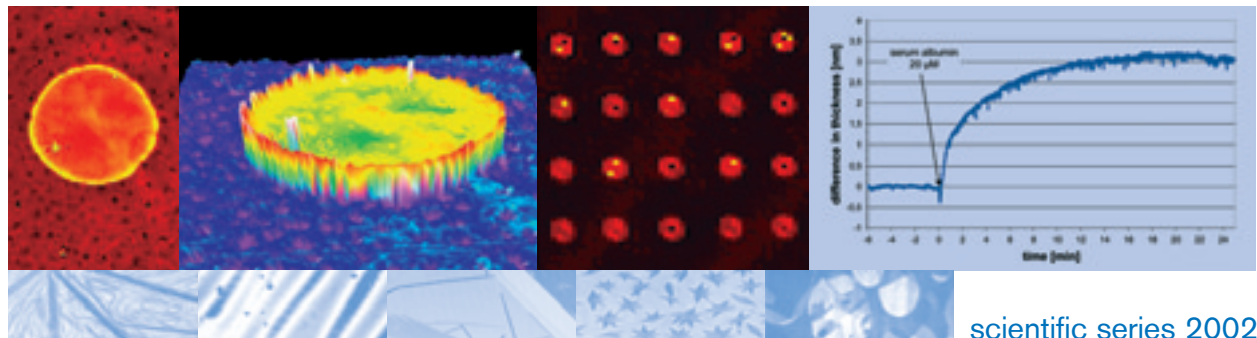


Imaging Ellipsometry in Biotechnology

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scientific series 2002



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(Second edition, July 2002)

Printed in Germany

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ISBN 3-9807279-6-3

Abstract

The imaging ellipsometer I-Elli2000 from Nanofilm is a versatile tool to analyze biochips without any labeling of the probes. You can examine the surface structure of biological macromolecules, e.g. DNA and proteins, on substrates like glass, silicon or gold with high spatial resolution. Due to real-time data acquisition it is possible to measure adsorption reactions by using diverse solid-liquid cells for in vitro conditions.

Imaging ellipsometry gives you the opportunity to measure the thickness of multiple spots simultaneously and to monitor hundreds of reaction channels in parallel. This makes the instrument perfectly suited for high throughput screening (HTS).



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1. Introduction: Analytics of biochemical reactions

1.1 From genomics to proteomics

When the decoding of the human genome was completed some time ago, it seemed as if this was the key milestone on the way to understand the “construction” of life. A new discipline was born called “genomics” – the investigation of all genes of one organism, their functions and activities. But now it is becoming more and more obvious that only a small part of the exploration is done and that the investigation tools have to be enhanced.

Current understanding of the genome and the fact that drugs normally target proteins that are encoded by genes, but not the genes themselves, has spawned the new science called “proteomics” – the large-scale study of all proteins encoded by an organism’s genome.

Genomics in combination with proteomics are fields in biotechnology which have a huge impact on everybody’s lives. They will inspire the medicine by supplying better diagnostics and drugs.

1.2 The biochip technology

Currently, the most common tools for the rapid characterization of different biological samples are biochips with oligonucleotides or proteins.

A conventional DNA chip is developed by selecting oligonucleotide probes corresponding to specific genes of interest and immobilizing them on a solid surface (glass or plastic slides coated with functional layers) to form an array. RNA samples with potential target activity are reversely transcribed to cDNA, amplified, tagged with a e.g. fluorescent label, added to the array, and allowed to hybridize with the corresponding oligonucleotide probes. By stimulating with the correct excitation light, only the spots where the hybridization took place will appear as bright stains. These DNA microarrays have been utilized to generate databases of gene expression patterns and changes in these patterns associated with a certain phenotype or physiological state.

Nowadays, the protein chip technology is a powerful tool for high throughput assays of protein expression and of protein interaction with other macromolecules. Unlike DNA, proteins are much more sensitive to their physiological environment and can easily be degraded by physical or chemical effects. Marker binding (like a fluorescent label) and also the construction of fusion proteins (e.g. with the green fluorescent protein) will most times restrict the natural activity of a protein in some ways. This leads to a demand for label-free detection techniques.

1.3 Analytical methods in genomics and proteomics

1.3.1 Fluorescence in comparison to ellipsometry

The microarrays based on fluorescent materials can be evaluated with instruments called fluorescence readers. Normally proteins and DNA do not fluoresce. Therefore, a marker is needed which has to be attached to the biological material. Thus, a time consuming labeling-step is required besides expensive labeled substances and detection equipment. Moreover, the three dimensional structure of the biological polymer may change due to the attached marker so that the natural activity is disturbed (e.g. proteins). This labeling-step can be avoided by using the imaging ellipsometer I-Elli2000 because it uses a label-free and non-destructive optical measuring technique [Chapter 1.4].

The spatial resolution of a fluorescence reader is between 5-100 μm and thus worse than the one of the I-Elli2000 (1-35 μm). It takes up to 12 minutes to scan a 22 x 73 mm area by fluorescence, where as the I-Elli2000 needs less than a minute at the same resolution (field-of-view 20 x 30 mm).

The sensitivity of the imaging ellipsometer is 0.05-0.2 nm or 0.05-0.2 ng/mm^2 and is thus in the same range as fluorescence spectroscopy [Product specifications of the fluorescence reader: *GenePix 4 000B* from **Axon**].

The imaging ellipsometer allows real-time kinetic measurement of the interaction of unlabeled biological molecules at surfaces which is important for the quality control of microarrays.

1.3.2 2D gel electrophoresis in proteomics

Classical proteomic approaches have relied upon searching whole-cell lysates by 2D gel electrophoresis. In fact, 2D gel electrophoresis was almost synonymous with proteomics until some years ago. In this method the proteins are separated in an electrical field first by their isoelectric point (pI) and finally by their molecular weight.

Except for simpler organisms such as *E.coli*, the hope that the combination of molecular weight and pI of the protein will be sufficient for identification of the protein has not been realized. The chief disadvantages of 2D gels are lack of reproducibility, failure to resolve most proteins greater than approximately 100 kDa, failure to routinely detect more than 1000 spots and the inability to separate most membrane proteins [Mann M.,

Hendrickson R.C., Pandey A., *Annu. Rev. Biochem.* **2001** 70: 437-473].

1.3.3 Mass spectrometry

Mass spectrometry (MS) is a venerable technique to identify peptides and proteins by their mass or the mass of their decay products. After the fragmentation and ionization of the biological material the mass of the charged species is recorded.

There are two possibilities to ionize the sample: First, a laser is used to ionize the probes which are embedded inside of a special matrix (matrix assisted laser desorption ionization (MALDI)).

Second, a liquid containing the analyte is pumped through a needle at high voltage to electrostatically disperse micrometer sized droplets, which rapidly evaporate and which impart their charge onto the analyte molecules (electrospray ionization (ESI)). This ionization process takes place in atmosphere and is therefore very gentle (without fragmentation of the analyte ions in the gas phase) [Mann M., Hendrickson R.C., Pandey A., *Annu. Rev. Biochem.* **2001** 70:437-473].

The MS is often combined with different separation techniques like high performance liquid chromatography (HPLC) and 2D electrophoresis.

Moreover, there are imaging mass spectrometers available which work label free. But these instruments are 10 times more expensive than the I-Elli2000.

1.3.4 Atomic force microscopy

Atomic force microscopy (AFM) is a technique to analyze the topography of a solid surface. A small needle scans over the surface and if a structure is on top of the surface the needle increases its distance to the surface to avoid the hitting to the structure. This movement of the needle is recoded in conjunction with its position.

AFM is the method with highest spatial resolution (0.1 nm) on cost of time. It takes several minutes to scan an area of 100 x 100 μm . Thus, if a high resolution down to 0.1 nm is not necessary, imaging ellipsometry is much faster and still has a medium resolution (1.2 μm).

In contrast to using imaging ellipsometry to measure the film thickness, an edge or a step on the surface is required with AFM. Imaging ellipsometry can even obtain profiles and maps of inner layers of a multi-layer stack. Moreover, it gives maps of material properties like the refractive index and extinction coefficient of the layers.

1.4 Imaging Ellipsometry – Nulling Ellipsometry

Ellipsometry is a non-destructive, label-free optical method for determining film thickness and optical properties. It measures the change in the state of polarization of the light reflected off the film's surface. Fast ellipsometry methods, single or multi wavelength, have been adopted for monitoring film growth in situ, allowing the precise control of film deposition processes [Arwin H. in: "*Physical Chemistry of Biological Interfaces*", Baszkin A., Norde W. (ed.), **2000**, 577-607, Marcel Dekker Inc., New York]. The development of imaging ellipsometry (Figure 1), which combines the power of ellipsometry with microscopy, has an x/y-resolution of approximately 1 μm . The enhanced spatial resolution of imaging ellipsometers potentially expands ellipsometry into new areas of bioanalytics and microelectronics.

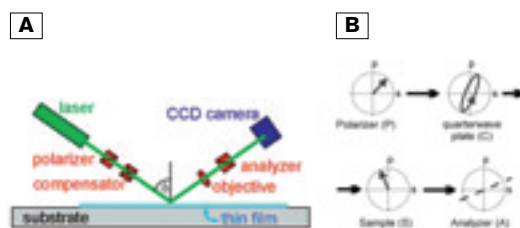


Figure 1: Set-up of the imaging ellipsometer I-Elli2000 (A) and the principle of nulling ellipsometer (B).

The commercial imaging ellipsometer I-Elli2000 operates on the principle of classical null ellipsometry and real-time ellipsometric contrast imaging (Figure 1 B). The laser beam is elliptically polarized after it passes through a linear polarizer (P) and a quarter-wave plate (C). The elliptically polarized light is then reflected off the sample (S) into an analyzer (A) and imaged onto a CCD camera through a long working distance objective. In this PCSA configuration, the orientation of the angles of P and C is chosen in such a way that the elliptically polarized light is completely linearly polarized after it is reflected off the sample. As shown in Figure 1 B, the ellipsometric null condition is obtained when A is perpendicular with respect to the polarization axis of the reflected light, i.e., the state at which the absolute minimum of light flux is detected at the CCD camera. The angles of P , C and A that determine the null condition are related to the ellipsometric parameters Δ and Ψ . The tangent of the angle Ψ gives the ratio of amplitude change for the polarization components, while Δ denotes the relative phase shift of these polarization components upon reflection. Reduction of the measured Δ and Ψ with computerized optical modeling leads to a deduction of the optical properties of the sample (complex refractive indices) and the film thickness.

2. Quality control of biochips

All spots of immobilized biological macromolecules on a biochip should have a homogeneous shape and the same size. If these requirements are fulfilled, the amount of material that can hybridize is quantified correctly and the results are reliable. Ideally, you would monitor the quality of the spots before a hybridization process to avoid the loss of expensive probes on non optimal biochips. Unfortunately, current techniques for quality control either need very time consuming staining processes or destroy the biochips. By using the I-Elli2000 with the non-destructive and label-free method of imaging ellipsometry, you have the opportunity to check the shape and the size of all spots without staining or before the hybridization takes place, and additionally evaluate the results afterwards. As an example, a non-hybridized oligonucleotides spot is displayed in Figure 2. In another example we have observed non-specific binding of DNA with the imaging ellipsometer.

The spot detection with the I-Elli2000 does not require any marker and therefore, reducing your expenses for labeled nucleotides and measurement hardware. Besides, the natural activity of proteins are not affected by an attached marker.

Figure 2: Images of a spot of immobilized non-hybridized 50-mer oligo nucleotides produced by Advalytix AG (Brunnthal, Germany). The spot diameter is 150 μm (Ellipsometric contrast (A), thickness-map [z in nm and x/y in pixel] (B) and the corresponding 3D-profile (C)).

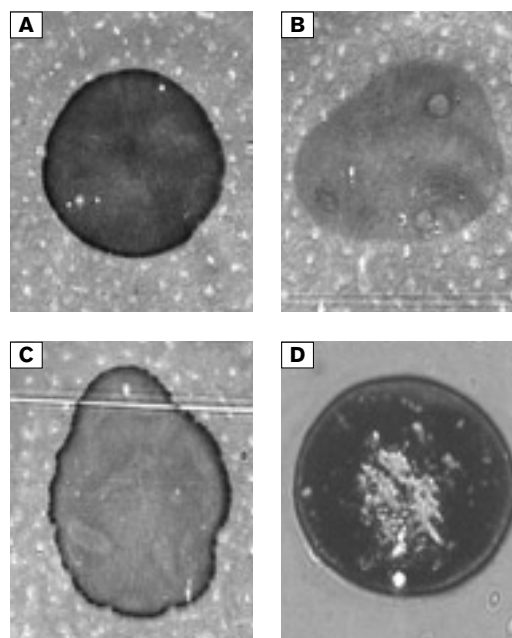
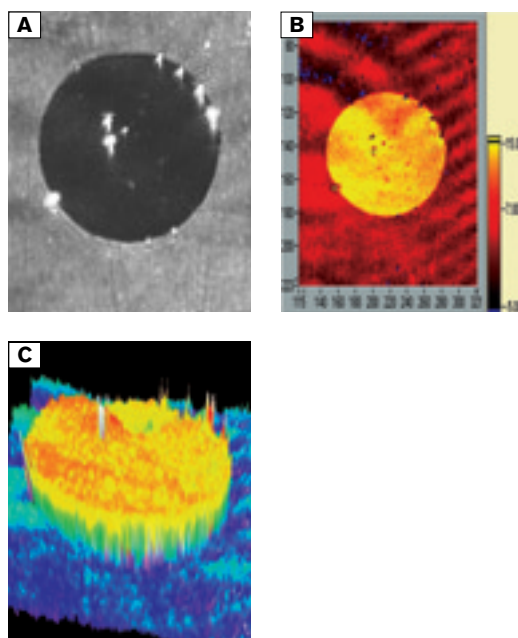


Figure 3: Ellipsometric contrast images of DNA spots with different shape, thickness, homogeneity and diameter (about 200 μm).

2.1.1 The power of imaging with I-Elli2000

The diameter, homogeneity, and shape of four DNA spots on glass have been measured and are displayed as ellipsometric contrast images in Figure 3. The first spot is round and homogeneous in contradiction to the second, third and fourth one. The darkness of the spot is related to the thickness and gives you a qualitative impression of the thickness distribution. Because the thickness is related to the amount of immobilized material this method can be used to evaluate the amount of immobilized material on the surface.

2.1.2 Thickness and mass quantification

To quantify the thickness distribution it takes typically less than a minute. You get a thickness-map of your spot (Figure 4 A, C) which can be shown as a 3D-profile with the I-Elli2000 software (Figure 4 B, D).

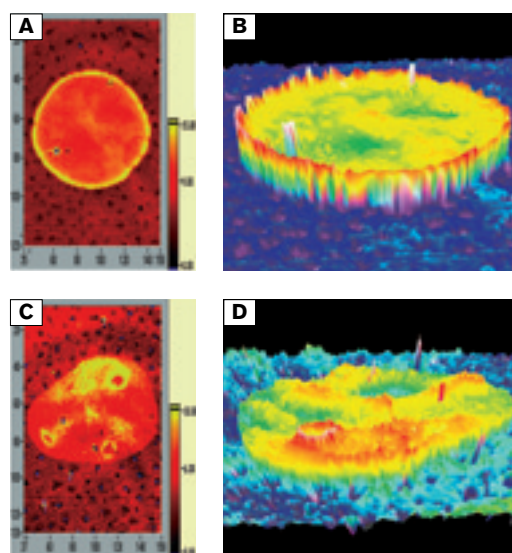


Figure 4: Thickness-map [z in nm and x/y in pixel] (A) and the corresponding 3D-profile (B) of the DNA spot shown in Figure 3 A and underneath to it (C and D) the similar style of representation of the DNA spot of Figure 3 B.

A monolayer of bovine serum albumin with the molecular weight 67 kDa typically has a surface capacity of ~ 3 ng/mm² and a thickness between 2 to 3 nm depending on the surface density (18 000-27 000 molecules/ μm^2) [Arwin H. in: "Physical Chemistry of Biological Interfaces", Baszkin A., Norde W. (ed.), 2000, 577-607, Marcel Dekker Inc., New York]. This yields to the response ~ 1 nm/(ng/mm²).

The surface capacity of immobilized oligonucleotides (fragments of single stranded DNA) is in the range of 70 000 molecules/ μm^2 [Sojka B., Piunno P., Wust C., Krull U., *Appl. Biochem. Biotechnol.* 2000 Oct, 89(1): 85-103; Chrisey L., Lee G., O'Ferrall E., *Nucleic Acids Research*, 1996 24(15): 3030-3039; Strother T., Cai W., Zhao X., Hamers R., M Lloyd, *J. Am. Chem. Soc.*, 2000 122(6): 1205-1209]. Thus, depending on the strand length the thickness of such a layer is 0.8 nm (20-mer oligo), 1.9 nm (50-mer oligo) and 5.8 nm (150-mer oligo). Assuming a hybridization yield of 33% [Chrisey L., Lee G., O'Ferrall E., *Nucleic Acids Research*, 1996 24(15): 3030-3039] the medium thickness increase due to the binding of the complementary oligonucleotides is 0.3 nm (20-mer oligo), 0.6 nm (50-mer oligo) and 1.9 nm (150-mer oligo).

2.1.3 Detection of Hybridization with the I-Elli2000

The thickness of non-hybridized and DNA spots, which have been hybridized with complementary oligonucleotides (fragments of single stranded

DNA), has been evaluated with the I-Elli2000 (Figure 5). The spots have been spotted on glass slides with different test-oligonucleotides concentrations in the spotting solution. Previous to the hybridization the slides have been treated with blocking reagents to minimize the unspecific binding of the hybridizing DNA to the surrounding.

As a control non-complementary oligonucleotides have been spotted, where no hybridization occurred as known from fluorescence scanning (data kindly supplied by PicoRapid Technologie GmbH, Bremen, Germany).

In the experiment with the non-hybridized spots the thickness is defined as the difference in thickness between the spot and the surrounding surface. In the hybridization experiment the control spot is defined as the reference, because the control spot is not be affected by the hybridization process as proved by fluorescence measurements [unpublished results; PicoRapid Technologie GmbH, Bremen, Germany]. The surrounding area is filled up during the blocking process and its layer thickness increases, because relative large molecules like proteins adsorb to the surrounding surface. Therefore, the thickness of the hybridized test-oligo spots are adjusted to the offset of the control.

The hybridization is proved by comparison the thickness of the test-spots, where hybridization took place (4.4, 4.3 and 3.3 nm, depending on the amount of immobilized oligos) (Figure 5, red columns 1-3), with the control spots (1.7 nm) (Figure 5, red column 4).

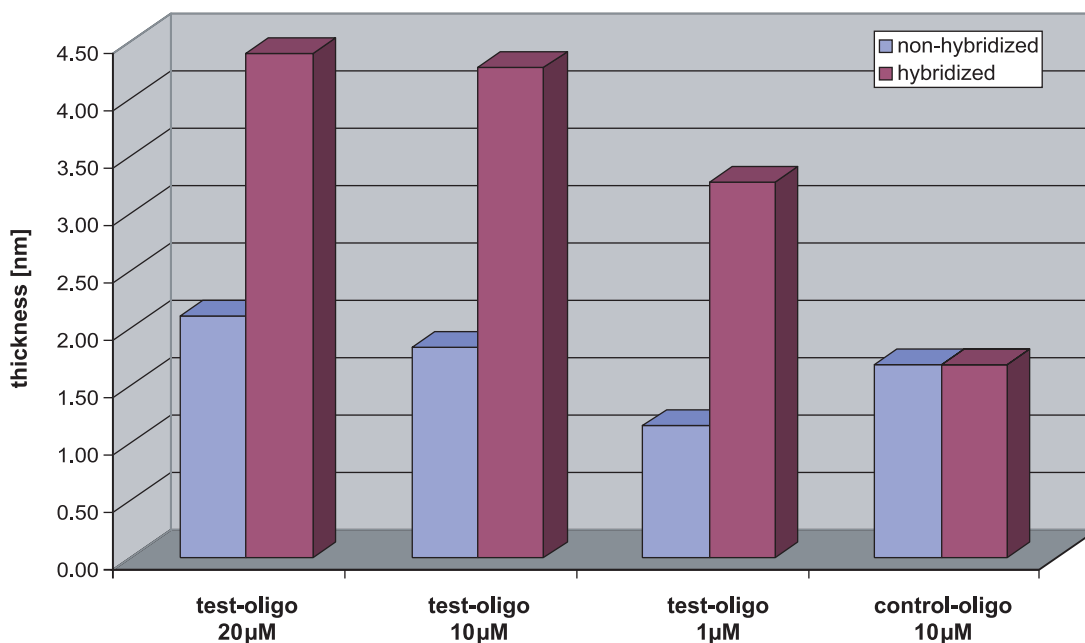


Figure 5: The thickness of non-hybridized test-oligo-nucleotides spots and the thickness of spots, that have been hybridized with complementary DNA. The test-spots have been spotted with different concentrations in the spotting solution (columns 1 to 3). As a control, oligonucleotides have been spotted, where no hybridization occurred (column 4) (Displayed is the average thickness of minimum 15 spots). (Samples kindly supplied by PicoRapid Technologie GmbH, Bremen, Germany).

In addition, the thickness of the hybridized DNA is larger (4.4, 4.3 and 3.3 nm, Figure 5, red columns 1-3) than the one of the non-hybridized DNA spots (2.1, 1.8 and 1.2 nm, Figure 5, blue columns 1-3).

Moreover, the more oligonucleotides were in the spotting solution the more oligonucleotides are immobilized on the glass-slide (Figure 5, blue columns 1-3) and the more complementary DNA can hybridize to them and the thicker the spots finally appear (Figure 5, red columns 1-3). The amount of immobilized test-oligo can be observed at the non-hybridized spots (Figure 5, blue columns). The thickness of the 20 μM test oligo spots is just slightly larger (2.1 nm) than the one of the 10 μM spots (1.9 nm). The spots produced with the test-oligo concentration of 1 μM have a lower thickness (1.2 nm). Therefore, the saturation on the surface is reached at a DNA concentration of about 10 μM in the spotting solution.

The amount of control-oligo (1.7 nm) on the surface of the non-hybridized is in the same range than the amount of the test-oligo with the concentration above 10 μM . The control-oligo was spotted with 10 μM and only differs from the others in its sequence.

In these two experiments, a difference between the thickness of hybridized and non-hybridized control spots has been observed. This demonstrates, that the imaging ellipsometer can be used to measure the hybridization of DNA.

2.1.4 Comparison of hybridized DNA spots visualized with fluorescence and imaging ellipsometry

In the conventional evaluation of microarrays the fluorescence signal of the control spot is compared with the one of the test spots where the hybridization takes place. At the control no hybridization occurred because the oligonucleotides were not complementary. The fluorescence intensity is proportional to the amount which binds to the spot.

To determine whether the ellipsometry is comparable to fluorescence, identical DNA spots have been evaluated with both methods. With the ellipsometer the parameter Delta (section 1.4) yields the signal. The difference in Delta between the control spot (section 2.1.3) and the diverse hybridized test-spots is displayed versus the relative fluorescence intensity of the identical spots (Figure 6). It is observed that the shift in delta is proportional to the relative fluorescence intensity.

The proportionality between the fluorescence signal and the ellipsometric parameter demonstrates, that both methods are equivalent. But the fluorescence signal cannot be transferred into the amount of bound material directly. In contrast, from the ellipsometric parameter Delta the layer thickness can be calculated. The layer thickness is related to the mass of adsorbed material, which can be transferred into molecules per area (section 2.1.2). Thus, the ellipsometer gives additional information about the hybridization process.

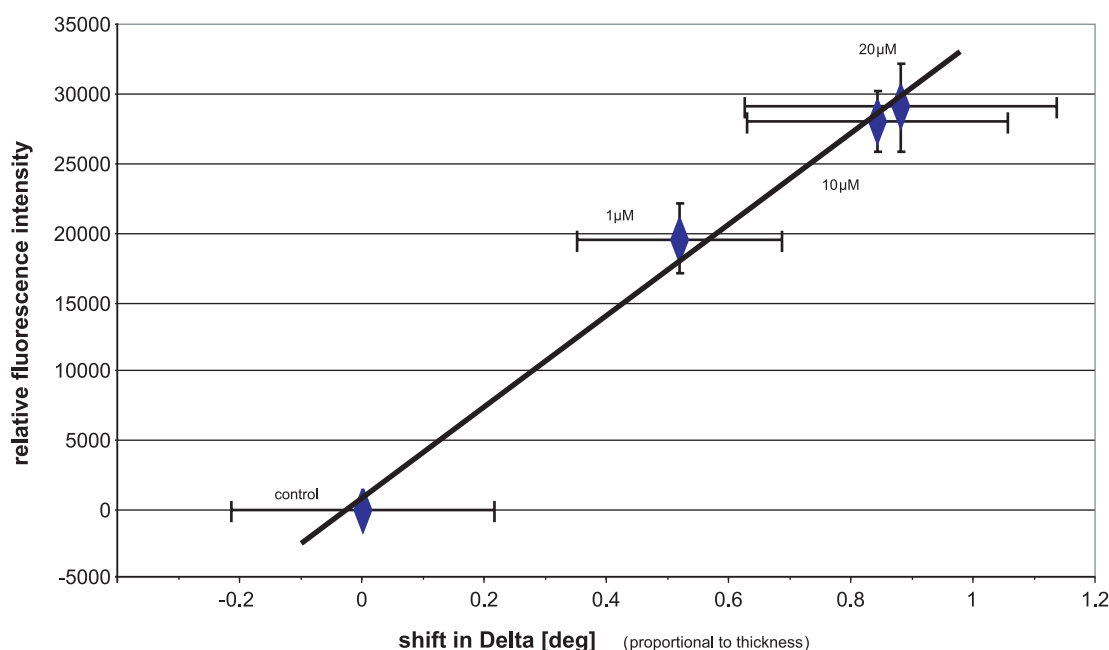


Figure 6: Linear regression of the ellipsometric parameter Delta with the relative fluorescence intensity of hybridized DNA spots. The DNA for the hybridization has been labeled with Cy 5. The measurement of the control spot is defined as the reference in both techniques. Error bars represent standard deviation of minimum 15 spots. The standard deviation of the linear regression is much smaller. (Samples and data kindly supplied by PicoRapid Technologie GmbH, Bremen, Germany).

2.2 Protein spots and the influence of the spotting procedure on the spot-shape

Besides the evaluation of DNA spots, the I-Elli2000 allows the examination of protein spots as well. Figure 7 represents an example of protein-spots with the typical "donut-shape" which results from the applied spotting or printing technique. This kind of non perfect spotting is still a problem and the imaging ellipsometer helps you to control the quality of the spots.

The influence of additives in the spotting solution on the shape or the homogeneity of the resulting protein spot can be evaluated and gives you versatile information about the optimal spotting conditions right after the spotting process (Figure 7). Time consuming and expensive steps to visualize the spots like the hybridization with fluorescent substances are not required.

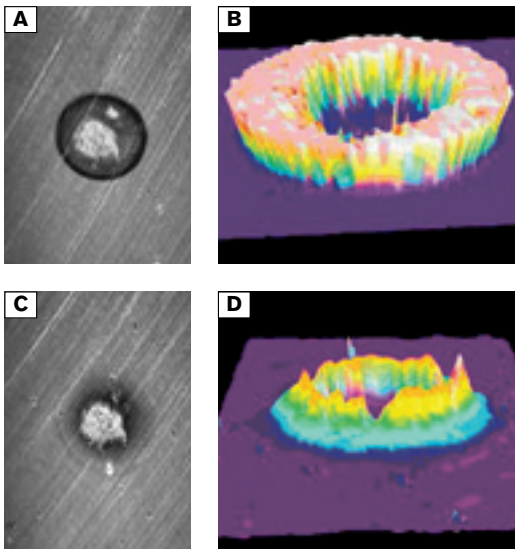


Figure 7: Images of protein-spots on gold (diameter 200 μm) without (A and B) and with an additive in the spotting solution (C and D). (Ellipsometric contrast images (A and C) and the corresponding 3D-profile of the thickness (B and D)).

Unlike other detection techniques the imaging ellipsometer gives you the opportunity to monitor the homogeneity of the surface-layer, which can consist of e.g. a streptavidin-layer (Figure 8). Its homogeneity is a quality criteria of your surface because it influences the amount of the substance which can bind to the surface. It should be monitored by an instrument like the imaging ellipsometer.

2.3 Large Area I-Elli2000

The field-of-view of the large area I-Elli2000 is 20 x 30 mm. All spots at this area can be imaged in one step, which takes typically seconds to get a qualitative image with ellipsometric contrast. In this

case the darkness of the spots is related to its thickness. To get a quantitative result, a thickness map like it is shown in Figure 9 is recorded in 30-60 seconds.

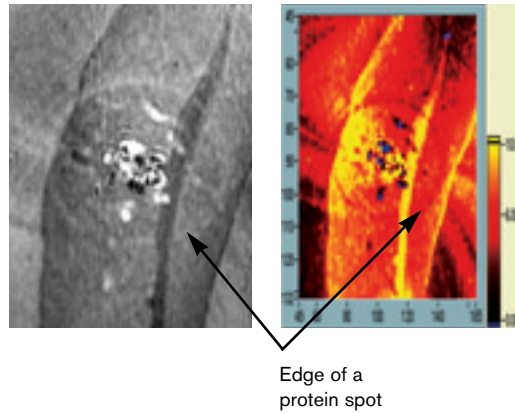


Figure 8: Ellipsometric contrast image and the corresponding thickness-map [z in nm and x/y in pixel] of a protein spot (diameter 200 μm) on a streptavidin surface.

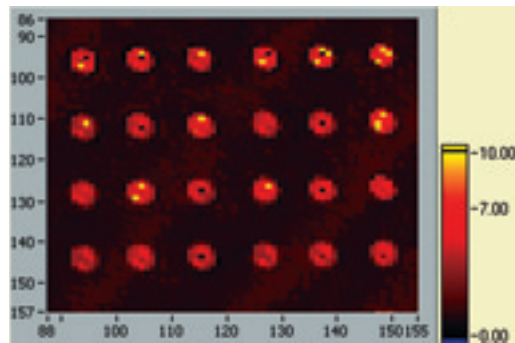


Figure 9: Thickness-map [z in nm and x/y in pixel] of protein array on a gold surface (field of view 3 x 2 mm).

3. Adsorption of proteins on solid surfaces in liquid ambient

The imaging ellipsometer I-Elli2000 can record the adsorption of substances in vitro to a variety of solid surfaces in real-time. It is able to detect the growth of the layer thickness during the binding process label-free. Therefore, the adsorption and desorption kinetics of your proteins can be evaluated and the natural activity of any protein is not affected by an attached marker.

With this instrument the examination of the distribution and the homogeneity of the adsorbed material is possible. Hence the I-Elli2000 can obtain the thickness of multiple spots simultaneously and monitor hundreds of reaction channels in parallel. So the instrument is perfectly suited for high throughput screening (HTS).

3.1.1 Example: Adsorption of serum albumin on a non transparent sample

To measure the adsorption of a protein like bovine serum albumin (BSA) on TiO_2 on a non-transparent substrate (e.g. silicon) the solid-liquid-cell (SL-cell) is versatile (Figure 11). After the addition of the protein to the liquid inside of the chamber it binds to the TiO_2 -surface. Thus, a protein layer is formed and the layer thickness increases (Figure 10).

Figure 10: Adsorption of bovine serum albumin on a TiO_2 -surface on a silicon wafer in phosphate buffered saline.

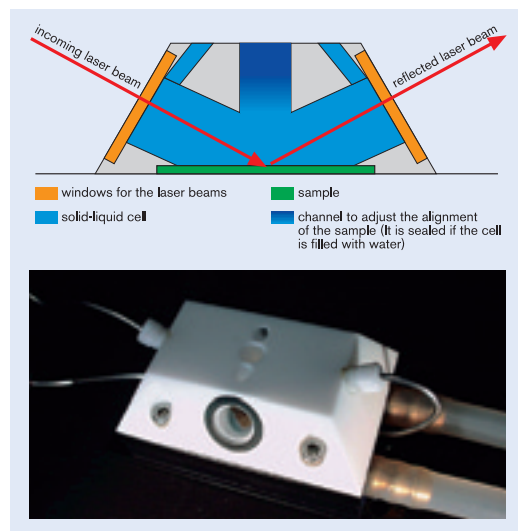
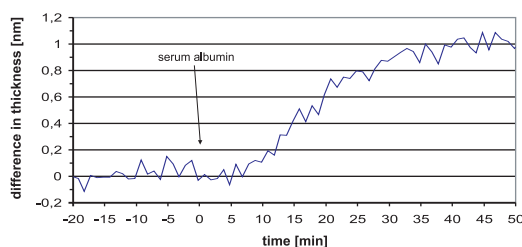


Figure 11: Sketch and image of the Nanofilm SL-cell.

The determined increase in thickness 0.9 nm (Figure 10) is less than the published value of 2.4 nm [Arwin H. in: "Physical Chemistry of Biological Interfaces", Baszkin A., Norde W. (ed.), 2000, 577-607, Marcel Dekker Inc., New York] because there was not enough protein in solution to cover the surface totally.

The response for a protein like serum albumin is

about 1.0 nm/ng/mm². The standard deviation is 0.1 nm or 0.1 ng/mm². Hence a small peptide with the molecular weight 5000 Da and the surface capacity 1.9 ng/mm² like insulin can be detected easily.

3.1.2 Example: Adsorption of serum albumin on a glass-slide

The adsorption of proteins on a conventional glass-surface can be observed in the inverse solid liquid cell (ISL-cell) (Figure 13). The binding of the bovine serum albumin to a glass-surface covered with aminosilane leads to an increase in the protein layer thickness (Figure 12). The layer thickness and the kinetics were different in this experiment compared to the one shown in Figure 10 because the protein concentration was larger.

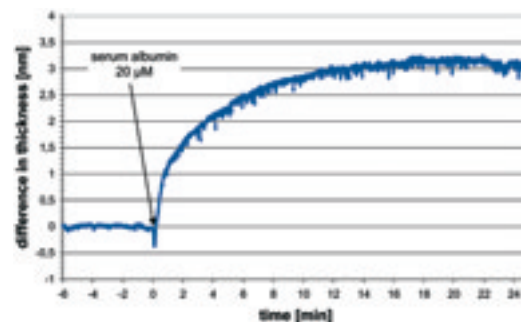


Figure 12: Adsorption of the bovine serum albumin on a glass-surface covered with aminosilane in buffer solution.

The measured increase in thickness is 3.1 nm, which yields to a response of 1.0 nm/ng/mm² and corresponds the published value 2.4 nm [Arwin H. in: "Physical Chemistry of Biological Interfaces", Baszkin A., Norde W. (ed.), 2000, 577-607, Marcel Dekker Inc., New York]. The slight difference might be due to different layer densities between these experiments. The standard deviation is 0.04 nm or 0.05 ng/mm². Thus the adsorption of the peptide β -amyloid with the surface capacity (0.07 ng/mm²) and the molecular weight 4331 Da can be measured.

In the ISL-cell an inverse set up is established, which means that the liquid is underneath the glass-slide (Figure 13). There are five separate measuring channels in this cell to use one glass slide for five experiments simultaneously.

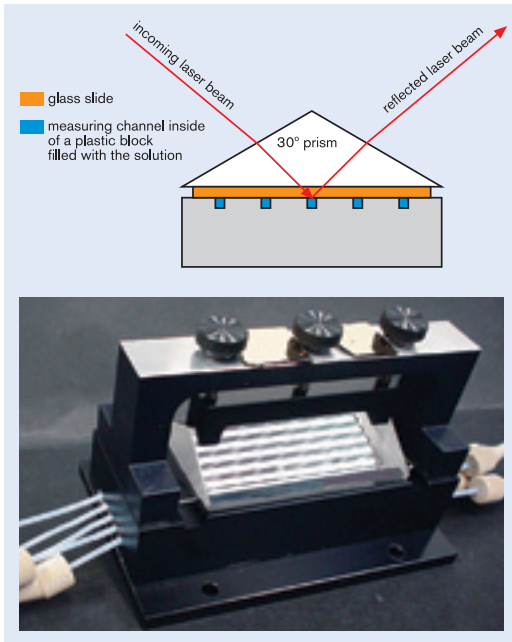


Figure 13: Sketch and picture of the inverse solid liquid (ISL-cell) with five measuring channels (visible through the prism).

3.2 Specifications of the cells

The main advantage of the SL-cell is, that any kind of substrate, transparent and non-transparent substrates, can be examined. Any surface chemistry can be used. The drift of the signal due to temperature gradients is negligible. The sensitivity with this cell is less (section 3.1.1) than the one with the ISL-cell (section 3.1.2) or with the SPR-cell (section 4.1). The cell volume in the SL-cell is smaller than 3 ml.

In the ISL-cell (section 3.1.2) the cell volume is minimized to 50 μl . Typically, there are five measuring channels in the cell. The sensitivity with the ISL-cell is in between the one with the SL-cell and with the SPR-cell. The drift of the signal due to temperature gradients is very small and fulfills most applications. Any transparent substrate, like a glass-slide, can be evaluated and the frequently used surface chemistry established for glass slides can be applied.

In the SPR-cell (section 4.1) the substrate consists of a glass-slide covered with a thin metal layer. This set-up is more restricted in comparison to the SL-cell or the ISL-cell. In the SPR-cell the method surface plasmon resonance (SPR) is combined with imaging ellipsometry (section 4). The advantage of this combination is the better sensitivity. The volume of the SPR-cell is 50 μl .

4. SPR-imaging ellipsometry

4.1 The combination of ellipsometry and SPR

Conventional surface plasmon resonance (SPR) technology is a very sensitive method to measure the adsorption kinetic of ligands to immobilized substances. It can be used to detect the binding of antibodies to their antigens or the binding of proteins to their reaction partners. The versatile information you get can be used in biotechnology, medicine and pharmacology.

In a traditional SPR-cell the angle of incidence ϕ (Figure 14) as a function of the reflected light intensity is recorded (Figure 15) [Silin V., Plant A., *Trends in Biotech*, 1997 15: 353-359]. The minimum of the reflected light is shifted if a substance adsorbs on the surface, a new layer is formed and its thickness increases.

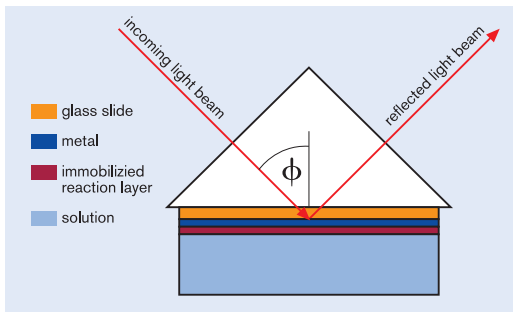


Figure 14: Sketch of a SPR-cell with the incoming and outgoing light beam and the angle of incidence ϕ .

The ellipsometer measures the ellipsometric parameters Psi and Delta instead of the reflected light intensity. The tangent of the angle Psi gives the ratio of amplitude change for the polarization components, while Delta denotes the relative phase shift of the polarization components upon reflection. The parameter Psi is analogous to the reflected intensity in classical SPR whereas the Delta provides additional information exceeding classical SPR. Thus the light intensity vs. angle of incidence ϕ (Figure 15) corresponds to the curve Psi vs. angle of incidence ϕ (Figure 16).

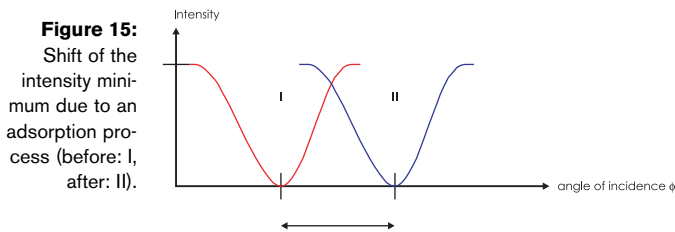


Figure 15: Shift of the intensity minimum due to an adsorption process (before: I, after: II).

Sensitivity on thickness or on mass change respectively is proportional to the slope of the measured parameter. While the slope in Psi ($\delta \text{Psi} / \delta \phi$) like the one in intensity is limited,

($\delta \text{Delta} / \delta \phi$) can be infinite (Figure 16). Thus ellipsometric Psi has the same sensitivity like classical SPR where Delta can be 10 to 100 times more sensitive.

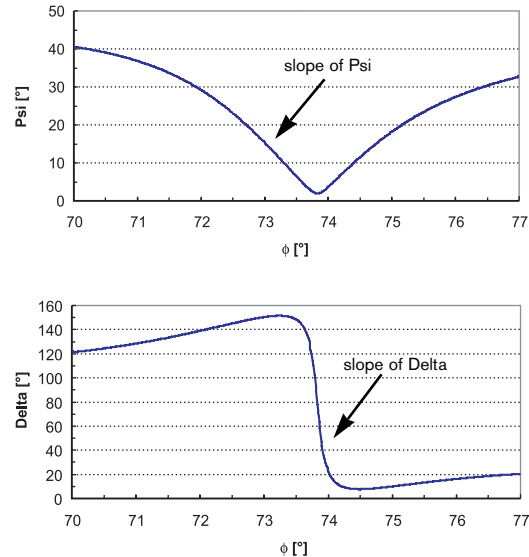


Figure 16: The slope of Psi corresponds to the slope in intensity in classical SPR. While the slope in Psi or in intensity is limited, the slope in Delta can be infinite. The sensitivity is the slope of Delta and is optimal if the minimum in Psi is close to zero. This is the case if the wavelength and the gold-layer thickness are optimal.

The slope ($\delta \text{Psi} / \delta \phi$) in ellipsometric SPR or in ($\delta \text{intensity} / \delta \phi$) in classical SPR respectively are material constants of the layer system and equivalent to the sensitivity in thickness or in the amount of adsorbed material. Therefore the sensitivity in the classical SPR approach is restricted and cannot be increased.

4.2 The SPR-cell from Nanofilm Technologie

To get the best sensitivity a glass substrate with a 50 nm gold layer is optimal. The experimental set up is shown in Figure 17.

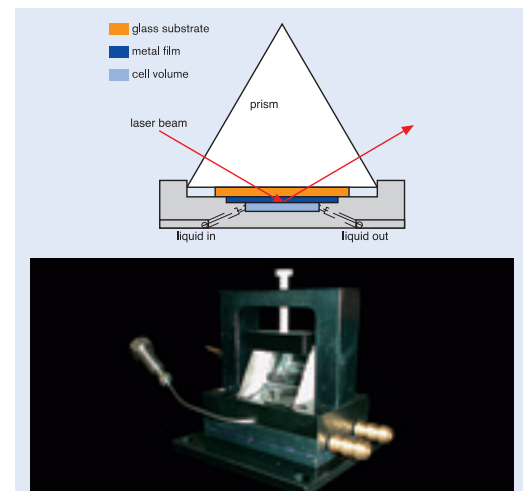


Figure 17: Sketch and image of the SPR-cell.

5. Conclusion

In this report the broad possible applications of imaging ellipsometry are demonstrated. It is perfectly suited for the quality control of biochips, the detection of DNA hybridization and the observation of adsorption reactions in vitro and in real-time. The instrument uses a marker-free, non-destructive and fast optical detection technique.

The results of this report have been measured with the imaging ellipsometer I-Elli2000. To satisfy the demands in biotechnology the next generation of imaging ellipsometers has been developed. Now, the EP³ or ellipsometric platform Number 3 is available.

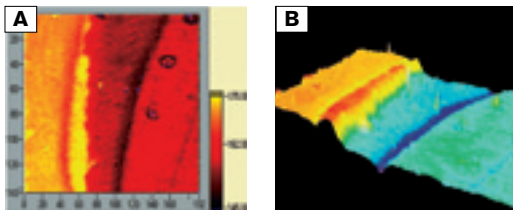
The EP³ has a modular set-up where you can choose the light source and the field-of-view. The components of the EP³ are further improved and therefore, the instrument is more accurate. The EP³ can observe the layer thickness at multiple spots simultaneously in real-time. The EP³ has a remote control and can be serviced via LAN.

Like for the ISL-cell an inverse set up, with the liquid underneath the sample, is used for the SPR cell. The gold layer is directed to the cell volume which can be filled with any liquid. The laser beam passes through the prism and the substrate. It is reflected by the metal layer. The light stimulates surface plasmon resonance in the metal film. This creates an evanescent field at the metal surface which extends into the liquid medium. The changes of optical parameters like refractive index or thickness inside the evanescent field are in conjunction with the ellipsometrical parameters Delta and Psi. These values are recorded.

4.3 The imaging ellipsometer in SPR-geometry

In comparison to traditional SPR technology, the combination of SPR and imaging ellipsometry provides information about surface morphology (Figure 18). In addition it is one of the methods with the highest sensitivity available.

Figure 18: Thickness [z in nm and x/y in pixel] of a sensorpixel on a gold SPR chip (A) and the corresponding 3D-profile (B).



4.4 Example: Adsorption of a thiol on a gold surface

The gold layer can be modified with a small molecule like alkanethiol. The adsorption of a substance on the gold surface can be monitored by measuring the ellipsometric parameters Delta and Psi, which can be transferred into the layer thickness. In Figure 19 the adsorption of an alkanethiol to a gold layer is shown. The adsorption of larger molecules like DNA or proteins is even easier to detect.

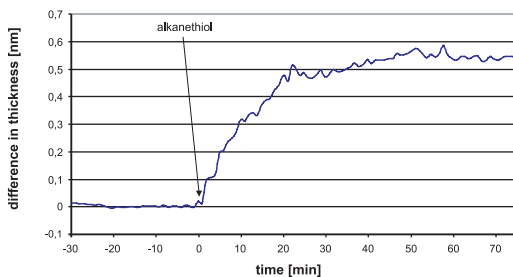


Figure 19: Adsorption of alkanethiol on a gold surface.



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ISBN 3-9807279-6-3