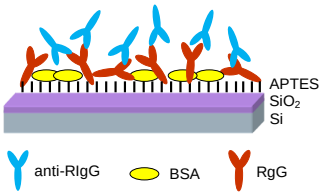
ThetaMetrisis APPLICATION NOTE #002

Biomolecular Layer Thickness Evaluation Using White Light Reflectance Spectroscopy (WLRS)



Goal: The development of a fast & accurate methodology for the biomolecular layer(s) thickness evaluation.

Means & Methods: WLRS is introduced for the evaluation of the effectiveness of biomolecules immobilization onto solid surfaces as well as their subsequent reaction with counterpart biomolecules via measuring the respective layers thickness. In particular, the adsorption of rabbit (RgG) and mouse gamma-globulins (MgG) as well as their reaction with the complementary antibodies were investigated. The measurements were performed by an FR-Basic equipped with a VIS-NIR spectrometer with 0.35 nm optical resolution and a white light Halogen lamp. The substrate is Si wafer with a thermally grown SiO_2 film with a thickness of ~1000nm.

Results: In fig. 1, the spectra of the various layers in the case of RgG – antiRIgG system are depicted where the spectral shifts due to the binding of the various biomolecular layers are shown. The average layers' thicknesses values based on these spectral shifts as calculated by the FR-Monitor are provided in fig. 2.

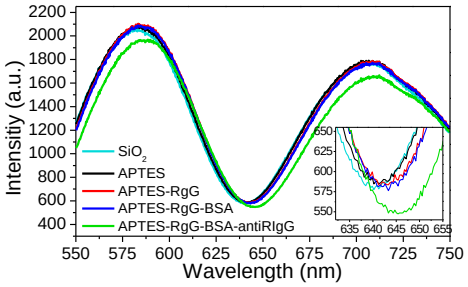


Figure 1: Shifts in the reflectance spectrum after deposition of biomolecular layers on the substrate.

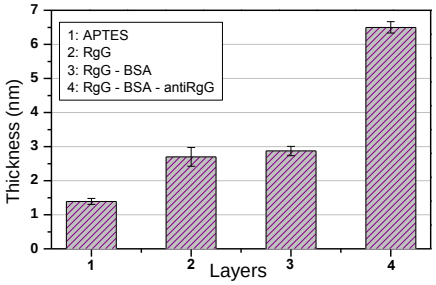


Figure 2: Thicknesses of the biomolecular layers created with the RgG - antiRgG system.

In fig. 3a the reflectance spectra of MgG before and after the reaction with non-complementary binding molecules (RgG) are presented. Fig 3b shows the respective thicknesses, where no thickness increase is observed after the reaction with RgG as it was expected. This result verifies the method's accuracy for thin biomolecular layers evaluation.

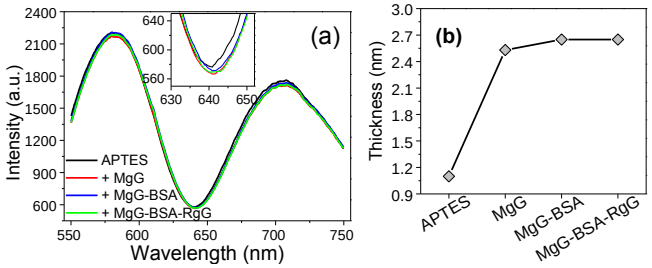


Figure 3: a) Spectra before and after the reaction of MgG coated surface with non-complementary binding molecules (RgG), b) the respective layers thickness.

Conclusions

Thus WLRS methodology is a simple, non destructive approach for ultra-thin biomolecular layers thickness estimation. These features make the proposed methodology appropriate as a fast tool to evaluate new surface activation/biofunctionalization protocols.

Reference: M. Kitsara, P. Petrou, D. Kontziampasis, K. Misiakos, E. Makarona, I. Raptis, K. Beltsios, Microelectron. Eng. 87, p. 802, 2010.