A simple implementation of an optical biosensor based on Raman Spectroscopy

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In this work we present the implementation of a biosensors integrating optical waveguides and Surface Enhanced Raman scattering (SERS) surfaces for the label-free detection of biological compounds.
Biomarker detection at low concentration in body fluids (blood, serum, saliva…) is a key point for early diagnosis of severe diseases and to set appropriate therapies. This remains a challenge due to the complexity of the media to be analysed, to the non specific detection and to the sensitivity of the current methods [1]. Raman spectroscopy has gained a lot of attention because it is able to provide various biochemical information about a compound without the need of complicate sample pretreatment protocols [2]. However in classical Raman spectroscopy there is the need to use bulky optical set-ups. The aim of this work was to miniaturize the detection device and to have more flexible optical functions and interfaces [3].
For such a purpose, we present a SERS surface integrated on top of an ITO waveguide fabricated over a glass substrate and coupled to an external optical fiber by means of a prism coupler (for the excitation) and to an optical microscope (for the signal collection) (Figure 1). Using such a configuration, the SERS surface can be excited by a laser beam through the optical fiber connected to a laser source. The advantage of such excitation is that it will be done through an evanescent wave that is confined at the surface and limited to the sensing surface. This will confine the excitation of the biological media reducing consistently the background signal and increasing the signal to noise ratio. In addition the developed sensor can be easily integrated in microfluidic devices, due to the fact that the optical waveguides allow reducing the size of the device and exciting particular regions of the substrate without the need of complicate alignments of huge optical set-ups, which would create several issues to be coupled into a microfluidic device. Moreover, such a device is fabricated by a simple lithographic process. First, a positive photoresist S1813 is spun on a glass substrate (20mm x 30mm x 0.17mm) coated with 300 nm of Indium Tin Oxide (ITO) at 4000rpm for 1 min. Subsequently, it is exposed under UV light for 26 s after being protected by an optical mask reproducing the layout of the optical waveguide. After exposure, the S1813 is developed to remove the part which were not exposed. The ITO is then etched for 1 min in HCL and acetone was used to remove the remaining S1813 to reproduce the optical waveguide. Finally gold was sputtered on the ITO for 30s after having protected it with an aluminum mask to reproduce a SERS surface at the end of the waveguide (Figure 2). For the characterization of the biosensor, a beam of HeNe laser (632nm) was collimated from an optical fiber coupler (Achromatic FiberPort,FC/APC, f = 4.00mm, 400 - 700nm, Ø0.65mm Waist, PAFA-X-4-A from Thorlabs) to the prism coupler (λ/4 Rutile TiO_2 prism from Thorlabs 45-45-90) and guided into the ITO waveguide to acquire an evanescent wave in correspondence of the SERS surface. A λ/4 Rutile prism characterized by higher refractive index (n=2.6 acted as a trap of light, resulting in total internal reflection of the incident laser beam. The light incident angle was optimized to eliminate reflection losses. At a particular angle, incident beam at the prism interface tunnels through the small air gap into ITO film (n=1.82) that acts as waveguide over the glass substrate (n=1.42). Thereby due to guiding effect the SERS surface received a large intensity of laser beam and scattering happened. Scattered light is captured for Raman Analysis (Figure 3). A preliminary set of experiments are carried out on the developed device by using a sample of phenylalanine. Raman spectra are recorded for these samples with concentration down to 0.01mM and compared to the measurements obtained by conventional Raman Spectroscopy on glass slides at a concentration of 0.1mM. The results shown in Figure 4 demonstrate that conventional Raman detects the main vibrations of phenylalanine, such as the peak at 1003cm^{-1} from the ring breathing vibration, the one at 1032cm^{-1} basically from in phase-motion of C atoms, that at 1207cm^{-1} mainly assigned to side chain vibrations, at 1586cm^{-1} basically assigned to the out-phase motion of N atoms and the 1606cm^{-1} from the in-phase motion of C atoms of the phenyl ring. Also waveguide+SERS Raman is able to detect the main Phe peak at 1003cm^{-1}, the Raman band around 1207cm^{-1} and the peak at 1586cm^{-1}. Furthermore other intense peaks are observed at 1369 and 1648cm^{-1}. These two peaks could arise due to SERS effect: as well known in SERS experiments the binding of biomolecules with Au nanoparticles could enhance the Raman signal coming from vibrations non-detectable with conventional Raman. However, these to peaks require further investigation before suitable assignment. The fabricated device has competitive advantages over other biosensor systems exploiting the unique characteristics of a SERS surface integrated with an optical platform.

[1] G. Perozziello, P. Candeloro, F. Gentile, A. Nicastri, A. M. Perri, M. L. Coluccio, E. Parrotta, A. De Grazia, M. Tallerico, F. Pardeo, R. Catalano, G. Cuda, E. Di Fabrizio, Microelectronic Engineering, 144 (2015) 37-41;
[2] G. Perozziello, P. Candeloro, F. Gentile, A. Nicastri, A. Perri, M. L. Coluccio, A. Adamo, F. Pardeo, R. Catalano, E. Parrotta, H. D. Espinosa, G. Cuda, E. Di Fabrizio, RSC Advances 4 (98) (2014) 55590-55598
[3] G. Simone, G. Perozziello, Journal of Nanoscience and Nanotechnology, 11 (2011) 2057-2063.

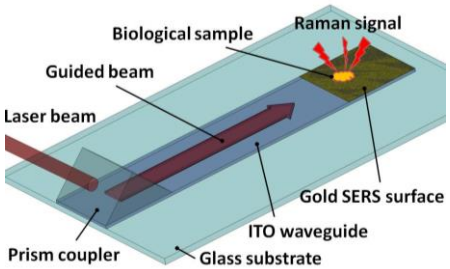

Figure1. Schematic representation of device layout and its working principle.

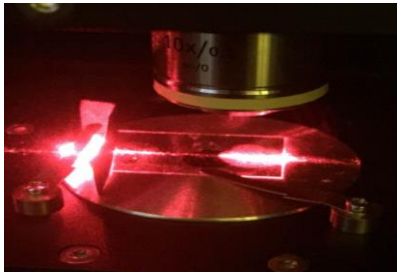

Figure 2. Optical set-up displaying the incident laser beam, the prism, the waveguide and the light scatteringfrom the SERS surface.

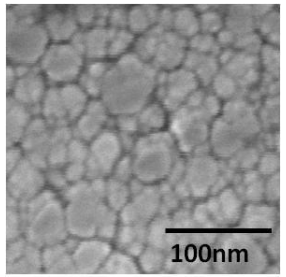

Figure 3. Scanning Electron Microscopy image of the gold nanoclusters sputtered on ITO performing SERS effects.

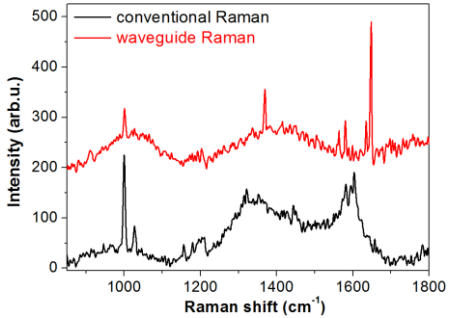

Figure 4. Raman spectra of phenylalanine: taken by conventional Raman spectroscopy (red curve), and by using the developed device (black curve).