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Sensors and Actuators B 104 (2005) 276–281
SENSORS AND ACTUATORS B CHEMICAL
www.elsevier.com/locate/snb

Following fast adsorption processes with surface plasmon spectroscopy: reflectivity versus mismatch tracking

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Available online 2 July 2004

Abstract

Surface plasmon resonance (SPR) spectroscopy is a widely used optical reflection technique for the characterization of thin films. The central quantity of SPR spectroscopy is the surface plasmon coupling angle as a characteristic signature of the prevailing interfacial architecture. Adsorption processes lead to a shift of the surface plasmon resonance which is in the thin film limit directly proportional to the corresponding mass coverage. The aim of any SPR instrument is a precise measurement of the coupling angle with a sufficient high time resolution that fast kinetic processes can be monitored. In this paper, we compare two promising methods, an established one, the reflectivity tracking and a fairly new one, the mismatch tracking. Reflectivity tracking simply records the intensity of the light in the vicinity of the coupling angle. The shift of the plasmon modifies the intensity at the detector which can subsequently be used for data analysis. Mismatch tracking is more complex. Light is focused with a lens onto the prism base and the reflected light is detected via a bicell detector. The upper and lower cell integrate over a well-defined angular range of the fan of rays produced within the focus. The mismatch in the intensity between the upper and lower segments is evaluated and used to retrieve the unknown film parameter. In this contribution we suggest some decisive variations of the originally proposed scheme and demonstrate that the modified mismatch scheme yields a significantly higher sensitivity than the original one. Furthermore, it is demonstrated that this scheme is a superior alternative to reflectivity tracking.

Keywords: Surface plasmon resonance (SPR); Reflectivity tracking; Mismatch tracking

1. Introduction

An affinity biosensor faces two major challenges. The first one concerns the underlying chemistry as the sensor requires the design of a surface functionalization that shows only specific binding to the target molecule and suppresses unspecific adsorption. The second challenge is the monitoring of the binding events with a sufficient sensitivity and time resolution. The latter task is quite frequently addressed by various configurations of surface plasmon spectroscopy [1–5].

SPR spectroscopy is an optical reflection technique with a high sensitivity to the prevailing interfacial architecture [6]. A surface plasmon is a charge density oscillation that may exist at the interface of two media with dielectric constants of opposite signs, for instance, a metal and a dielectric. The charge density wave is associated with bound TM-polarized electromagnetic wave at the metal dielectric interface. The electric field of this wave has its maximum at the interface and decays evanescently into both media. Any change in refractive index of the bulk or binding events lead to a shift in the SPR resonance. The shift in the resonance coupling angle is for thin films (≈50 nm) directly proportional to the mass coverage of the adsorption layer [7]. The central quantity in SPR spectroscopy is the resonant coupling angle. The aim of SPR instrumentation is a fast and precise determination of the resonance position.

The excitation of a surface plasmon requires a special geometry. A good overview is given in the review articles [8,9]. The most common configurations are angular resolved techniques based on the ATR technique [10,11] as outlined in Fig. 1. The projection of the wavevector k_x changes during a Θ–Θ angle scan and the resonant conditions are fulfilled at a specific angle, the so-called surface plasmon coupling angle Θ_0. The formation of the plasmon shows up as a dip in the reflectivity curve. The energy of the laser light is then converted in a collective charge density oscillation of the electrons at the metal dielectric interface. A nice side effect of the high localization of the electric field at the interface is

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doi:10.1016/j.snb.2004.05.017

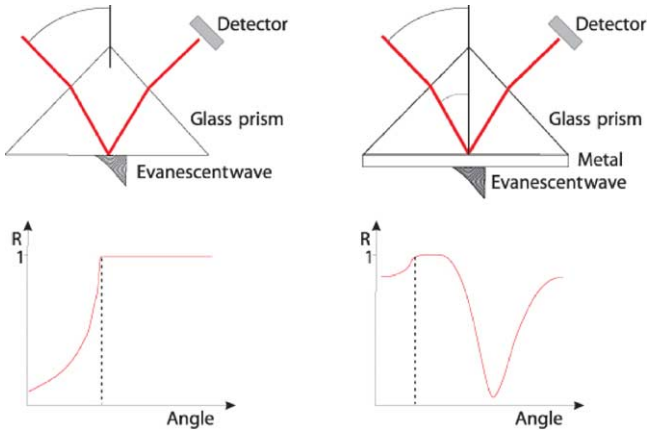

Fig. 1. Kretschmann configuration for the excitation of surface plasmons. The reflectivity of p-polarized light is measured in a Θ–Θ scan as a function of the angle of incidence Θ. If the light reflects on a prism, total reflection ($R = 1$) occurs above a critical angle Θ_{tot}. If the prism base is coated with a thin metal layer, a higher reflectivity is observed below the total reflection while Θ_{tot} remains unchanged. The prominent new feature is the formation of the surface plasmon in the total reflection regime. The use of the prism changes the slope of the dispersion $\omega = c \times k$ of the incident wave so that an intersection with the plasmon dispersion relation may exist. The plasmon formation shows up as a dip in the reflectivity curve.

the robustness of SPR measurements to changes occurring within the bulk phase. For instance, it has been demonstrated that SPR spectra work even in highly scattering turbid solutions [12].

An SPR experiment is fairly simple and requires the measurement of the reflectivity of p-polarized light as a function of the angle of incidence. The classical experimental set-up uses a two circle goniometer to perform a Θ–Θ scan. Obviously, this is rather slow since the movement of mechanical parts is required. Hence several alternatives have been suggested.

The aim of this paper is to compare two promising measurement schemes, an old and widely used one, reflectivity tracking and a rather new one, the so called mismatch tracking. The comparison is mainly based on Fresnel modeling, but the paper will also address various experimental error sources and how they affect both measurement modes. Both schemes are able to follow the adsorption process with a time resolution determined by the characteristics of the electronics which is typically in the microseconds range. Furthermore, we suggest some decisive modifications of the originally proposed mismatch scheme which boost the sensitivity.

2. SPR in kinetic mode

In order to compare the different techniques, all our simulations are considering the following experiment, the binding of a protein layer with a refractive index of $n = 1.42$ and a layer thickness of $t = 2.5$ nm onto a gold surface $\varepsilon = -12.5 + i1.5$ out of an aqueous solution $\varepsilon = 1.777$. The calculation was done using a wavelength of 633 nm and a 90° high index prism $n = 1.84$. This experiment is the most common application for SPR spectroscopy and a routine experiment in many bio-sensing laboratories.

All simulations are based on Fresnel theory of stratified media. The numerical algorithm follows a matrix method outlined in greater detail in [13,14]. No simplifying approximations are used and the algorithms have been optimized to account for truncation and round off errors in the numerical representation as some of the calculations require derivatives [15].

3. Reflectivity tracking

Fig. 2 shows the reflectivity as a function of the angle of incidence. The solid line refers to the state before adsorption and the dashed line corresponds to the state after adsorption. As a result of the adsorption there is a shift of the coupling angle which is directly proportional to the mass coverage.

A convenient and widely used way to follow in situ fast adsorption processes is the reflectivity tracking [16–18]. The intensity at the detector is monitored at fixed angle of incidence in the vicinity of the plasmon coupling angle Θ_0. The shift of the plasmon resonance $\delta\Theta$ leads to a decrease or increase of the intensity at the detector and can be used for the calculation of the mass coverage. The time resolution of this arrangement is given by the characteristics of amplifier and AD converter and an upper limit of a nanosecond has been reported [19]. The experiment should be carried out at an angle of incidence which provides the highest sensitivity in detecting sub-monolayer coverage. In order to identify the best setting, we calculate the difference in the reflectivity

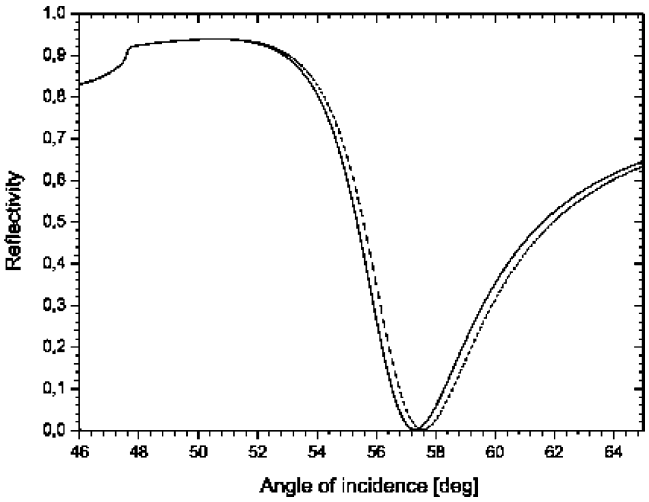

Fig. 2. The reflectivity before and after protein adsorption (90° prism with $n = 1.84$, gold: $\varepsilon = -12.5 + i1.5$ and $t = 45$ nm, protein: $n = 1.42$ and $t = 2.5$ nm, water $n = 1.333$, $\lambda = 633$ nm). The adsorption leads to a shift of the plasmon resonance Θ_0 which is the essential parameter of an SPR curve.

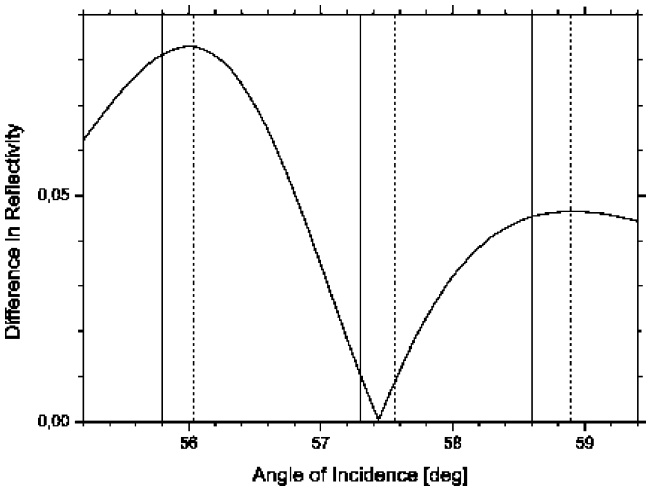

Fig. 3. Difference in the reflectivity before R_o and after protein adsorption R_f as a function of the angle of incidence. The solid vertical line refers to the point of inflection, reflectivity minimum and second point of inflection of the reflectivity scan before (solid) and after adsorption (dashed). Due to the asymmetry of the SPR curve the best choice is the point of inflection at lower angles.

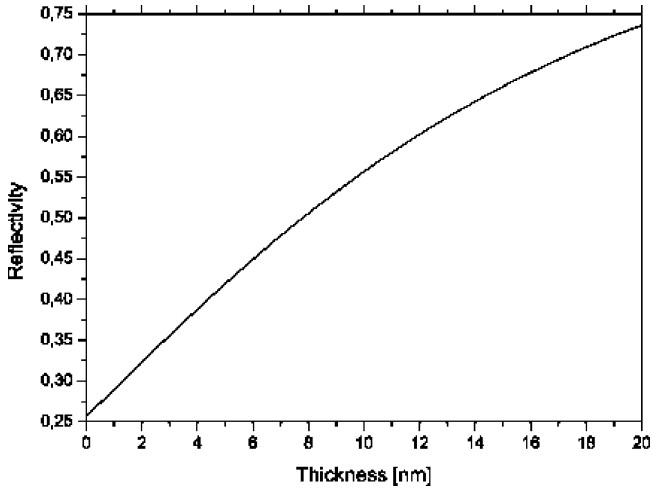

Fig. 4. Dependence of the reflectivity as a function of the layer thickness for a fixed setting of the refractive index $n = 1.4$. The angle of incidence Θ matches the point of inflection at lower angles. The reflectivity increases in a monotonous fashion and a direct proportionality holds for a layer thickness up to 10 nm.

before R_0 and after adsorption R_f as a function of the angle of incidence. The result is displayed in Fig. 3.

The functional relation reveals two maxima which correspond to the points of inflection in the reflectivity curve. The reflectivity curve is not symmetric and the point of inflection at smaller angles is the best choice. The vertical lines in Fig. 3 indicate the points of inflection and the minimum of the corresponding reflectivity curve before and after adsorption. The difference in the reflectivity is about 0.08 and this value can be used for a comparison with the mismatch scheme. Reflectivity tracking is a fast and convenient measurement scheme with no special demand on the alignment. However, several experimental errors sources have to be considered. The first concern is that a drift of the laser light intensity or intensity fluctuation caused by the turbidity of the cell may be misinterpreted as a drift in the coupling angle. This problem can be circumvented by a simultaneous detection of s- and p-light. In this mode, one irradiates the sample with mixed s- and p-light and splits the beam into s- and p-light after the sample. The s-light, which does not display a surface plasmon, serves as the reference channel and is used for the normalization of the data. Another process that has to be ruled out is the broadening of the surface plasmon. The optical constants of gold may change with time and this can cause a broadening of the resonance. In a simple intensity measurement this would be misinterpreted as an adsorption or desorption process. For this reason it is mandatory to characterize the final state in a conventional Θ–Θ reflectivity scan to rule out this unwanted scenario.

If all this is properly considered reflectivity tracking is an easy mean to follow fast adsorption processes. The relation between reflectivity and layer thickness is linear for thickness up to 10 nm and then it levels off as outlined in Fig. 4.

4. Mismatch tracking

In [20,21] an elegant and simple solution for the measurement of fast adsorption processes has been suggested and successfully applied [22]. The measurement scheme takes advantage of a bicell or a four quadrant photodiode. These detectors are fast, cover a broad dynamic range and show a linear response with respect to the light intensity.

The underlying concept is illustrated in Fig. 5. The Kretschmann configuration is used for plasmon excitation. The incident p-polarized light is focused with a lens onto the prism base. The beam covers therefore a defined angular range $\delta\phi$ at the focus. The reflected light can be regarded as

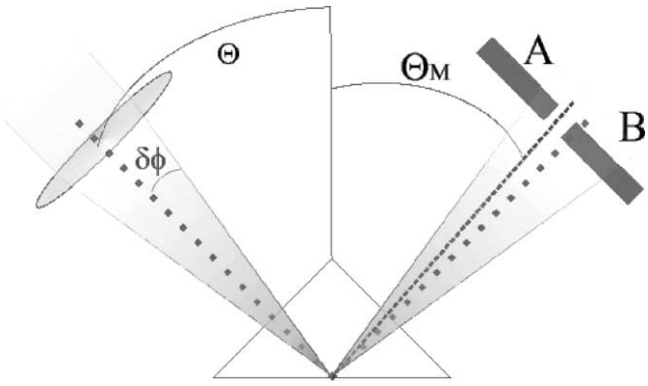

Fig. 5. Illustration of the experimental arrangement for mismatch tracking. The main ray is set to the angle Θ and a lens produces a convergent beam of a defined angular range $\delta\phi$. The reflected light is then detected via a two quadrant diode which is mounted on an translation stage. The setting of Θ and $\delta\phi$ defines the mismatch cut-off angle Θ_M, which is basically the angle where the upper and lower half of the bicell detector records the same intensity. To fulfill this condition experimentally the diode can be moved using a translation stage until the intensity reading of the upper and lower segment matches.

a fan of rays with an intensity given by the corresponding reflectivity $R(\Theta)$. The light is then detected via a two quadrant diode which is mounted on a translation stage. The upper segment A integrates over the angular range Θ–$\delta\phi$ until Θ_M, whereas the lower segment B integrates over Θ_M and $\Theta + \delta\phi$. For the understanding of the calculations and the experiment it is important to distinguish the following experimental parameters: the main ray setting Θ, which is determined by the goniometer, the angular range $\delta\phi$, which is given by the focal length of the lens and the laser beam diameter, the plasmon coupling angle Θ_0, which is given by the reflectivity minimum of the SPR curve and the mismatch cut-off angle Θ_M, which is basically the angle where the upper and lower half of the bicell detector record the same intensity.

The intensity reading of both photodiode segments defines the mismatch M:

$$M = \frac{A - B}{A + B} \propto \Delta\Theta \tag{1}$$

The suggested experimental protocol goes as follows [20]: Before each measurement the prism was rotated so that there was a dark line located at the center of the laser beam. This dark line corresponds to the plasmon coupling angle Θ_0. Then the intensity falling on the two cells of the detector was balanced by moving the diode until the mismatch M vanishes. In other words, the plasmon coupling angle Θ_0 matches the goniometer setting Θ.

Fig. 6 illustrates the experiment using a lens producing an angular range of $\delta\phi = 2.0^\circ$. The SPR resonance curve is asymmetric and for this reason the minimum of the SPR reflectivity Θ_0 does not coincide with the mismatch cut-off angle Θ_M. The mismatch vanishes if the upper segment integrates over the horizontally striped angular range and the lower segment integrates over the angular range marked by the diagonal stripes are equal. Upon adsorption the reflectivity curve is modified (shifted) and as a result a non-vanishing mismatch is detected. The dotted line refers to the reflectivity after adsorption. The magnitude of the mismatch is then used for the interpretation of the adsorption process as outlined in the next section.

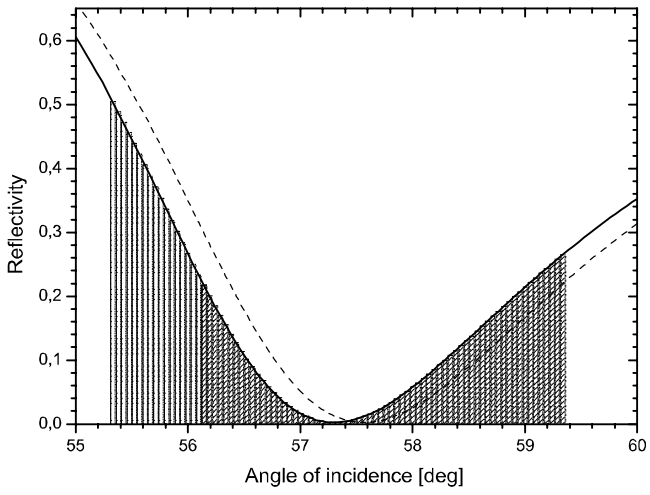

Fig. 6. Illustration of the mismatch concept using a goniometer setting Θ_0 and an angular range of $\delta\phi = 2.0^\circ$. The vertically striped area matches the diagonal striped area and the mismatch M vanishes. All settings are then kept fixed and a mismatch is observed as a consequence of the adsorption. The solid line refers to the reflectivity before and the dashed one after adsorption.

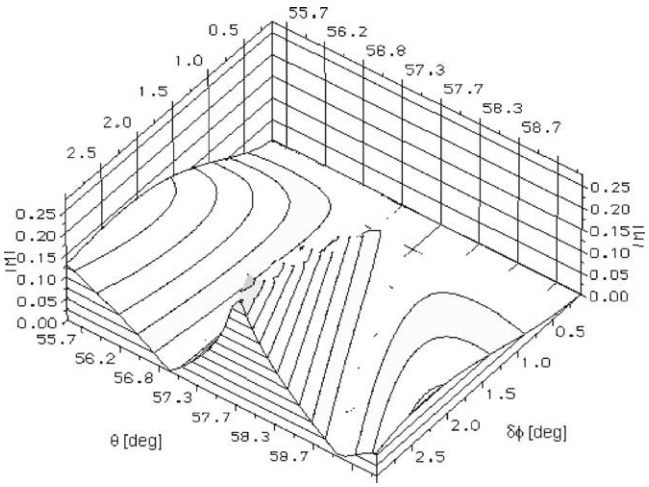

Fig. 7. Magnitude of the mismatch as a function of the angular range $\delta\phi$ produced by lens and the main ray setting Θ of the goniometer.

The mismatch depends on the angle of incidence of the main ray Θ and the angular range $\delta\phi$ produced by the lens. Fig. 7 illustrates a simulation of the magnitude of the mismatch $|M|$ in dependence of the main ray setting Θ and $\delta\phi$. The underlying relation reveals a non-monotonous behavior with a pronounced maximum.

Obviously, it is critical to adjust both quantities Θ and $\delta\phi$ carefully in order to maximize the sensitivity. In our example an angular range of $\delta\phi = 2.5^\circ$ works out best. Furthermore, it is decisive that the main ray setting Θ does not coincide with the plasmon coupling angle Θ_0 as suggested in the original protocol.

Fig. 8 shows the dependence of the mismatch versus the angular setting of the main ray using the lens that provides the highest sensitivity. The dependence reveals a pronounced maximum. The critical points of the Θ–Θ reflectivity scans are indicated by vertical lines. The maximum in Fig. 8 does not correlate with the points of inflection nor with the SPR coupling angle Θ_0. The right angle choice provides a factor of four higher sensitivity as compared to the setting where the main ray coincides with the coupling angle Θ_0. There is a simple physical interpretation for the optimized angular setting. Once the angle of incidence is fixed the integration limits are defined by the angular range of the lens. The maximum sensitivity is obtained, when the integration limits are such, that the mismatch cut-off angle Θ_M equals the minimum of the reflectivity curve Θ_0. In other words the best sensitivity is achieved if the angle of incidence Θ is chosen such that minimum in the reflectivity Θ_0 coincides

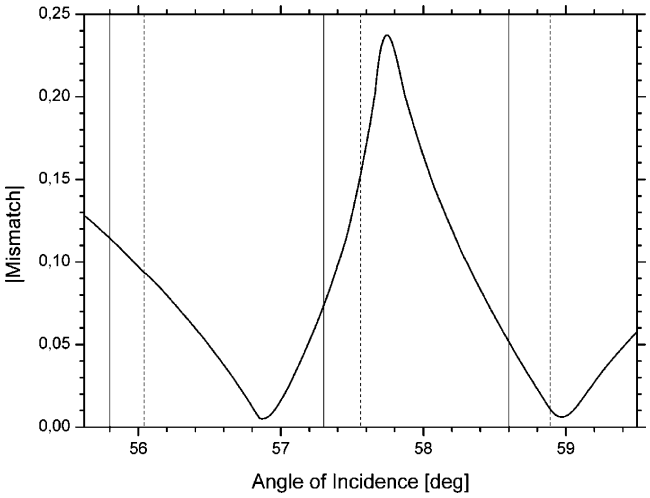

Fig. 8. Dependence of the mismatch versus the angular setting of the main ray using the lens that produces the highest sensitivity. The vertical lines indicate the points of inflection and the minimum of the reflectivity curve before (solid) and after protein adsorption (dashed).

with the mismatch cut-off angle Θ_M. The main ray is then not at the minimum of the SPR curve!

This optimized setting offers a further advantage. The mismatch increases in a monotonous fashion and a direct proportionality holds for thin films ($\approx$10 nm). If the main ray setting Θ coincides with the reflectivity minimum Θ_0 a non-monotonous behavior is observed. Fig. 9 shows the mismatch versus thickness of the adsorbed layer for a lens with an angular range of 2.5° and the two different settings of the main ray Θ. Obviously, this arrangement limits the use of the scheme to very thin films. Secondly, the boost in the sensitivity between both settings is obvious.

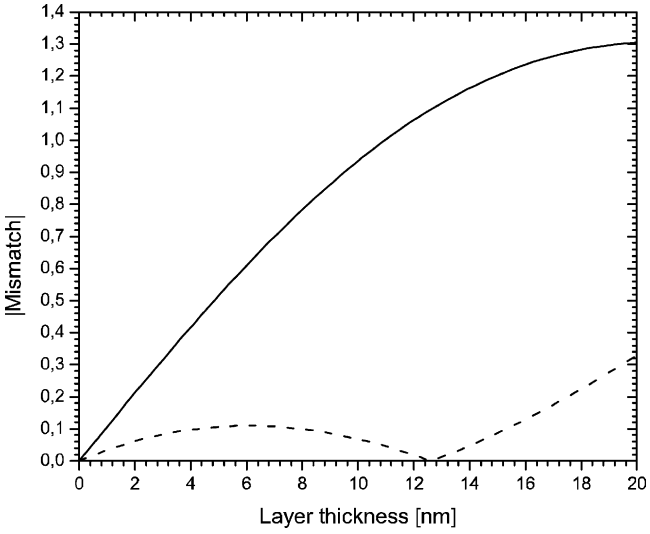

Fig. 9. Mismatch in dependence of the thickness of an adsorption layer with a fixed refractive index of $n = 1.4$. The magnitude of the mismatch and the underlying functional relation depends critically on the main ray setting Θ. The solid line refers to a goniometer setting Θ so that the mismatch cut-off angle Θ_M coincides with the reflectivity minimum Θ_0 of the state before adsorption. The dashed line refers to a goniometer setting so that the main ray Θ coincides with the reflectivity minimum, $\Theta_M = \Theta_0$. An angular range of $\delta\phi = 2.5°$ has been used for the simulation.

The magnitude of the mismatch can be compared to the sensitivity analysis of the reflectivity tracking scheme. Both numbers basically represent differences in reflectivities which are translated in a voltage at a photodetector. Using a detector with a similar characteristics we can compare the smallest change in the refractive index of the ambient media which can be detected by both schemes. The mismatch scheme offers a factor of 3 in its sensitivity as compared to reflectivity tracking provided that all parameters are optimized.

Experimentally, the mismatch scheme is more demanding and requires a careful alignment of a lens (proper focal length and the right angle of incidence). An experimental benefit of the mismatch scheme is the intrinsic normalization that eliminates laser light fluctuations. However, we would like to point out that also the mismatch scheme yields wrong results if the plasmon half-width changes. This is due to the asymmetry of the SPR curve. In other words, both schemes require a measurement of a Θ–Θ scan before and after the adsorption in order to rule out a broadening of the plasmon.

For comparing different SPR detection techniques, the SPR resolution is often described in terms of the smallest detectable change in the refractive index of an analyte. In order to test this we performed a simple experiment in a Kretschmann configuration consisting of prism, gold and air using an SPR spectrometer which is described in great detail in [12]. Using a specially designed flow cell, the temperature of the air was varied by a couple of degrees and the mismatch monitored. The result is presented in Fig. 10. The mismatch varies in a linear fashion with the temperature. Using the known refractive index increment of air $dn/dT = -8.7 \times 10^{-7}\,°C^{-1}$ we can estimate that the smallest detectable refractive index increment which can be resolved is about $\Delta n/n = 10^{-7}$.

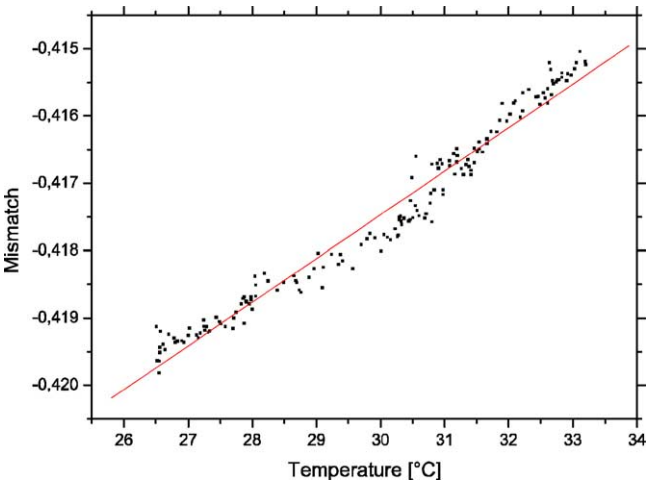

Fig. 10. Measurement of the mismatch M as a function of the temperature of the air. The smallest detectable change of the refractive index of the analyte can be estimated to be in the order of 10^{-7}.

5. Conclusion

We discussed and compared two schemes for detecting the shift of the surface plasmon resonance in a kinetic mode. The relevant parameters that maximize the sensitivity have been worked out. The mismatch scheme works out best if the cut-off angle Θ_M coincides with the minimum of the reflectivity Θ_0 and if the lens produces a well-defined angular range determined by the optical layer model. A linear relation between mismatch and adsorbed layer thickness is then given. Assuming that all parameters are optimized the mismatch scheme provides a higher sensitivity as compared to the conventional reflectivity tracking carried out at the point of inflection of the reflectivity curve.

Acknowledgements

The authors thank Prof. H. Möhwald for his continuous and steady support and stimulating discussions.

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