PhD Course: Physics and Nanosciences, curriculum Physics (XXXIX cycle)

PhD Student: Ilaria Micol Baldi

Supervisor: Francesco De Angelis (IIT)

Tutors: Maria Blanco Formoso (IIT), Kirill Khabarov (IIT)

PhD SECOND YEAR REPORT – September 2025

Research activity

The main goal of my Ph.D. research project, belonging to Nanotechnology and Biophysics areas and realized at the Italian Institute of Technology (Center for Convergent Technologies, CTT), is the high-sensitive identification of biomolecular sequences, as proteins and nucleic acids, by inducing their translocations through plasmonic nanopores and collecting light–matter interactions as optical spectra via ultrafast Raman spectroscopy[1, 2].

During the second year, I focused my attention on optimizing our devices at the nanoscale and performing experiments based on SERS, in order to detect translocation events of biomolecules.

Our nanodevices are commercial Si_3N_4 solid-state membranes with a central 500x500 μm^2 window on top of the Si_3N_4 1x1 cm^2 frame. After coating with Au 20 nm, I milled them by FIB, to get a nanoarray, composed precisely of 10 pores (500 nm in diameter) with a triangle disposition.

To achieve a better resolution in the molecular sequence identification, we increased the translocation dwell time, by reducing the nanopore's diameter until reaching the cross-section size of DNA and proteins, which upon linearization and unfolding process is around 1-2 nm.

With this aim, I've explored the application of agarose, a natural polymer which, by forming in water a 3D gel matrix, allows the free passage of biomolecules through its pores and channels[3] (**Fig. 1**). Specifically, I put 1% liquid agarose on the groove cavity of the membrane (backside), by successfully reducing the holes' diameter up to 3.8 nm of average size[4].

After that, to reduce water adsorption and improve adhesion between sputtered metals and dielectric surfaces, as Si_3N_4, I deposited the photolithographic agent hexamethyldisilazane (HMDS)[5] on the membrane (topside). Then, to induce better the SERS effect, I coated further the surface with Ti 3 nm and Ag 20 nm.

With these advanced strategies, I obtained efficient devices, characterized by a polycrystalline structure with nanogaps, due to Ag granules and the agarose porosity[4], exhibiting plasmonic properties for biomolecular detection (**Fig. 2**).

Initially, I performed electric current measurements as positive control to assess the presence of translocation events.

Then, I've passed the specific training for confocal Raman microscope (InVia Renishaw®), equipped with a SPAD camera (64×32 pixels), of our laboratory, to exploit Surface-Enhanced Raman Spectroscopy (SERS) which, by inducing local enhancement of electromagnetic fields (plasmonic hotspots), intensifies the Raman scattering of molecules[4] adsorbed on metallic nanostructures.

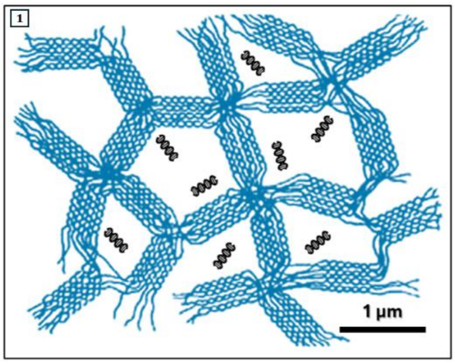
In particular, we have merged SERS with the application of a very low electrical bias (30 mV)[4] to detect and distinguish low-abundance single DNA nucleosides (1 nM).

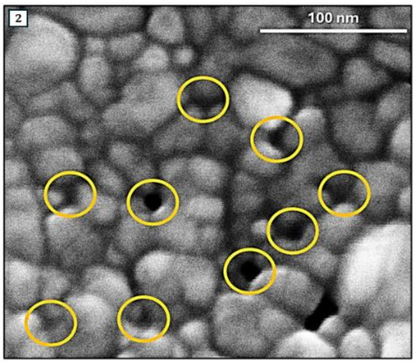
In our typical experiment, a bias voltage, supplied by an external source, is applied to a solution, containing linearized molecules, which are forced to move through the nanopore of the fixed metallic device. The laser source is placed exactly on the plasmonic hotspots to provide the SERS effect (**Fig. 3**). At the end, the optical signal is collected by a SPAD camera and converted to digital data by a computer.

I've carried out these measurements, by exploiting 532 nm laser radiation and 63x dipping objective on our optimized plasmonic nanodevices[4]. As biological sample, I considered very short molecules to limit the variability of the experiments and facilitate the optical signal interpretation, like single DNA nucleosides (adenosine, guanosine, thymidine and cytidine), diluted to the concentration of 1 nM in a NaCl 150 mM buffer solution[4].

The successful acquirement of the average spectra related to 1 nM single nucleosides at a 20 μs time resolution[4], demonstrated the efficiency and feasibility of our approach in discriminating biomolecules, based on their Raman fingerprints.

Because of similar size and translocation behaviour of nucleosides and amino acids, next step will be the application of our optical method to identify protein sequences[4].





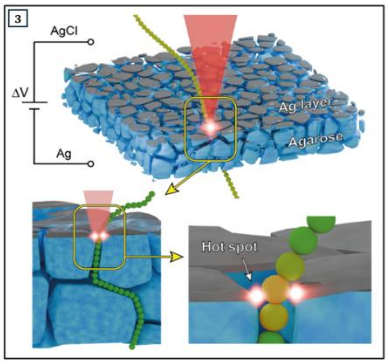


Fig. 1 (*left*). Scheme of 3D agarose matrix, allowing the free passage of biomolecules through its pores and channels.
Fig. 2 (*centre*). SEM image of Ag polycrystalline structure with nanopores.
Fig. 3 (*right*). Nanodevice, coated on top with Ag, showing the passage of a linear molecule through a plasmonic hot spot.

References

1. Zhao Y., et al. "Plasmonic Bowl-Shaped Nanopore for Raman Detection of Single DNA Molecules in Flow-Through". *Nano Letters*. Vol. 23 (2023).

2. Iarossi M., et al. "High-Density Plasmonic Nanopores for DNA Sensing at Ultra-Low Concentrations by Plasmon-Enhanced Raman Spectroscopy". *Advanced Functional Materials*. Vol. 33, p. 2301934 (2023).

3. Jun-Ying Xiong J.Y., Narayanan J, Liu X.Y., Chong T.K., Chen S.B., and Chung T.S. Topology Evolution and Gelation Mechanism of Agarose Gel. *The Journal of Physical Chemistry B*. Vol 109 (12), 5638-5643 (2005).

4. Khabarov K., Blanco-Formoso M., Baldi I. M., Khozeymeh Sarbishe F., Marongiu R., Bruno G., Bhandari B., Storari V., Haka H., Dipalo M., Canepa P., Gentile F., Goldman N., Villa F., Tantussi F., and De Angelis F. Raman identification of single nucleotides flowing through permeable plasmonic films. *Nature Communications* (2025).

5. Vishnevskiy A., Vorotyntsev D., Seregin D., and Vorotilov K.. Effect of surface hydrophobisation on the properties of a microporous phenylene-bridged organosilicate film. *Journal of Non-Crystalline Solids*. Vol 576 (2022).

Attended PhD courses and approved exams

- *Nanophotonics and Nanofabrication* [Prof. Maria Caterina Giordano (UniGe), 3 CFU]
- *Optics for Microscopy and Spectroscopy* [Dr. Alessandro Zunino and Dr. Eli Slenders (UniGe - IIT), 1.8 CFU]
- *Atomic Force Spectroscopy* [Prof. Annalisa Relini (UniGe), 3 CFU]

- -

- *Biosensing* [Prof. Elena Angeli, Paolo Canepa and Ornella Cavalleri (UniGe), 3 CFU] -> APPROVED on 28th October 2024
- *Microscopic and spectroscopic techniques for the analysis of surfaces and interfaces* [Prof. Letizia Savio (UniGe), Renato Buzio (CNR) and Andrea Gerbi (CNR), 3 CFU] -> APPROVED on 10th February 2025
- *Optics for Microscopy and Spectroscopy* [Dr. Alessandro Zunino and Dr. Eli Slenders (UniGe - IIT), 2 CFU] -> APPROVED on 6th May 2025 (1.8 CFU) + 2h LAB VISIT on 29th May 2025 (0.2 CFU)

Schools

- *BIOCUBE 3rd edition* – International Winter School on Bioelectronics, Biophotonics and Biomechanics (Sestriere Italy, 9th-15th December 2024] -> POSTER PRESENTATION "SERS based DNA Sequencing on Permeable Plasmonic Membranes"

Conferences

- *NANOSERIES 4th edition* – Global Conference on Nanotechnology (Valencia Spain, 17th-20th June 2025) -> POSTER PRESENTATION "DNA Sequencing via SERS Technique on Permeable Plasmonic Membranes"
  Award winner of:
  - **"NanoSeries Innovation Foundry" - Best Poster Award**
  - **"Small Structures (Wiley-VCH)" - Best Poster Award**

Publications

Khozeymeh Sarbishe F., Khabarov K., Blanco-Formoso M., ***Baldi I. M.***, Storari V., Haka H., Mastrangeli M., Difato F., Armirotti A., Villa F., Tantussi F., and De Angelis F. High-Speed Raman Readout of Single Polypeptides Via Plasmonic Nanopores (2025). Advanced Materials.

Khabarov K., Blanco-Formoso M., ***Baldi I. M.***, Khozeymeh Sarbishe F., Marongiu R., Bruno G., Bhandari B., Storari V., Haka H., Dipalo M., Canepa P., Gentile F., Goldman N., Villa F., Tantussi F., and De Angelis F. Raman identification of single nucleotides flowing through permeable plasmonic films (2025). Nature Communications.