White Paper

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How to analyze combined QCM-D and ellipsometry data

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[Progress Together]

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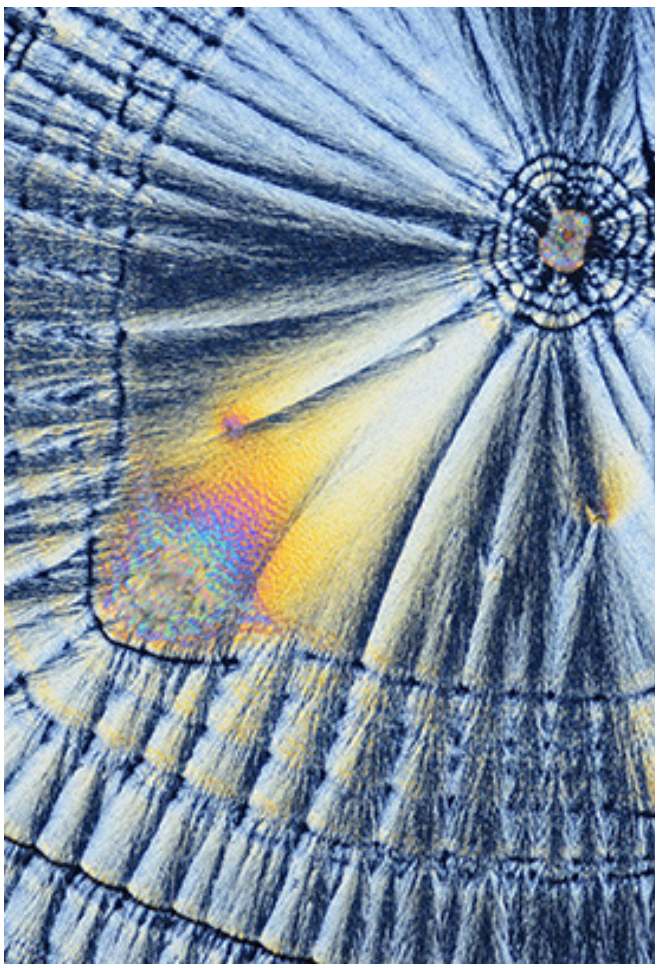
The porosity, conformation, or swelling state of organic layers can be especially important for their functionality and performance. One approach to characterize these properties is to use a combination of the complementary techniques quartz crystal microbalance with dissipation monitoring (QCM-D) and ellipsometry, which allows for the quantification of the surface mass density and porosity of such adsorbate layers. This paper outlines the theory behind this quantification and also outlines the step-by-step procedure on how to do such an analysis.

Ellipsometry for the characterization of layer thicknesses and optical properties

What is ellipsometry?

Ellipsometry is an optical surface characterization technique that can measure material properties, such as the thickness profile and optical response, of thin films and bulk materials. Ellipsometry is widely used, and perhaps most well-known, in the semiconductor industry. Application examples in this field are numerous and include quality control (thickness of deposited layers, sample uniformity, attainment of desired optical property) for coatings on Si wafers, glass panels, photovoltaics, etc.; in-situ process control for chemical or physical vapor deposition, atomic layer deposition, or plasma etching; and characterization of semiconductor and conductive organic device properties.

Ellipsometry has been applied since the 1980s to characterize the optical properties and quantify the amount of protein adsorbed on surfaces [1]. Since then, technical and automation improvements in ellipsometry, and an increased interest in biological materials, have ushered in many new studies to monitor the real-time formation and modification of organic or biomolecule adsorbate layers, such as proteins, polymers, nucleic acids, lipids, and surfactants, under liquid ambient conditions. Information can be obtained such as thickness/mass from the visible spectrum [1-4]; intramolecular bonds and molecular alignment from the infrared spectrum [5]; and intermolecular bonds from the terahertz spectrum [6, 7].



How does ellipsometry work?

Ellipsometry relies upon the measurement of the polarization state of a light beam, where light with a known polarization is generated and directed at the sample substrate, see **Figure 1**. The light may be transmitted through the sample or reflected off the sample. For the combined ellipsometry and QCM-D application, the surface is opaque, and the light is reflected off the surface. When the light interacts with the sample, the polarization state of the light is modulated. The reflected, modulated beam is then measured by a polarization state detector, and the collected data can be used to extract information about the sample geometry and material properties responsible for modulating the light beam.

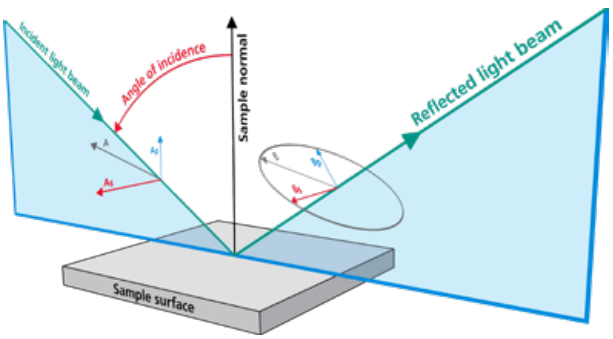


Figure 1
Schematic illustration of the plane of incidence (***p*** plane) and the angle of incidence of incident and reflected light beams. The incident light beam is here linearly polarized, and after interaction with the sample, the reflected light's polarization state is modulated and becomes elliptical. A_p, A_s, B_p, and B_s denote the complex amplitudes of the ***p*** and s components before and after reflection, respectively. ***p***-component vectors lie along the plane of incidence and are normal to the light beam propagation vector. ***s***-component vectors are normal to the ***p*** plane.

Ellipsometry measures polarized light forming the shape of an ellipse

The light is considered to have two orthogonal polarization axes, which are both normal to the light beam propagation vector, see Figure 2. One axis, ***p*** (from the German word for parallel), lies along the measurement **plane of incidence** (POI), while the other axis, ***s*** (from the German word for perpendicular), is normal to the POI. The polarization state of the light beam, ϱ, is expressed by the ellipsometry equation

$$\varrho = \frac{r_p}{r_s} = \tan\Psi\, e^{i\Delta} \qquad (1)$$

where r_p and r_s denote the complex reflection p and s coefficients, respectively, and Ψ and Δ are the ellipsometric parameters. Ψ is related to the relative amplitude magnitudes between the p- and s-component waves, and $\tan\Psi$ is the absolute value of the real part of ϱ. $e^{i\Delta}$ is the relative phase shift between the p- and s-component waves [8].

If one could look down a randomly polarized light beam propagation vector and observe the shape that the amplitude makes as the electromagnetic wave cycles over time, in almost all cases that shape would be an ellipse, see **Figure 2**. The only exceptions are linearly and circularly polarized light. Ellipsometry gets its name because Ψ and Δ describe the shape of the ellipse.

To summarize, the sample and experimental system optical properties determine the change in the polarization state of the light beam. Because the incoming light beam polarization state is known (generated by the instrument) and the outgoing light beam polarization state is measured, ellipsometry determines the change in the polarization state. This is the information used with data analysis techniques to yield physical properties of interest. These parameters include the thicknesses and optical properties of oxide layers grown as a stack on a silicon wafer or the thickness of a monolayer of proteins on a surface in liquid. Dynamic, **in-situ** ellipsometry measurements allow for monitoring the growth and modification of adsorbate layers at the solid-liquid interface in real time.

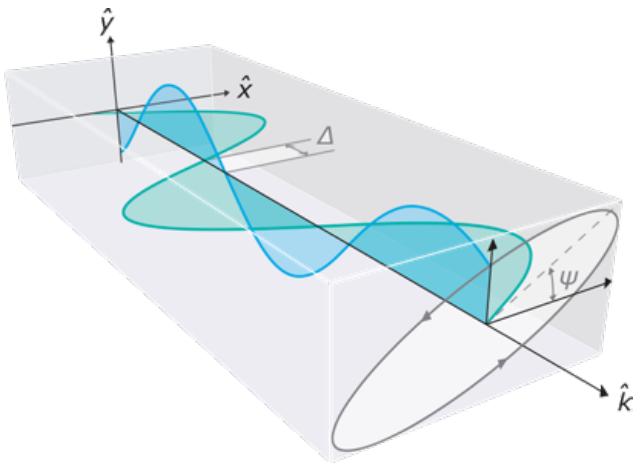


Figure 2
Schematic illustration of elliptically polarized light and description of what Ψ and Δ are related to. The light beam propagation vector lies along the k-axis, and the arbitrary ***x***- and ***y***-axes are redefined as the ***p***- and ***s***- axes depending on what the plane of incidence is.

An approach to ellipsometry data analysis

The equations that relate the ellipsometry parameters Ψ and Δ to physical properties of interest, e.g., the thickness and mass of an adsorbate layer, are nonlinear and therefore require data modeling techniques. A physically meaningful optical model thus needs to be developed by the user for each experiment, as described thoroughly in the literature, for example Refs.[8, 9]. The unknown model parameters, such as the adsorbate layer thickness, are given guess values, and Ψ and Δ data are calculated from the modeling equations and compared with measured Ψ and Δ data. When the model-calculated and measured Ψ and Δ data best-match, the parameters of the optical model are considered to describe the physical system [8, 9]. If one compares ellipsometry data analysis with the analysis of QCM-D data using the Voigt-Voinova viscoelastic model, Ψ and Δ are analogous to the frequency (f) and dissipation (D) QCM-D parameters, and the wavelength of light (λ) is analogous to the harmonic overtone order (n).

Laser ellipsometers only measure Ψ and Δ at one wavelength of light, but since the 1980s, commercially available spectroscopic ellipsometers (SE) have become available with data analysis software. Additional measured wavelengths allow for more rigorous data analysis [8, 9].

Spectroscopic Ellipsometry approaches to quantify the adsorbate surface mass density

Before the experiment and data collection of the adsorption process start, the optical properties of the substrate and ambient liquid need to be determined, **Figure 3**. Once this information is known, a suitable approach must be selected to obtain the thickness of the solvated adsorbate layer. Here, two possible choices are described:

i) **the de Feijter Equation**.

ii) **the Virtual Separation Approach**

Additional details may be found in Refs.[2-4, 10].

i) The de Feijter Equation to quantify the surface mass density

The de Feijter equation allows one to directly obtain the adsorbate mass from the optical model [3] and is given by

$$m_o = \frac{d_{eff}^{SE}(n_{eff} - n_a)}{dn/dc} \quad (2)$$

where m_o is the "dry" adsorbate surface mass density with units of mass/area, d_{eff}^{SE} is the "effective" thickness of the porous adsorbate layer as determined by the SE optical model, n_{eff} is the index of refraction of the porous adsorbate layer, n_a is the index of refraction of the ambient liquid, and dn/dc is an adsorbate material parameter known as the refractive index increment. The de Feijter equation should be used in most cases unless the adsorbate layer is very thin, for example, on the order of less than 10-30 nm when using the visible spectrum (see Virtual Separation Approach).

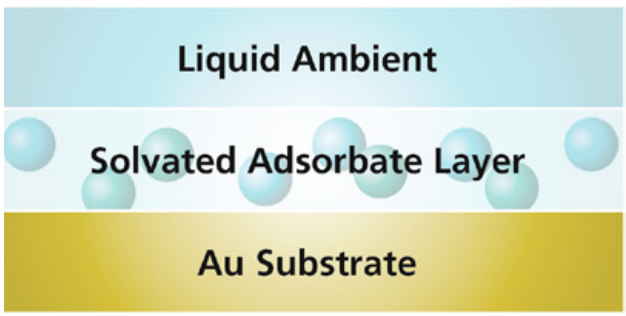


Figure 3
A schematic illustration of the optical model. The optical properties of the substrate and ambient liquid are determined before the experiment, and the thickness of the solvated adsorbate layer may be obtained from the de Feijter Equation or the Virtual Separation Approach.

If the difference of indices of refraction of the adsorbate (***no***) and ambient materials is small, ***i.e.***, $|n_o - n_a| \ll n_a$, then ***dn/dc*** is assumed to be constant with respect to the adsorbate volume fraction, f_o^V, of the porous layer. ***dn/dc*** may be experimentally measured via refractometry of reference solutions with known solute concentrations. ***dn/dc*** can be dependent on the surface and ambient solution (e.g., ionic strength, temperature). When obtaining a ***dn/dc*** value from the literature, **Table 1**, note the laser wavelength and environmental conditions of the measurement. Work has also been done to predict ***dn/dc*** for a protein if the amino acid composition is known [11].

Material	dn/dc (mL/g)	Reference
General protein	0.185	12
Human serum albumin	0.18	13, 14
Bovine serum albumin	0.187	3
β-lactoglobulin A	0.168	15
Bovine γ-crystallin	0.203	16
Polyacrylic acid	0.133	17
General nucleic acid	0.170	12

Table 1. *Reported **dn/dc** of select example materials.*

Thus, the two remaining unknown parameters are n_{eff} and d_{eff}^{SE}, which are both fit parameters of the SE optical model. n_{eff} may be more specifically yielded by the parameterized Cauchy model. The Cauchy model describes the optical response of dielectrics and is valid for optically transparent materials, such as many oxides and organics.

$$n_{eff}(\lambda) = A + \frac{B}{\lambda^2} + \cdots, \quad (3)$$

where λ is the wavelength, and A and B are tabulated optical constants of the material used. When the ***C***auchy equation is substituted for n_{eff}, A and B replace n_{eff} as fit parameters. With these values, the de Feijter equation yields the dry surface mass, m_o.

ii) The Virtual Separation Approach to quantify surface mass density

The Virtual Separation Approach is a simplification of the optical modeling mathematics that are used to treat very thin, transparent layers that meet the ultra-thin film criterion [2]

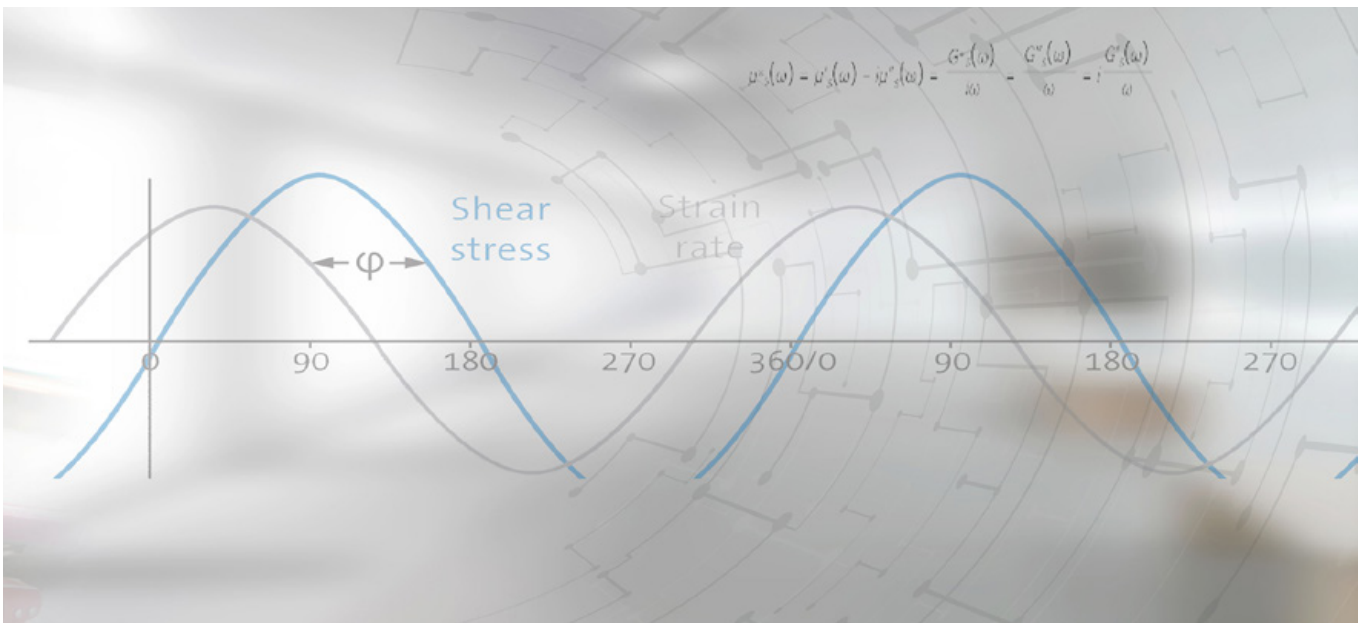
$$d_{eff}^{SE} \ll \frac{\lambda}{2\pi n_{eff}}. \quad (4)$$

In this case, ellipsometry loses sensitivity to the parameter Ψ relative to Δ [2,4,10]. For the visible spectrum, the ultra-thin film thickness limit is on the order of 10-30 nm. Thus, one is left with only Δ as a reliable measurement parameter to determine the two unknowns n_{eff} and d_{eff}^{SE}. Hence one is left with

$$C * \delta\Delta = n_{eff} d_{eff}^{SE}, \quad (5)$$

where C is a constant and $\delta\Delta$ is the shift in measured Δ between before and after layer formation.

It is clear that in this case, n_{eff} and d_{eff}^{SE} are correlated. Increasing one parameter and decreasing the other parameter can yield an equivalent modeling result. If one does not know the index of refraction of the porous layer, the sensitivity to the layer thickness is lost.



However, it has been shown that when the ultra-thin film criterion has been met, one may "virtually separate" the organic adsorbate and liquid ambient inclusions into separate, pure sublayers from an ellipsometric modeling point of view [2]. In this scenario,

$$n_o d_o^{SE} \approx n_{eff} d_{eff}^{SE} \quad (6)$$

where d_o^{SE} is the thickness of a hypothetical sublayer where all the adsorbate of the measured, effective layer is perfectly compact and homogeneous. We see that in this case, SE loses sensitivity to the liquid ambient contribution to d_{eff}^{SE} and directly yields d_o^{SE} upon assumption of n_o. Finally, knowledge or assumption of the adsorbate density ρ_o allows for the determination of the dry mass, m_o.

Approaches to analyze the QCM-D data

The analysis of QCM-D and the extraction of mass, m_{QCMD}, and thickness, d_{QCMD}, is outside the scope of this white paper. Resources on QCM-D data analysis may be found in Refs.[4] and [18] or at the Biolin Scientific website.

Adsorbate fraction parameters

One can describe the quality of a solvated organic layer by an adsorbate fraction parameter f_o. The adsorbate volume fraction parameter f_o^V can be simply expressed by

$$f_o^V = \frac{d_o^{SE}}{d^{QCMD}}, \quad (7)$$

where d_{QCMD} is the thickness of the layer determined via QCM-D.

The adsorbate mass fraction parameter is given by

$$f_o^m = \frac{\rho_o d_o^{SE}}{\rho_{eff} d_{QCMD}} = \frac{m_o^{SE}}{m_{QCMD}}, \quad (8)$$

where ρ_o is the adsorbate density, ρ_{eff} is the effective layer density, m_o^{SE} is the adsorbate surface mass density obtained from SE, and m_{QCMD} is the effective layer surface mass density obtained from QCM-D.

Example: Optical model setup in practice

Here we will consider the adsorption of a solvated protein layer onto a bare gold surface as a reference experiment. Other scenarios may require different optical and mechanical modeling approaches or a variation on the experimental steps provided here. However, the general sequence of events should be very similar. Here we describe how a sequence of SE measurements is taken to build and adjust an ellipsometry optical model. The execution and data analysis of the QCM-D experiment is beyond the scope of this paper and is described in more detail by references [19-20].

The modeling of the SE data requires SE reference measurements that provide information on the optical properties of the substrate, ambient medium, adsorbate, and other effects caused by the experimental setup at all stages of the measurement. The following Ψ and Δ spectra hence must be captured:

1. **Bare surface**
2. **Bare surface** + windows in air/void
3. **Bare surface** + windows in liquid (i.e., background solution)
4. **Bare surface** + windows in liquid, exposed to the molecule under study.

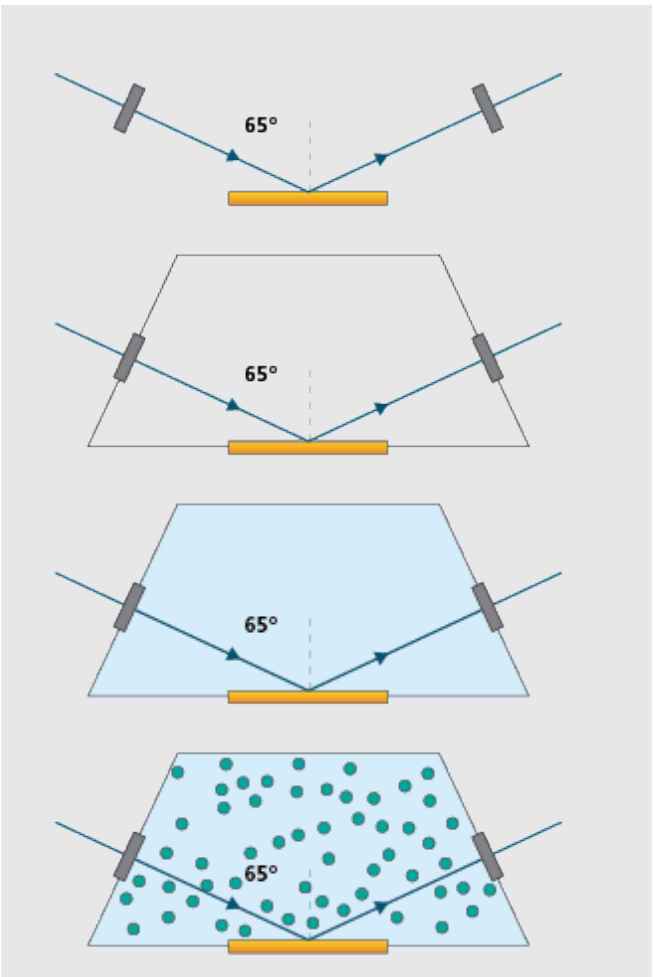


Figure 4
A schematic illustration of the optical model setup in practice.

1. Spectroscopic Ellipsometry (SE) characterization of sensor substrate

The first step is to take an SE measurement of the substrate, which is the metal-coated QSensor. We should consider the following questions about the substrate so that a well-fitting model can be prepared: Is the substrate transparent or absorbing? Is the substrate made up of sublayers? If so, can the probing light beam penetrate one or more of the top sublayers and thereby reveal sensitivity to underlayers? In this example we use Au. In the visible spectrum, Au is absorbing. Because the Au layer is sufficiently thick, the reflected light is sensitive neither to the underlying adhesion layer between the Au and the quartz nor the quartz. Thus, the Au can be considered as a semi-infinite substrate. **Note** that for oxide surfaces, accurate characterization of the oxide layer thickness and optical properties is essential for analyzing the in-situ experiment.

2. SE characterization of effects due to liquid handling (window, angle offsets)

When the Ellipsometry Module is loaded with the QSensor and mounted onto the Explorer chamber, the ellipsometry probing light beam is able to travel through the module windows and reach the detector. In practice, it is sometimes necessary to manually adjust the AOI to perfectly realign the beam with the detector. The module windows may not have been perfectly aligned against the beam due to how they are mounted via O-rings. Changing the AOI from its idealized set point of 65 degrees has to be compensated for as a modeled effect on Ψ and Δ. Additionally, the windows, themselves, have an effect on the ellipsometry spectra. Window and AOI offsets are used as fit parameters in the optical model and should be determined via the capturing of a second SE spectrum before proceeding. Typically, the window effects will only modulate Δ and so are parameterized as Δ offsets.

3. SE characterization of solid-liquid interactions

Once the two pre-experiment Ψ and Δ spectra have been captured, the next step is to introduce the solvent that will be used as background solution. In our example, we use buffer solution. Now, the optical model must be adjusted for the change. The ambient material in the model is hence changed from air (or void) to the liquid ambient buffer. The optical properties of the liquid must be known a ***priori***, and may be measured, for example, by the beam deviation method [14]. A colored liquid, with metallic ions, for example, will have non-zero k at wavelengths where light is being absorbed. Ellipsometry data at these wavelengths will commonly be much noisier than for a transparent region.

A third SE measurement is taken to ensure that the adjusted

optical model describes the experimental system. Discrepancies between the experimental and model-calculated data may be caused by the rinsing off or attachment of contaminants and for our purposes may be considered by the optical model as substrate modification. The substrate optical properties should be refit at this step.

We now assume that there is a good match between the model-calculated and experimental SE data sets. From this point, differences between the data sets may be attributable to the introduction of the protein solution and the formation of the adsorbate layer. If necessary, the optical properties of the protein solution can be used for the liquid ambient in the optical model during protein solution exposure. Otherwise, we solely attribute modulation of the Ψ and Δ spectra to formation of the adsorbate protein layer.

4. Prepare optical model for protein adsorption measurement

Now, we are ready to begin the measurement of protein adsorption onto the gold. In the optical model, add an adsorbate layer above the substrate and allow the model to vary the optical properties and/or the thickness of the layer during the experiment. The initial guess value for the thickness of a protein layer can usually be allowed to be 0 nm. As already mentioned, the choice of optical model for the adsorbate layer depends on the expected properties of the layer. In this example, we are adsorbing protein to Au, and will use the de Feijter model. Modern ellipsometry software packages support "dynamic measurements" where ellipsometry spectra are periodically, automatically measured.

During signal baseline stabilization with the background liquid, prior to protein introduction, create a time stamp in QSoft and record the current measurement time for the dynamic ellipsometry measurement. This can be used to synchronize the data for calculating the adsorbate fraction parameters later.

5. After protein adsorption measurement

After the experiment has ended, remodeling the ellipsometry data is generally possible if one wishes to try an alternate approach. QCM-D data is also analyzed at this point. Adsorbate thickness or mass versus time data from both instruments may then be exported to obtain the adsorbate fraction parameters versus time.

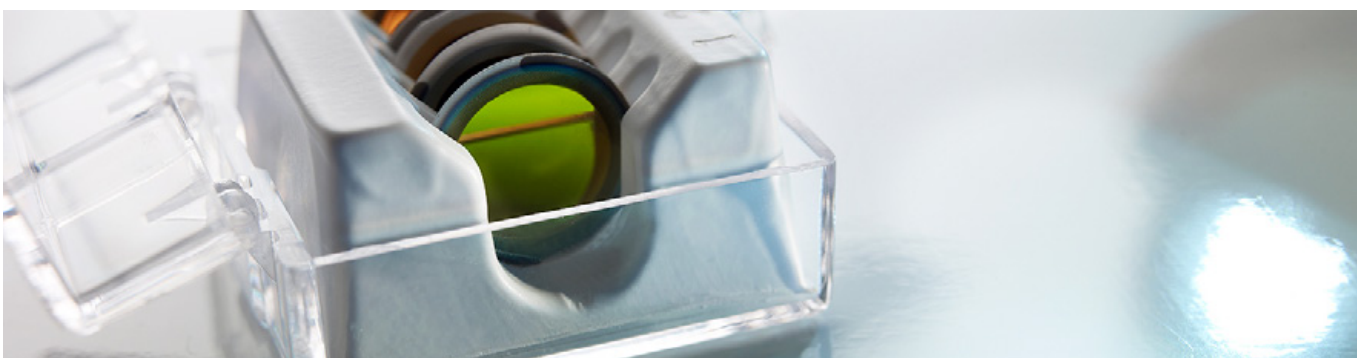
The ellipsometry and QCM-D measurement start times may be slightly different. To solve this issue, use the time stamp taken by QSoft to synchronize the data so that time t = 0 is the same for both data sets.

Measurement alignment with respect to time

The adsorbate fraction parameter is calculated as a function of time, and so the ellipsometry- and QCM-D-obtained thickness or mass parameters need to align closely with respect to time. As the ellipsometry and QCM-D data point resolution with respect to time almost certainly is going to be different, this requires one of the data sets to be reduced. Usually the QCM-D takes data points much more frequently than the SE instrument. Thus, one matches each slow instrument (ellipsometry) data point with the closest fast instrument (QCM-D) data point with respect to time. The excess fast instrument data points are ignored. Described another way, if one has a set of x data points from the slow instrument and a set of y data points from the fast instrument ($x < y$), the set of y data points is reduced to x data points that align as closely as possible with the data set from the slow instrument.

Final remarks

QCM-D and ellipsometry can be used together to study the formation and modification of thin layers at the solid-liquid interface to yield information about the mass, thickness, mechanical and optical properties, and even the organization or structure of layers in real time. Here, we have presented the theory behind the data analysis as well as outlined a step-by-step procedure on how to set up the data capture and modelling. Ellipsometry data analysis is a very broad topic and is traditionally concerned with semiconductor device physics. Fortunately, most organic and biomolecule adsorbates are simpler materials, and this article described two methods to use for ellipsometry data analysis: the de Feijter equation and the Virtual Separation Approach. Generally, if one is able to fit the thickness and index of refraction of the adsorbate layer without parameter correlation, the de Feijter equation should be used. If there is parameter correlation due to the adsorbate layer being too thin, then the Virtual Separation Approach can be a useful alternative.



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