In situ comparison of immunosensor performances based on dually functionalized Au-SiOx chips

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Context of the study

Dielectric film thin layers grown using plasma technology are widely used by miniaturized devices. This plasma technology is becoming increasingly attractive for the deposition of dielectric films such as silicon nitride and amorphous silicon oxide (SiOx) which are the most common materials used for manufacturing integrated optical biosensors. The inductively coupled plasma enhanced chemical vapor deposition (ICPECVD) paves the way for the development of chemical and biological sensors.

We decided to deposit SiOx thin films on top of Au Surface-Plasmon-Resonance (SPR) sensor chips in order to study the intrinsic properties of such films and compare them with the "gold standard".

From a chemically point of view, SiOx surface has several advantages over Au as a biosensor surface material, such as the thermal stability of the most commonly used surface biocoupling agents employed for immobilization of captured molecules. Silanes show larger thermal stability than thiols, which are typically used for Au-biofunctionalization, because it forms stronger bonds with the SiOx compared than gold.

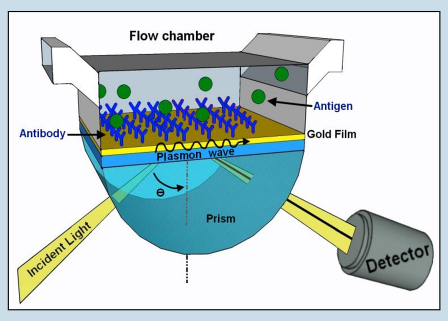
Such Au-SiOx chips can be considered as versatile chips compatible with SPR instrumentations offering a new platform of measurement for silane based chemistry. It is believed that the results presented here are useful and inspiring for engineers interested in plasmonic biosensor applications compatible with the chemical functionalization of oxidized substrates.

Instrumentations

❖ **Surface plasmon resonance imaging**

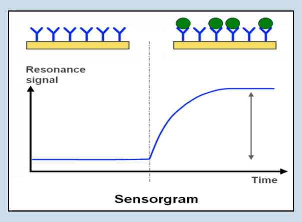
XelPlex apparatus
(HORIBA Scientific)

- Real-time monitoring of bio-molecular interactions in a multiplex format
- Optimized fluidic systems
- Working at various angle



Gold/SiOx biochip:

- Home-made
- Functionalized either with thiols or with silanes
- Specific ligands arrayed onto the functionalized surfaces



Conclusion / Prospects

The present study demonstrates that thin layers of amorphous silicon oxide (SiOx) grown by inductively coupled plasma enhanced chemical vapor deposition (ICPECVD) technology at lower temperatures can be successfully combined with biosensors.

In particular, gold-amorphous silica (Au/SiOx) interfaces were investigated for their potential applications as a low-cost Surface Plasmon Resonance (SPR) sensor chip.

Chemical functionalization procedures have been developed and validated for both substrates on the same chip.

With the same protocol of activation (with EDC-Sulfo NHS) we reach very high rate of antibody grafting (up to 35 fmoles/mm^2) in monolayer.

Finally, the functionality of this "2 in 1" immunosensor has been tested and validated with a biological target.

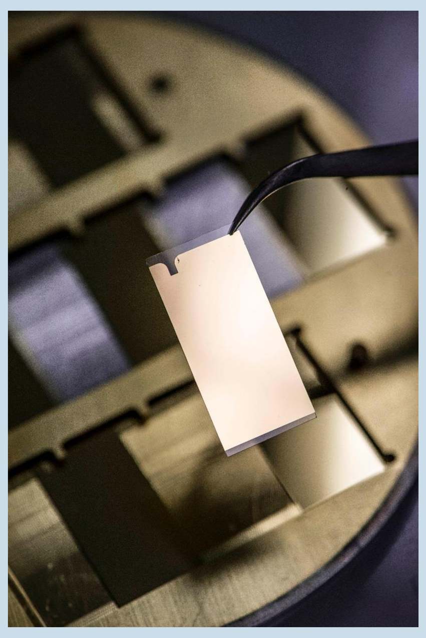
Hybrid chips and SPR instrumentation appear as a new analytical platform in the field of biosensing and could bring new knowledges for the dual chemical funtionalization of microsystems and lab-on-chips.

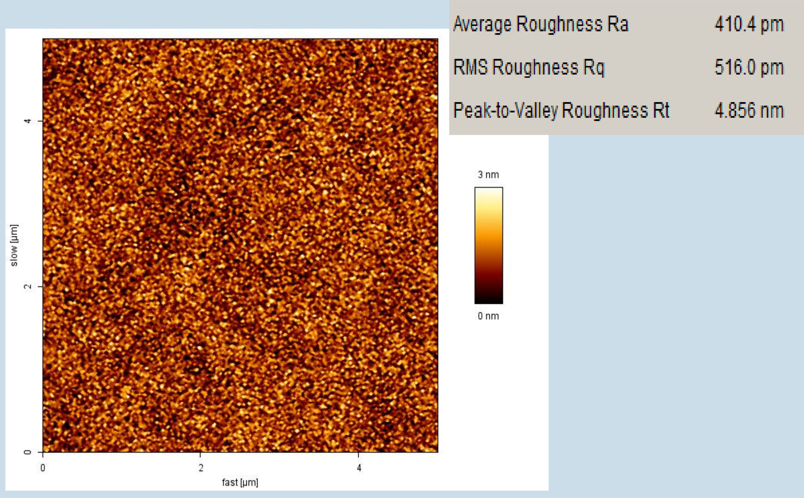
Bibliography

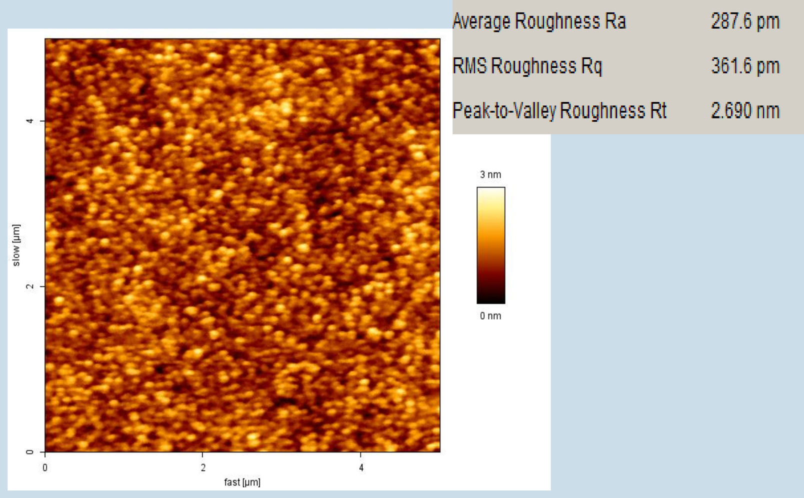
Herth et al. 2016 Microelectronic Engineering 163, 43–48
Meillan et al. 2014 RSC Advances 4, 11927-11930
Remy-Martin et al. 2012 Analytical & Bioanalytical Chemistry, 404 (2), 423-432

Experimental part

❑ Chip fabrication, characterization and plasmonic properties

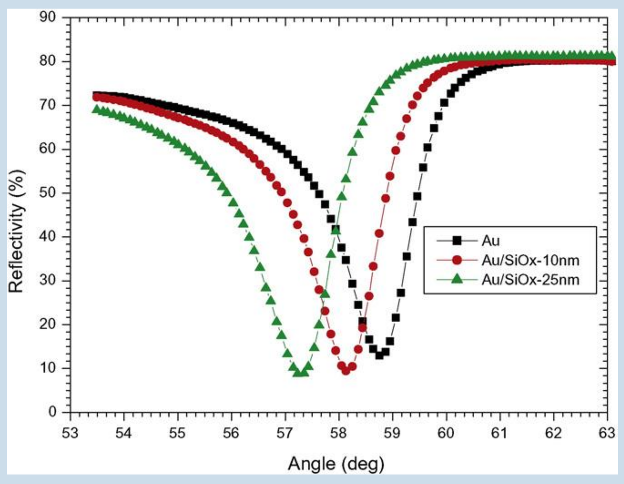


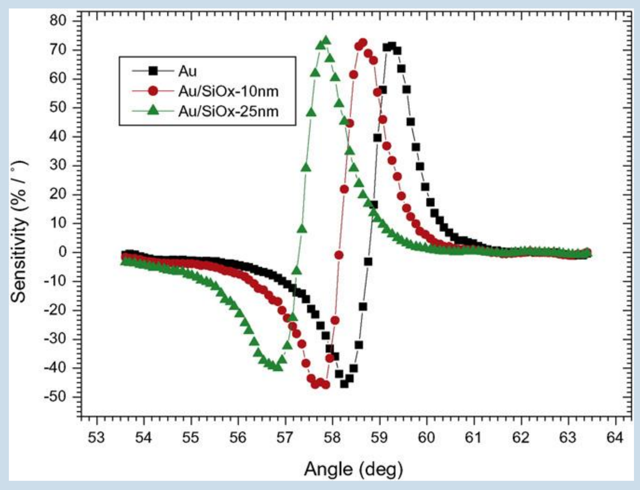




AFM measurements on naked gold (left) and SiOx/Au (right) surfaces.

We present original Au-SiOx sensorchips that have high surface plasmon resonance (SPR) performances. Such SPR chips present thin layers of amorphous silicon oxide (SiOx) grown onto gold chips by ICPECVD technology (Herth et al., 2016). AFM measurements demonstrate the homogeneity of the thin layers depositions and a very low RMS roughness.



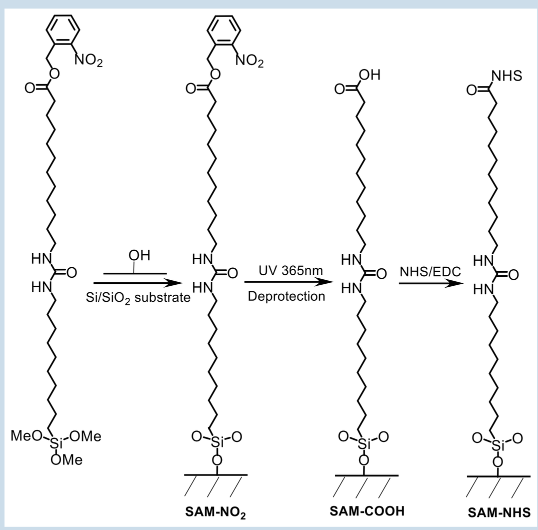


Reflectivity curves (left) and sensitivity spectrum (right) versus incident angle for different thicknesses of deposited SiOx on 48 nm thick gold layers.

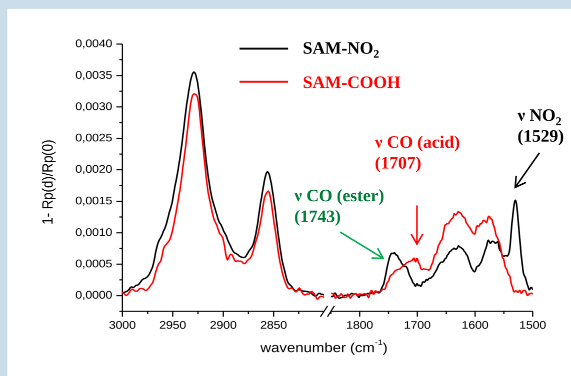
❑ Chemical Functionalizations

The silylated coupling agent was chemisorbed onto SiO_2 surface in optimized conditions in order to obtain a homogeneous and smooth surface (Meillan et al., 2014). The identification of the protected carboxylic acid groups-terminated monolayers onto the surface as well as the subsequent chemical surface modifications were performed using PM-IRRAS, and contact angles measurements.

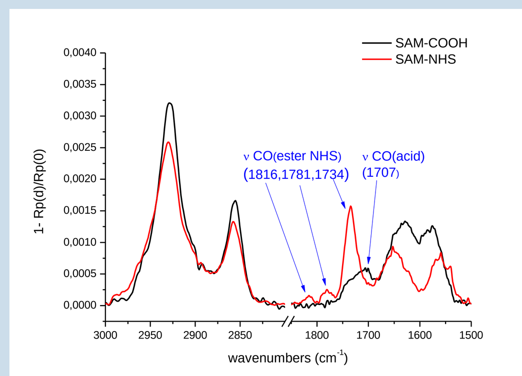
The contact angle values of 60° was observed for SAM-NO_2 and decrease to 55° after deprotection for SAM-COOH. These values showed that the surface became more hydrophilic due to the presence of acid carboxylic groups.



Infrared spectroscopy (PM-IRRAS) was used to monitor the formation of the SAMs



Before and after photodeprotection



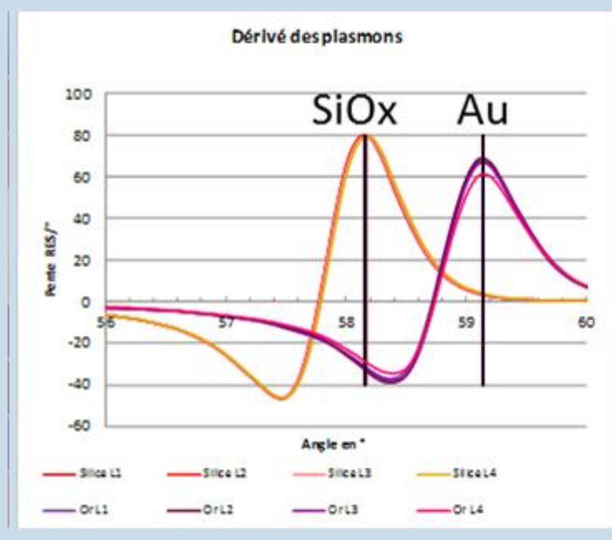
Activation of the SAM with EDC/NHS

Gold Standard Chemistry:

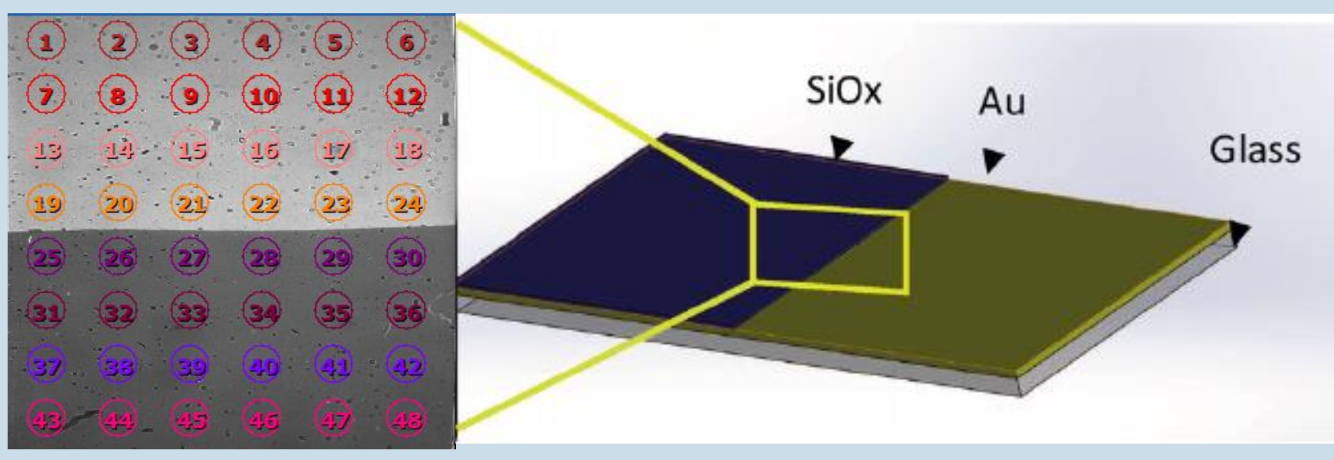
To functionalize the gold chip surface, we used a solution composed of a mixture of thiolated coupling agents that were chemisorbed onto Au surface in optimized conditions in order to obtain a homogeneous surface allowing efficient Ab grafting with good properties to prevent non-specific adsorption in complex biological fluids (Remy-Martin et al., 2012).

❑ Hybrid SPR chip with two different surface chemistries

The deposition technique allows the constitution of hybrid biochips bearing simultaneously Au and SiOx surfaces that open the way to a comparative study of Self Assembled Monolayers (SAMs) based on thiol and silane chemistries.

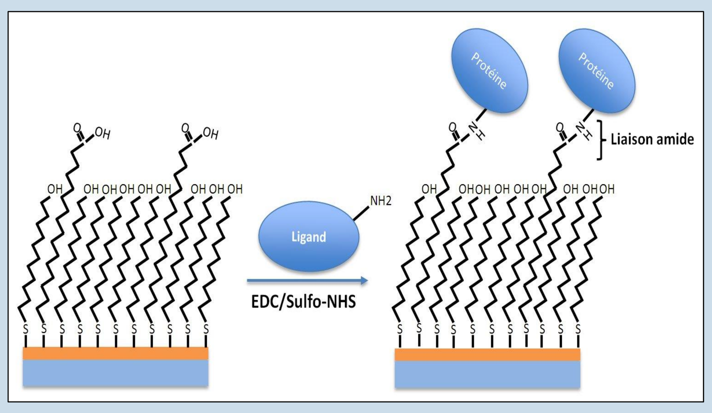


	Family name	Color
SiOx	L1	
	L2	
	L3	
	L4	
Au	L1	
	L2	
	L3	
	L4	

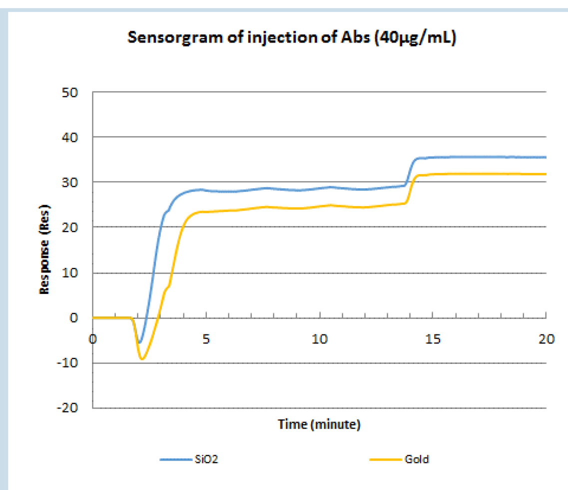


❑ Biochemical graftings on chip bearing two different surface chemistries

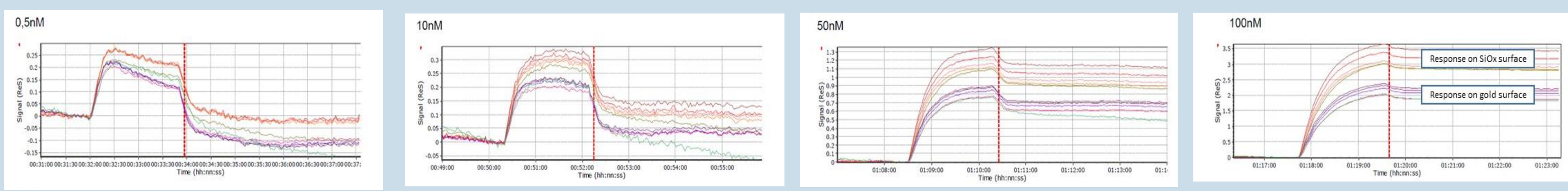
Scheme of a monolayer of antibodies covalently grafted onto chemically activated thiolated SAM onto gold chip



Sensorgramm of Ab immobilization onto 2D silanized and thiolated SAMs leading to a surface coverage of 32 to 35 fmoles/mm^2 for both surface chemistries



❑ Proof of concept of simultaneous Biomolecular Interaction Analysis on hybrid biochip



Good reproducibility of immunocapture with the gold standard chemistry.
Promising preliminary results with the SiOx biochip

Sensorgramms of simultaneous immunocapture of LAG 3 protein (from 0.5 to 100nM) with two different surface chemistries