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Reusable plasmonic biosensor for the rapid detection of *Escherichia coli* cells in potable water

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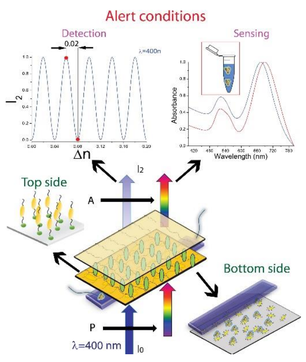
Abstract

NANO-LC project



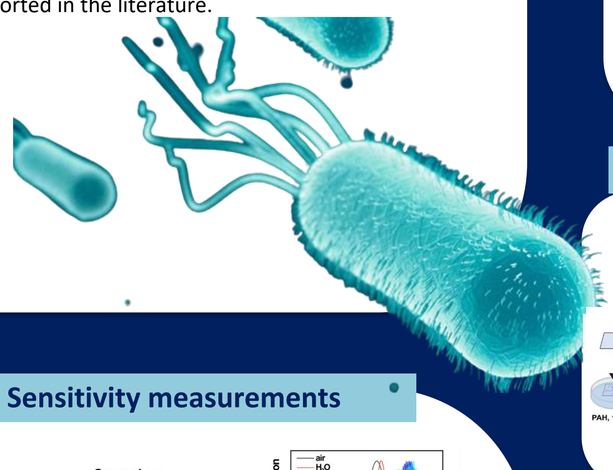
This work focuses on the fabrication and characterization of a label-free reusable localized surface plasmon resonance (LPSR) biosensor for the on-site detection of *Escherichia coli* cells in potable water.

After selecting the suitable gold nanorods (Au NR)dimensions, Au NRs are immobilized on a glass substrate through the polyelectrolyte-mediated electrostatic layer-by-layer assembly method. The morphological and optical properties of the resulting Au NR functionalized substrates are investigated, and specific studies are performed to assess the preparation protocol's repeatability and quantify the bulk sensitivity. Au NR functionalized substrates are then bioactivated by incorporating a monoclonal antibody (Ab or anti-*E. coli* 1011) for achieving the specific recognition of *E. coli*. Several techniques, including contrast phase microscopy, fluorescence microscopy, and absorption spectroscopy, demonstrate how the bioactivated Au NRs substrate exploits the high sensitivity of Au NRs to refractive index variations for *E. coli* spectroscopic detection and quantification.

In the presence of *E. coli* cells, the univocal recognition between the Ab and the *E. coli* antigens alters the local refractive index resulting in a variation of the LSPR wavelength. Therefore, a specific biochemical phenomenon is converted into an optical signal that a UV-Vis spectrophotometer promptly reveals.

The proposed biosensing nanoplatform shows a detection limit of 8.4 CFU/mL, which is one order of magnitude lower than other LSPR-based biosensors reported in the literature.

More importantly, it shows a low environmental impact. As Au NRs are excellent light-to-heat transducers, the multicolor thermo-plasmonic properties of the Au NRs-based substrates are here exploited to promote the photothermal substrate disinfection carried out after the biosensing experiment. Following the photothermal disinfection, a suitable washing step allows to fully recover the bioactive substrate's spectroscopic properties, thus enabling the biosensor reuse.



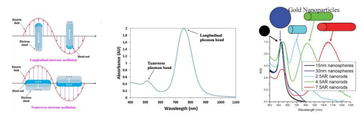
State of art

Bioweapons

***E. coli*: indicator of drinking water quality**

Counting bacteria

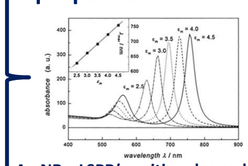
Au NRs



Gans theory

$$\sigma_{abs} = \frac{\omega}{3c}\varepsilon_m^{3/2} V \sum_j \frac{\left(1/P_j^2\right)\varepsilon_2}{\left[\varepsilon_1 + \left\{\left(1-P_j\right)/P_j\right\}\varepsilon_m\right]^2 + \varepsilon_2^2},$$

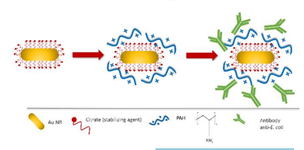
Biosensing properties

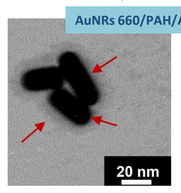


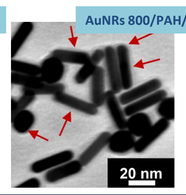
Au NRs: LSPR/ position depends from the refractive index

Selection of suitable AuNRs dimensions for the biosensor fabrication

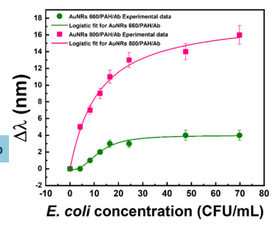
AuNR bioconjugation



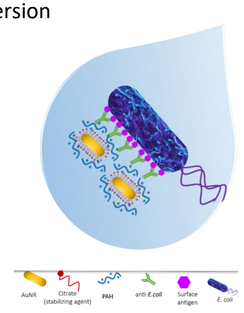




Recognition of *E. coli* cells in water dispersion

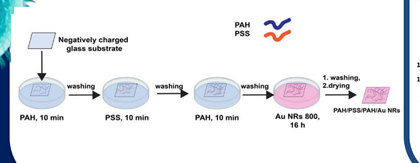


IC_{10} values: 5.07 CFU/mL for Au NR 660/PAH/Ab; **1.4 CFU/ mL** for Au NR 800/PAH/Ab.

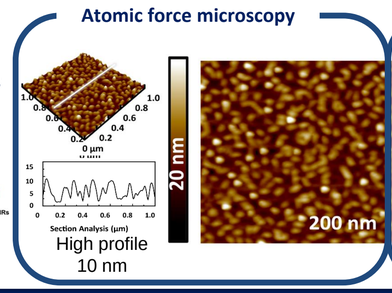


Fabrication and characterization of AuNRs 800 functionalized substrates

Electrostatic Layer-by-layer assembly: G/PAH/PSS/PAH/AuNRs

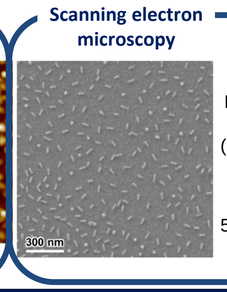


Atomic force microscopy



High profile 10 nm

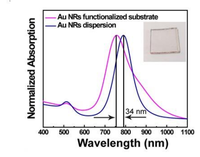
Scanning electron microscopy



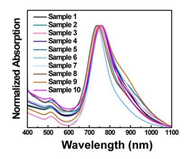
Interparticle distance (142±50) nm

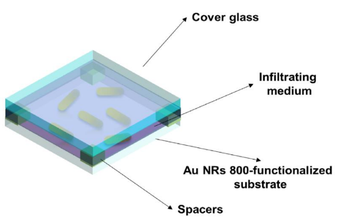
Fill fraction 5.9% ± 0.2%

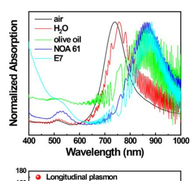
Optical characterization of AuNRs 800 functionlized substrates

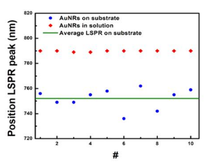


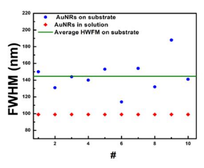
Sensitivity measurements

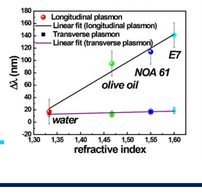








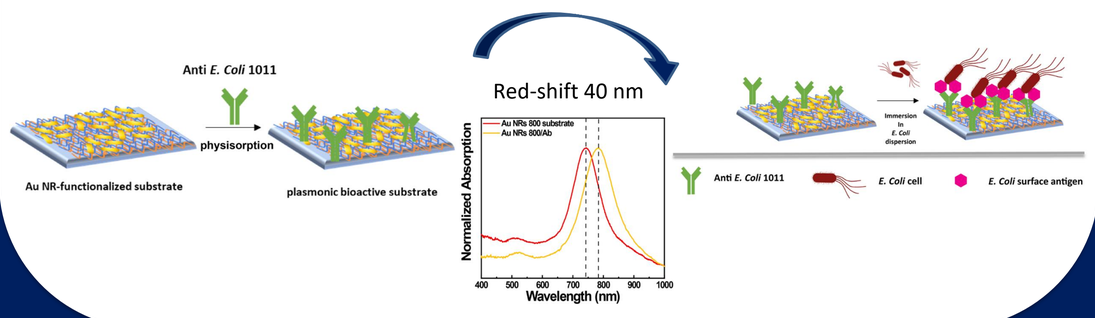




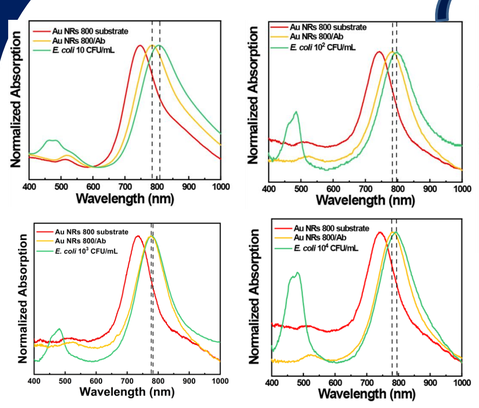
Sensitivity= 456 nm/RIU

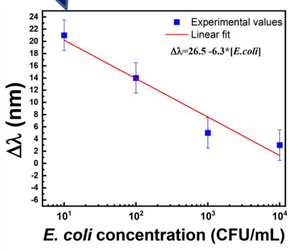
FOM = S/FHMW = 3.1

Fabrication and characterization of biologically-active AuNRs functionalized substrates



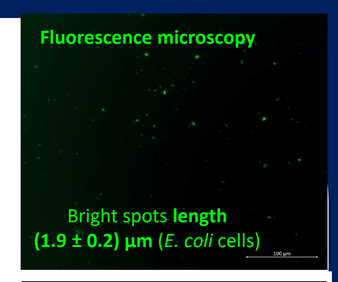
Detection of *E. coli* cells

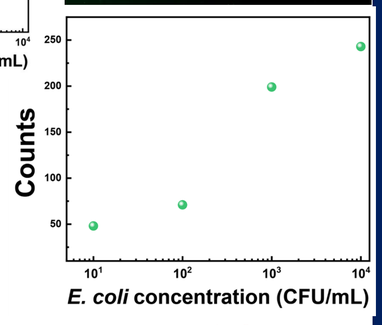




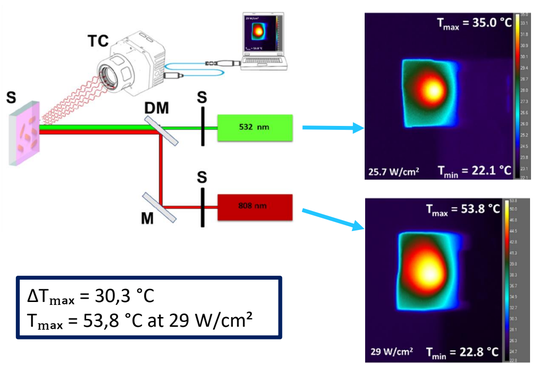
LOD = 8,4 CFU/mL

Fluorescence microscopy

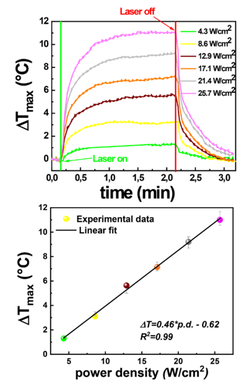




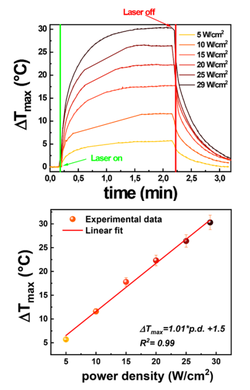
Multicolor photothermal disinfection



ΔT_{max} = 30,3 °C
T_{max} = 53,8 °C at 29 W/cm²

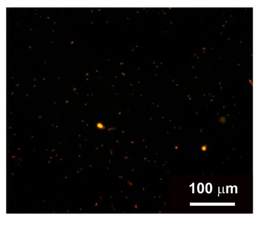


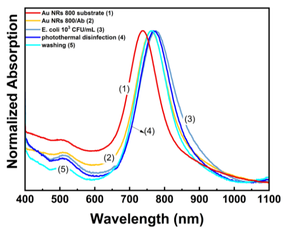
CW laser beam 532 nm



CW laser beam 808 nm

dead *E. coli* cells





Reusability

Conclusions

- **Label-free LSPR biosensor for the detection of *E. coli* cells in potable water.**
- **Selective recognition of *E. coli* cells through the antigen/antibody interaction that results in the change of the local refractive index and thus in the variation of the LSPR/ position. The peak shift value is proportional to the cell concentration.**
- **High sensitivity (456 nm/RIU) and low LOD (8.4 CFU/mL).**
- **Time response in the minutes range.**
- **Thermoplasmonic heating: 53.8 °C, suitable to inactivate the *E. coli* cells.**
- **Reusable biosensor via multicolor thermoplasmonic disinfection and a suitable washing procedure.**

Ackowledgements

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End user:

The end-user is continuously assisting the consortium during the research and development process. In addition, it helps to share the results with defence customers and other relevant industrial partners. The end-user is helping to drive the technology towards higher TRLs and potentially taking care of the future manufacturing process of the NANO-LC biosensor.

Bibliography

F. Petronella, D. De Biase, F. Zaccagnini, V. Verrina, S.I. Lim, K.U. Jeong, S. Miglietta, V. Petrozza, V. Scognamiglio, N. P. Godman, D. R. Evans, M. McConney and L. De Sio, **Label-free and reusable antibody-functionalized gold nanorod arrays for the rapid detection of *Escherichia coli* cells in a water dispersion,** *Environmental Science: Nano, 2022,* https://doi.org/10.1039/D2EN00564F

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