PhD in Physics and Nanoscience-XXXVII Cycle

3rd Year Report

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Research activity:

In the first two years 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), employing the RdRp/Helicase of Sars-CoV-2 as the target sequence. This study was conducted by coupling different methods, Spectroscopic Ellipsometry (SE), Surface Plasmon Enhanced Ellipsometry (SPEE), Quartz Crystal Microbalance with Dissipation (QCM-D), X-Ray Photoemission Spectroscopy (XPS), and Atomic Force Microscopy (AFM).

During my third year I focussed on:

- Designing an optical model for SPEE measurements. Testing the system with different tDNA concentration
- Performing nanografting experiments with AFM, testing the platform selectivity, and deriving a binding affinity curve after exposing the system to different tDNA concentration.
- Coupling SE with QCM-D.

SPEE model

Previously, I implemented the SPEE method, which couples SE with Surface Plasmon Resonance (SPR) in an internal reflection configuration known as Kretschmann configuration. As well as for SE spectra, the analysis of SPEE data requires an optical model. The implemented model is structured as follows:

- BK7 glass layer which accounts for the prism and the glass support of the gold film.
- A titanium layer (2 nm) and a gold layer (50 nm) that model the metallic substrate.
- A Bruggerman Effective Medium Approximation (BEMA) layer that accounts for the interaction between the molecules and the substrate (supported by previous XPS results).
- A transparent molecular layer described by a Cauchy equation.
- The TE Buffer solution, described by another Cauchy layer.

The model accurately reproduced the acquired spectra. Unlike SE model, no absorbing layer is required since analysis is performed away from the UV absorbing region of DNA. The simulation parameters used to model the interface reveal an increase in the interface itself following the incubation of the thiolated DNA and MCH. No increase is detected upon the hybridization with non-thiolated tDNA, as expected since tDNA does not directly interact with gold. Interestingly, the incubation of MCH produces a vertical shift of ψ (that can be modelled by a change in BEMA parameters), highlighting the method sensitivity to modifications of the interface structure.

A deeper study of SPEE dynamics as a function of the target concentration highlighted the presence of two regimes in the hybridization process: a reaction-limited regime, with a time scale under the minute, and a diffusion-limited regime, with a time scale that increases when the target concentration decreases. Exposing the system to different target concentrations allowed to obtain a binding affinity curve fitted with Hill equation, obtaining a dissociation constant $K_D=(70\pm10)$ nM.

Nanografting

Starting from grafting results obtained last year, I prepared arrays of DNA patches for the Sars-CoV2 sequence detection, testing the system sensibility, which reaches the nM level, and selectivity towards previous Sars-CoV. Furthermore, I obtained a binding affinity curve fitted with a Hill equation, obtaining a

dissociation constant K_D=(15±3)nM. The difference between the K_D obtained with SPEE and nanografting is due to the different system under scrutiny. In fact, different DNA densities are analyzed with the two methods, and the binding affinity curve properly reflects this difference. The accurate control of the DNA density achieved by nanografting paves the way for promising results through the combination of nanografting, and optical detection through Imaging Micro-Ellipsometry.

SE+QCM

Finally, I performed experiments coupling SE and QCM-D. This provides the integration of optical data with mass deposition information, therefore enabling the combined analysis of optical thickness variation with mass and viscosity data. I previously reported (first year summary) the detection of hypochromism for immobilized DNA strands. Coupling SE and QCM-D paves the way for a deeper understanding of this phenomena, not yet studied for DNA molecules immobilized on a surface, where the ordering of the nucleotide bases can be a key element. To further investigate hypochromism, I studied the hybridization of pDNA with a fully complementary target sequence to which a tail of extra bases at the 5' end is added. Compared with the tDNA fully complementary sequence studied so far, the extra tail increases the number of immobilized absorbers, adding bases that are not affected by hypochromism since the extra tail does not hybridize. Hybridization with the new sequence brought an increase in film thickness in SE and a decrease in frequency in QCM-D larger than the one obtained with the fully complementary target, with an increase in dissipation connected to the non-hybridized tail. Data processing is still in progress.

List of courses:

Spettroscopie e Materiali per la fotonica: passed
AFM BioMed Summer School: passed

List of publications:

Rotondi, S. M. C., Canepa, P., Canepa, M., & Cavalleri, O. (2024). *Design of a multiplex sensing platform: AFM as a nanolithographic tool*. Proceedings. Vol. 104. No. 1. MDPI, 2024.

Canepa, P., **Rotondi, S. M. C**., & Cavalleri, O. (2024). *Atomic Force Microscopy as a nanolithography tool to investigate the DNA/gold interface*. Current Opinion in Electrochemistry, 101444.

List of attended conferences and presentations:

Rotondi, S.M.C. , Dante, S., Bisio, F., Canepa, P., Canepa, M., & Cavalleri, O. *Optical label-free DNA based biosensor for Sars-CoV2 sequence detection.* **Oral communication** at 110th national congress SIF, September 9-13 2024, Bologna, Italy.

Rotondi, S.M.C., Canepa, P., Canepa, M., & Cavalleri, O. *Exploiting AFM as a nanolitographic tool: designing DNA patches for parallel detection of oligonucleotide sequences.* **Poster** presented at XXVII National Congress SIBPA, June 16-20 2024, Genova, Italy.

Rotondi, S.M.C., Dante, S., Canepa, P., Canepa, M., & Cavalleri, O. *Detecting diseases-related oligonucleotides sequences: a multi-technique approach for label-free sensing.* **Flash talk** at ESAB2024, June 04-07 2024, Donostia-San Sebastian, Spain. Winner of EBSA bursary.

Rotondi, S M.C., Canepa, P., Canepa, M., & Cavalleri, O. *Design of a multiplex sensing platform: AFM as a nanolithographic tool*. **Oral communication** at IECB2024, May 20-22 2024, Online.

Other activities:

Didactic tutor for "Fisica e laboratorio di misure fisiche" course, 1st year of B.Sc. in Biology.
Orientation tutor for high school students.
PNRR project "Le discipline e le lingue nella realtà" with Daneo primary school.