Babar Ali

Self-Assembled Nanostructured Substrates for ATR-SEIRA Applications

Tutor: Prof. Cutolo Antonello
Co-Tutor: Prof. Marco Pisco

Cycle: XXXVI

Year: 3^{rd}

My background

- MSc. degree:Electronics and Communication Engineering
- Research group/laboratory: Information Photonics and Optical Communication
- PhD start date: November,2020
- Scholarship type: UNINA

	Courses	Seminars	Research	Tutorship	Total
1st	33.1	18.65	45	0	**96.75**
2nd	15	4.1	44	0	**63.1**
3rd	0	0	72	0	**72**
Total	48.1	22.75	161	0	**231.85**
Expected	**30 - 70**	**10 - 30**	**80 - 140**	**0 – 4.8**	

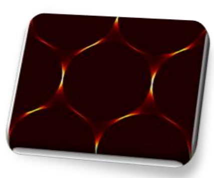
Summary of Study Activities

- ***Briefly summarize the study activities of the academic year***
  - *Attended (Courses, Seminars, PhD Schools)*
  - *The optimization of numerical simulations of proposed SEIRA substrates and the experimental (Fabrication, Morphological characterisation by SEM and spectral characterization by ATR-FTIR) SEIRA substrates are the main areas of my third-year research activity.*

Ad hoc PhD courses / schools

- *Software Defined Radio Applications for Radar and Localization*
- *Ultra-High Field Magnetic Resonance Imaging*
- *Virtualization technologies and their applications*
- *Machine Learning for Science and Engineering Research*

- ***Conferences / events attended***
  - *Bio Photonics Conference*



FTIR instrument

ATR Module

- Microscopy ,Optical Coherence Tomography ,Biomedical Imaging, ,Photoacoustic Imaging, Lasers & Therapeutics and Spectroscopy

Research activity: Numerical Simulation

- ***Designing of close packed array of Nano disks-based Antenna and Simulation of the SEIRA substrate***
- ***Parametric Analysis (Gap, Thickness of Nano Disk)***
- ***SEIRA Gain Calculations***

Research activity: Experiment

- ***Fabrication Procedure***
- ***Morphological characterization by SEM***
- ***Spectral characterization by ATR-FTIR***
- ***SEIRA Enhancement Factor Calculation***
- ***Comparison between numerical and experimental results Results***

Research activity: PhD Thesis Overview and Objective

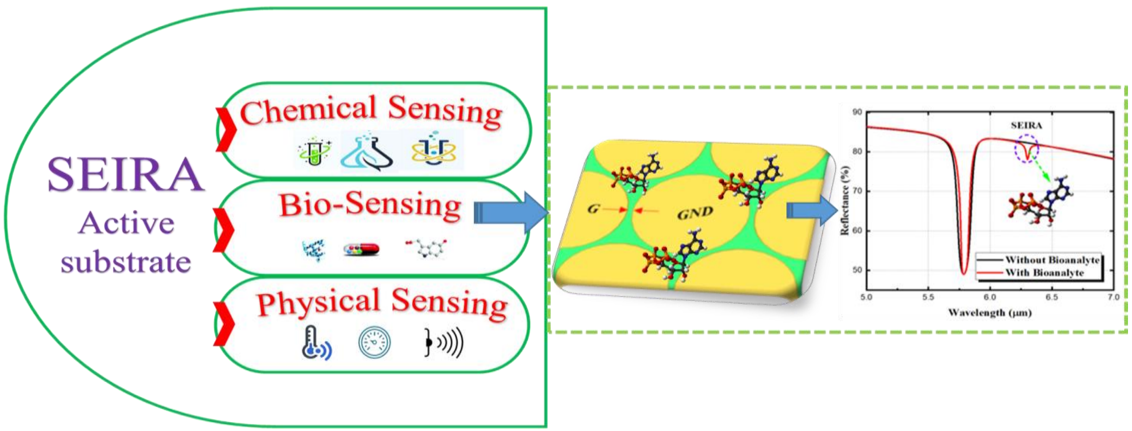
ATR-FTIR: Attenuated Total Reflectance Fourier-Transform Infrared SEIRA: Surface-Enhanced Infrared Absorption

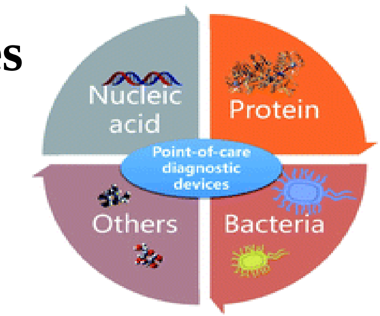
Developtmnet of atctive SEIRA substate with ATR-FTIR

- To access the rich vibrational information of biomolecules and enables the investigation of uniqe structural chracteristic of biosamples
- Identify and develop novel and cost effective and highly efficient plasmonic nanostructures exhibiting good SEIRA properties (namely, the gain factor) in order to improve the detection characteristic of an ATR-FTIR instrument for biological analysis.

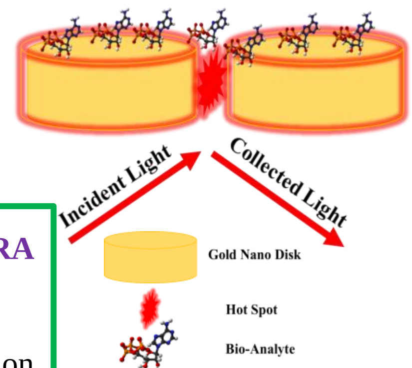
Motivations

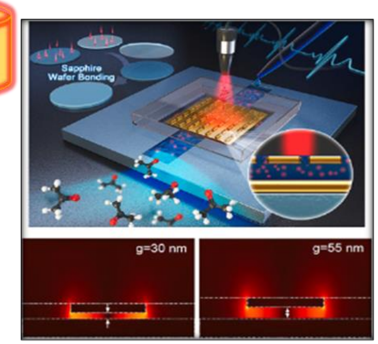
▪ Point of Care diagnostics devices





Michael J. Serpe (2021), DOI:10.1039/D1AY01643A





Jikai Xu, ACS Nano 2020 DOI:10.1021/acsnano.0c05794

- **Our Proposal** (Gold Nano disks and "Otto" configuration) **ATR-FTIR** and **SEIRA** are combined for biosensing applications
- **Numerical Results:** Parametric Analysis devoted to identify the best configuration in terms of SEIRA gain factor
- **Experimental Results:** Fabrication, Morphological characterization by SEM. and Spectral characterization by ATR-FTIR, SEIRA gain factor calculation)
- **Numerical fitting of the experimental results**

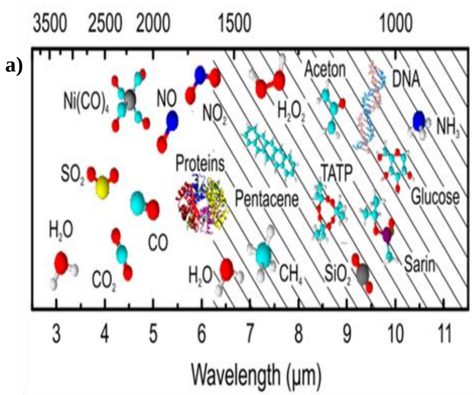
- Simple design and Efficient
- Cost effective
- Sensitivity and reliability
- Tunability
- Hot spot accessibility
- Repeatability

Research activity: SEIRA and State of Art

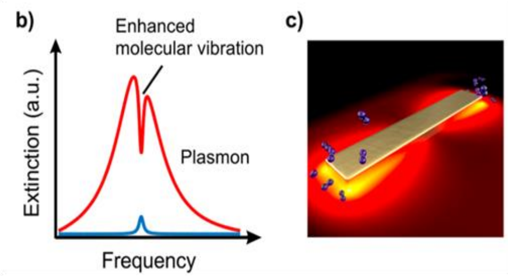
- SEIRA-active substrates are a crucial component in biosensing, greatly enhancing the sensitivity of infrared spectroscopy.

- **Electromagnetic Enhancement (EME)**
- **Chemical Enhancement (CE)**

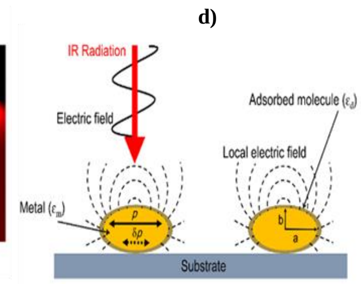
- The **EME** mechanism (Enhancement effect of electric field intensity on the substrate) and **CE** (orientationally substrate–molecule interaction at the interface).
- Localized enhancements of the electromagnetic field (EMF) are responsible for the amplification of the signals, and **hot spots** are defined as spatial regions of highly intense local field enhancement.



Different biomolecule in the midinfrared



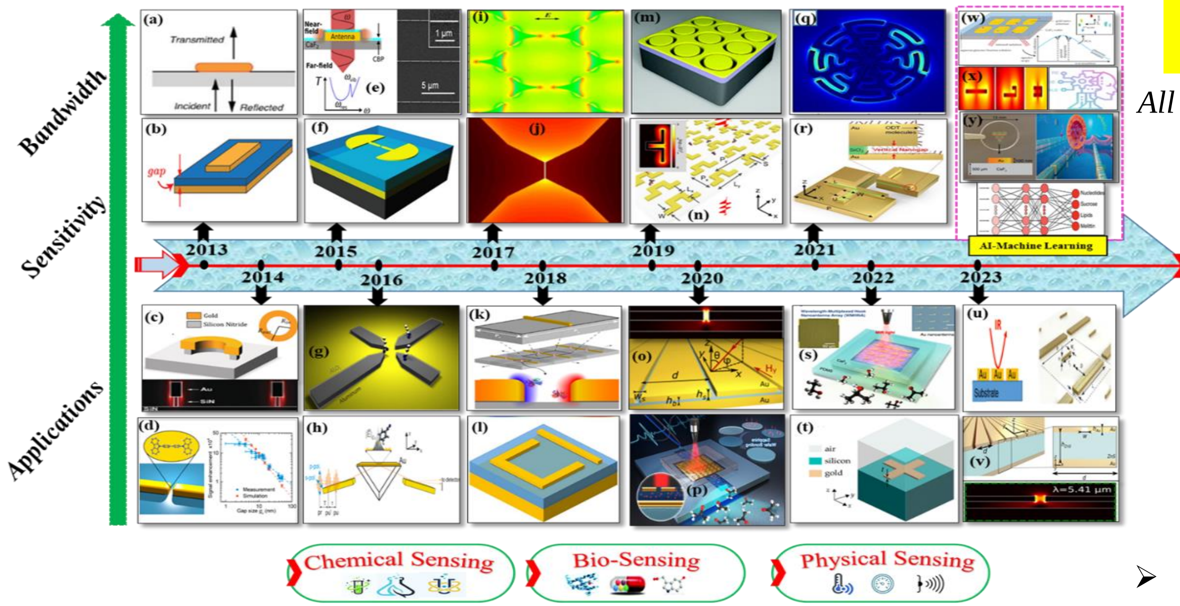
Characteristic infrared vibrations of selected molecular species. The fingerprint region containing skeletal vibrations is hatched. **(b,c)** Principle (SEIRA)



Schematic representation of the electromagnetic mechanism of SEIRA on metal island film

(a-c) Frank Neubrech (2017, 117 (7), 5110-514) https://10.1021/acs.chemrev.6b00743

(d) Wu, L., Jiang, X. (2018), https://10.1007/978-981-10-6823-2_11



SEIRA Active Substrate Road Map

SEIRA enhancement factor (EF) $= \frac{\Delta R}{\Delta R_0} \times \frac{A_0}{A_{SEIRA}}$

All these studies have been done with FTIR coupled with optical microscope

Reference	Molecule Studied	Enhancement Factor (EF)
Tao Wang(2013), DOI:10.1364/OE.21.009005	PMMA	10^3
Lisa V. Brown(2013), https://doi.org/10.1021/ja312694g	1-Octadecanthiol	10^4
Jochen Vogt(2014),https://doi.org/10.1039/C4CP04851B	4,4′-bis(N-carbazolyl)-1,1′-biphenyl	10^3
Benjamin Cerjan(2016) , DOI:10.1021/acsphotonics.6b00024	Stearic acid	10^4
Brown(2015) https://doi.org/10.1021/nl504455s	Zeptomoles of octadecanethiol	10^5
Meo(2019), DOI:10.1016/j.snb.2019.02.014	PMMA (Methyl acrylate)	10^4
Shuyan Zhang(2023), DOI:10.1039/D3NR01369C	miRNAsbohydrates	10^3

Self-assembly		
Bibikova (2017), DOI: 10.1039/c6an02596j	Dimercapto polyethylene glycol	10^3
Levin, Carly S. et al. DOI: 10.1039/b909080k	Proteins and DNA	10^4
Emanuel Pfitzner (2018), DOI: org/10.1021/acssensors.8b00139	Proteins	10^4

- The SEIRA near filed enhancement $(|E_{loc}/E_0|^2)$ is defined as the SEIRA surface capacity to increase the EMF close to the target analyte.

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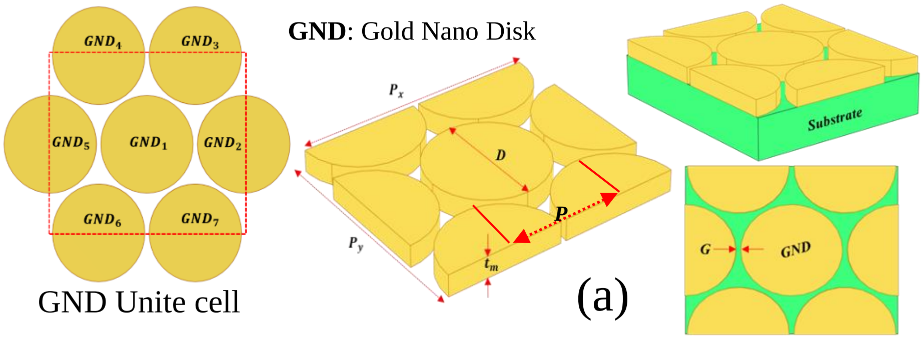
ΔR =Difference of Resonance peak with and without Molecule presence on NA

ΔR_0 =Difference of Resonance peak with and without Molecule on presence Flat Gold

A_0=Area of the Unit cell (Nano Antenna) exposed to light , A_{SEIRA}=Effective Area of Nano Antenna(Where the filed is localized)

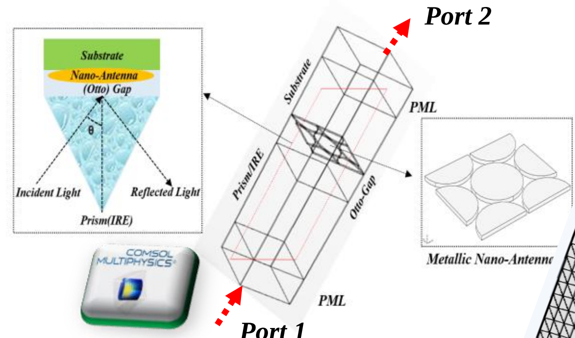
Research activity: Design and Simulation of the SEIRA substrates

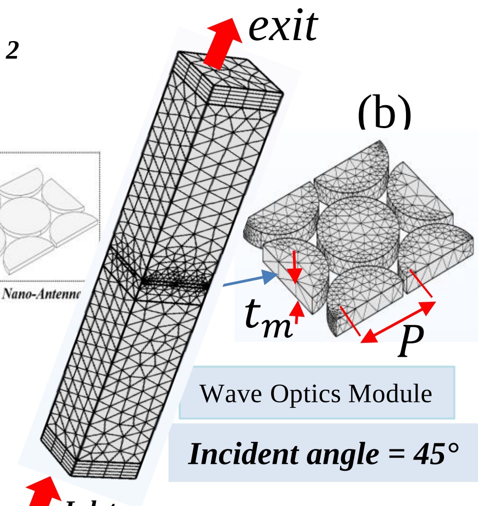


- D = Diameter between Nano Disk = 757nm,
- t_m = Thickness of Nano Disk = 30nm
- G = Gap between Nano Disk = 15nm
- P= Period=772nm
- Refractive Index of Glass =1.5
- Refractive Index of Diamond= n=2.65

$I_0 = 1[MW/m^2]$

$Power = I_0 \times Width(P_x) \times deth(P_y)$





Numerical Tool

***Port 1**:Incident /Reflection (Inlet)*

PML (Perfectly matched layer)

Wave Optics Module

Incident angle = 45°

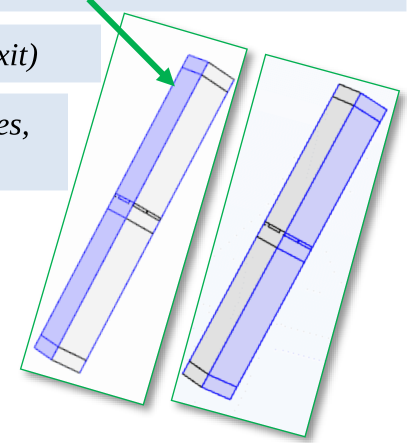
The Finite Element Method (FEM)

Floquet PBC were imposed on the side walls

***Port 2**:Transmission (exit)*

Electromagnetic Waves, Frequency Domain

- Importantly, adaptive meshing is required for PBC to work accurately. Thus, an exact meshing scheme on the opposite side walls was imposed. The model of the SEIRA substrate in COMSOL Multiphysics use free triangular mesh on the opposite side walls and free tetrahedral mesh (For inside the walls and Nano disks (Nanoantenna)) between the PML layers. PML layers are meshed by using the swept option (Distribution based mesh). See in (b)



PBC (Periodic boundary conditions)

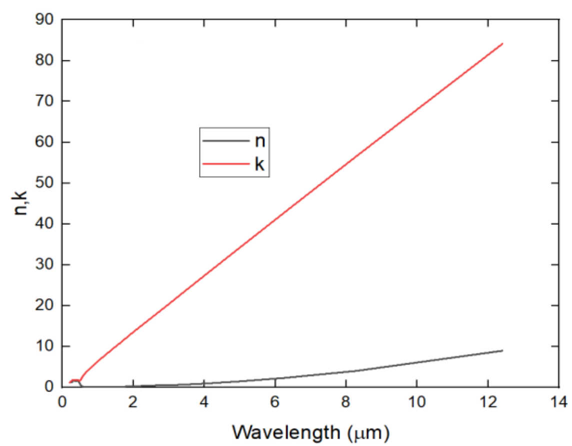
Drude model for the dielectric function of Gold

$$\varepsilon_{Au}(\omega) = 1 - \frac{\omega_p^2}{\omega(\omega + i\omega_c)}$$

Plasma frequency: ω_P *Collision frequency*: ω_c

$$\theta c = sine^{-1}\,(n_{Ref}/n_{Inci})$$

- The critical angle (θc)
- Incident angle : n_{Inci}
- Reflection angle : n_{Ref}



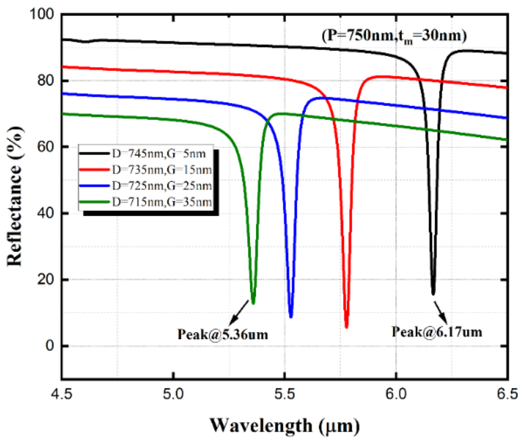
Cheng Shi, DOI: 10.1039/C8NA00279G (Communication) Nanoscale Adv., 2019, 1, 476-480.
W.-G. Yeo, "Far-IR multiband dual-polarization perfect absorber for wide incident angles," Microw. Opt. Technol. Lett. 55(3), 632–636 (2013).

Research activity: Simulation Results (Parametric Analysis)

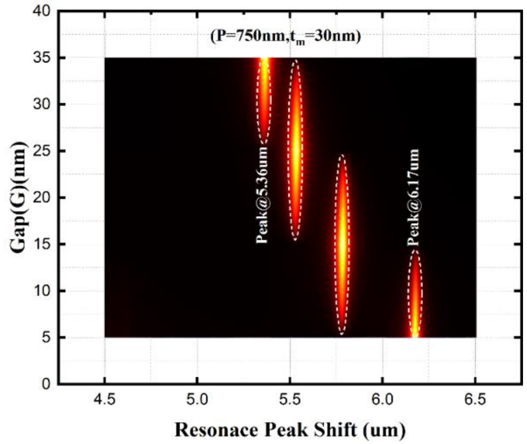
Influence of GND Diameter(D) and Gap(G) on tunability . Period (P) =750nm.

NANO Disk Period (p)= 750 nm

- P is fixed ,and D varies from 745nm to 715nm with 10nm Steps, and G varies from 5 to 35nm.



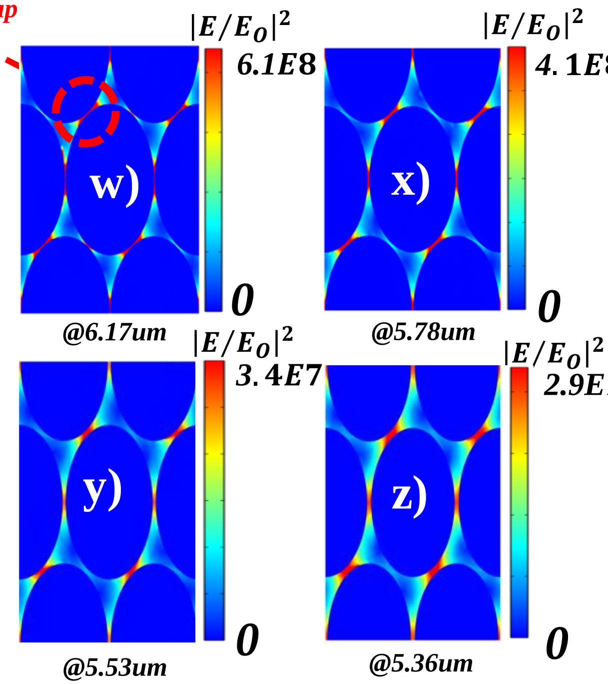
a) Influence of D and G at Resonance Peak



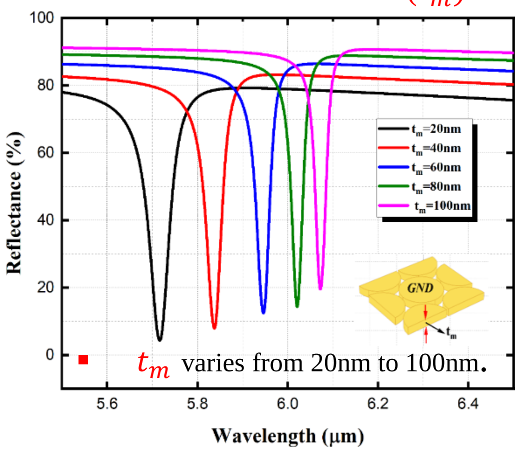
b) Influence of D and G at Resonance Peak (**2d-Surface Plot**)

Period **Fixed**	Nano Disk Diameter **Varies**	Gap between Nano Disk **Varies**	Resonance Peak Shift
750nm	745nm	5nm	6.17um
750nm	735nm	15nm	5.78um
750nm	725nm	25nm	5.53um
750nm	715nm	35nm	5.36um

Hot-Spot at Nano gap

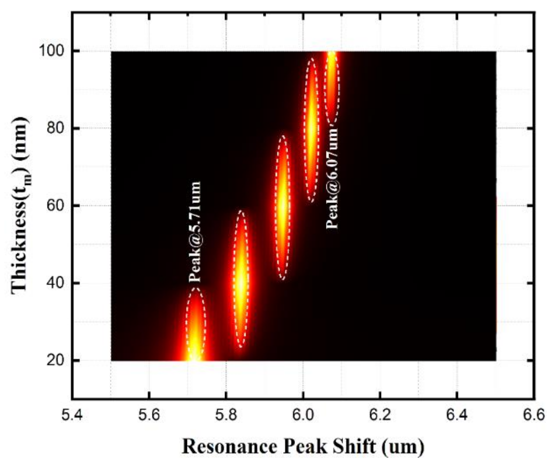


Influence of GND thickness (t_m) on tunability . Period (P) =750nm.



- t_m varies from 20nm to 100nm.

a) Influence of t_m at Resonance Peak



b) Influence of t_m at Resonance Peak (**2d-Surface Plot**)

GND thickness (t_m)	*Resonance Peak Shift (um)*
20nm	5.71
40nm	5.86
60nm	5.96
80nm	6.03
100nm	6.07

Near-Field Enhancements When GND D , G varies, and P is fixed

- Near E-field confinement with Hot-Spot created by coupling between GND are clearly visible between the Nano gap (Between Nano disks) as seen in figure (w-z)

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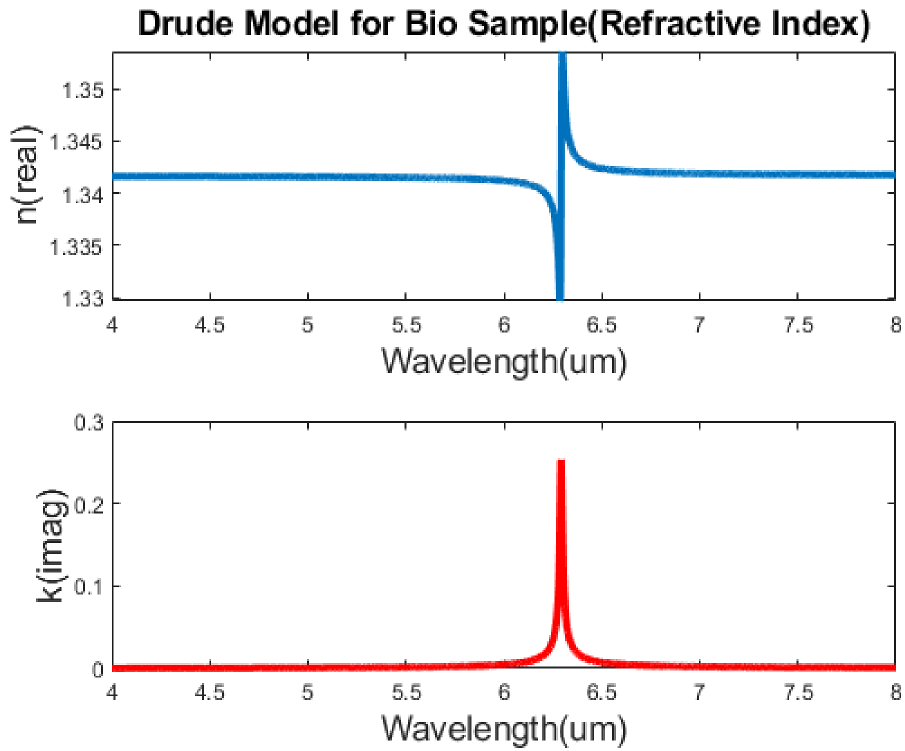
Research activity: Simulation Results

Absorbing Material model

A permittivity model for bio sample is established .The both real(n) and imaginary(k) part of the refractive index, as $n^{\sim} = n + ik$, are shown in figure.

$$\varepsilon_{\omega} = \varepsilon_{\infty} + \frac{A\omega_0^2}{\omega_0^2 - \omega^2 - i\gamma\omega}$$

Equation of Drude-Lorentz describes the relative permittivity ε_{ω} of the bio sample . An oscillator with amplitude A is used to imitate absorption. While ω_0 ,γ are eigenfrequency and damping factors, respectively. Hence, the parameters we chose to determine the permittivity of the bio sample in MATLAB, are $A = 10^{-5}, \varepsilon_{\infty} = 1.8$ and $\gamma = 2.8 \times 10^{11}$.



Research activity: Simulation Results (Parametric Analysis)

NANO Disk Period (p)= 750 nm

- Impact of Gap(G) on Absorbance peak . G varies 5nm to 35nm , D change and with fixed P and t_m

a) Influence of G on Absorbance Peak. When using the absorbed bio analyte

b)The near filed SEIRA enhancement as a function of the G

Numerical SEIRA Gain Calculations

Figures (**c-f**) illustrates the changes in reflectance spectra due to an artificial bio-Analyte.

- D varying from 745 nm ,715nm
- G varies 5 nm-35 nm,
- P is Fixed=750 nm
- t_m=30nm

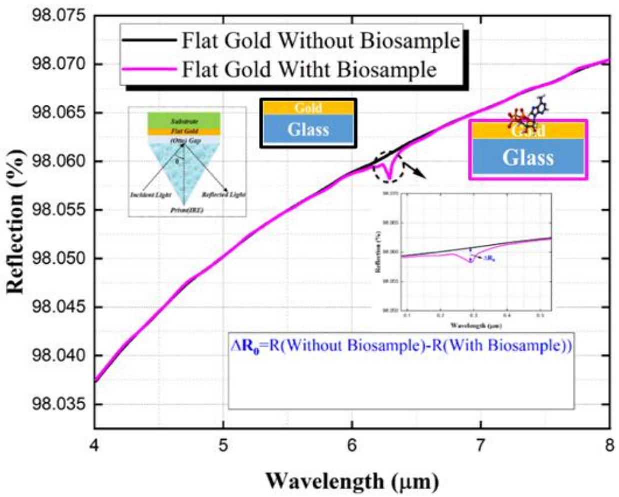
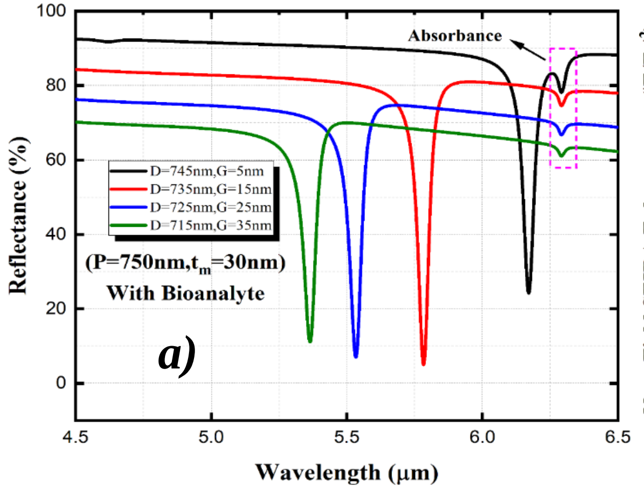
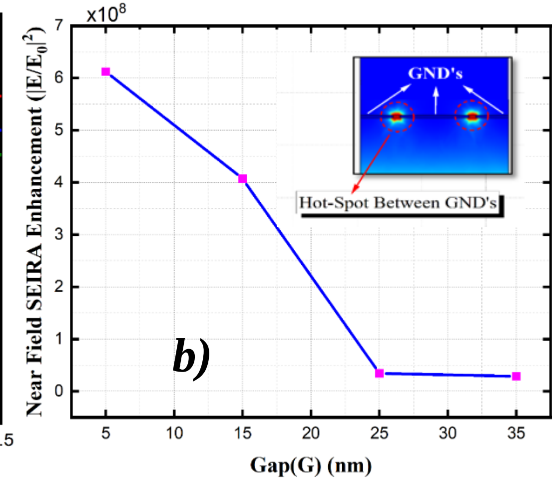
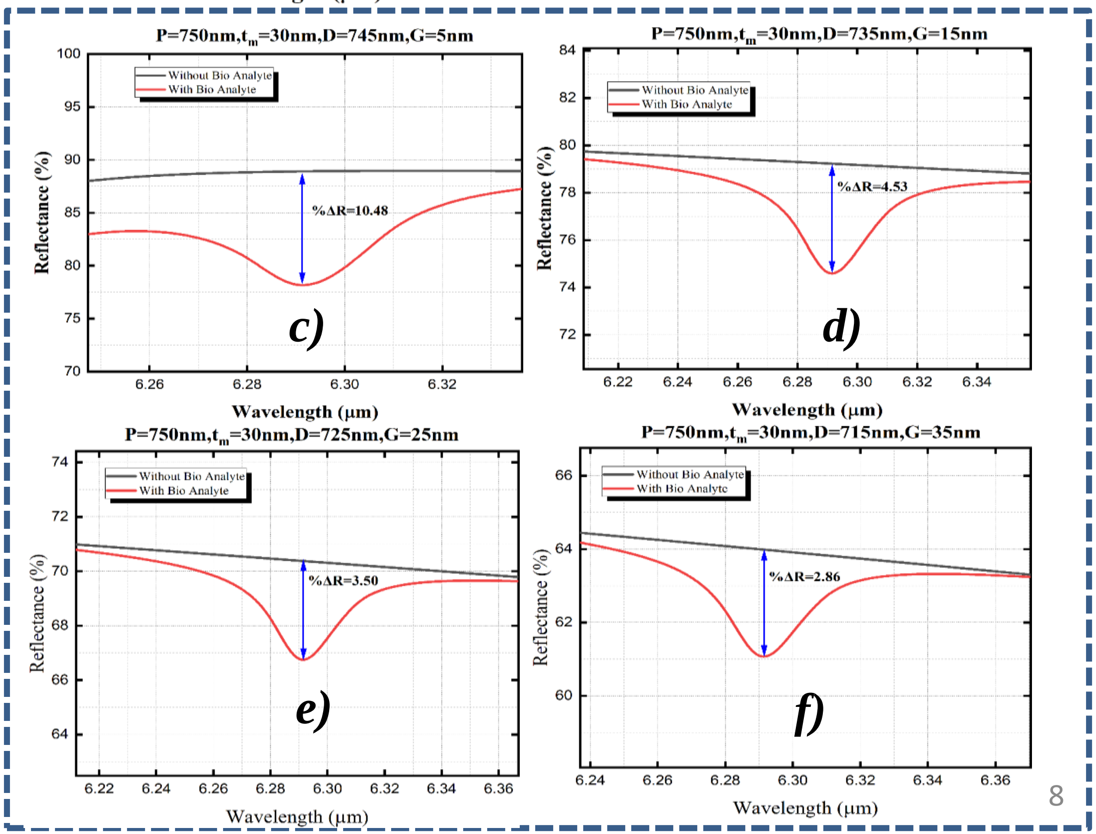


Figure: Illustration of a reference design with a thin Gold layer deposited on a Glass substrate in the presence and absence artificial bio-Analyte. Changes in reflectivity (ΔR0)







Research activity: Simulation Results (Numerical SEIRA Gain Calculations)

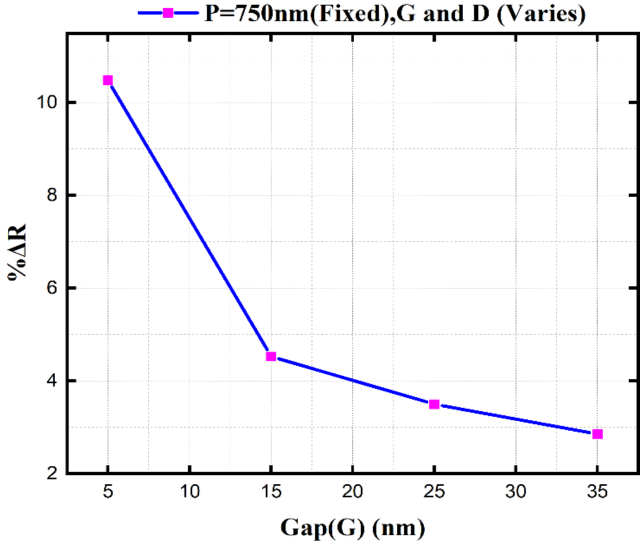
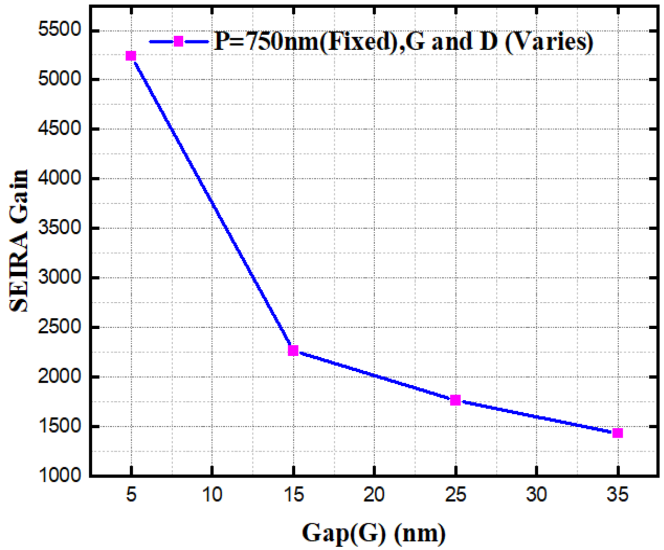
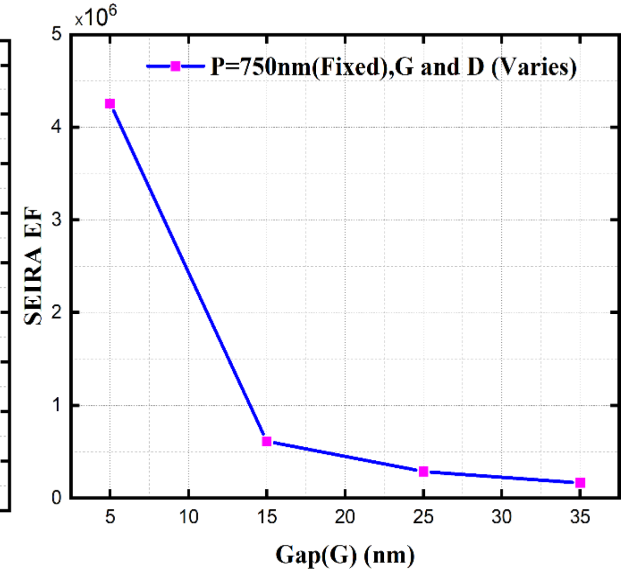


Figure illustrates the changes in reflectivity (ΔR) as a fu of the G and G varying from 5nm -35nm. P is fixed and D varies 745nm-715nm



The SEIRA gain as a function of the D , Here G and D varies. But P is fixed.



The SEIRA EF as a function of the G , Here G ,D varies. But P is fixed.

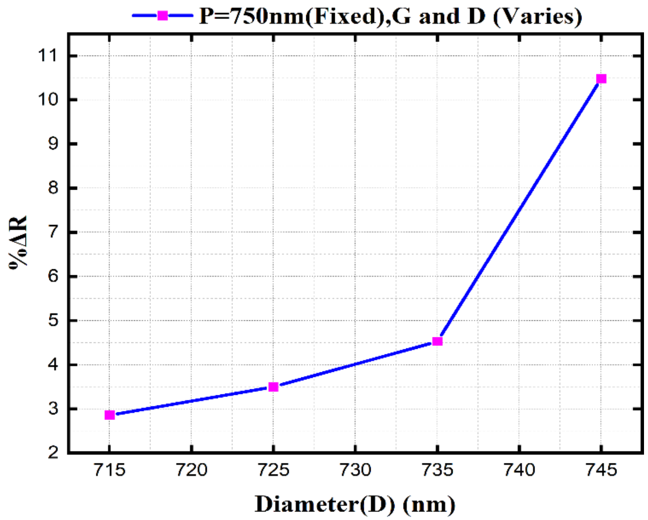
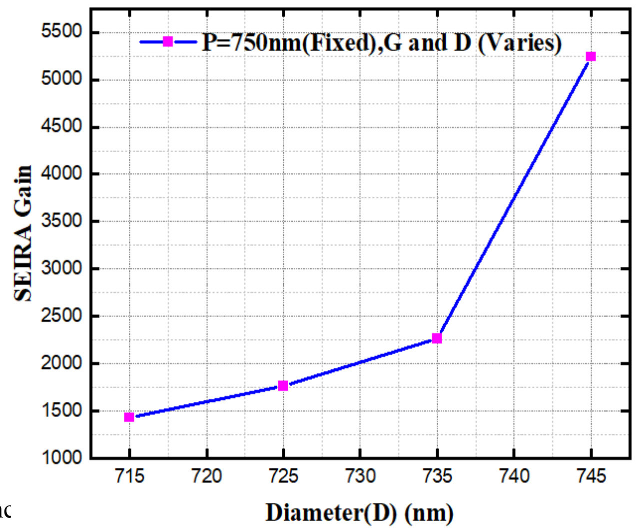
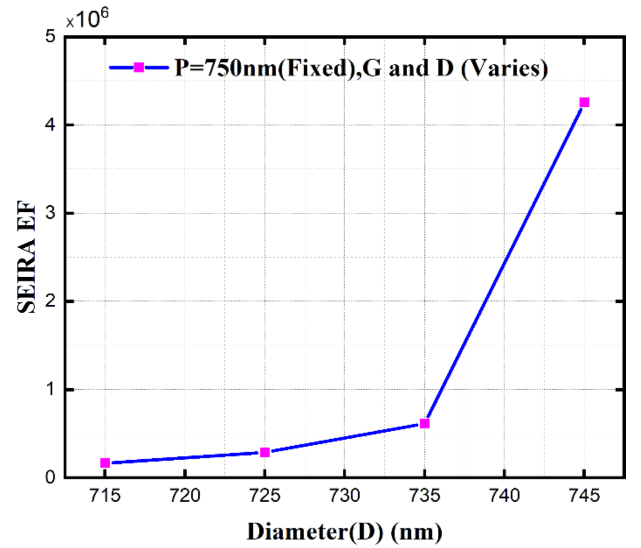


Figure illustrates the changes in reflectivity (ΔR) as a func of the D. G varying from 5nm -35nm. P is Fixed and D Varies 745nm-715nm



The SEIRA gain as a function of the D , Here G varies between GND. But P is fixed.



The SEIRA EF as a function of the D , D and G varies. But P is fixed.

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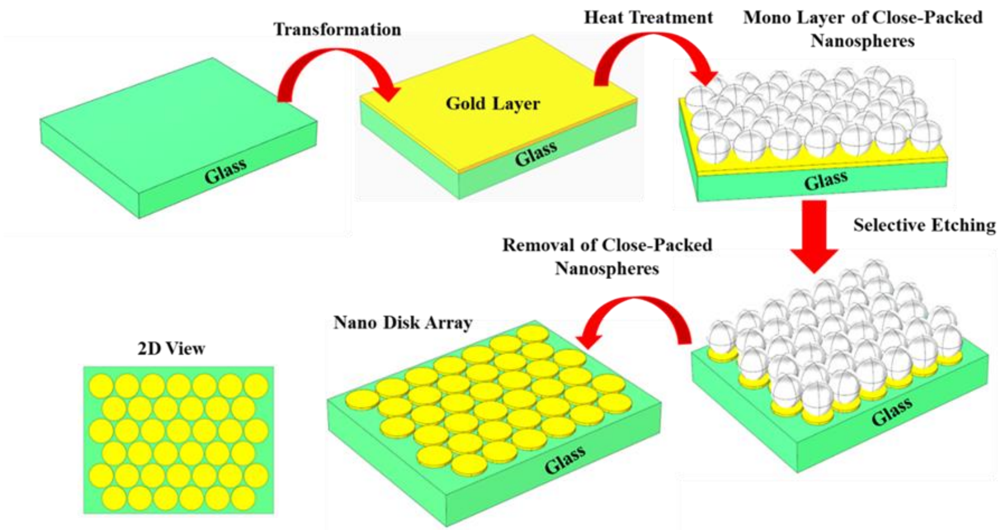
Research activity: Experiment(Fabrication and SEM Characterization)

Fig. Schematic Representing the Fabrication Procedure **(Self Assembly)**



Fabrication Samples

Diameter Nano Disk	*Thickness of Nano Disk*	*Annealing*
757nm	*30nm*	*110°c*

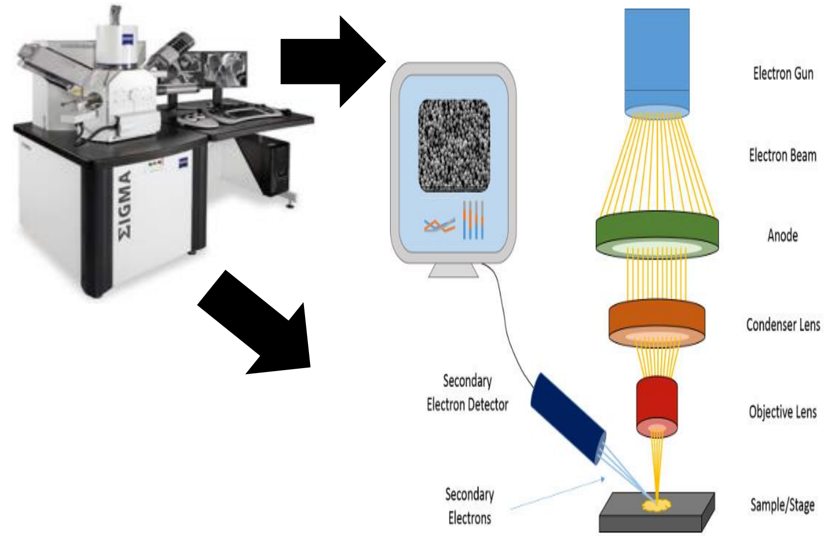
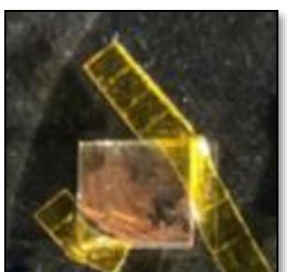


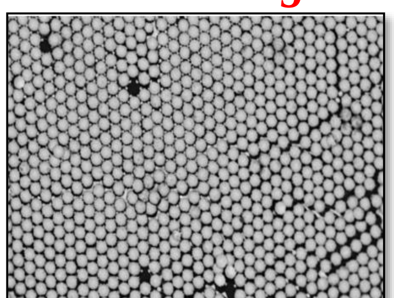
Illustration of SEM Setup

Morphological characterization by SEM

SEM Image

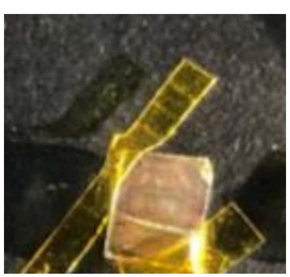
GND-A2

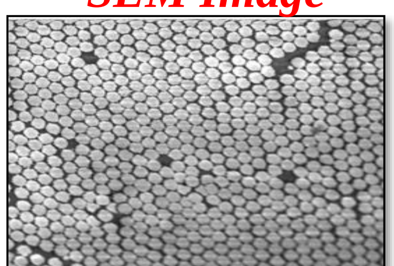




SEM Image

GND-A3





It provides detailed, topographical images, providing versatile data.

The instrument used for SEM includes these components:

- Electron source
- Anode
- Condenser lens
- Scanning coils
- Objective lens

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Research activity: Experimental characterization

Spectral characterization by ATR-FTIR

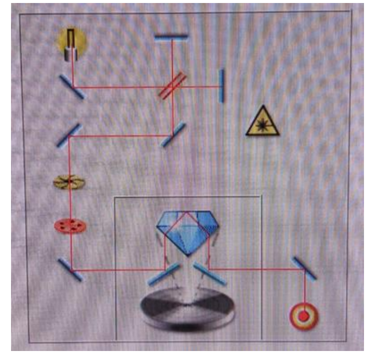
The PerkinElmer Spectrum™ 3 FT-IR spectrometer

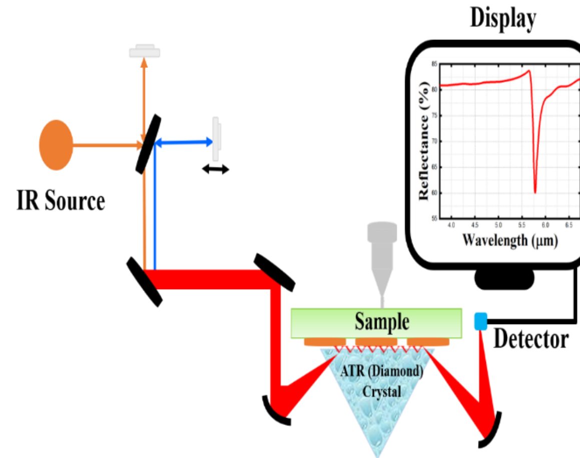
- Model: L1280127
- Wavelength range:8500 - 30 cm-1
- Exceptional signal-to-noise ratio and photometric performance
- High reproducibility of spectral data without spectral interferences
- Best-in-class sensitivity, even when using room temperature detectors
- Characterize fast reactions with scan speed up to 100 scans/sec
- Optimize sensitivity and spectral resolution performance

FTIR instrument

ATR Module

Scheme IR beam path

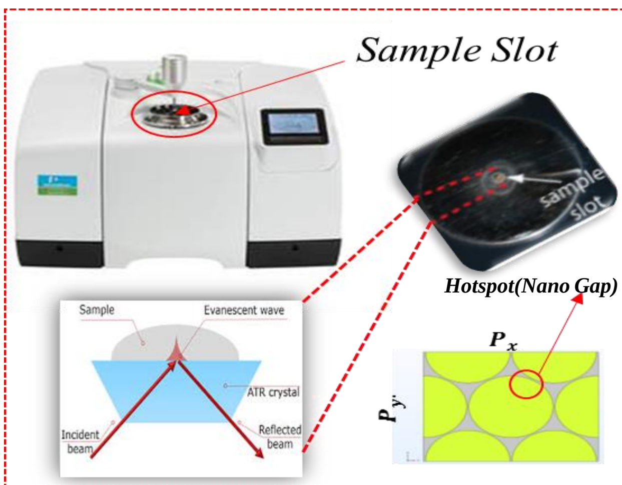




ATR-FTIR (at CeRICT SCRL-Benevento)

Instrument settings:

- Resolution=4cm^{-1}
- Measurement range = 4-7micrometers
- Measured=Reflectance
- Accumulation: 16
- CO_2/H_2O settings
- Scan speed (cm/s)=0.2
- Phase correction=magnitude
- Apodization=strong



Experimental ATR FTIR- Reflectance Spectra

$P = 772nm, t_m = 30nm, D = 757nm$

Resonance Peak at 5.78um

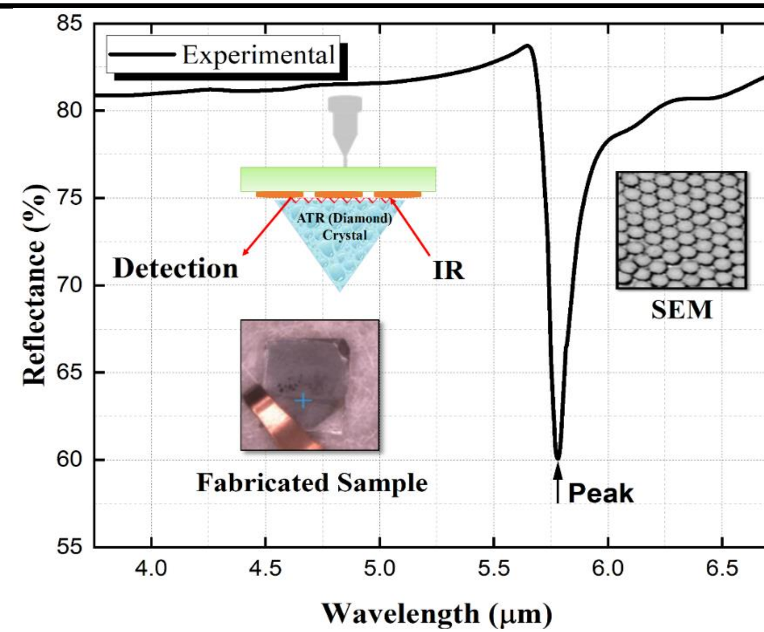
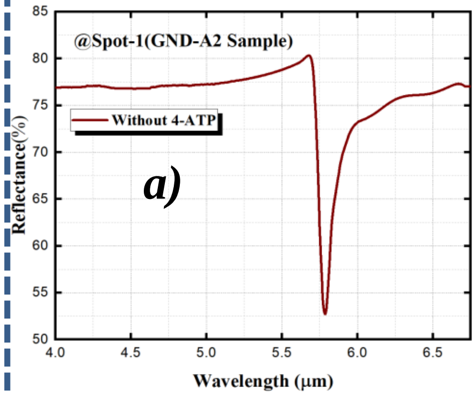
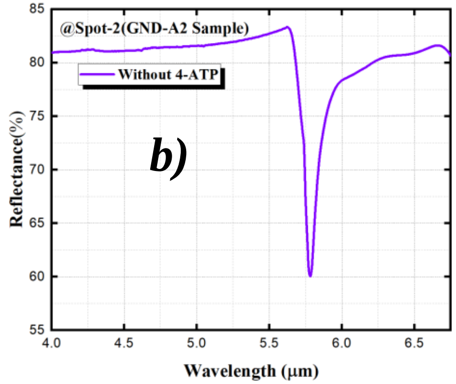


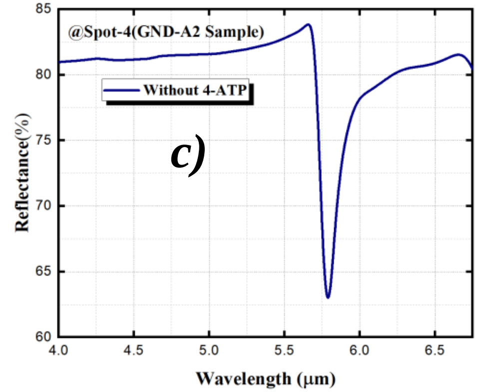
Figure ATR-FTIR reflection spectrum obtained from the fabricated sample. Reflection peak at 5.784 um (1728.91 cm^{-1}).

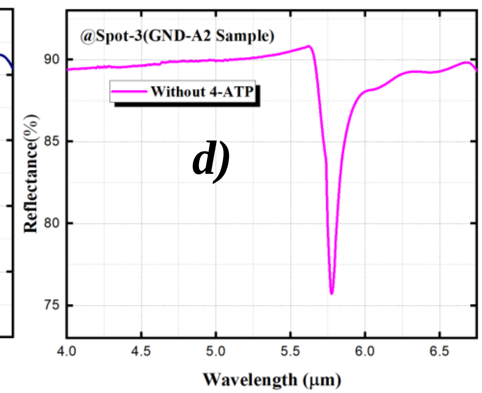
Research activity: Experimental characterization

Spectral characterization different Spots(Uniformity)

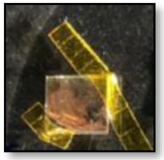


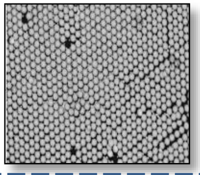




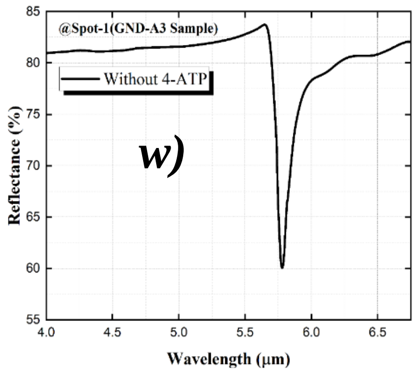


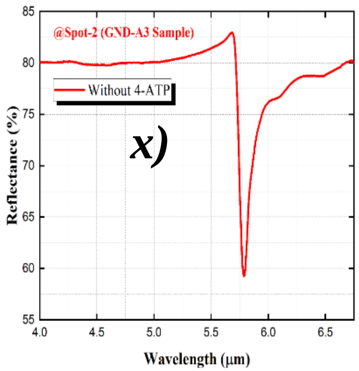
Reflectance spectra for proposed GNDs closed array-based NA (Sample-A2) at different spots without 4-ATP. We see the uniformity of between the different spot's reflection spectra in term of resonance peak (Figure (**a,b,c,d**))

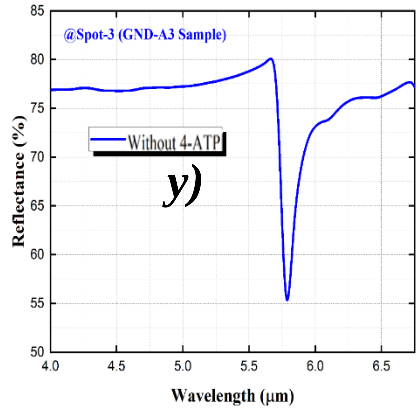




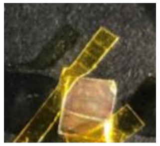
Sample-A2







Sample-A3



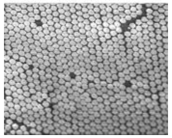
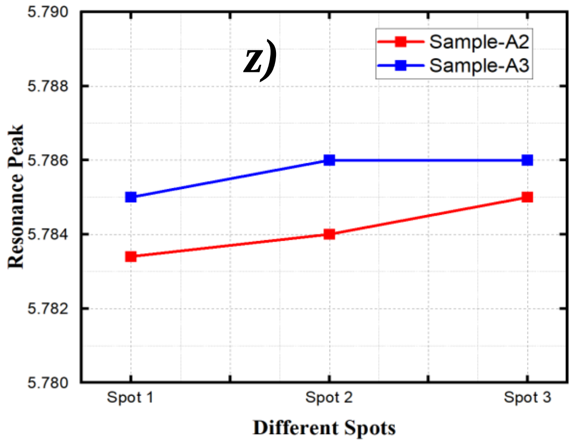


Figure (**w,x,y**)Reflectance spectra for (Sample-A3) at different spots without 4-ATP.

We see the uniformity of between the different spot's reflection spectra in term of resonance peak



Repeatability

- ATR-FTIR characterization (Sample-A2 and A3) at different spots without 4-ATP. We see the Repeatability in term of Resonance Peak for Samples.

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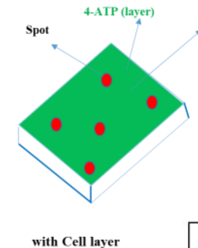
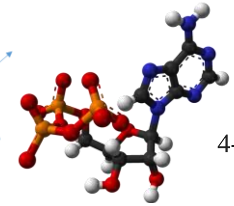
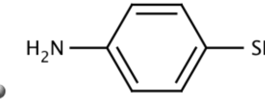
Research activity: Experimental Characterization

Monolayer Preparation on the SEIRA substrate

- Immersed in a 1 mM 4-aminothiophenol (4-ATP) (Merck-Millipore Milan, Italy) solution in ethanol for 16 hours, washed with pure ethanol to remove the excess unbound molecules, and air dried.
- Uniform self-assembling monolayer of 4-ATP (thickness=30nm), covalently bound to the NA

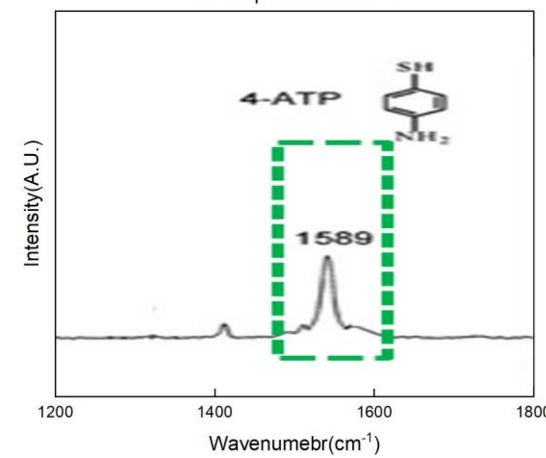




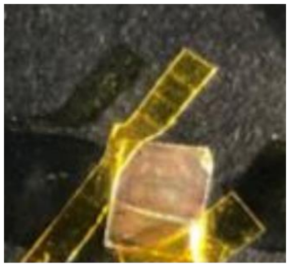


4-ATP Molecular structure

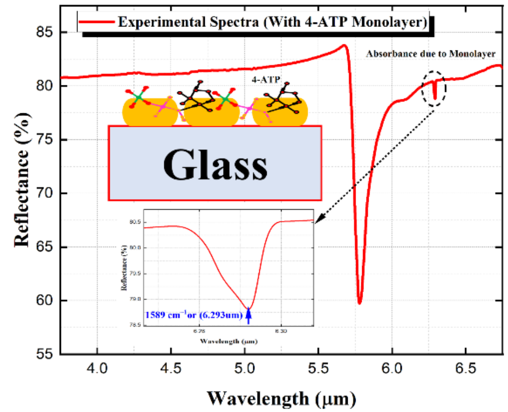
FTIR Spectra for 4-ATP



Ming Li(2012) DOI: 10.1021/ac203325z

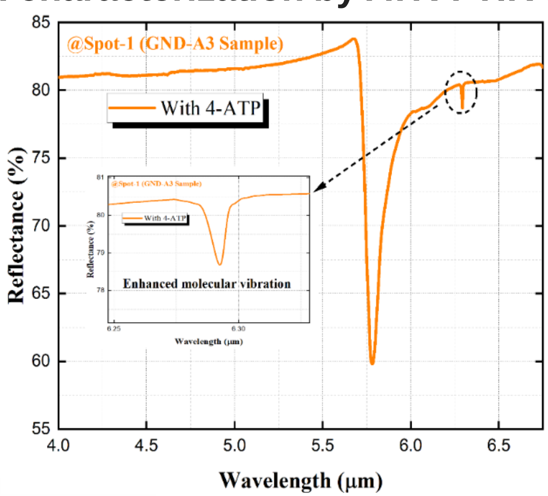


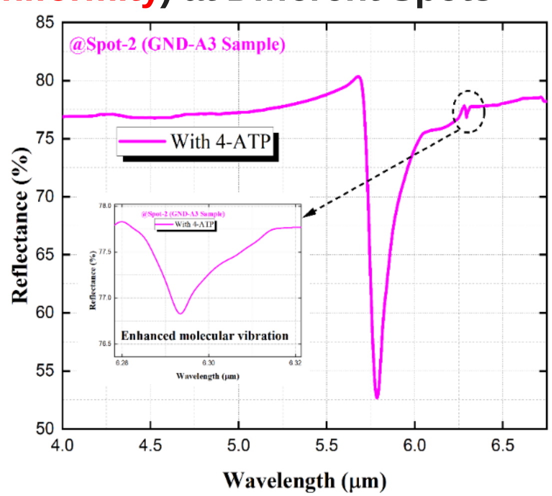
Sample with 4-ATP

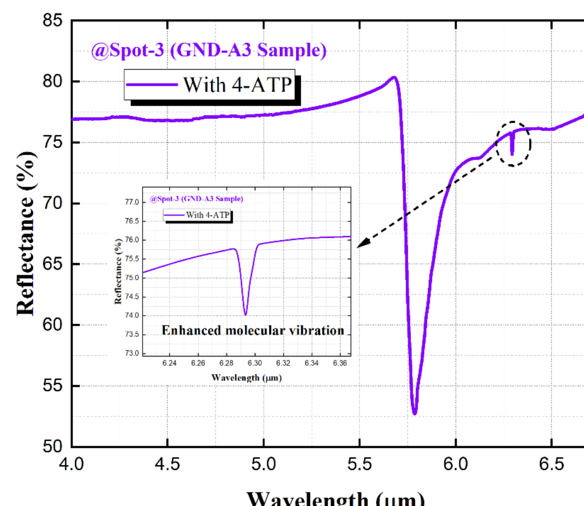


FTIR-ATR Spectra for Fabricated Sample With Bio Sample

Spectral characterization by ATR-FTIR (Uniformity) at Different Spots

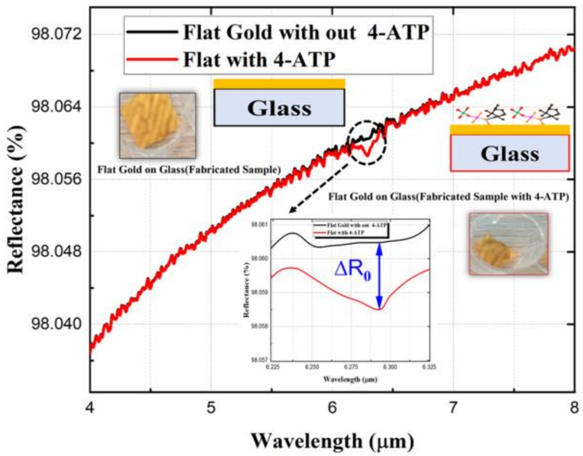






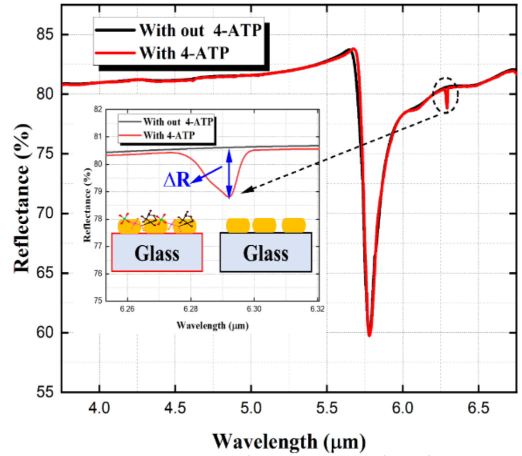
ATR-FTIR characterization in terms of reflectance spectra for our proposed GNDs closed array-based NA at three different spots with 4-ATP.

Research activity: Experimental characterization



Experimental ATR-FTIR reflection spectra for a fabricated sample with flat gold on glass (for reference) in the presence(Red) and absence (Black) of a 4-ATP monolayer.

SEIRA Enhancement Factor(EF)Calculation



Experimental ATR-FTIR reflection spectra for a fabricated sample with NA on glass in the presence(Red) and absence(Black) of a 4-ATP monolayer.

$$\text{SEIRA EF} = \frac{\Delta R}{\Delta R_0} \times \frac{A_0}{A_{SEIRA}}$$

$A_{SEIRA} = 0.0072 um^2$

$A_0 = 2.04 um^2$

$A_0 = 2 \times (\sqrt{3} \times a^2)\ ; a = D + G$ $\quad t_G = Thickness\ of\ Gap = 30nm$

$A_{SEIRA} = Number\ of\ Gaps\ in\ Unit\ Cell \times G \times t_G$

ΔR =Difference of Resonance peak with and without Molecule presence on NA
ΔR_0 =Difference of Resonance peak with and without Molecule on presence Flat Gold
A_0=Area of the Unit cell (Nano Antenna) exposed to light , *A_{SEIRA}=Effective Area of Nano Antenna(Where the filed is localized)*

$$\Delta R_0 = R_0(Without\ Substance) - R_0(With\ Substance)$$

$$\Delta R_0 = 0.002$$

$$\Delta R = R(Without\ Substance) - R(With\ Substance)$$

$$\Delta R = 1.995$$

SEIRA Gain	SEIRA EF
997.5	2.83×10^5

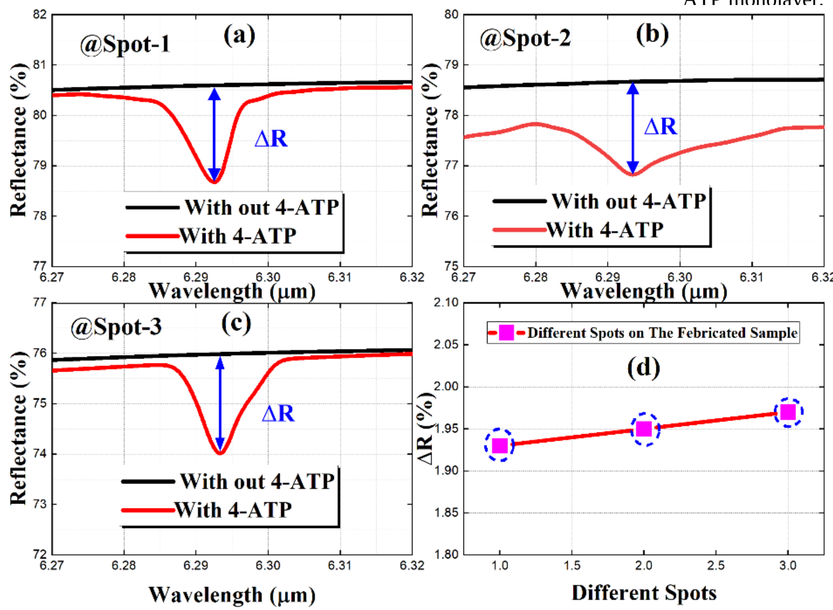
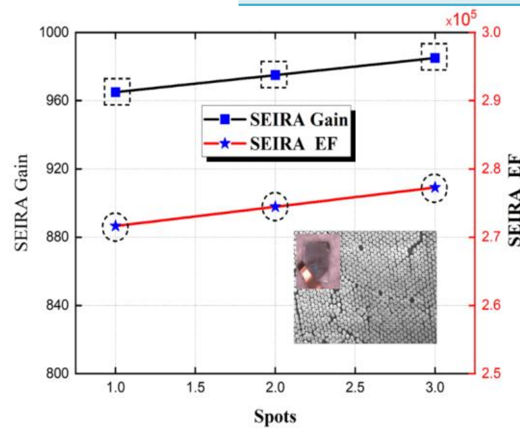
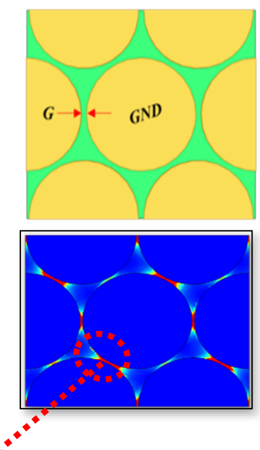


Figure (a-d) illustrates the change in reflectance spectra ΔR calculations for our proposed GNDs closed array-based NA at three different spots in the presence of 4-ATP monolayer. (d) 2D representation of changes in reflectance is ΔR as a function of different spots.



Relation of SEIRA Enhancement Factor and SEIRA Gain for Different Spots of Unit Cell



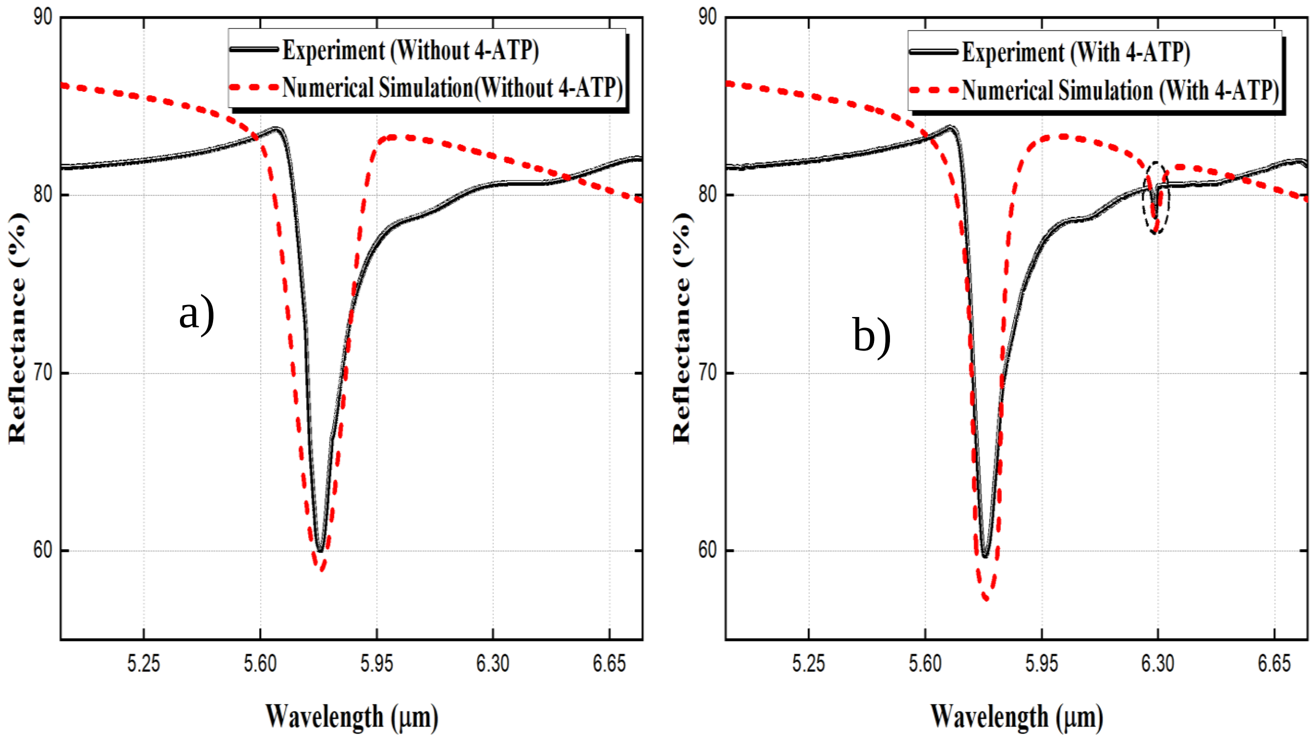
Hot-Spot at Nano gap

Name	SEIRA Gain Factor	SEIRA EF
Spot 1	993	2.72×10^5
Spot 2	995	2.75×10^5
Spot 3	997	2.77×10^5

Research activity: Experimental characterization

- **Comparison between numerical and experimental results** $P = 772nm, t_m = 30nm, D = 757nm$

Figure (a-b) represents the fitting of experimental ATR-FTIR reflection spectra with simulated spectra in the presence and absence of absorbance analyte 4-ATP monolayer.



(a) ***Without 4-ATP Reflectance (Simulation and Experiment) Spectra Fitting***

(b) ***With 4-ATP Reflectance (Simulation and Experiment) Spectra Fitting***

Conclusion

- We developed novel SEIRA substrates to be used in ATR/FTIR instruments for biological sensing applications
- The SEIRA substrate is composed of a regular pattern of gold nano disks exhibiting hotspots nanogaps.
- Numerical analysis was used to identify the geometric constraints which leads to an optimized SEIRA substrate featuring a regular pattern of nanogaps with intense hotspots
- SEIRA substrates have been fabricated by using a self-assembling approach to provide cost-effective SEIRA substrates
- ATR FTIR instruments has been used to characterize and assess the fabricated substrates performances
- SEIRA Enhancement Factor as high as 10^5 has been obtained
- Experimental results have been compared with numerical predictions showing a good matching
- Overall, the proposed SEIRA substrate can provide a viable tool to perform IR absorption spectral analysis with superior performance with respect to standard FTIR approaches

Future Prospective

- Future developments pertain to
- Use of the proposed approach to detect specific biomarkers in liquid biopsy
- Functionalize the substrate to get further boost the detection specificity


Questions


Thank You