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)

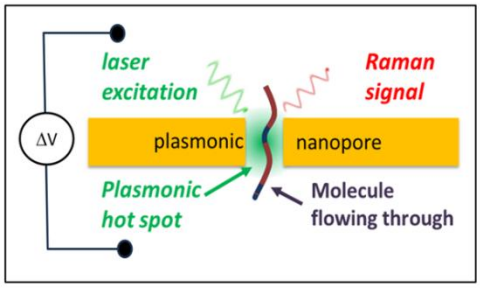
PhD FIRST YEAR REPORT – September 2024

Research activity

My Ph.D. research project lies in Nanotechnology and Biophysics areas. Specifically, it's focused on exploiting nanostructures featured by surface plasmons, which are collective electron oscillations confined at the dielectric–metal interface.
The main purpose is to identify with extremely high sensitivity the sequence of biomolecules, like proteins and nucleic acids, by forcing their translocations through plasmonic nanopores and collecting light–matter interactions as optical spectra via ultrafast Raman spectroscopy (**Fig. 1**).
Proteins and nucleic acids are biopolymers, composed of repeated amino acids or nucleotides, respectively, that play an essential role in many biological processes, including metabolism, DNA replication, and cell signaling[1]. The sequencing of these biomolecules is crucial for identifying responsible mutations for a wide spectrum of genetic and metabolic diseases, and therefore speed up and improve their diagnosis, treatment, and prevention.
During the first year, I focused my activities on preparing devices at the nanoscale and performing experiments, based on electrical measurements, with the aim of distinguishing translocation events of biological molecules.
To perform the hole milling of our nanodevices I've passed various trainings in the Clean Room facility at the Italian Institute of Technology (Center for Convergent Technologies, CTT):
- Dual Beam system (FEI®HeliosTM NanoLabTM 650), including Focused Ion Beam (FIB) together with Scanning Electron Microscope (SEM), to make patterning on the surface of samples and for visualising the surface respectively;
- Oxygen-based Plasma Cleaner (Gambetti® Tucano Plasma Reactor) for the removal of organic impurities on the samples' surfaces;
- Sputtering machine (Quorum® Q150T) to coat our devices with metal layers as Au;
- Profilometer (Veeco® Dektat 150 Surface Profiler) to estimate the thickness of metal layers on the devices.

The Dual Beam system, composed of an ion column and an electron one, combines the FIB-milling with high resolution of SEM. In particular, the first one allows to produce accurately holes or cavities on a surface, by removing materials at the desired location via beam of accelerated liquid gallium ions. The second one, instead, is used to control the precision level and get images of nanostructured samples[2].
In particular, our devices are commercial solid-state membranes, composed of silicon nitride (Si_3N_4) and having a 500x500 μm^2 window, centered on top of a Si_3N_4 frame, having a 1x1 cm^2 area (**Fig. 2A**). After coating the surface with gold (Au), I've carried out the FIB milling, producing single or multiple arrays (**Fig. 2B**) of pores with 500 nm in diameter (**Fig. 2C**), as main way to allow the translocation of molecules.
Then, I have proceeded to learn working principles and applications of the patch clamp, a well-known and developed method, as propaedeutic approach to study and detect translocation events of biomolecules through solid state nanopores in a very fast, easy and low-cost way.
This technique, based specifically on electric current measurements, applies an electrical bias across the membrane, which separates two chambers of a Teflon holder containing an electrolyte solution. Ions flow freely through the pore, producing a constant open pore current. When biomolecules translocate through the pore from one side to the other under the influence of the electric field, they induce a temporary blockage of the ionic current level, which can be monitored by the instrument[3].
To carry out these experiments, I have exploited different biological molecules, such as the protein Bovine Serum Albumin (BSA), and DNA molecules like the plasmid pBR322 and the virus bacteriophage λ, at different concentrations and buffers, and by applying different voltages, between 100 and 500 mV.
In our experiments, patch clamp was used as positive control even though the presence of disadvantages, as limited discrimination power, limited bandwidth (fast molecular translocation), and limited capture rate (low molar sensitivity). The next step will be the application of optical sensing, which benefits from the utilization of plasmonic nanostructures to exploit local enhancement of electromagnetic fields, as plasmonic hotspots[4]. With this aim, I have

started to use Surface-Enhanced Raman Spectroscopy (SERS) to identify and analyse biological sequences with unmet sensitivity level. SERS is a surface-sensitive technique that enhances Raman scattering by molecules adsorbed on rough metal surfaces or by nanostructures that are used to detect the presence of low-abundance biomolecules in biological systems.



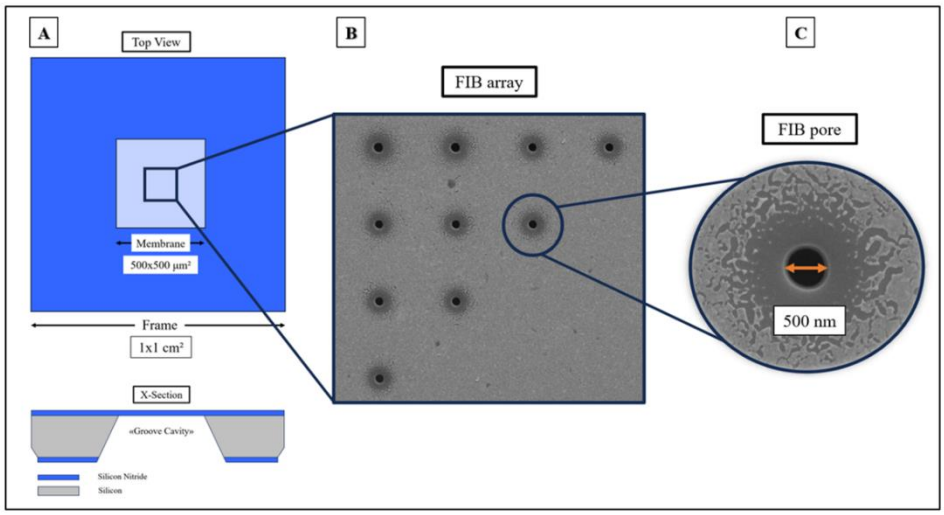


Fig.1 (*left*). Translocation events of biomolecules through a plasmonic nanopore.
Fig.2 (*right*). Schematic representation of a Si_3N_4 device (A), multiple array (B) and pores' diameter (C), performed at FIB.

References

1. Tagami S., Why we are made of proteins and nucleic acids: Structural biology views on extraterrestrial life. *Biophysics and Physicobiology*. Vol. 20 (2023).

2. Faber T., McConville J.T, and Lamprecht A., Focused ion beam-scanning electron microscopy provides novel insights of drug delivery phenomena. *Journal of Controlled Release*. Vol. 366, p. 312-327 (2024).

3. Xue L., Yamazaki H., Ren R., Wanunu M., Ivanov A. P., and Edel J. B., Solid-state nanopore sensors. *Nature Reviews Materials*. Vol. 5 Issue 12, p.931-951 (2020).

4. Li W., Zhou J., Maccaferri N., Krahne R., Wang K. and Garoli D., Enhanced Optical Spectroscopy for Multiplexed DNA and Protein-Sequencing with Plasmonic Nanopores: Challenges and Prospects. *Analytical Chemistry*. Vol. 94, p.503−514 (2022).

Attended PhD courses

- *Applied Cryogenics* [Prof. Riccardo Musenich (UniGe), 3 CFU]
- *Biosensing* [Prof. Elena Angeli, Paolo Canepa and Ornella Cavalleri (UniGe), 3 CFU] -> exam in 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].

Schools

- *BIOCUBE 2nd edition* – International Winter School on Bioelectronics, Biophotonics and Biomechanics (Sestriere, 10th-16th December 2023] -> APPROVED on 19th July 2024

Workshops

- *8th NIC@IIT Nanoscopy 2.0 – Workshop on Advanced Microscopy* (Genoa, 27th November-1st December 2023)
- *PLASMONICA 2024 – Workshop on Plasmonics and Nano-optics* (Messina, 9th-12th July 2024).

Publication (in progress)

Khabarov K., Baldi I. M., Blanco-Formoso M., Khozeymeh F., Tantussi F., and De Angelis F. *Single nucleotide identification in hybrid electrophoretic SERS on permeable plasmonic membranes*; 2024. In preparation.