Abstract

Enhanced Raman spectroscopies such as Tip Enhanced Raman Spectroscopy (TERS) and Surface Enhanced Raman Spectroscopy (SERS) are vibrational spectroscopic techniques characterized by an amplification of orders of magnitude in Raman signal intensity, thanks to the excitation of Localized Surface Plasmon Resonances of metal nanostructures. Due to their high sensitivity and molecular specificity, in the last decades such techniques have found important applications in many different fields, including biosensing and materials science. For this reason, the first part of the Thesis is devoted to the characterization by TERS and colocalized Raman-AFM (Atomic Force Microscopy) spectroscopy of dielectric nanostructures such as zinc oxide nanowires that can be exploited for technological applications like memristors. On the other hand, in the second part of the Thesis, SERS is applied using highly performing plasmonic substrates for biosensing applications in the field of food safety and biomedicine.

More in detail, in the first part of this Thesis, the Raman-AFM coupling of an advanced experimental setup able to perform both colocalized Raman-AFM and TERS measurements was firstly optimized. Afterwards, such setup was employed to carry out both Raman and colocalized Raman-AFM measurements on zinc oxide nanowires. Subsequently, Ag-coated plasmonic nanotips aimed to the TERS characterization of such dielectric nanostructures were developed and the growth on AFM nanoprobes was tentatively optimized by DC sputtering. Finally, the performance of the fabricated tips was evaluated by performing TERS measurements on a thin film sample of malachite green.

In the second part of this Thesis, porous silicon membranes covered with Ag nanoparticles and supported on a polydimethylsiloxane slice (Ag-PSD) were exploited as SERS-active substrates for the detection of both antibiotics in food matrices and micro-RNAs (miRNAs) as tumor biomarkers. For what concerns the analysis of antibiotics, the Ag-PSD substrates were functionalized with aptamers for the detection of tetracyclines, some of the most known and used antibiotics that can be found in food matrices like milk, honey and eggs. At first, the immobilization conditions of the aptameric sequences on the surface of the SERS substrates and the conditions for the detection of the antibiotics were optimized. Afterwards, the sensitivity of the bioassay was assessed by analyzing several concentrations of different tetracyclines in water-based solutions. The specificity of the bioassay, instead, was evaluated by testing other antibiotics widely used in animal breeding belonging to the family of sulfonamides.

Concerning the analysis of miRNAs, the Ag-PSD substrates were functionalized according to both a one-step and a two-step hybridization bioassay exploiting complementary DNA probes and Raman reporters for the specific and sensitive detection of the target miRNA. At first, the influence of the reporter-to-surface distance on the sensitivity of the bioassays was deeply investigated and the experimental findings were corroborated by the results of 3D Finite Element Method simulations carried out on a simplified model of the SERS substrates employed. Afterwards, the optimized two-step assay was used for the label-free detection of several concentrations of different miRNAs involved in lung cancer. The same protocol was then exploited to detect miRNAs in real biological samples consisting of total RNA extracts obtained from transfected cancer cell lines.

Finally, the Ag-PSD substrates functionalized for the detection of both antibiotics and miRNAs were integrated into microfluidic chips, in order to further improve the sensitivity of the bioassays and the reproducibility of the results. The *in-chip* measurements were performed by using also a portable Raman spectrometer aimed to *in-field* analyses. Moreover, custom scripts that enable the automation of both the measures carried out by the portable apparatus and the analyses of the acquired SERS spectra were developed.