Modular multichannel surface plasmon spectrometer

G. Neuert,[a] S. Kufer, M. Benoit, and H. E. Gaub
Lehrstuhl für Angewandte Physik & Center for Nanoscience, Ludwig-Maximilians Universität, Amalienstrasse 54, 80799 München, Germany

(Received 3 December 2004; accepted 6 March 2005; published online 22 April 2005)

We have developed a modular multichannel surface plasmon resonance (SPR) spectrometer on the basis of a commercially available hybrid sensor chip. Due to its modularity this inexpensive and easy to use setup can readily be adapted to different experimental environments. High temperature stability is achieved through efficient thermal coupling of individual SPR units. With standard systems the performance of the multichannel instrument was evaluated. The absorption kinetics of a cysteamine monolayer, as well as the concentration dependence of the specific receptor-ligand interaction between biotin and streptavidin was measured. © *2005 American Institute of Physics.* [DOI: 10.1063/1.1899503]

I. INTRODUCTION

Receptor ligand interactions are the hallmark of life. Surface plasmon resonance (SPR) spectroscopy was established in recent years as a standard method for the quantification of such interactions. This optical technique uses an evanescent wave to measure changes in the refractive index at a metal—typically gold—surface. One of the binding partners is immobilized at this metal surface. Binding of the other partner results in an increase of the surface concentration, and as a consequence, in a change of the refractive index. Such measurements are performed in real time and the amount of bound ligand as well as association and dissociation rates are determined.[1]

Several commercial instruments are available,[2] which may be operated with little training on day to day basis with acceptable throughput.[3] However, these instruments can hardly be modified to suite the needs in a combined experimental setup, e.g., in combination with an atomic force microscope (AFM) or a second optical device accessing the same metal surface. The SPREETA-sensor from Texas Instruments (Dallas, Texas) is a fully integrated one-chip surface plasmon device.[4] As such it is easy to modify and may therefore be used for a wide range of application.[5] Furthermore it is inexpensive compared to the established standard systems.

Here we describe the design of a multichannel SPR spectrometer based on such sensor chips. This spectrometer is modular and the entire half space above the gold surface is available for additional experiments. The performance of our instrument is demonstrated with binding assays of different standard systems.

II. DESCRIPTION OF THE EXPERIMENT

A. SPR-sensor chip

The basis of this SPR system is the SPREETA-Sensor from Texas Instruments [4,5] (Fig. 1). This sensor consists of a light-emitting diode (LED) whose light is reflected from the gold film onto a linear camera. The camera signal is digitized with 12-bit resolution by a digital signal processor (DSP) (Normadics, Stillwater, Oklahoma) and transferred via a serial interface to a personal computer. The initiation and data collection is controlled with EVM software (Normadics, Stillwater, Oklahoma).

The sensor is initially covered by a gold layer, which was removed by dipping the sensor into solution out of 3/4 hydrochloric acid and 1/4 nitric acid. Afterwards, it was rinsed extensively with double deionized water (ddH_2O). The sensor was now cast with an epoxy resin (Robnor Resins, UK) into an aluminium block. Multiples of these units are combined to form a multichannel block. For multichannel operation each chip was operated by its own DSP controller, and analyzed in multiple windows of EVM software.

B. Gold-coated cover slips and surface functionalization

In order to allow for the sensor to be reused also with different surfaces, the initial single-use gold surface of the sensor was removed. Instead gold-coated glass cover slips were optically coupled with index matching oil to the surface of the sensor. These gold-coated cover slips are prepared as follows: cover slips (Roth, Karlsruhe, Germany) were cleaned once in 2% Helmanex-solution (Helma, Germany) for 15 min. and then two times for 15 min. in ddH_2O. All steps were performed in an ultrasonic bath. Afterward, the cleaned cover slips were dried in an oven at 75 °C overnight. The clean and dry glass cover slips were covered with 10 Å chrome/nickel (80% Cr / 20% Ni, GoodFellow, GB) as adhesive layer and 500 Å gold (99,99% pure, Leybold Optics, Germany) by thermal evaporation.

For surface functionalization the coated glass slips were transferred immediately after evaporation into a ddH_2O solution containing 20 mM cysteamine (2-aminoethanethiol, Sigma-Aldrich) stored overnight to allow a self-assembled monolayer (SAM) to form onto the gold surface.[3,6–8] After 12 h of incubation (as can be seen later on in Fig. 2, already

[a]Electronic mail: gregor.neuert@physik.uni-muenchen.de

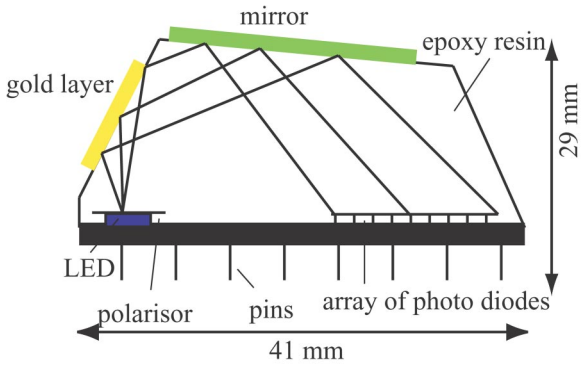

FIG. 1. (Color online) Schematics of the SPREETA sensor. A LED emits light at 840 nm, which passes a polarizer, illuminating the whole sensor surface. The reflected light is mirrored onto the array of photo diodes.

an incubation time of one hour would be sufficient), the SAM-coated cover slips were washed extensively with ddH_2O and placed into 1 M NaOH for 5 min to deprotonate the NH^{3+} groups of the SAM. Afterwards, the cover slips were washed with ddH_2O, dried in a stream of N_2 and processed immediately.

As a result of this procedure the gold-coated cover slips have a high density of NH_2 at the surface, which are used to couple carboxymethyl cellulose (CMC, Sigma).[3] A CMC solution was prepared and added to the solid form of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochlorid (EDC, Sigma)/N-hydroxysuccinimid (NHS, Sigma) to reach a final concentration of 5% CMC with 50 mM EDC/NHS in 10 mM hepes. 200 μl of this solution was pipette onto one cysteamine coated gold slide and covered by a second cysteamine coated gold slide in a sandwich like structure. Previous AFM studies had shown that a covalent attachment of the polymer to the gold surface is achieved this way.[9] These slips were then stored in an incubation chamber with a ddH_2O atmosphere at room temperature for 2 h. After the CMC coupling, the cover slips were washed extensively with ddH_2O and stored in ddH_2O for later use. Before use, the functionalized slips were well dried in a N_2 stream.

C. Fluid cell

The flow chambers were made from poly (dimethylsiloxane) (PDMS, Sylgard 184, Dow Corning) a fluid silicon

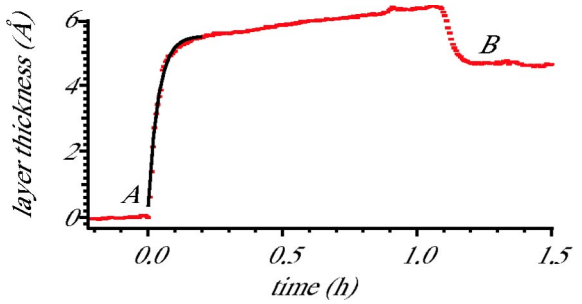

FIG. 2. (Color online) Binding kinetics of cysteamine layer on gold. (*A*) Start of the absorption. (*B*) Rinse with PBS. The thickness of $d=4.8$ Å is measured between points (*A*) and (*B*). The index of refraction of the cysteamine layer was assumed to be $n=1.525$. The black line is a guide for the eye.

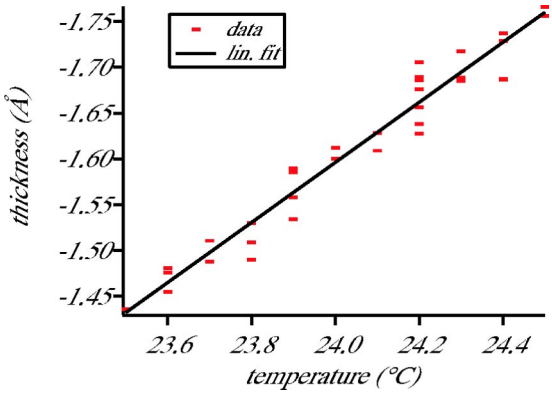

FIG. 3. (Color online) Linear drift of the surface plasmon plotted as thickness change ($n=1.5$ in water $n=1.33$) dependent on the temperature (°C) over a time period of 5.5 h. Linear least-squares line fit results in an average thickness error of 0.33 Å per °C. Red dots: measured data, black line: linear fit.

elastomer.[5] After mixing the elastomer with a catalyst, the mixture was degassed and cast into a special form. The silicon tubes, which later on allow the fluid exchange of the sample are already inserted and polymerized into the elastomer. The polymer was cured for 24 h at 60 °C. The approximately 2 mm thick fluid chambers were finished by cutting a 10 mm×3 mm sample volume out of the PDMS. Before each measurement, the fluid chambers were cleaned with Helmanex, ddH_2O, ultra pure ethanol, and dried in a stream of N_2. The fluid chambers were then placed on top of the coated cover slip and sealed with a microscope slide. This assembly was fixed with a metal bar from the top.

To ensure a continuous flow of the buffer, the fluid cell was connected to a peristaltic pump. It is also possible to introduce sample liquid via a valve [Fig. 4(a)]. The flow was controlled down to values as low as 30 μl/min. This allows measurements of sample volume as small as 100 μl per channel in a stop flow manner. Before each measurement, the sensor was calibrated in air and buffer.

D. Cysteamine monolayer adsorption

The quantification of the online adsorption of cysteamine onto a freshly evaporated glass cover slip was the first proof of reliable operation of the instrument as shown in Fig. 2. The gold cover slip was prepared and placed into the instrument as described above. As a running buffer we used (PBS). After equilibration of the instrument, we switched to the sample liquid system that contained a 10 mM cysteamine solution dissolved in PBS and inject it into the fluid chamber. The thickness of the adsorbed layer in units of angstroms as a function of time in hours is plotted. The index of refraction n is set to $n=1.525$. The adsorption of the cysteamine starts at $t=0$ h (A) and rises rapidly, indicating the strong adsorption of the mercapto group to the gold surface. After 15 min. a plateau was reached which indicates a saturation of the gold surface with cysteamine. After one hour no significant increase of the signal was observed any longer, so that the adsorption of the cysteamine was stopped (B) by switching to the running buffer. The latter washed away unbound cysteamine, which resulted in a baseline shift of 4.8 Å,

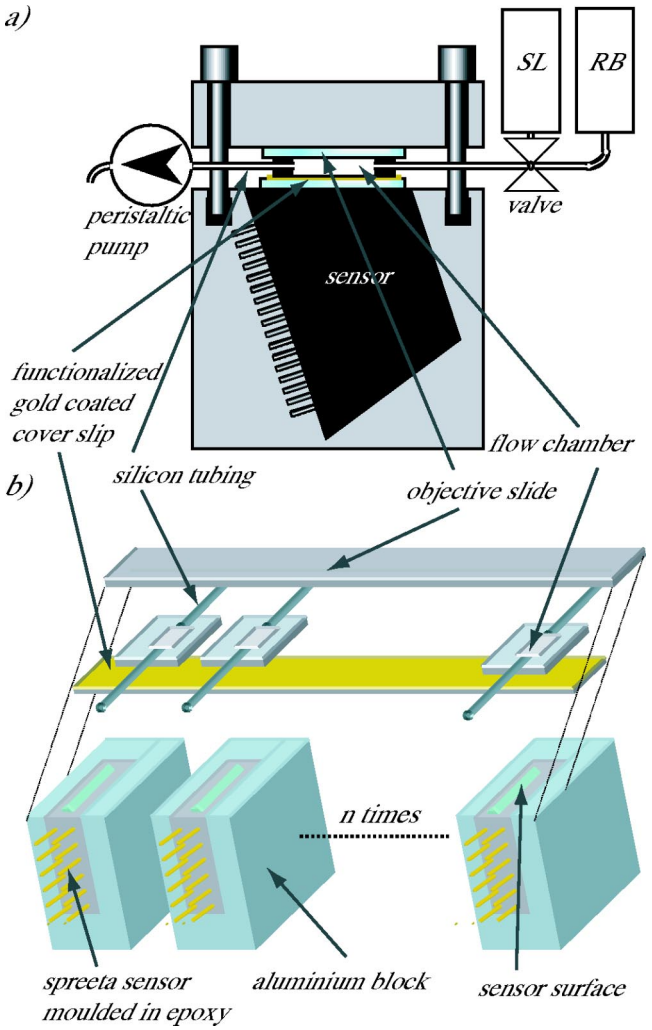

FIG. 4. (Color online) (a) Compiled setup contains the molded sensor with functionalized gold coated glass cover slip and fluid cell. The fluid cell is connected via a valve to the sample liquid (SL) or the running buffer (RB). To ensure a continuous flow of the buffer, the fluid cell was connected to a peristaltic pump. (b) The factory-made gold surface of the SPREETA sensors was removed with nitro hydrochloric acid. The bare sensor was molded with epoxy resin into an aluminium block. A cover slip, which was evaporated with 15 Å CrNi and 500 Å gold was optically coupled with index matching oil to the glass surface of the sensor. A flow chamber, made of PDMS, was placed onto the active sensing region of the sensor and sealed with a microscope slide.

which corresponds to a dense monolayer of cysteamine. This is in very good agreement with the theoretical value of 4.83 Å.

E. Temperature dependence of the sensor signal

Surface plasmons are very sensitive to temperature. Therefore we measured the temperature dependence of the plasmon resonance after encapsulation into the aluminium block (Fig. 3). The experiment was performed in ddH_2O. Plotted is the resonance angle in values of the thickness of a hypothetical film with a refractive index of $n=1.5$ in water ($n=1.33$) as a function of the temperature. This measurement was taken over 5.5 h and shows a linear thickness drift of 0.33 Å per °C.

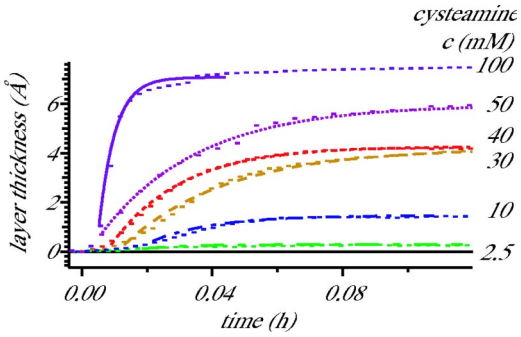

FIG. 5. (Color online) Simultaneous multichannel SPR measurements of the absorption of cysteamine onto the gold surface. Concentrations in PBS ranged between 100 and 2.5 mM. Thickness was measured based on an refractive index of $n=1.5$. Solid lines are first order exponential fits of the measured data.

III. MULTICHANNEL OPERATION

Because of this pronounced dependence of the surface plasmon resonance on the temperature we coupled several sensor units together to form a solid block [Figs. 4(a) and 4(b)]. This solid block connects all sensors thermally, ensuring the same temperature for each sensor. Having the same temperature at all sensors, it is therefore possible to use one sensor as a reference for the others. The temperature dependence can later be subtracted from the other sensor signals.

Another benefit of arranging single sensor units in parallel is the possibility to build a modular multichannel SPR device [Fig. 4(b)]. In our experimental setup it is possible to cover six SPR sensors at the same time with one gold slide. To demonstrate the ability of multichannel operation, we measured the absorption kinetic for cysteamine in a concentrations range between 2.5 and 100 mM.

In Fig. 5 the layer thickness is plotted as a function of absorption time in hours for six different channels in parallel. The cysteamine was solved and diluted in PBS. After the instrument was thermally equilibrated, we injected several solutions of cysteamine at different concentrations (2.5, 10, 30, 40, 50, and 100 mM) in the six flow channels. The measured concentration dependence in the absorption kinetic in the different channels is a convincing demonstration of the stable, reliable, and parallel operation of the instrument.

To explore the potential range of applications of this instrument in biophysical research we investigated the interaction of biotin with streptavedin as shown in Fig. 6. For this experiment we used a CMC surface as described before, which provides a carboxyl functionalized surface. All measurements in this experiment were performed in degassed 10 mM hepes buffer (Sigma) under a constant flow rate of 30 µl/min. After equilibration of the experiment 10 mM biotinhydrazid (Sigma–Aldrich) was mixed with an equal volume of 10 mM hepes with 100 mM EDC / NHS (channel 1,2). In channel 3 only biotinhydrazid without EDC/NHS was injected into the fluid system (*A*). The strong increase in signal is related to the large change of the refractive index related to EDC/NHS. By switching all three channels to hepes buffer after 32 min of incubation (*B*), we washed unbound biotinhydrazid and EDC/NHS away. This resulted in a strong decrease of the signal until a new baseline was reached. We

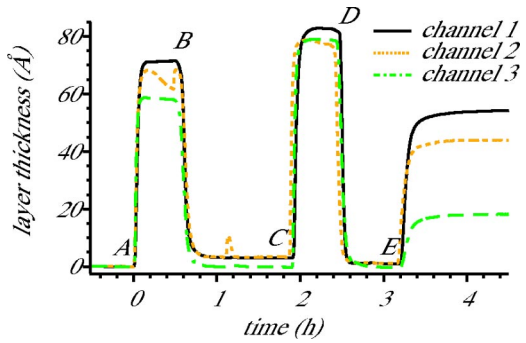

FIG. 6. (Color online) Competitive binding assay for streptavidin to biotinhydrazid. (*A*) Immobilization of biotinhydrazid with EDC/NHS (channel 1,2) and without EDC /NHS (channel 3); (*B*) rinse with 10 mM hepes; (*C*) deactivation of the remaining activated carboxyl groups with 1 M ethanolamine; (*D*) rinse with 10 mM hepes; (*E*) channel (1,3) streptavedin (0.1 mg/ml), channel 2: streptavedin 0.1 mg/ml preincubated with 1:25 biotinhydrazid.

then deactivated the surface by injecting a solution of 1 M ethanolamine (Sigma) (channel 1–3) (*C*) for 30 min. Since not all the EDC/NHS activated carboxyl groups have reacted with biotinhydrazid it is necessary to deactivate the remaining activated carboxyl groups with an excess of ethanolamine, so that no protein binds covalently to the surface, later on.

Afterward, we washed again with 10 mM hepes (*D*) until a stable baseline was reached. At point (*E*) we injected 0.1 mg/ml streptavedin in 10 mM hepes (channel 1,3). Preincubated streptavedin (0.1 mg/ml) with a 25-fold molar excess of biotinhydrazid was injected in channel 2 until saturation was reached. It can be clearly seen that the strongest interaction occurs between the biotin functionalized surface and streptavedin in channel 1. The coverage decreased for the preincubate streptavedin (channel 2). This was to be expected because the majority of the binding sites of streptavedin were blocked with free biotinhydrazid and binding of streptavedin to the surface is thus largely suppressed. For the surface that was not activated before (channel 3) the interaction of the streptavedin bound only non-specifically at much lower levels.

IV. DISCUSSION

Three major benefits helped this instrument to become a workhorse in our laboratory: multichannel operation, free access to the active gold surface with other techniques, and ease of operation. High end commercial instruments like the Biacore have a better thickness resolution but the accuracy of 1% of a protein monolayer, reached with our setup is more than sufficient for most applications. Also the option to design, test, and implement new surface functionalization protocols on the gold films with great ease and moderate costs has helped to standardize surface chemistry in our lab.

Having multiple channels running in parallel not only speeds up screening steps. Since all traces run on the same chip with the same history and chemistry, standard deviations between the traces came down drastically. Being able to design the fluid chamber, e.g., with an optical window to the upper side has allowed to combine SPR measurements with optical excitation and light induced chemistry. The integration of an AFM became possible and initiated a different set of experiments not reported here. The block design helped markedly to increase the temperature stability of the setup. An additional external thermostat, also controlling the sample fluids may help to improve this stability further if needed.

ACKNOWLEDGMENTS

The authors thank E. K. Sinner, Ch. Albrecht. K. Blank, T. Nickolaus, and E. Sackmann for helpful discussions. Financial support by the Deutsche Forschungs Gemeinschaft (SFB 486) is gratefully acknowledged.

[1] W. Knoll, Annu. Rev. Phys. Chem. **49**, 569 (1998); E. K. Sinner *et al.*, Anal. Biochem. **333**, 216 (2004); E. L. Florin and H. E. G. Gaub, Biophys. J. **64**, 375 (1993).
[2] W. M. Mullett, E. P. C. Lai, and J. M. Yeung, Methods **22**, 77 (2000).
[3] B. Johnson, S. Löfås, and G. Lindquist, Anal. Biochem. **198**, 268 (1991).
[4] J. Meléndez *et al.*, Sens. Actuators B **35–36**, 212 (1996).
[5] A. R. Wheeler *et al.*, Sens. Actuators B **98**, 208 (2004).
[6] C. D. Bain *et al.*, J. Am. Chem. Soc. **111**, 321 (1989).
[7] S. Löfås *et al.*, Sens. Actuators B **5**, 79 (1991).
[8] G. Brink *et al.*, Biochim. Biophys. Acta **1196** (2), 227 (1994).
[9] M. Grandbois *et al.*, Science **283**, 1727 (1999).