# *PhD in Physics and Nanoscience-XXXVII Cycle*

## 2nd Year Report

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*Supervisors*: Prof.ssa Ornella Cavalleri, Dr. Paolo Canepa

*Reasearch activity*:

During the first year of my Ph.D., I investigated the self-assembly of thiolated single-stranded DNA molecules (probe DNA, pDNA) on gold substrates, followed by the insertion of a molecular spacer (mercaptoexanol, MCH) and the detection of the complementary sequence (target sequence, tDNA). My research focussed on using the RdRp/Helicase of Sars-CoV-2 as the target sequence. This study was performed by coupling different methods, Spectroscopic Ellipsometry (SE), Surface Plasmon Enhanced Ellipsometry (SPEE), X-Ray Photoemission Spectroscopy (XPS), and Atomic Force Microscopy (AFM).
In this second year, I continued the study of uniform self-assembled monolayers (SAMs) and started developing a patterned functionalized interface, paving the way for a multiplex sensing platform.

***Uniform sensing platforms:***
During the second year, I continued the study of this sensing platform by testing the system response using different concentrations of tDNA, for the two optical methods (SE and SPEE). For both techniques, I obtained a sensitivity calibration curve. SPEE resulted to be a more sensitive method compared to SE, as expected since this technique couples SE with Surface Plasmon Resonance (SPR), allowing the detection down to 1nM.
I performed Quartz Crystal Microbalance with Dissipation (QCM-D) experiments in collaboration with Dr. Silvia Dante (IIT, Genova). From the QCM-D analysis I could derive information about the surface density of pDNA molecules on the surface and the hybridization efficiency. By coupling QCM-D measurements with real time SE analysis, I could demonstrate the reusability of the sensing platform and its selectivity (capability to discriminate between different coronaviruses).
In collaboration with Prof. Karsten Hinrichs at the ISAS Institute in Berlin, I performed IRSE experiments together with Vis-Raman (in collaboration with Dr. Joerg Rappich from HZB) and UV-Raman (with Dr. Julian Plaickner from HZB). UV-Raman measurements were performed also for molecules in solution, in particular for pDNA, tDNA, and then dsDNA with two different hybridization ratios, 1:1 and 1:2. The latter is the condition that we could derive for pDNA immobilized on the surface based on QCM-D experiments. The analysis of the measurements performed in Berlin is currently underway.

***Patterned sensing platforms:***
For the development of a multiplex DNA sensing platform, I studied the patterning of the surface with DNA strands. To this aim, I used the AFM in a nanolithography mode, called nanografting. On a previously formed film of MCH, by applying a high load, it is possible to remove chemisorbed molecules and substitute them with thiolated DNA molecules present in solution. In this way,

micrometric DNA patches can be obtained in well-defined regions. The DNA density within the patches can be tuned by changing two parameters: the density of the scanned lines and the line scan rate. The former can be quantified as the S/A ratio, where S is the scanned area, and A is the actual area of the final patch. This parameter indicates how many times the same line is rescanned, while the line scan rate is related to the tip scan velocity. Patches obtained with high S/A parameters were higher and denser than patches obtained with low S/A, while increasing the line scan rate decreases the patch height, i.e. the pDNA molecular density.
The thickness of grafted DNA patches was compared to the thickness of uniform DNA SAMs evaluated using nanoshaving, another AFM nanolithography mode. In nanoshaving, molecules are selectively removed from a defined area, thus exposing the gold underneath. The depth of the hole left behind by the removed molecules provides an estimation of the film thickness. The thickness of the uniform film was found to be comparable to that of the thinner grafted patches. Therefore, nanografting allows to increase the DNA density compared to self-assembly in solution. Moreover, patches obtained with high grafting efficiency (i.e., high S/A and low line rate) are more uniform than patches obtained with low grafting efficiency.
Following exposure to the target, all patches increased in thickness. Patches with low density had a higher relative height increase, indicating them as the most suitable for sensor use. The height increase was measured exposing the system to a concentration of tDNA down to the nanomolar level, showing a sensitivity similar to SPEE experiments.
Using a non-target sequence, no increase in the patch height was observed, confirming that the increase after the exposure to tDNA was due to hybridization and not to aspecific adsorption, thus highlighting the selectivity of the platform.
This patterning technique can be used also on larger areas, up to 10 μm in lateral size. Under the perspective of a multiplex optical detection, I patterned the surface with patches between 5 μm and 10 μm and conducted some preliminary ex-situ studies with a Micro Ellipsometer on the p-DNA patches. Patches were prepared with different scanning parameters, resulting in different nanoscale heights. The combined AFM and Micro Ellipsometry analysis showed a very good correlation between the patch nanometer thickness measured by AFM and the optical thickness derived from ellipsometric measurements, thus proving the sensitivity of the optical measurement.
Finally, I started some preliminary tests patterning the surface with three different pDNA, for the detection of RdRp/Helicase of other Corona Viruses, such as the previous Sars-CoV and MERS-CoV (Middle East Respiratory Syndrome Corona Virus), aiming to the parallel detection of different sequences.

## *List of courses*:

Force Spectroscopy: attended.

## *List of publications*:

Canepa, P., Gregurec, D., Liessi, N., **Rotondi, S. M. C.**, Moya, S. E., Millo, E., Canepa, M., & Cavalleri, O. (2023). *Biofunctionalization of Porous Titanium Oxide through Amino Acid Coupling for Biomaterial Design*. Materials, 16(2), 784.

**Rotondi, S. M. C.**, Canepa, P., Angeli, E., Canepa, M., & Cavalleri, O. (2023). *DNA Sensing Platforms: Novel Insights into Molecular Grafting Using Low Perturbative AFM Imaging*. Sensors, 23(9), 4557.

**Rotondi, S. M. C.**, Ailuno, G., Mattioli, S. L., Pesce, A., Cavalleri, O., & Canepa, P. (2023). *Morphological Investigation of Protein Crystals by Atomic Force Microscopy*. Crystals, 13(7), 1149.

## *List of conferences and presentations*:

**Rotondi, S. M. C.**, Dante, S., Pinto, G., Canepa, P., Canepa, M., & Cavalleri, O.
*A DNA sensing platform for the multiplex and label-free targeting of oligonucleotide sequences.*
**Oral communication** at ICOMF18, August 21-25 2023, Frankfurt, Germany.

**Rotondi, S. M. C.**, Dante, S., Pinto, G., Canepa, P., Canepa, M., & Cavalleri, O.
*Coupling SE and QCM-D for label-free detection of oligonucleotides sequences.*
**Poster** presented at CMD30 FisMat 2023, September 4-8 2023, Milano, Italy.

## *Other activities*:

Tutor of Mathematics and Physics for the 1$^{st}$ year of B.Sc. in CTF and Pharmacy.