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Protein adsorption monitored by plasmon-enhanced semi-cylindrical Kretschmann ellipsometry

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ABSTRACT

The Kretschmann–Raether geometry is widely used to investigate the properties of various biological samples and their behavior on different substrates [1] (mostly on gold surface with/without different functionalization). In this configuration the surface plasmon polaritons (SPPs) are used to enhance the sensitivity of the measurement. Recently, the combination of this method with spectroscopic ellipsometry (SE) became more and more popular. In our work protein adsorption was monitored *in situ* using this configuration. The performance of the configuration was investigated for different thicknesses of the plasmonic layer. The best measurement parameters were identified in terms of layer thickness, angle of incidence (AOI) and wavelength range. It was shown that the spectroscopic capability over a broad wavelength range, the possibility to adjust the AOI accurately, as well as the phase information from the measurement proves to be a significant advantage compared to standard configuration and surface plasmon resonance configurations.

1. Introduction

Among the numerous techniques for thin film characterizations, the special features of ellipsometry are its high sensitivity, high speed and the non-destructive nature that makes it capable for *in situ* monitoring of biological processes. Spectroscopic ellipsometry is based on the measurement of the amplitude- and phase change of the light beam, which is reflected from the surface of the sample. The measured quantities are defined by the equation [2]:

$$\rho = \tan \Psi e^{i\Delta}, \quad (1)$$

where ρ is the complex reflectance ratio, Ψ and Δ express the amplitude ratio and the phase difference between p- and s-polarizations, respectively. Spectroscopic ellipsometry is an indirect method. That means that to obtain a physical property of the investigated material, we have to build an optical model and then fit the parameters. To obtain the fitted values of these parameters we minimize the root mean square error (RMSE) [3]:

$$\mathrm{RMSE} = \sqrt{\frac{1}{N-P-1}\sum_{j=1}^{N}\left[\left(\frac{\Delta_j^m - \Delta_j^c}{\sigma_{\Delta_j}^m}\right)^2 + \left(\frac{\Psi_j^m - \Psi_j^c}{\sigma_{\Psi_j}^m}\right)^2\right]}, \quad (2)$$

where N is the number of the measured (Ψ, Δ) pairs, P is the number of the unknown parameters of the model, ('m') and ('c') denote the measured and the calculated Ψ and Δ ellipsometric angles, while σ is the standard deviation of the measured values.

Real-time ellipsometry has been used for many decades not only in vacuum chambers for solid state processes [4], but also for studying of biointerfaces and chemistry [1]. Although waveguide sensors [5] offer a better sensitivity than bioellipsometry in the conventional configurations, the latter has also been developed toward new, higher-sensitivity spectroscopic configurations, in order to achieve multi-parameter modeling capabilities for the characterization of complex surface structures [6–9].

Most of the configurations for bioellipsometry use flow cells in which the solid-liquid interface is measured through the liquid at a certain AOI [9]. The two major limitations of this configuration are the restricted wavelength range due to the absorption of the water, and the fixed AOI. Recently, we have demonstrated dual-channel measurements using a plasmon-enhanced Kretschmann–Raether

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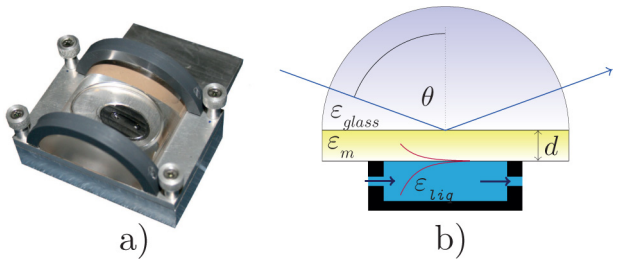

Fig. 1. (a) Photograph and (b) the schematic arrangement of the flow cell. Here ε_{glass}, ε_m and ε_{liq} are the dielectric functions of the prism, the gold layer and the ambient in the flow cell, respectively. θ is the angle of incidence and d is the thickness of the gold layer.

configuration (Fig. 1) [10,11], in which the interface is measured from the substrate [2] through a plasmonic gold layer [12]. This configuration can utilize a larger range of both wavelengths and angles of incidence, as well as an additional plasmon enhancement. Furthermore, the construction is less sensitive to bubbles, and the small volume allows to follow quick processes, because of the little time needed to completely flush the whole volume of the cell.

The aim of this study is to investigate different gold layer thicknesses and configurations for the *in situ* measurement of protein adsorption, and to identify the best conditions and the corresponding parameters, such as the sensitivity and the limit of detection. This will be compared to results by standard flow cell [8] and surface plasmon resonance (SPR) [13] configurations.

2. Experimental details

We constructed a 10-μL Kretschmann–Raether flow cell [14,12], equipped with a glass (BK7) semi-cylinder (Fig. 1) that allows the use of incident angles in the range of 45–70°. The cell was placed on the mapping stage of a Woollam M-2000DI rotating compensator spectroscopic ellipsometer having a wavelength range of 190–1690 nm. For the beam path of both the illumination and detection arms, lenses were designed to focus the light of the ellipsometer on the solid-liquid interface at the bottom of the semi-cylinder. The variation in the angle of incidence caused by the lenses is below 1°.

The plasmon-enhanced measurement was realized by attaching a ++150 μm thick microscope slide covered by gold films with thicknesses from 5 nm to 50 nm. The gold layer was created by e-beam evaporation. Between the gold layer and the glass, a 2-nm Cr_2O_3 layer was evaporated to enhance the adhesion of gold film to the glass. The glass slide was attached to the bottom part of the semi-cylinder with the gold layer facing down to the flow cell. Between the glass slide and the glass semi-cylinder there are an immersion oil (Zeiss Immersion Oil™ 518) layer to match the refractive indices of these materials and to avoid the formation of an air gap.

The freshly prepared samples were cleaned using a rinse of ethanol and high purity water. Then the rest of the water on the surface was removed using nitrogen gas. This cleaning process was done on all the samples. It is a very important step, because hydrocarbons may adsorb to the gold surface from the air that have to be removed. The cleaning step increases the reproducibility of the measurement, because after the process we always get the same surface. The protein used for the adsorption studies was 0.3 mg/mL fibrinogen (FGN) from bovine plasma (Sigma–Aldrich F8630-1G) in 10 mM phosphate-buffered saline (PBS) in high purity MQ water.

The measurement with the flow cell required a careful alignment procedure for the sample plane and the focusing lenses. The optical model consists of a BK7 ambient, a stack of Cr_2O_3, gold, protein layer and the PBS as a substrate (Fig. 2).

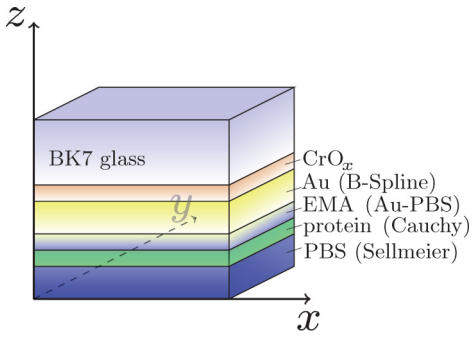

Fig. 2. Optical model used for the evaluation in the plasmonic Kretschmann–Raether configuration.

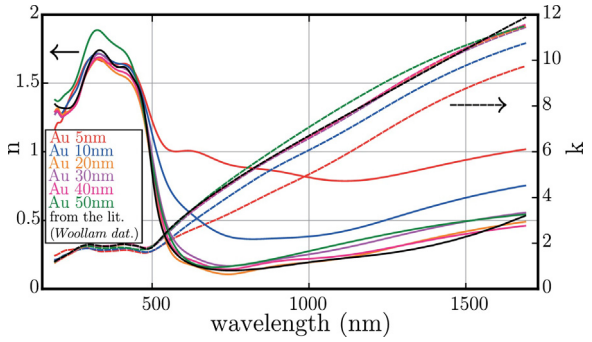

Fig. 3. Dielectric functions of gold layers of different thicknesses measured *ex situ*, from the gold side. The literature data are from the Kramers–Kronig fit for thin gold films from the Woollam database.

In order to achieve an accurate characterization, the dielectric function of the gold layers of different thicknesses have been measured *ex situ*, from the gold side, and fitted using the B-spline parameterization. Fig. 3 shows that the dielectric functions of the evaporated gold films are close to the reference values from the literature (Kramers–Kronig fit for thin gold films from the database of the Woollam CompleteEASE software), except for the 5-nm film, which was not a compact monolayer – as verified by atomic force microscopy.

Most of the gold layers were thin enough to have backlight from the backside of the 150-μm thin substrate. Therefore, absorbing tapes were used to avoid backside reflection [15]. The obtained thicknesses are listed in Table 1.

We put the sample into the cell and made a measurement in the whole AOI range. Then we filled the cell with PBS buffer. The next step was to obtain the optical properties of this ambient. It was shown that a thin interface appears between the gold layer and the PBS buffer since the evaporated layers are not strictly smooth (Fig. 2). This interface was modeled using the Bruggeman effective medium theory (EMA) [2].

Table 1
Gold layer thicknesses fitted using the B-spline model. Here d and d_r are the thicknesses of the gold layer and the surface roughness, respectively.

Sample	d (nm)	d_r (nm)	RMSE
'5 nm'	5.44 ± 0.79	0.97 ± 0.05	10.26
'10 nm'	11.82 ± 0.42	1.03 ± 0.07	4.71
'20 nm'	21.36 ± 0.17	0.74 ± 0.04	2.96
'30 nm'	30.37 ± 0.56	0.64 ± 0.07	3.01
'40 nm'	38.75 ± 0.26	1.68 ± 0.03	2.75
'50 nm'	46.96 ± 0.85	1.60 ± 0.03	3.54