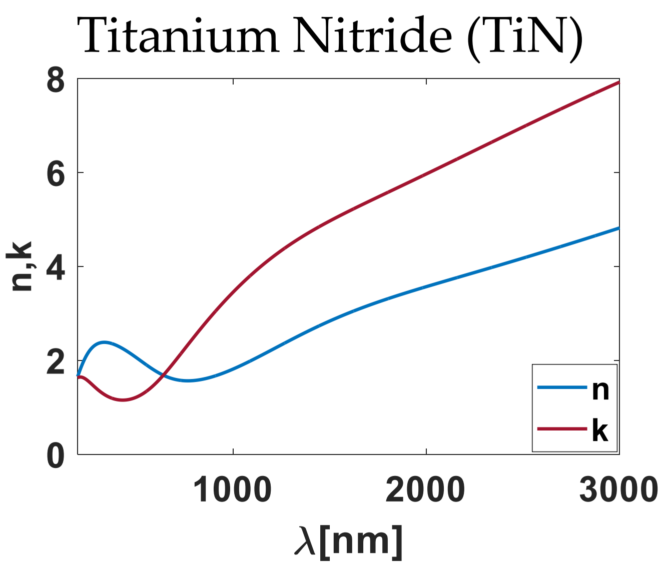
Innovative plasmonic material: CMOS-Compatible

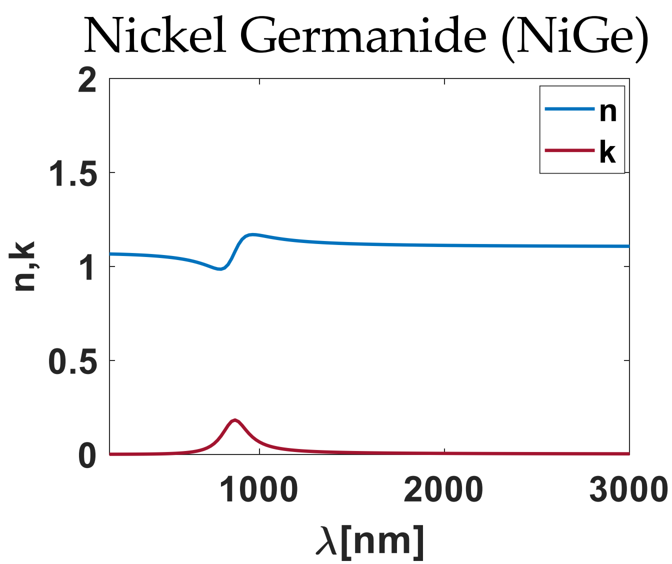
Refractive index acquisition

01-06-2023 to 31-08-2023









My work

Innovative plasmonic material: CMOS-Compatible

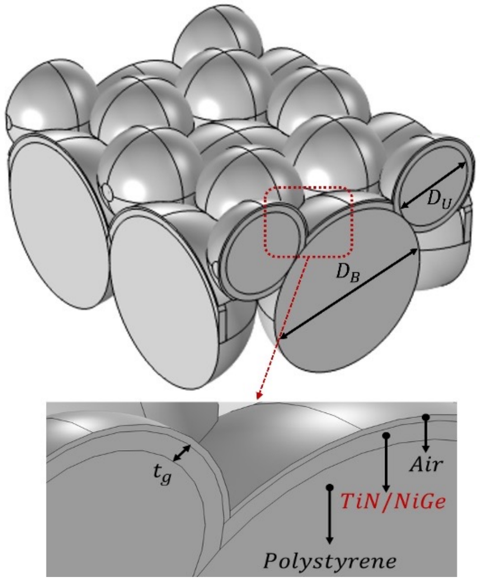
Refractive index acquisition

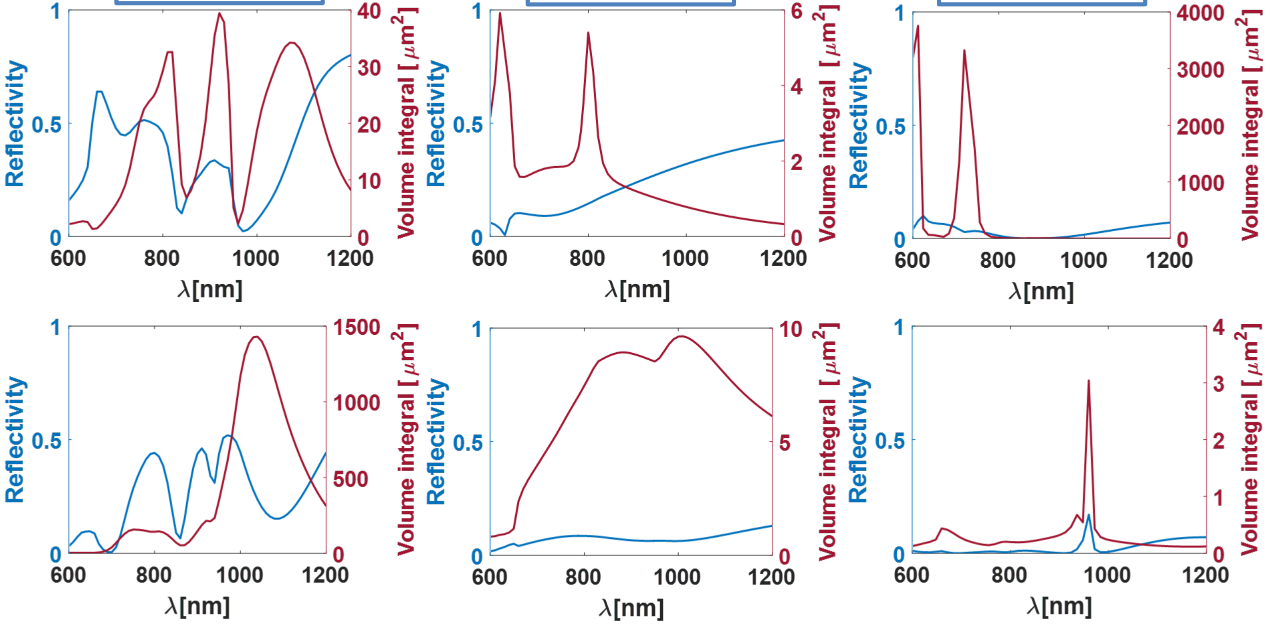
01-06-2023 to 31-08-2023

Numerical Analysis

CPA = D_B =757 nm

HSN= $\begin{cases} D_B = 757 \text{ nm} \\ D_U = 350 \text{ nm} \\ \delta = 0.48 \end{cases}$





Sample	Material	Maximum Volume Integral (MVI) [µm³]	$\frac{MVI_{HSN}}{MVI_{CPA}}$	Average Volume Integral (AVI) [µm³]	$\frac{AVI_{HSN}}{AVI_{CPA}}$
CPA	Au	39.45	-	21.41	-
CPA	TiN	5.92	-	2.25	-
CPA	NiGe	3756.77	-	11.89	-
HSN	Au	1430.06	36.25	116.08	5.39
HSN	TiN	0.964	1.63	8.35	3.71
HSN	NiGe	3.04	0.001	0.23	0.02

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Conclusion

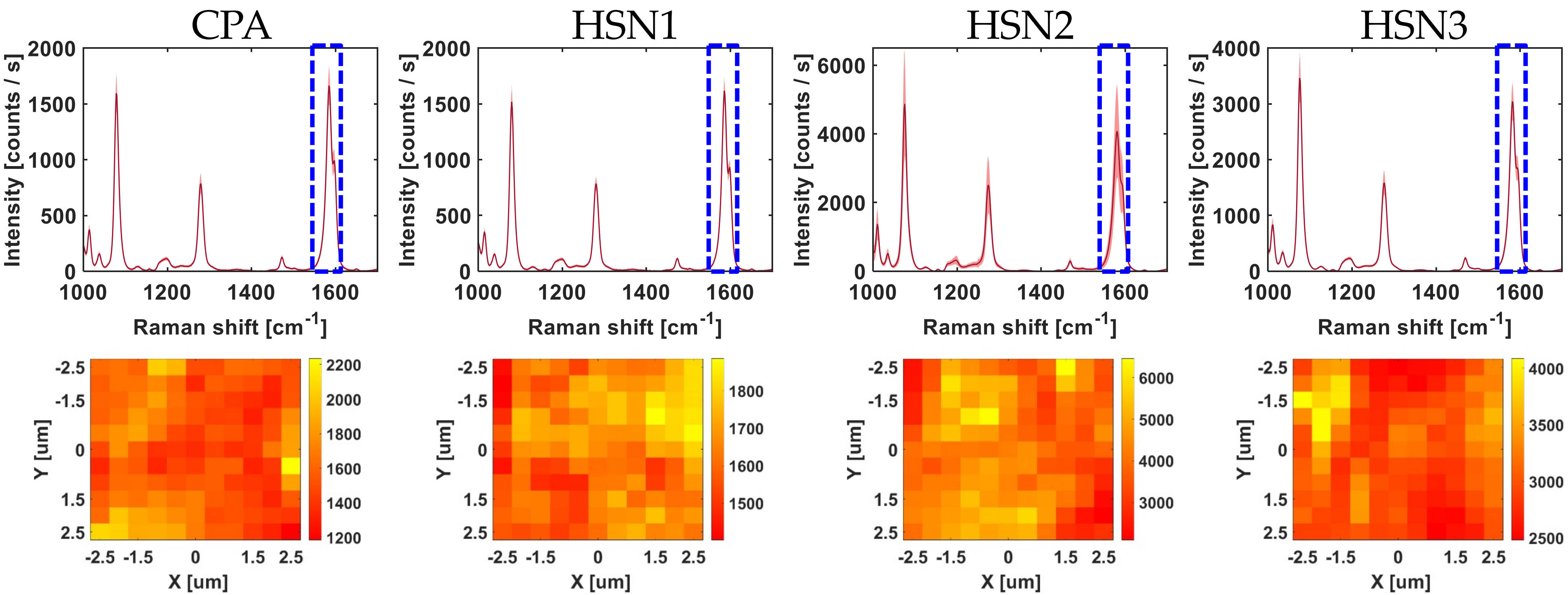
- ✓ Numerical analysis provided precise geometrical constraints to attain a regular pattern of nanogaps with intense hotspots
- ✓ Two experimental approaches suitable to provide a regular pattern of HSN have been demonstrated
- ✓ Full SERS substrates characterization have been carried out
- ✓ Experimental analysis of the SERS response demonstrates that a hierarchical approach can provide cost-effective SERS substrates with superior performances with respect to the simpler single layer configurations.
- ✓ The reported numerical and experimental investigation can suggest a general way to enhance the performances of existing SERS substrates and to create new SERS substrates with superior performances
- ✓ A comparative preliminary analysis on TiN and NiGe revealed the low performance compared to Au.

Future prospective

- ✓ Fabrication and testing of the hierarchical substrate on the optical fiber tips.
- ✓ Make a fine tuning of the innovative plasmonic material with the geometry.
- ✓ Design and fabricated a SERS active substrates with CMOS compatible materials.

Thank you for the Attention

SERS characterization and performance assessment



	Peak Intensity (PI) [counts/s], μ±σ	σ/μ [%]	$\frac{PI_{HSN}}{PI_{CPA}}$	Average Volume Integral (AVI) [μm^3]	$\frac{AVI_{HSN}}{AVI_{CPA}}$	Enhancement Factor (EF) [10^5]	$\frac{EF_{HSN}}{EF_{CPA}}$
CPA	1660.92 ± 243.12	14.61	1.00	2.14	1.00	0.69	1.00
HSN1	1691.47 ± 100.80	5.95	1.01	0.96	0.45	0.76	1.09
HSN2	4174.92 ± 864.80	20.71	2.51	11.61	5.40	1.64	2.36
HSN3	3037.10 ± 342.80	11.28	1.82	5.25	2.40	1.24	1.79

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