Adapting Bobbert-Vlieger model to spectroscopic ellipsometry of gold nanoparticles with bio-organic shells

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Abstract: We investigate spectroscopic imaging ellipsometry for monitoring biomolecules at surfaces of nanoparticles. For the modeling of polarimetric light scattering off surface-adsorbed core-shell nanoparticles, we employ an extension of the exact solution for the scattering by particles near a substrate presented by Bobbert and Vlieger, which offers insight beyond that of the Maxwell-Garnett effective medium approximation. Varying thickness and refractive index of a model bio-organic shell results in systematic and characteristic changes in spectroscopic parameters $\Psi$ and $\Delta$. The salient features and trends in modeled spectra are in qualitative agreement with experimental data for antibody immobilization and fibronectin biorecognition at surfaces of gold nanoparticles on a silicon substrate, but achieving a full quantitative agreement will require including additional effects, such as nanoparticle-substrate interactions, into the model.

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References and links

1. Introduction

Nanoparticles (NPs) are widely used in biomedical applications: biosensing, point-of-care diagnostics, in vivo sensing and imaging, cell tracking and drug delivery, among others [1, 2]. Their versatile physical and chemical properties make NPs one of the most promising components for developing chemical and biological detection systems [3], while highlighting the need for a robust characterization, particularly of functionalized and bioconjugated NPs. Nanoparticles are typically characterized in terms of specific parameters, such as surface area and porosity, solubility, particle size distribution, aggregation, solvent or ligand accessibility. For metallic NPs, the most commonly used techniques include transmission electron microscopy (TEM), UV-Visible (UV-Vis) absorption spectroscopy and dynamic light
scattering (DLS) [4]. The NPs used in biological systems are usually biofunctionalized, therefore, there is a pressing need for technical solutions to monitor the functionalization and any subsequent biorecognition processes, such as capture of target DNA or proteins [5]; few techniques are available for an effective characterization of functionalized NPs, particularly techniques that can be extended to measurements in biological solutions [6].

Spectroscopic ellipsometry has been recently used for biosensing, in combination with plasmonics, whereby even small changes in optical properties produced by biofunctionalization or biorecognition can be detected and sometimes amplified [7, 8]. Previous studies have shown that different polarization states of the light scattered and reflected by a sample can provide, with appropriate modeling, information about its optical properties, roughness and thickness [8–10]. In the context of scattering of light by a sphere placed on a surface, ellipsometric measurements are the most common and practical means of characterization.

Modeling reflection of light from a substrate decorated with functionalized spherical core-shell NPs (metallic core with bio-organic shell) requires a formalism that describes light scattering for a sphere placed on a substrate [11–13]. Different models have been proposed to describe this scattering, including the quasistatic approximation, small particle broadening, effective medium theories, anisotropic particle resonance effect, and Green’s functions [14–16]. An exact solution of Maxwell’s equations for the scattering problem of a core-shell particle placed on a substrate was studied by Bobbert and Vlieger [17, 18]. The Bobbert-Vlieger model was extended by Kim et al., who improved and developed a computational solution and included coated spherical particles [19]. Further extensions of the model, include slightly non-spherical particles [20] and coating(s) on the substrate [21], the latter is implemented in an open-access script. The optical scattering of core-shell nanoparticles with an anisotropic shell has been presented through a combination of the Bobbert-Vlieger model with advanced Mie scattering calculus [22].

Here, we report the Bobbert-Vlieger model that has been adapted to describe light scattering by core-shell metal-organic NPs (metallic core with bio-organic shell) placed on a substrate and thus can be used to determine their optical properties. This model offers a practical approach, an improvement on models based on the effective medium approximation (EMA), because Bobbert-Vlieger model describes an exact solution of the underlying Maxwell’s equations, while being less complex than the Green’s functions methods.

We first construct a theoretical formulation for light scattering by a core-shell metal-organic NP on a substrate, based on exact solutions of Maxwell’s equations provided by the Bobbert-Vlieger model. We then compare our theoretical predictions for an isolated nanoparticle fully covered by a shell with those of the conventional Maxwell-Garnett scattering theory [23,24]. Finally, we apply our model to predict the ellipsometry spectra (i.e., $\Psi$ and $\Delta$) for Fibronectin (protein) molecules attached to gold nanoparticles and compare them with corresponding experimental data. We anticipate that, with appropriate models developed and validated, spectroscopic ellipsometry could become a complementary characterization technique for nanoparticle systems, alongside the more traditional techniques such as TEM, UV-Vis and DLS.

2. Theoretical description

An exact solution of Maxwell’s equations for light scattering by a spherical particle on a surface, as shown in Fig. 1, was originally developed and presented by Bobbert and Vlieger [18].
Fig. 1. Light scattering geometry considered in the model. A particle with a core dielectric constant \( \varepsilon_p \) with a shell of multiple coatings (\( \varepsilon_a, \varepsilon_b, \ldots \)) is placed next to a substrate (\( \varepsilon_s \)) at a distance \( \gamma \). The angle of incidence of the light is \( \theta \); \( V^I \) and \( V^{IR} \) are the incident and reflected waves from the surface, respectively; \( W^S \) is the light scattered directly by the sphere and \( W^{SR} \) is the light scattered by the sphere and reflected by the substrate surface. The dielectric constant \( \varepsilon_0 \) corresponds to the environment surrounding the sphere.

The Mie theory [11] of the light scattering by a sphere was extended using the Weyl’s method [12] for the reflection of the dipole radiation by a flat surface. The formal solution of the problem of light scattering by a sphere on a surface (\( W^S \)) can be described as:

\[
W^S = (1 - B \cdot A)^{-1} \cdot B \cdot (V^I + V^{IR}),
\]

where \( V^I \) and \( V^{IR} \) are the incident and reflected waves, respectively. Matrices \( A \) and \( B \) represent the reflection of the spherical wave by the substrate and the Mie solution for a sphere in free-space, respectively.

We have used the computational method developed by Kim et al. [19] to solve the scattering problem for our core-shell metal-organic particle configuration. This idealized model applies to a sphere with any number of coatings, placed a distance \( \gamma \) above a substrate, which can also have any number of coatings. The sphere and its coatings must lie entirely above the substrate stack. The parameterization of this model in terms of physical parameters of the system (Fig. 1) makes it a convenient tool for systematically examining the evolution of spectral features as each parameter, e.g., a layer thickness, is varied across an experimentally relevant range. Such a theoretical survey of the parameter space will help to optimize the choice of experimental systems and indicate theoretical limits of sensitivity and resolution. In turn, any quantitative discrepancies between theoretical predictions and experimental data will provide insight into limitations associated with the model approximations.

3. Model system

We attempted to construct a model system that could be defined with realistic parameters for theoretical investigation and implemented experimentally in a configuration approximating the idealized theoretical one. The basis of this model system is a silicon substrate with native oxide and some adsorbed gold nanoparticles (AuNPs), which could be coated with a bio-organic shell.

For theoretical investigation, the values of refractive index were taken from the literature [25]. Parameters for the organic shell were adopted from the literature on protein measurements [27–29] assuming proteins to be out of the aqueous medium but not completely dried.

Experimental implementation of the model system started with a silicon substrate (Si[100]) with native oxide that was functionalized with Poly-L-Lysine (PLL) to promote
reproducible adsorption of AuNPs. These AuNPs were further functionalized sequentially with (1) a heterobifunctional crosslinker, (2) an antibody and (3) a protein. The protein chosen for this study is cellular Fibronectin (cFn). Apart from being commonly used as a model protein, biologically cFn plays an important role in cell adhesion, growth, migration and signaling processes. In biosensing and medical diagnostics context it is considered to be a promising biomarker [29–31]. Each AuNP functionalization step, including the capture of cFn targets, was characterized by spectroscopic ellipsometry to provide data for comparison with the model predictions.

3.1 Sample preparation

A CZ-grown, Boron-doped, 725 ± 25 µm thickness, p-type (1-30 Ω-cm resistivity) Si wafer with <100> (± 0.5 degrees) orientation was purchased from Siegert Wafer. The wafer was single-side polished and covered with ca. 3 nm of native oxide. AuNPs, stabilized in citrate buffer, were purchased from NanoGAP, Spain. Poly-L-Lysine (PLL), 0.1% (w/v) in H2O was acquired from Sigma-Aldrich. Sodium phosphate mono- and dibasic buffer components were acquired from Sigma-Aldrich; phosphate buffer (PB) was prepared at pH 7.4 by mixing 100 mM NaH2PO4 and 100 mM Na2HPO4. Sulfosuccinimidyl 6-(3′-(2-pyridyldithio)propioamido)hexanoate) (Sulfo-LC-SPDP) was purchased from Pierce, Thermo Scientific. Ultra-pure water (Milli-Q Element, Millipore) with resistivity of 18.2 MΩ·cm (at 25°C) was used for preparing all the solutions. Human Cellular Fibronectin (cFn) and the corresponding monoclonal antibody, purified Anti-Human Fibronectin (Anti-Fn), were purchased from Immunostep, Spain.

To prepare well-defined model substrates, numbered marks were added by photolithography to ensure that the same region could be located and measured after each functionalization step. After this process, the wafer was cut in 7 × 7 mm² pieces. The substrates were cleaned with acetone followed by isopropanol (IPA), rinsed with Milli-Q water and dried under flowing N2. To promote reproducible electrostatic adsorption of AuNPs, each substrate was functionalized for 30 min in PLL (Fig. 2(a)), a cationic polymer, rinsed with Milli-Q water and dried under flowing N2.

Fig. 2. Schematic view of the functionalization process and the final detection/biorecognition model system. a) Native oxide on Si surface functionalized with PLL; b) AuNPs adsorbed on the PLL-modified surface; c) AuNPs functionalized with a heterobifunctional linker; d) antibody immobilization on the AuNP surface; e) target protein capture/recognition by the antibody.
The nominally monodisperse AuNPs were deposited on each PLL-treated substrate by spotting 10 µL of the as-received solution (Fig. 2(b)). These samples were then treated with 1 mg/mL Sulfo-LC-SPDP heterobifunctional crosslinker in phosphate buffer (PB), with sufficient volume to completely cover the sample surface; the linker treatment was carried out for 20 min in humid atmosphere (Fig. 2(c)). Prior to antibody immobilization, the sample surface was rinsed with PB, then with Milli-Q water, and dried under flowing N₂. Anti-Fn solution (250 µg/mL) was contacted for 1 h with the SPDP-modified surfaces of AuNPs (Fig. 2(d)). After the Anti-Fn immobilization, 40 µg/mL solution of cFn was spotted onto each sample and left in contact with antibody-functionalized AuNPs for 1 h (Fig. 2(e)). After this biorecognition step, each sample was washed several times with PB, then with Milli-Q water, and dried under flowing N₂.

3.2 Sample characterization

After adsorption of the AuNPs, each sample was inspected by Scanning Electron Microscopy (SEM) to analyze the distribution and density of the particles on the surface. The SEM characterization was carried out using a Quanta 650 FEG (FEI) field-emission SEM in high-vacuum mode, with a voltage of 5 kV. The surface density for all the samples was in the range of 15-20 particles/µm², and the particles were randomly scattered across the surface, with very few clusters observed in each field of view (Fig. 3).

The ellipsometric measurements were made using a commercial spectroscopic imaging ellipsometer from Accurion (Accurion nanofilm_EP4_se, Accurion GmbH, Göttingen, Germany) equipped with a supercontinuum laser that provides broad excitation between 450 and 1100 nm; experimental data were acquired from 500 to 600 nm, with an angle of incidence (AOI) of 65°. This imaging ellipsometer enabled us to select the same region of interest (ROI) consistently after each sample preparation step, while registration markers ensured that distribution and density of AuNPs in each ROI was independently and directly established by the SEM measurements described above.

In ellipsometry, the polarization of electric field before and after the reflection of light by the sample is measured. The resulting measured ellipsometric parameters, \( \psi \) and \( \Delta \) (the magnitude and phase of the ratio of p- and s-polarized reflectivity), provide rich information about the sample under investigation [32]:

![Fig. 3. SEM image of AuNPs (ca 20 nm diameter) on PLL-functionalized Si substrate. Surface density is ca 20 particles/µm².](image-url)
\[ \frac{R_p}{R_s} = \tan(\Psi) e^{i\Delta} \]

where \(R_p\) and \(R_s\) are the complex-valued reflection coefficients for the polarization parallel (p) and perpendicular (s) to the plane of incidence; \(\tan(\Psi)\) is the amplitude ratio upon reflection and \(\Delta\) is the phase difference \((\delta_p - \delta_s)\).

To provide the parameters of experimental substrates for the Bobbert-Vlieger model, a PLL-functionalized substrate was measured by ellipsometry and determined to be Si covered with a 3-nm layer of SiO\(_2\) and a 1.5 nm layer of PLL with a refractive index of 1.45.

3.3 Numerical computation

Computation results for ellipsometric parameters \(\Psi\) and \(\Delta\) are based on an open-source C++ light scattering tool—SCATMECH (version 7.00, Jan 2015)—developed by Thomas A. Germer at the National Institute of Standards and Technology (NIST) [21]. As indicated above, for direct comparison with experimental data the substrate is in all cases modeled as Si covered with 3 nm of native oxide and 1.5 nm of PLL. In each calculation, the AuNPs are placed on the substrate at a specific surface density \((d)\). Initially, AuNPs are assumed to have no organic shell, with shell layers subsequently added to model the attachment of antibodies and proteins to the particles.

4. Results and discussion

4.1 Numerical results

We took advantage of numerical computations to systematically investigate the changes in the ellipsometric parameters \(\Psi\) and \(\Delta\) as a function of characteristics of the core-shell NPs—surface density, shell refractive index and thickness—varied within ranges realistically accessible in experiments; all calculations assumed a fixed gold core diameter of 20 nm and a fixed AOI of 65°. We find that of the two ellipsometric parameters, the phase shift \((\Delta)\) exhibits the higher sensitivity to changes in the configuration of core-shell NPs.

With increasing surface density of particles, the AuNP-specific signal increases for both \(\Psi\) and \(\Delta\), but the trend is more pronounced for \(\Delta\) (Fig. 4). In the following calculations, the surface density will be fixed at 20 particles/\(\mu\)m\(^2\), which corresponds to the experimental value (Fig. 3) and apparently is close to the practical limit of detection, based on the data in Fig. 4.

To examine the effect of adding a bio-organic shell around the gold core, we have to consider a range of refractive index values, because the most appropriate value to use for protein shells around NPs is not clear \textit{a priori} [26–28]. Accordingly, we varied the refractive index of the shell from that of pure water (1.33) to that of dried protein (1.57), assuming air as the surrounding medium. Ellipsometric parameters \(\Psi\) and \(\Delta\) calculated assuming particles
with 20 nm diameter gold core (same as in Fig. 4) and a putative bio-organic shell of 10 nm in thickness are shown in Fig. 5. There is a clear (and readily measurable) red-shift for the minima in both $\Psi$ and $\Delta$, as indicated by arrows in Fig. 5, suggesting that with appropriate modeling and calibration it may be possible to estimate the effective degree of hydration for bio-organic shells on AuNPs. For the following calculations, the refractive index will be fixed at 1.4, a value commonly used to represent thin protein layers.

Fig. 5. $\Psi$ and $\Delta$ calculated through Bobbert Vlieger model of AuNPs covered with a 10 nm shell having different refractive index values. Surface density is 20 particles/µm², gold core diameter is 20 nm, AOI is 65°. Arrows are added as guides-for-the-eye to highlight the trend.

Shell thickness, in contrast to its refractive index, only affects the magnitude of the spectral features in $\Psi$ and $\Delta$, while their position remains the same (Fig. 6). Interestingly, the effect is both more apparent and systematic in different representations of the data for $\Psi$ and $\Delta$. For $\Psi$, the baseline shift is essentially monotonic with shell thickness, but not systematic across the range from 450 to 600 nm, while subtracting the baseline, $\delta_{\Psi} = \Psi(450\text{nm}) - \Psi(\lambda)$ shown as inset in Fig. 6, reveals a monotonic and systematic trend for the minimum around 520 nm. Conversely, for $\Delta$ the baseline shifts vertically in a monotonic and systematic fashion with increasing shell thickness, while the baseline-corrected representation in the inset reveals only minimal differences that are neither monotonic nor systematic (Fig. 6).

A useful comparison in terms of the magnitude of the shifts predicted as a function of the refractive index and thickness of the bio-organic shell (Figs. 5 and 6) is provided by the shifts associated with varying the diameter of the particles (Fig. 7). The systematic shifts in Fig. 7 also can be used to compare the trends in spectral features for ellipsometric angles to those in other size-dependent properties of gold nanoparticles, such as optical absorbance [22].
Fig. 7. $\Psi$ and $\Delta$ calculated through Bobbert Vlieger model of bare AuNPs having different diameters.

The numerical simulations summarized in Figs. 4–6 offer two useful predictions for experimental measurements. First, a minimal surface density of AuNPs would be required to observe AuNP-specific spectral features. This minimal density will be substrate-dependent because any substrate spectral features may overlap and otherwise interfere, but for a commonly used Si substrate with native oxide, the minimal density would be on the order of 20 particles/µm$^2$ (Figs. 3 and 4). Second, after a bio-organic shell is added to the AuNPs, we expect a red-shift on the order of 50 nm for the spectral features as well as measurable baseline shifts and/or magnitude changes for $\Psi$ and $\Delta$, with baseline shifts in $\Delta$ providing a systematic response that should be readily observed in realistic systems.

4.2 Validation of the model

The well-known Maxwell-Garnett model [23,24] provides a useful validation for our predictions from the extended Bobbert-Vlieger model. The Maxwell-Garnett approach is based on effective medium approximation (EMA), assuming a layer of one material with inclusions of another material. EMA models are commonly implemented in ellipsometry instrument software where they are used for interpreting data for nanostructured samples. For comparison with our Bobbert-Vlieger calculations for bare AuNPs of 20 nm diameter (Fig. 4), we use EMA models of gold with 10 or 20 nm thickness and 99% void fraction, the latter approximating the surface coverage observed by SEM in Fig. 3. The shapes of spectral features produced by the different models are qualitatively similar (Fig. 8), but of course the absolute values predicted for $\Psi$ and $\Delta$ differ, due to different underlying assumptions. Notably, for $\Delta$ positions of the minima are similar, but the baseline shift predicted by the Bobbert-Vlieger model is closer to the EMA result for 10 nm rather than 20 nm film thickness.

Fig. 8. Comparison between Bobbert-Vlieger model (AuNPs 20 nm diameter at 20 particles/µm$^2$) and Maxwell-Garnett model (layer of gold of 20 nm and 10 nm thickness and 99% void).
4.3 Ellipsometric experimental results

Our experimental model system had been prepared to closely approximate the idealized configurations assumed for numerical simulations. The commercial AuNPs we used are of approximately spherical shape and 20 nm in diameter. They were adsorbed on PLL-modified substrate (Fig. 2(b)) at surface density of 20 ± 3 particles/µm² verified by SEM measurements (Fig. 3). Lithographic markers ensure that the same ROI with the known AuNP distribution and density had been measured in SEM and all of the imaging ellipsometry experiments. As a model of biofunctionalized AuNPs, we chose the sample after antibody immobilization step (Fig. 2(d)), while the sample after cFn protein capture step (Fig. 2(e)) was chosen as a model of a finished biorecognition or biosensing assay.

Accordingly, ellipsometric measurements were performed for samples corresponding to steps b), d) and e) from the diagram in Fig. 2. As expected, the attachment and capture of different molecules induced changes in both \( \Psi \) and \( \Delta \) values (Fig. 9). In agreement with our numerical simulations (Figs. 4–6), the changes in \( \Delta \) spectrum are more pronounced than that in the \( \Psi \) spectrum, indicating a higher sensitivity to bio-organic coatings when using the \( \Delta \) signal.

![Fig. 9. Experimental ellipsometry data for a model implementation of an immunoassay based on AuNPs.](image)

The known and measured parameters of our experimental model system allowed us to perform numerical simulations for a realistic representation of the experimental conditions. The following parameters were used for the Bobbert-Vlieger model calculations summarized in Fig. 10: Si substrate with 3 nm of native oxide and 1.5 nm of PLL with a refractive index of 1.45 (matching ellipsometric measurements of the PLL-treated substrate), AuNPs with 20 nm diameter at surface density of 20 particles/µm² (matching SEM measurements), protein shells with a refractive index of about 1.45 [26, 27] and thicknesses calculated for anti-Fn (7.7 nm) and cFn (11.5 nm) using a protein size calculator (CalcTool) [33].
The overall shapes of the spectra in Figs. 9 and 10 are clearly similar, indicating that our implementation of the Bobbert-Vlieger model captures the relevant physical phenomena for the core-shell metal-organic particles in this size range. We adjusted the aspect ratios of the plots to be consistent with those in Fig. 5, to make the comparison of the features and shifts simpler.

The predicted $\Delta$ signal in Fig. 10 exhibits trends in two characteristics: a downward shift of the baseline with increasing thickness of the protein layer and a red-shift of the minimum from “bare” to coated AuNPs.

The baseline shift in the $\Delta$ signal is in a semi-quantitative agreement between the experimental (Fig. 9) and predicted (Fig. 10) spectra. From “bare” AuNPs to AuNPs + linker + Ab experimental shift is ca. 1.5 degrees vs predicted ca. 1 degree, from AuNPs + linker + Ab to AuNPs + linker + Ab + Fn the shifts are ca. 5.5 vs 3.7 degrees, respectively; the absolute $\Delta$ values remain in the range of 160 to 170 degrees in both cases. The predicted shifts are in both cases the same relative fraction of the experimental ones (ca. 30% smaller), suggesting a possible systematic bias. Specifically, simple estimates of protein sizes used as predicted layer thicknesses are not likely to be an accurate approximation of the effective layer thicknesses produced and observed experimentally. Notwithstanding the bias, the measurements confirm predictions from our systematic simulations (Fig. 6) that the baseline shift would be a robust experimental signature of increasing the thickness of the bio-organic shell. The consistent relative baseline shifts for $\Delta$ in Figs. 9 and 10 are also an important indication that the experimental signal is likely dominated by the protein coatings on AuNPs rather than by proteins nonspecifically adsorbed elsewhere on the substrate. Such a putative nonspecific contribution would produce a larger shift because the AuNPs occupy essentially a negligible fraction of the substrate surface. Furthermore, a nonspecific contribution would also effectively make the AuNPs-specific minimum less prominent, since thin bio-organic films do not have spectral features in this wavelength range.

The red-shift of the minimum in the $\Delta$ spectra is predicted in Fig. 10 between “bare” and coated AuNPs, but not observed in Fig. 9. The likely main cause of the predicted red-shift is the change in the refractive index, because both the shift and increased magnitude of the predicted dip are in a quantitative agreement with our systematic simulations in Fig. 5, taking into account that “AuNP” simulated line in Fig. 10 assumes the refractive index of 1 around the core. Conversely, the absence of this red-shift in the experimental data in Fig. 9 implies that the effective refractive index in the immediate vicinity of the AuNPs before functionalization is not very different from that of protein layers. In other words, the starting AuNPs should not be assumed to be completely “bare”, which could be related to the presence of organic shells on solution-synthesized AuNPs and their interactions with PLL chains on the substrate. Assuming a well-defined state for as-received AuNPs that are
nominally covered only with a weakly bound citrate shell is notoriously difficult, in part because they can easily become contaminated with other adsorbates at levels detectable by sensitive techniques [34–36]. Upon exposure to the ambient environment, adventitious organic contamination will be present on both nominally bare and peptide functionalized gold surfaces in the amounts at least comparable to those of the intentionally immobilized molecules [37,38], thus further diminishing the difference in refractive index surrounding the “bare” vs biofunctionalized AuNPs in the experiments.

The $\Psi$ signal in Figs. 9 and 10 behaves qualitatively similarly to the $\Delta$ signal, but the baseline shifts do not trend as clearly for $\Psi$ as they do for $\Delta$, in agreement with our systematic simulations (Figs. 5 and 6). Analogously to the above discussion for the $\Delta$ signal, the red-shift and amplitude enhancement of the minimum in the $\Psi$ signal from “bare” to functionalized AuNPs in Fig. 10 are consistent with predictions in Figs. 5 and 6, given that the “bare” AuNP simulated line assumes the refractive index of 1 around the core; the lack of such dramatic changes in Fig. 9 then also implies that the experimental AuNPs do not start completely bare. This brief analysis of the $\Psi$ signal thus supports our conclusion from systematic studies in Figs. 5 and 6 that $\Psi$ and $\Delta$ change in similar and consistent ways when parameters of the bio-organic shells are varied, but the signatures in the $\Delta$ signal are more easily interpreted.

While our implementation of the Bobbert-Vlieger model captures the main ellipsometric signatures of the bio-organic shells on AuNPs, we note that some additional effects related, for example, to interactions between NPs and the substrate or to NP properties being divergent from the idealized assumptions may be not fully included. In particular, various depolarization and asymmetry effects could affect experimental measurements, particularly the absolute positions and exact shapes of the spectral features, so the extent to which our results can be generalized to a broad range of realistic combinations of NPs, substrates, and corresponding materials will need to be explored in future investigations.

5. Conclusions

We have investigated spectroscopic ellipsometry as a technique for characterization of core-shell metal-organic nanoparticles adsorbed on a substrate. Specifically, we adapted the Bobbert-Vlieger model for light scattering by core-shell particles on a substrate for numerically computing predicted ellipsometric parameters $\Delta$ and $\Psi$ for realistic metal-organic particle configurations encountered in biomedical applications. In a limiting case of nominally bare gold nanoparticles, the predictions of the Bobbert-Vlieger model are consistent with those of the commonly used Maxwell-Garnett effective medium approximation (EMA). Advantages of the Bobbert-Vlieger model include its reliance on exact solution of Maxwell’s equations and the possibility to simulate nanostructured configurations more complex than those assumed in EMA calculations. We found, both theoretically and experimentally, that the changes in ellipsometric parameters associated with biofunctionalization and biorecognition events at nanoparticle surfaces can be detected under realistic experimental conditions. Our results also suggest that with further development and validation this approach may be extended to measurements of more complex parameters, such as the degree of hydration for bio-organic shells, or perhaps even to measurements of biofunctionalized nanoparticles in solution.

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