Ellipsometry on thin organic layers of biological interest: characterization and applications

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Abstract

The thickness resolution and in situ advantage of ellipsometry make this optical technique particularly suitable for studies of thin organic layers of biological interest. Early ellipsometric studies in this area mainly provided thickness quantification, often expressed in terms of surface mass. However, today it is possible to perform monolayer spectroscopy, e.g. of a protein layer at a solid/liquid interface, and also to resolve details in the kinetics of layer formation. Furthermore, complicated microstructures, like porous silicon layers, can be modeled and protein adsorption can be monitored in such layers providing information about pore filling and penetration depths of protein molecules of different size and type. Quantification of adsorption and microstructural parameters of thin organic layers on planar surfaces and in porous layers is of high interest, especially in areas like biomaterials and surface-based biointeraction. Furthermore, by combining ellipsometric readout and biospecificity, possibilities to develop biosensor concepts are emerging. In this report we review the use of ellipsometry in various forms for studies of organic layers with special emphasis on biologically-related issues including in situ monitoring of protein adsorption on planar surfaces and in porous layers, protein monolayer spectroscopy and ellipsometric imaging for determination of thickness distributions. Included is also a discussion about recent developments of biosensor systems and possibilities for in situ monitoring of engineering of multilayer systems based on macromolecules. © 2000 Elsevier Science B.V. All rights reserved.

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1. Objective and outline

Quantification of adsorption of macromolecules and parameterization of microstructure and optical properties of thin organic layers are of high interest in research areas like biomaterials, biosensors, fabrication of devices using organic layers, interfacing electronics with biological systems, etc. The objective of this work is to discuss the role of ellipsometry as an optical diagnostic tool, as well as its use in device applications in these areas. The discussion will mainly be limited to layers of biological relevance. Restrictions are also made to thin layers, that is thicknesses typically below 100 nm and often down to molecular dimensions.

First some basic theory is given as a background to a description of tools and strategies for experimental work and data analysis. Instrumentation is summarized and then some selected applications are presented. Finally some future perspectives are discussed. Note, however, that the examples chosen are from the authors own research experience and a complete overview of the field is not attempted.

2. Theoretical framework

2.1. The ellipsometric principle

Ellipsometry is based on polarization changes occur-
ring upon reflection at oblique incidence of a polarized monochromatic plane wave [1]. The basic quantity measured with an ellipsometer is the complex reflectance ratio

\[ \rho = \frac{\chi_r}{\chi_i} \]

where \( \chi_r \) and \( \chi_i \) represent the state of polarization of the reflected and incident beam, respectively. For samples that can be described with a diagonal Jones matrix [1], like optically isotropic samples and a few special cases of anisotropy, Eq. (1) simplifies to

\[ \rho = \frac{R_p}{R_s} = \tan \psi \exp i \Delta \]

where \( R_p \) and \( R_s \) are the complex reflection coefficients for light polarized parallel and perpendicular to the plane of incidence, respectively. The two parameters \( \psi \) and \( \Delta \) are called the ellipsometric angles and are often said to be the experimentally determined quantities obtained from an ellipsometer. However, the actually measured quantities depend on the instrumental configuration used. For samples with non-diagonal Jones matrices, a full characterization requires measurements of at least three values on \( \rho \) at three different \( \chi_i \), and three pairs of \( (\psi, \Delta) \) are defined. This is referred to as generalized ellipsometry [2].

### 2.2. Which basic parameters are determined in ellipsometry?

Ellipsometry is an indirect technique and the relevant information is obtained by analysis in optical models with the goal to extract values of the parameters defined as unknowns. One basic quantity is the complex dielectric function \( \varepsilon = \varepsilon_1 + i\varepsilon_2 \) of a thin layer or a substrate. Sometimes it is more convenient to use the complex refractive index \( N = n + ik = \sqrt{\varepsilon} \). When doing spectroscopy these quantities are functions of photon energy \( E \): \( \varepsilon(E) \) and \( N(E) \), respectively. Another basic property is the thickness \( d \) of a layer. In multilayered samples several thicknesses may be unknown. For porous layers the void fraction \( f_v \) is the parameter of interest and in composite media in general the relevant parameters are the volume fractions \( f_i \) of each constituent.

### 2.3. The tools for optical modeling

The primary data, either expressed as \( \psi \) and \( \Delta \) or as some related parameters like \( \tan \psi \) and \( \cos \Delta \), are seldom of interest as such and analysis using appropriate models must be carried out to extract information in terms of parameters relevant for the study undertaken. Here we briefly describe microstructural models in terms of a matrix model for multilayer systems and an effective medium approximation for composite media. Dispersion models for describing optical properties are often employed as well but are not discussed here. Finally some simple models for conversion to parameters of biological relevance are described.

#### 2.3.1. Multilayer models

Here we generally deal with samples that can be described as a semi-infinite substrate with one or several layers on top. This optical model is called the \( n \)-phase model and is shown in Fig. 1. An efficient way to handle this model is to employ the scattering matrix formalism which is based on \( 2 \times 2 \) matrices [1]. Each layer and interface is represented by a layer matrix \( L_j \)

![Fig. 1. Isotropic stratified planar structures: (a) the 2-phase model; (b) the 3-phase model; (c) the \( n \)-phase model.](image-url)
and an interface matrix $I_{ij}$, respectively. A system with $m = n - 2$ layers can then be described by a scattering matrix $S$.

$$S = \begin{pmatrix} S_{11} & S_{12} \\ S_{21} & S_{22} \end{pmatrix} = I_{01}L_{1}L_{2}...L_{n-m-1}L_{n-2}L_{n-2,n-1}$$

(3)

The ratio of matrix elements $S_{21}$ and $S_{11}$ gives the reflection coefficients in Eq. (2). If at least one of the layers is anisotropic the preceding formalism must be extended. A general $4 \times 4$-matrix formalism has recently been developed for this purpose [3]. In this case one obtains the general transfer matrix $T$.

$$T = L_{0}^{-1} \prod_{i=1}^{m} T_{i}(-d_{i})L_{f}$$

(4)

where $L_{0}$ and $L_{f}$ are the incident and exit matrices, $T_{i}$, the partial transfer matrix for each layer $(i = 1 \ldots m)$ and $d_{i}$, the thickness of each layer. For a given $\chi_{i}$, the complex reflectance ratio $\rho$ can then be expressed in terms of the matrix elements $T_{ij}$ of $T$. The above extended model should be used whenever ordered systems are studied. Examples of such systems are layers of liquid crystals [4] and Langmuir–Blodgett films [5].

### 2.3.2. Modeling of composite and porous media

Composite materials can be modeled with an effective medium approximation (EMA). Examples of such materials are porous silicon layers where void and silicon are the two main constituents. In the studies presented here such layers are immersed in a liquid and water is filling the pores. If biomolecules are adsorbed in such layers they make up a third constituent. Several EMAs have been developed but here the Bruggeman EMA is used [6]. In this approximation the dielectric function $\varepsilon$ of a three-composite material is obtained from:

$$\frac{\varepsilon_{A} - \varepsilon}{\varepsilon_{A} + 2\varepsilon} + \frac{\varepsilon_{B} - \varepsilon}{\varepsilon_{B} + 2\varepsilon} + \frac{\varepsilon_{C} - \varepsilon}{\varepsilon_{C} + 2\varepsilon} = 0$$

(5)

where $\varepsilon_{A}$, $\varepsilon_{B}$, and $\varepsilon_{C}$ are the dielectric functions and $f_{A}$, $f_{B}$ and $f_{C}$ ($\Sigma f_{j} = 1$) the volume fractions of constituent $A$, $B$ and $C$, respectively. A layer with a density or compositional gradient can now be modeled. The layer is then divided in sublayers and the scattering formalism is employed with sublayer optical functions determined from Eq. (5).

### 2.3.3. From physics to biology

By using the tools described above one obtains values on parameters like refractive indices, layer thicknesses and volume fractions. However, when biological processes are discussed, these terms are not always appropriate and conversion to other forms or further modeling is, therefore, requested. In many cases it is sufficient to determine the surface concentration $\Gamma$, i.e. the adsorbed mass per unit area ($\mu$g/cm$^2$). If an independently determined value of the density of the layer material, $\rho_{\text{layer}}$, is available in the literature, $\Gamma$ is simply obtained from $d$ as

$$\Gamma = d\rho_{\text{layer}}$$

(6)

If the layer is assumed to be transparent and the ellipsometric data allow both $d$ and $n$ to be determined, one can use de Feijter’s formula [7]

$$\Gamma = \frac{d(n - n_{0})}{dn/dc}$$

(7)

where $n_{0}$ is the refractive index of the ambient and $dn/dc$ is the refractive index increment for the molecules in the layer.

However, in many applications it is of great interest to do further modeling to arrive at parameters of more biological relevance. In more advanced studies such parameters could be tilt angle of molecules in a monolayer, density of a protein layer, mass distribution (density gradient) over a protein layer, lateral mass distribution over a surface (clustering), etc. Such studies can be performed in a few cases and represent state-of-the-art of ellipsometric analysis on thin layers. Spectroscopic ellipsometry is here necessary to employ, and combined with advanced optical modeling, more details about the microstructure and optical properties of monolayers of organic films are obtainable [8,9].

### 2.4. The strategies

Layers of biological interest in general have a low refractive index and typical thicknesses are in the nanometer range. To resolve optical and microstructural details and go beyond simple detection of a layer, much is, therefore, to gain by a careful choice of methodology both for experiments and analysis.

#### 2.4.1. Getting the best data: experimental methodology

The information contained in single-wavelength ellipsometric data are in most cases insufficient and spectroscopy should be employed whenever possible. For measurements in air it is also advantageous to do measurements at multiple angles of incidence but this may be rather difficult in situ at a solid/liquid interface due to cell limitations. To reduce parameter correlation effects multiple sample [10], multiple ambient [11] or multiple substrate [11,12] procedures may be employed. Care must then be taken to assure that all parameters that are assumed invariant really remain
invariant. As an example it can be foreseen that the microstructure of a protein layer in most cases is different on different surfaces and a multiple substrate procedure will not work, whereas for samples with spin coated polymer films this may very well be an appropriate procedure. The possibilities and strategies will, thus, depend on the actual sample(s) under study and the objectives.

2.4.2. Getting the best out of the data: regression analysis

Once the optical model is specified, the ellipsometry data are fitted to the model. The fitting problem is basically non-linear and considerable knowledge and powerful mathematical tools are available. The general strategy is to define a figure of merit like the biased mean squared error

$$\chi^2 = \frac{1}{2P-M+1} \times \sum \left( \frac{(\psi_{\text{exp}} - \psi_{\text{calc}}(z))^2}{\delta \psi} + \left( \frac{\Delta_{\text{exp}} - \Delta_{\text{calc}}(z)}{\delta \Delta} \right)^2 \right)$$

where $2P$ is the total number of data points, $M$ the number of parameters in the optical model, $\delta \psi$ and $\delta \Delta$ are experimental errors and the sum is over all experimental data pairs $(\psi_{\text{exp}}, \Delta_{\text{exp}})$ and $\psi_{\text{calc}}(z)$ and $\Delta_{\text{calc}}(z)$ are data calculated with the optical model employed. The parameters to be determined are contained in the $M$-component vector $z$. The inclusion of the experimental errors in Eq. (8) has the advantage that data points with larger errors get lower weight and thus have lower influence on the results.

The next step is to use a fitting algorithm to minimize $\chi^2$, usually the Levenberg–Marquardt method is used [13]. With this method it is also possible to obtain the parameter correlation matrix and 90% confidence limits which, besides $\chi^2$ is very useful for estimating the quality of the data analysis. Further details about data analysis for ellipsometry is given by Jellison [14].

3. A brief review of instrumentation suitable for ellipsometric studies of biological layers

The most commonly used ellipsometer configuration for studying biological layers is the polarizer compensator sample analyzer system [1]. It is based on a nulling principle and is very robust and has a high precision. However, spectroscopy is generally not possible due to the compensator. The speed is also low with a time resolution of the order of a few seconds. For fast measurements operation in the off-null mode can be used [15]. The rotating analyzer ellipsometer (RAE) is considered to be the basic spectroscopic ellipsometer system [16]. The light source in a RAE system is a white light source like a xenon lamp and the wavelength is selected with a monochromator. A typical wavelength range is 200–1000 nm. By including a compensator it is possible to perform generalized ellipsometry. Time-resolved spectroscopy, also called multi-wavelength ellipsometry, requires optical multi-channel techniques [17] resulting in time resolutions of the order of tens of milliseconds. For surface mapping, scanning and imaging techniques have been developed [18].

Many studies are performed in situ and a cell for mounting the sample in a liquid is necessary to use. Several cell designs are found depending on volume, flow conditions, electrode arrangements, etc. [19,20,9]. Techniques for measuring in total internal reflection mode have also been developed and are useful for measurements in opaque liquids [19].

4. Applications

4.1. Unique features of ellipsometry useful in biological applications

Ellipsometry has several features that can be used in various applications for studying layers of biological relevance. First it does not require labeling of molecules like in fluorescence or radioimmunological measurements. It can be used in situ at solid/liquid interfaces maintaining surface selectivity and sub-nanometer thickness resolution. The in situ advantage allows kinetics to be monitored with a time resolution sufficient to resolve adsorption of macromolecules and surface dynamics. By including biorecognition, bimolecular phenomena like antigen–antibody binding, hormone–receptor binding and enzymatic reactions on surfaces can be monitored. More recent development includes development of sensor systems based on biorecognition and arrays of layers of sensing molecules in combination with imaging techniques. There is also a potential for developing ellipsometric systems for in situ optical diagnostics of multilayer growth.

4.2. Protein adsorption on planar surfaces: static and dynamic studies

Traditionally protein adsorption on solid surfaces has been monitored by single-wavelength null ellipsometry. Numerous proteins and their interaction with surfaces have been investigated [21,22]. Many studies are performed by measuring ex situ (in air) on a surface before and after protein adsorption. Such experiments involve rinsing with the uncertainty of possible desorption and denaturation of proteins before measuring. Such studies are very simple, allow many samples to be measured and can be performed by untrained personnel. More
precise information including kinetics of adsorption is obtained by in situ studies. This is illustrated in Fig. 2 which shows Δ vs. time during adsorption of the protein ferritin on a gold surface. If evaluated as thickness, the total change in Δ of approximately 3° corresponds to 9.5 nm, which is close to a monolayer of ferritin [23]. Given the noise level in this particular experiment, it is clear that very small thickness changes can be resolved. In careful experiments a resolution in Δ of 0.01° or better can be obtained which corresponds to 0.03 nm in the example above.

With the possibility to monitor the kinetics of adsorption follows an opportunity to model the adsorption and desorption with rate equations [24,25]. However, such modeling is often hampered by diffusion limitations and uncertainty in flow conditions at the interface. Several attempts to get these effects under better control have been made by developing special flow cells for ellipsometric measurements. Such cells allow comparative studies of adsorption and layer dynamics under static conditions with those under flow conditions [20].

4.3. Protein adsorption in porous layers

In biomaterials research new materials are continuously being tested and developed. Besides the physical and chemical properties of an interface, also its microstructure influences the interactions with biomolecules. Textured surfaces are for example developed using the strategy that surface features with dimensions of the same order as cells or macromolecules may improve biocompatibility [26]. Also porous surfaces are of interest in this context. A porous interface may provide a smooth transition between the organic and the inorganic phases. Possibilities for mechanical anchorage is another potential advantage in the case of bone implants. Controlled drug release from porous layers can also be envisaged.

We have earlier studied protein adsorption in porous silicon dioxide layers [27]. Here we report results from protein adsorption studies in layers of porous silicon. Ellipsometry was used to quantify the amount of adsorbed protein and also to follow the kinetics of adsorption from a liquid. Furthermore, depth profiling is possible by employing spectroscopic ellipsometry. Fig. 3 shows the kinetics recorded during adsorption of the protein human serum albumin on a porous silicon surface. The rate of adsorption in such layers are general lower than for adsorption on a flat surface (see Fig. 2) which most probably is due to slow diffusion of protein molecules in the porous structure.

In Fig. 4a ellipsometric spectra measured before and after protein adsorption in a porous silicon layer are shown. The difference between the two spectra contains information about the amount of protein adsorbed and also information about the adsorption depth. Quantification is achieved by analysis using multilayer models and EMA modeling and a microstructural parameterization as shown in Fig. 4b can be obtained. Results from albumin adsorption from citrate-HCl buffer (pH 4) in a porous silicon layer are summarized in Table 1. These results show that albumin penetrates into the porous matrix. Similar studies on fibrinogen showed adsorption only on top of the porous layers. Several factors influence this but of major importance is the larger size of fibrinogen molecules (MW = 340 kDa, typical dimension 46 × 7 nm) compared to albumin molecules (MW = 66 kDa, typical dimension 8 × 3 nm). Other factors are pore size, surface energy of the internal walls of the pores and the flexibility of the protein molecules.

![Fig. 2. Δ vs. time for ferritin adsorption on a gold substrate measured at a wavelength of 620 nm at two concentrations in solution (0.02 M phosphate buffer, pH 7.3, 0.15 M NaCl).](image1)

![Fig. 3. Kinetics of adsorption of human serum albumin in porous silicon in citrate-HCl buffer (pH 4) measured by in situ ellipsometry at a wavelength of 750 nm. The thickness of the porous layer is 288 nm and the average porosity is 70%.](image2)
4.4. Protein monolayer spectroscopy

With ellipsometry it is possible to determine the optical functions of thin layers. Early measurements were done by recording spectra in air (or nitrogen) on surfaces before and after protein adsorption [28]. However, such studies should preferably be carried out in situ at the liquid/solid interface. The main reason is that the adsorption must be done from a liquid. Removing the surface from the liquid phase, drying it and then measure in an ellipsometer may introduce uncertainties of the same order as the thickness of the layers.

Fig. 5 shows one example of an in situ measurement of the optical properties of a monolayer of ferritin on a gold surface [29]. The procedure is basically to first measure an ellipsometric spectrum of the substrate in solution. From this spectrum the optical properties of the gold substrate are derived by simple inversion in a two-phase model. After ferritin adsorption (see Fig. 2) a new spectrum is recorded and the properties of the layer can be extracted.

Characteristic for most protein layers are that their optical functions are rather featureless in the photon energy range used here. The value of the results is, besides filling our materials data base with optical functions, that by studying the time evolution of protein layer spectra as well as the dependence on parameters like surface energy, pH, protein concentration during adsorption, etc., it is possible to draw conclusions about the microstructure of the protein layer and understand details in the interaction between the protein molecules and the surface. In the case of ferritin, our studies gave support for proposing a two-state model for protein adsorption [29].

4.5. Protein layer imaging

Most ellipsometric studies are used with millimeter-sized light beams and beam-profile weighted average properties over the area projected on a surface are thus obtained. Microellipsometry is possible down to a lateral resolution of 50 μm but requires scanning methods for mapping a larger area. An alternative is ellipsometric imaging whereby a large diameter beam is employed [18]. Using a CCD-camera and a beam diameter of the order of 20 mm, it is possible to image areas up 15 × 25 mm. The lateral resolution depends on the number of pixels and the optics used but lateral

Table 1
Amount of adsorbed human serum albumin (HSA) in a porous silicon layer

<table>
<thead>
<tr>
<th>Sublayer</th>
<th>Thickness (nm)</th>
<th>Porosity (%)</th>
<th>HSA mass (μg/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>45.7</td>
<td>89</td>
<td>0.37</td>
</tr>
<tr>
<td>2</td>
<td>156.1</td>
<td>71</td>
<td>2.29</td>
</tr>
<tr>
<td>3</td>
<td>57.4</td>
<td>66</td>
<td>0.33</td>
</tr>
<tr>
<td>4</td>
<td>28.6</td>
<td>47</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. 4. Δ vs. wavelength before (solid curve) and after (dashed curve) adsorption of human serum albumin in a 288-nm thick porous silicon layer. The measurements were done in a citrate-HCl buffer (pH 4) at a protein concentration of 1 mg/ml. The angle of incidence was 68°. (b) Four-layer model of a porous silicon layer with human serum albumin (HSA) adsorbed in the pores. The voids are filled with buffer solution. The numbers to the left are the thicknesses of the sublayers.

Fig. 5. Ellipsometrically determined dielectric function ε and refractive index n for a 9.5-nm thick layer of ferritin on a gold substrate.
resolutions down to 5 μm with nanometer thickness resolution has been achieved. Fig. 6 shows an image of a silicon surface on which three different types of proteins have been adsorbed in selected areas. This image is recorded with null ellipsometry operated in the so-called off-null mode [15]. The intensity from each pixel is then a measure on the layer thickness in the corresponding area on the surface. In first approximation a square-root dependence is found. Observe that the image shows monolayers of the three proteins.

4.6. Diagnostics of multilayer engineering of biomolecules

From an engineering perspective biomolecules have an enormous potential. It can be foreseen that new materials based on biomolecules will be developed as well as new strategies to fabricate inorganic materials using methodology found in biology. A well-known example is spider threads which have mechanical strengths superior to man-made materials. An interesting parallel is growth of GaN [30] using growth methods mimicking those of molluscan shells and pearls [31]. We are only in the beginning of this development where ideas and/or molecules are obtained from nature.

An area in which ellipsometry can provide an in situ diagnostic tool is multilayer epitaxy of biomolecules using biorecognition. Pioneering work by Spaeth et al. [32] has shown that layer-by-layer growth of streptavidin and biotinylated albumin can be studied with ellipsometry. They grew films of 20 sublayers using the specific binding properties of the streptavidin–biotin system. After each sublayer an analysis using spectroscopic ellipsometry was carried out for quality assessment and thickness determination. We have performed similar studies using real-time spectroscopic ellipsome-

try whereby the layer growth was monitored at 88 wavelengths simultaneously during deposition of nine sublayers of streptavidin and biotinylated albumin on a silicon substrate. The optical analysis showed that the first few layers grew layer-by-layer whereas the following layers show intermixing. Fig. 7 summarizes the results in terms of surface concentration and layer thicknesses. In conclusion, under the experimental conditions used, we could not achieve the same layer quality as Spaeth et al. [32] but our experiments show that in situ diagnostics of multilayer growth is feasible with ellipsometry.

4.7. Affinity biosensor systems

Several concepts of biosensor systems based on optical readout have been proposed [33]. One successful development is real-time biospecific interaction analysis based on the surface plasmon principle and biorecognition [34]. Also ellipsometric readout has been proposed [35,36].

In the context of biosensors, ellipsometry has been used for detection of antibody–antigen binding, monitoring of enzymatic reactions, etc., but not being analytic, ellipsometry relies on utilizing biospecificity. However, one way to increase specificity is to use multisensor data in the detection and discrimination between different molecules. Imaging ellipsometry is then very useful in combination with samples as displayed in Fig. 6. Test surfaces, here called biochips, with several areas with different biospecificity can be used. In the particular case of antigen–antibody binding studies, a series of antigens (or antibodies) are preadsorbed in selected areas on a surface. The number of areas are determined by the method used but can be of the order of several thousands if procedures similar to those in fluorescence-based biosensors are used. In a fully developed application, the smallest dot size is most probably determined by the imaging system. Such chips

![Fig. 6. Ellipsometric image of a 15 × 25-mm silicon substrate with the three proteins fibrinogen (Fib), human serum albumin (HSA) and human gammaglobulin (h-IgG) adsorbed in 4-mm spots in duplicate.](image)

![Fig. 7. Surface mass per layer in a multilayer structure of streptavidin (SA) and biotinylated bovine serum albumin (BSA) as determined by multi-wavelength ellipsometry.](image)
with preadsorbed sensing layers are then incubated in a solution, normally a serum, in which the antibody (or antigen) under study is to be detected or quantified. By comparing images taken before and after incubation, the thickness increase in each dot can be quantified and by using multi-parameter analysis techniques, like principle component analysis, discrimination between the different adsorbing molecules can be done. The actual number of dots necessary for such discrimination is probably rather low if molecules are selected carefully and detection of several compounds on the same chip is possible. Applications of an affinity biosensor can be, e.g. screening tests in immunology or specific analysis of a blood sample to determine if a patient has a certain disease or not.

To verify that the principle above works, a pilot study was done using two different preadsorbed antigens [35]. The molecular systems used were human immunoglobulin (h-IgG) and fibrinogen (Fib) and their corresponding antibodies present in antisera. Fib and h-IgG were adsorbed in millimeter sized areas in duplicate on silicon substrates, the biochips. After rinsing an ellipsometric image was recorded, followed by incubation in antisera. Finally an additional image was recorded. The evaluation can be done by a simple image subtraction pixel-by-pixel followed by averaging over predetermined areas. Table 2 shows the result when a biochip was incubated in antisera containing antibodies to h-IgG. It is seen that the thickness increase in areas with specificity is considerably larger than in the other areas.

5. What will be next?

Infrared spectroscopy is used extensively in biology. However, only recently infrared ellipsometers have become commercially available. The analytic capability added by infrared studies will certainly give exciting new results. On the instrumental side we will probably also see small, specially designed measurement system dedicated to biosensor and chemical sensor applications. Specificity will be enhanced by multisensing either by using a discrete number of sensing layers or by using gradients and imaging ellipsometry.

The recently developed theories for studying anisotropic layers have not been fully implemented in the area of thin organic layers and many new results are expected here. In surface biology water plays an important role and ellipsometry with its surface sensitivity can here provide new information.

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