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RESEARCH PAPER

Direct immunosensing by spectral correlation interferometry: assay characteristics versus antibody immobilization chemistry

Alexandr G. Burenin · Alexandr E. Urusov · Alexei V. Betin · Alexey V. Orlov · Maxim P. Nikitin · Tatiana I. Ksenevich · Boris G. Gorshkov · Anatoly V. Zherdev · Boris B. Dzantiev · Petr I. Nikitin

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Abstract A 3-channel biosensor based on spectral correlation interferometry (SCI) has been adapted for direct optical detection of antigens by measuring changes in thickness of a biolayer on functionalized glass slips employed as affordable single-use sensor chips. The instrument is insensitive to the bulk refractive index of a solution under test and provides signals in metrological units (pm or nm). Using real-time monitoring with the SCI, protocols for fabrication of sensor chips with different functional (epoxylated, carboxylated, and biotinylated) surfaces for antibody immobilization have been developed and optimized to minimize chip-to-chip variations and achieve better limit of detection (LOD), shorter assay time, and longer shelf life. The optimized coupling surfaces have been compared for detection of human serum albumin (HSA) used as a model agent of medical significance. The dynamic ranges for measuring the HSA concentration were 0.07–20, 0.12–30, and 0.25–10 μg/ml, and the assay durations were less than 20, 15, and 30 min for the epoxylated, carboxylated, and biotinylated chips, respectively. The advantages of each type of sensor chip have been shown, namely, the carboxylated chips feature the shortest assay time, the epoxylated ones demonstrate the best LOD, and the biotinylated chips exhibit the longest shelf life in an unprotected environment. The developed protocols of antibody immobilization can be used in different biosensors and assay techniques including those based on fluorescent, magnetic or plasmonic labels, etc. The SCI is well compatible with various partially transparent layers used in biosensing and with microarrays for multi-analyte detection.

Keywords Biosensors · Interferometry · Reflectometry · Label-free immunosensors · Immobilization of biomolecules · Human serum albumin

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A. G. Burenin · A. V. Betin · A. V. Orlov · M. P. Nikitin · T. I. Ksenevich · B. G. Gorshkov · P. I. Nikitin
Prokhorov General Physics Institute, Russian Academy of Sciences, 38 Vavilov Str., 119991 Moscow, Russia

A. G. Burenin · M. P. Nikitin
Moscow Institute of Physics and Technology (State University), 9 Institutskiy per., Dolgoprudny, 141700 Moscow, Russia

A. E. Urusov · A. V. Zherdev · B. B. Dzantiev
A.N. Bach Institute of Biochemistry, Russian Academy of Sciences, Leninsky Prospekt, 33, Build. 2, 119071 Moscow, Russia

P. I. Nikitin (✉)
National Research Nuclear University MEPhI (Moscow Engineering Physics Institute), Kashirskoye sh. 31, 115409 Moscow, Russia
e-mail: nikitin@kapella.gpi.ru

Introduction

Nowadays, the increasing importance of biosensing has been demonstrated by the exponential growth of publications. A significant part of the biosensing techniques is represented by optical label-free systems originating more than three decades ago [1, 2], which include numerous refractometric sensors based on grating couplers [2], surface plasmon resonance (SPR) on flat gold films [1, 3] and on Schottky structures [4], waveguided and interferometric biosensors [5], as well as several reflectometric devices based on reflectometric interference spectroscopy (RIfS) on thin film biochips [6], spectral phase [7], and spectral correlation interferometry (SCI) [8] that employ glass slips as the sensor chips, etc. The direct optical biosensing systems have become the leaders for measuring kinetic constants of interacting biomolecules and very

effective tools for a variety of fundamental and applied research [9–12].

However, for routine analytical or medical applications, these methods are still behind the widely used lateral flow assays [13, 14] originated at about the same time [15] or enzyme-linked immunosorbent assay (ELISA) proposed 12 years earlier [16, 17]. Among the factors that are to be addressed to make the direct biosensors competitive for these applications, single-used consumables are of primary importance. Commercially available biochips, which employ thin-film structures with precisely deposited metal (gold or silver) or dielectric films, are rather expensive to be single-used. The biochip regeneration increases the maintenance cost of equipment, and each different analyte would require either another biochip or another expensive instrument. For medical diagnostics, the biochip regeneration is practically unacceptable because of contamination issues. In order to compete in medical applications with, for example, nitrocellulose immunochromatographic strips [13], the biochips should be of comparable cost.

Another factor concerns the refractometric biosensors, which use evanescent waves to measure changes in the refractive index n of the medium adjoined to the biochip surface. It is strong dependence of $n(T)$ upon temperature $\Delta n=10^{-4}$ per 1 K [18] and density of water (or salt concentration of buffers) $\Delta n=10^{-2}$–10^{-3}, whereas the bioreactions to be measured yield only $\Delta n\approx10^{-6}$–10^{-7}. The latter value was measured, for example, by a thermostating phase-jump SPR system with precision of 0.01 K and with additional one order compensation of the drift by 2-channel referencing [19]. However, the strict thermostating is inconvenient for many real-life tasks, and the higher level compensation by referencing might be tricky or influenced by enthalpy of some reactions on $n(T)$. In contrast, in reflectometry, the signal is nearly independent of temperature [9], which gives preferences to the related biosensors for some applications.

The third factor is the limit of detection (LOD) of direct biosensors, which is to be improved for better coverage of the clinically relevant ranges for various analytes. The simplest way to improve LOD in the existing biosensors is additional amplification steps with non-labeled tracer antibody for sandwich assays [20] or with various nanoparticles [10, 11, 21–23]. Although these steps diminish the beauty of "reagentless" assays; however, we believe that such biosensors may be attractive because of much shorter assay time as compared with ELISA and due to their compatibility for multi-analyte quantitative assays in contract to current lateral flow tests [13]. In addition, the sandwich format is a cost-effective way to determine antigens in complex matrices such as undiluted plasma, serum, etc. by recoding the signals while binding of the tracer antibody.

Earlier, it was shown [8, 20] that the spectral correlation interferometry allows avoiding some of the mentioned factors: (i) it uses low-cost microscopic glass slips without deposition of any metal or dielectric films as disposable sensor chips and (ii) it is not sensitive to the bulk refractive index of solutions and, hence, to the $n(T)$ dependence.

In this research, human serum albumin (HSA) was chosen as a model system for demonstration of a novel assay technique based on the SCI method. Previously, the HSA detection in urine was reported based on a 2-channel SPR biosensor, and a differential method was adopted to subtract the matrix effect [24]. This antigen in buffer was also used for testing an integrated optical immunosensor [25] and for measurements of affinity constants by a Reichert's SR7000DC SPR dual-channel system [26].

The HSA is synthesized in liver and is known to be the most abundant protein in blood plasma. Monitoring of urinary excretion of HSA is a convenient tool widely used for medical diagnostics of metabolic disorders [27–29]. The HSA concentration in urine is a diagnostic marker of kidney disorders such as nephrotic syndrome and uremia [30]. In addition, microalbuminuria, which is urinary excretion of HSA in concentrations of 20 μg/ml and higher, serves as a specific marker for identification of patients with high risk of diabetes and complications of cardiovascular diseases. Modern medical practice considers appropriate periodic monitoring of albumin in the urine [31].

The purpose of this research was to propose a novel direct assay that allows development of affordable single-use functionalized sensor chips and their optimization to minimize chip-to-chip variations to achieve better LOD, shorter assay time, and longer shelf life. An SCI biosensor has been adapted for monitoring each step of functionalization of the biochip surface and for comparison of the performance of the optimized biochips for detection of HSA employed as the agent of medical significance.

Materials and methods

Reagents

The following reagents were used in the study: human serum albumin, biotin, and casein (Sigma, USA); 3-aminopropyltriethoxysilane, 3-glycidoxypropyltrimethoxysilane (GLYMO) and succinic anhydride (Aldrich, USA); *N*-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), and methanol (Fluka, USA); biotin-*N*-hydroxysuccinimide ester, *N*,*N*-diisopropylethylamine, bovine serum albumin (BSA), dimethylformamide, dimethylsulfoxide, glycine, and streptavidin-horseradish peroxidase conjugate (MP Biomedical, USA); sodium chloride, potassium dihydrophosphate, potassium hydroxide and hydrochloric acid, triton ×100, sulfuric acid, and 30 % hydrogen peroxide (Chimmed, Russia); streptavidin

(Imtek, Russia); antispecies antibodies conjugated with peroxidase label (Medgamal, Russia); 96-well microtiter plates (Corstar, USA); and monoclonal antibodies against HSA (Russian Research Center for Molecular Diagnostics and Therapy, Moscow).

Antibody biotinylation

Biotinylation was done according to the standard method [32]. Antibody was placed in 2× phosphate buffer saline (PBS, 50 mM phosphate buffer, pH 7.4, 100 mM NaCl) and diluted to the concentration of 2.8 mg/ml. To this solution, biotin-*N*-hydroxysuccinimide ester dissolved in dimethylsulfoxide (DMSO) in concentration 10 mg/ml was added to reach 20× molar surplus on protein. After a 2-h incubation at room temperature with gentle stirring, the unreacted small molecules were removed by trice dialysis against PBS.

Characterization of biotinylated antibody with ELISA

The HSA was immobilized on the surface of polystyrene microtiter plate wells from 0.5 μg/ml solution in PBS. Then, the wells were four times washed with PBST (PBS+0.5 % *v*/*v* Triton X100) followed by adding a solution of the biotinylated antibody at concentrations from 2.5 to 100 ng/ml and 1-h incubation at 37 °C. After washing, avidin–peroxidase conjugate (1:3000 in PBST, 100 μl for each well) was added and incubated for 1 h at 37 °C. After four times washing with PBST, a substrate solution which contained 0.42 mM 3,3′,5,5′-tetramethylbenzidine and 1.8 mM H_2O_2 in 0.1 M citrate buffer saline, pH 4.0 (100 μl for each well) was added. After 15-min incubation at room temperature, the reaction was stopped by adding 100 μl of 1 M H_2SO_4. The optical density of the product was measured at 450 nm with a Zenyth 3100 (Anthos, Austria) microtiter plate reader. The results of verification of the biotinylation are given in Supplementary Note 1.

Biosensors based on the spectral correlation interferometry

The method of spectral correlation interferometry [8, 20] allows real-time measurements of changes in optical thickness (the product of the refractive index and physical thickness Δd) of a biolayer on a surface of a sensor chip as a result of a biochemical reaction. Briefly, the SCI optical scheme employs two Fabry-Perot interferometers and a wide-band radiation source. One of the interferometers has its inter-mirror distance periodically scanned by a piezoelectric actuator. The solutions under test are pumped along a surface of a glass cover slip, which serves simultaneously as a sensor chip and the second interferometer. The interference, which is produced by a reference beam reflected from the bottom surface of the slip and a beam reflected from the biolayer/water interface, depends upon the optical thickness of the slip with the biolayer (Fig. 1). The change of this optical thickness is measured by a phase change of correlation signals from photodetectors or a CCD camera. The SCI optical scheme is well compatible with multi-channel biosensing systems [8, 20].

The SCI method has been embodied in the family of optical label-free biosensing devices Picoscope® [20, 33]. The Picoscope® biosensor used in this study had three independent measuring channels (Fig. 1). For each channel, the reagents were supplied independently by a 3-channel peristaltic pump. The central channel was supplied with PBS, and the respective signal was used for drift compensation. The other channels were used for carrying out of two independent experiments simultaneously. The functionalized 100-μm microscope cover slips were used as the sensor chips.

Functionalization of the glass sensor chips

It is known that assay characteristics are strongly affected by immobilized bioreceptor molecules and by their orientation [34], sometimes resulting in more than 10-fold improvement in LOD [35]. In the present study, we further extend the protocols initially tested in [35] for the well-reproducible fabrication of epoxylated, biotinylated, and carboxylated sensor chips according to the scheme shown in Fig. 2.

1. Cleaning
   The glass cover slips used as the sensor chips were cleaned by 40-min incubation at 80 °C in piranha solution obtained by mixing 30 % hydrogen peroxide and concentrated sulfuric acid in 1:3 ratio by volume. After washing twice with deionized water, the glass slips were incubated in deionized water at 80 °C for 30 min followed by washing twice with deionized water. After washing thrice with isopropyl alcohol, the solvent was removed in a stream of dry air at 50 °C.
2. Preparation of epoxylated sensor chips (E-chips)
   After the abovementioned cleaning, the cover slips were placed for 18 h into 5 % solution of GLYMO in dry toluene at 90 °C. Then, they were rinsed three times with dry toluene and baked in dry air at 100 °C for 40 min.
3. Amination of the glass cover slips
   After cleaning, the cover slips were placed for 16 h in 3 % solution of 3-aminopropyltriethoxysilane in dry methanol at room temperature. Then, they were rinsed three times with dry methanol and baked in dry air at 105 °C for 60 min. The ready-for-use chips were stored at room temperature.
4. Preparation of biotinylated sensor chips (B-chips)
   A solution of 1 mg/ml biotin, 2 mg/ml EDC, 5 mg/ml NHS, and 30 μl/ml triethylamine in DMF was prepared. Two hundred microliters of this solution was placed on each aminated cover slip. After incubation in an evaporation-preventing chamber for 2 h at room

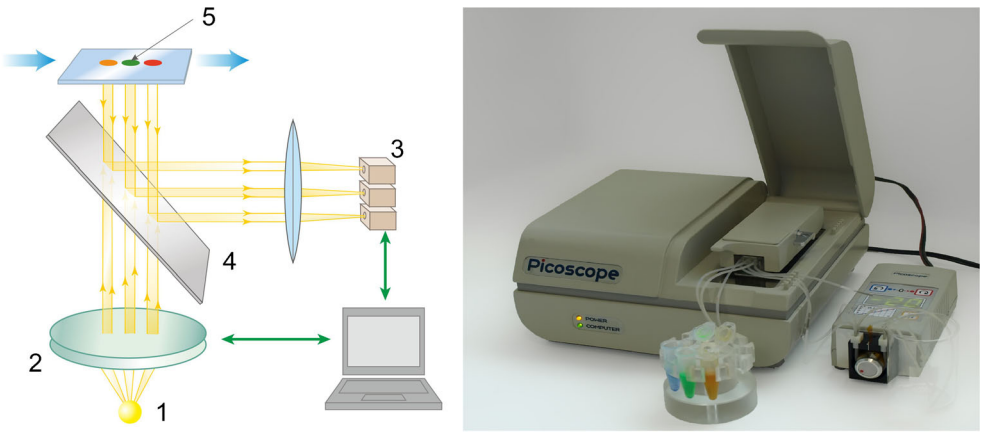

Fig. 1 The SCI principle (*on the left*) and a photo of 3-channel Picoscope® biosensor (*on the right*): *1* superluminescent diode, *2* scanned Fabry-Perot interferometer, *3* photodetectors or CCD camera, *4* semitransparent mirror, *5* channels (reaction spots)

temperature, the slips were rinsed five times with DMF. After solvent evaporation, the ready-for-use chips were stored at room temperature.

5. Preparation of carboxylated sensor chips (C-chips)

   For carboxylation, 200 μl of 50 mM solution of succinic anhydride in DMF was placed on the surface of each aminated sensor chip. After incubation in an evaporation-preventing chamber for 2 h at room temperature, the slips were rinsed three times with DMF. After solvent evaporation, the chips may be stored at room temperature, but in this study, antibody was immediately immobilized on them as follows.

6. Immobilization of specific antibody on the surface of the C-chips

   Two hundred microliters of solution of 2 mg/ml EDC and 5 mg/ml NHS in DMF was placed on the surface of each chip. After 15-min incubation, the solution was removed and the slips were rinsed twice with DMF and twice with PBS. Then, the chips were placed in 10 μg/ml solution of antibody in PBS and incubated for 2 h at room temperature. After two times washing with PBS, the slips were placed in a blocking buffer, which contained 0.1 % of glycine and 100 μg/ml of casein in PBS. Then, the chips were rinsed three times with deionized water and stored at room temperature.

Measuring procedure for optimization of surface modification protocols

All the solutions, unless otherwise indicated, were pumped until the signal saturation, i.e., until the signal change did not exceed 30 pm/min. As the antibody immobilization time, we considered the period between the start of significant growth (≥50 pm/min) of the signal and its saturation. The blocking step was controlled by pumping of the solution containing 50 μg/ml BSA and 30 μg/ml casein. The surface considered as being blocked if the signal growth after 5 min did not exceed 100 pm.

Specific antibody, streptavidin, and HSA were diluted in 150 mM PBS, pH 7.4. The pumping speed was 6 μl/min.

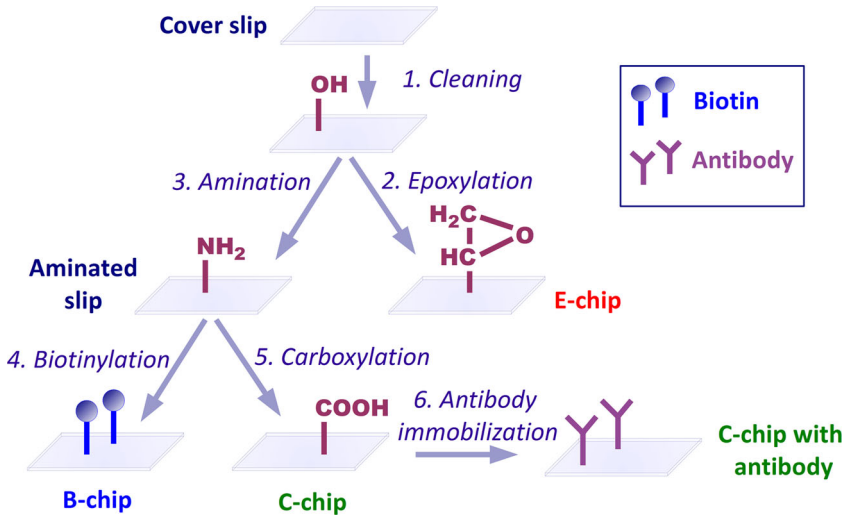

Fig. 2 General scheme for fabrication of the sensor chips with different surface chemistry

To carry out an immunoassay with the E-chips, 10 μg/ml of antibody solution, blocking buffer (0.1 % glycine and 100 μg/ml casein in PBS), PBS, and the sample were consecutively pumped along the chip surface in the biosensor fluid system. The signal change measured during the latter step was defined as the assay result.

During the assay on the B-chip, 10 μg/ml streptavidin solution, 10 μg/ml of antibody solution, blocking buffer (0.1 % glycine and 100 μg/ml casein in PBS), PBS, and the sample were consecutively pumped along the chip surface in the biosensor fluid system. Similarly, the signal change measured during the latter step was defined as the assay result.

Since the C-chips already possessed the immobilized specific antibody and the surface blocking was also done at the preparation step, only PBS was pumped for 5 min in the biosensor fluid system before pumping of the sample and measuring the result.

The measurements for each HSA concentration were done at least three times with the 3-channel SCI biosensor. Two channels of the biosensor were used for two independent experiments simultaneously as described in "Materials and methods." The result was calculated as the arithmetic mean, and the error as the standard deviation.

To optimize the key parameters while surface modification of the slips, the signals were compared using control solutions containing 3 μg/ml HSA and 3 μg/ml BSA. The difference between them was considered as the specific signal proportional to the specific sorption capacity of the glass chip surface. The dependences of the specific signal upon triethylamine concentration (for B-chips), upon duration of carboxylation and succinic anhydride concentration (for C-chips), and upon temperature and duration of epoxylation (for E-chips) have been investigated.

To examine the shelf life for each chip type, the initial measurement of the specific signal was done immediately after the chip preparation followed by weekly measurements during the first month and then monthly. No special protecting from humidity in the lab air was used at this stage. The measurements were performed until the specific signal decreased more than 5 % with respect to the initial measurement, and the time elapsed since that initial measurement was considered as the shelf life.

Calibration of the SCI biosensor for measurements of HSA concentration

For calibration curves, a signal from the test samples, which contained HSA in the concentration range from 0 to 100 μg/ml in PBS with addition of 10 μg/ml BSA, was measured as described above. A new chip was used for each measurement without regeneration. Based on these data, the dependence of the signal upon HSA concentration was plotted as arithmetic means of at least five measurements for each concentration with errors calculated as their standard deviation σ. The calibration curves were obtained by fitting the experimental data using the 4PL nonlinear regression model [36]. The LOD was calculated by the 2σ criterion as the minimal antigen concentration at which the recorded signal exceeded the signal of the negative control in the absence of antigen for at least two values of the standard deviation of the signal of the negative control.

Results and discussion

The three mentioned types of glass sensor chips, which differ in the method of antibody immobilization, the scheme and time of the assay, and potential for the surface stability, have been optimized toward the target characteristics, which are maximum sorption capacity of the surface for specific HSA binding and better LOD. Typical sensograms $\Delta d(t)$ of antibody immobilization and/or HSA binding on all chip types are shown in Fig. 3. The sensograms were measured independently and combined in the single figure for better comparison.

Optimization of E-chips

To minimize the portion of epoxy groups that hydrolyzed during antibody immobilization and to reduce the assay time as well, we used the minimal concentration of specific antibody of 10 μg/ml at which the immobilization completed (the sensogram saturated) within not more than 5 min. The time of pumping of the blocking buffer until sufficient reduction of non-specific sorption (see "Measuring procedure for optimization of surface modification protocols") has been

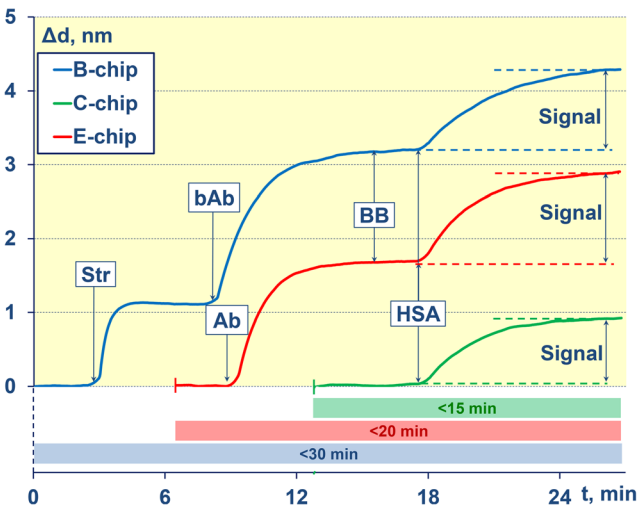

Fig. 3 Sensograms $\Delta d(t)$ of the assays while detection of 10 μg/ml HSA on B-chips (*top blue curve*), E-chips (*middle red curve*), and C-chips (*bottom green curve*): *Str* streptavidin, *bAb* biotinylated specific antibody to HSA, *Ab* specific antibody to HSA, *BB* blocking buffer saline, *HSA* sample

experimentally estimated to be 2 min. It can be seen from the figure that the blocking buffer did not cause any noticeable changes of the signal. This may be due to the lack of centers for non-specific protein sorption and to the fact that mainly glycine, not BSA, binds to the unreacted epoxy groups. Thus, the total procedure of the sensor chip preparation takes 10 min. An extra 10 min is required for the signal saturation during pumping of the sample. The resulting assay time is less than 20 min.

Since the target characteristics of the surface obtained by epoxylation in methanol according to the earlier developed method [32] strongly depend on the ambient humidity and temperature, in this study, we used toluene as a solvent for epoxylation. Due to lower hygroscopicity, toluene provides much more stable conditions for epoxylation and enhances the reproducibility of the E-chip surface.

The main parameters to optimize for the epoxylation procedure in toluene are temperature, time, and GLYMO concentration. The measured dependence of the specific signal upon incubation time for different temperatures at 5 % GLYMO concentration is shown in Fig. 4. The studied temperature range of 40–90 °C was, on one hand, surely higher than the room temperature to provide sufficiently stable conditions and, on the other hand, significantly less than the boiling point of toluene to prevent evaporation and boiling of toluene.

Since the maximum signals at different temperatures were almost equal, there was no reason for using lower temperatures, so the dependence of the signal for different GLYMO concentrations and incubation times was investigated at 90 °C (see details in Supplementary Note 2).

Although the specific signal increased with GLYMO concentration, using the concentrations above 5 % was unreasonable because signal increase is not that substantial at higher concentrations. Thus, 6-h epoxylation in 5 % GLYMO in toluene at 90 °C was found to be optimal for the further investigation. The shelf life of E-chip was 1 month.

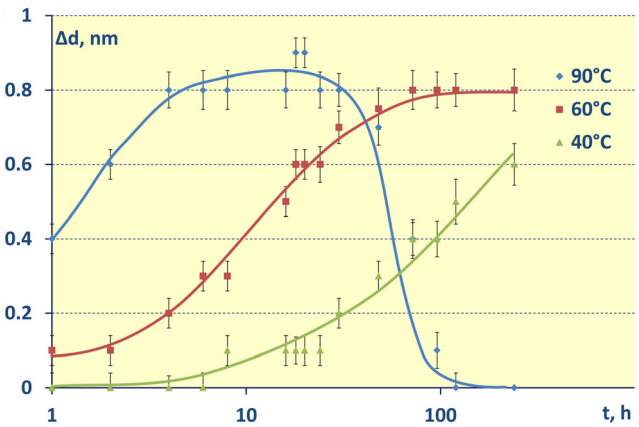

Fig. 4 Dependence of the observed specific signal on epoxylation time at different temperatures and 5 % GLYMO concentration

Optimization of B-chips

A typical sensogram of the assay on a B-chip is shown in Fig. 3 by the top (blue) curve. The concentration of specific antibodies was the same as in the case of E-chips. Because of an additional step, i.e., passing of streptavidin and biotinylated antibody, the preparation time for B-chips was about 18 min and the total assay time was less than 30 min.

The biotinylation was performed in an aprotic medium by activation by carbodiimide of carboxy group of biotin and its binding to amino group of the surface. We believe this method is more competitive for fabrication of disposable chips with inexpensive reagents rather than with widely used preactivated biotin derivatives such as biotin-*N*-hydroxysuccinimide ester [32]. To accelerate the reaction rate in the aprotic medium, a base compound that cannot react with an activated carboxy group should be added. Trialkylamines may be used. In this study, we used triethylamine with optimization of its concentration (see Supplementary Note 3). The optimal concentration of this compound is in the range of 1–5 % (see Fig. S3). Lower concentrations are insufficient for proper acceleration of the reaction, and higher concentrations deactivate carboxy groups too fast so that they cannot react with amines on the surface. The concentration of triethylamine of 3 % has been chosen for further experiments. The shelf life of B-chips obtained according to this protocol was more than 6 months.

Optimization of C-chips

A typical sensogram of the assay on a ready-to-use C-chip is shown in Fig. 3 by the bottom (green) curve. Since the antibody immobilization was done at the preparation stage, the assay time was less than 15 min. This could not only be crucial for some practical applications but may also decrease reliability of the procedure because the antibody immobilization is not controlled directly by the biosensor.

For carboxylation of the glass sensor chips, a reaction of amino groups with succinic anhydride was used. The reaction was carried out in an aprotic anhydrous medium to prevent anhydride hydrolysis. The main parameters of the carboxylation are reaction time and concentration of succinic anhydride. However, the parameters are related by a simple equation for the first order reactions, so the real parameter is the product of time and concentration. Based on the obtained dependence of the specific sorption capacity of the C-chips on the "time-concentration" parameter (see Supplementary Note 4), 2-h incubation at 50 mM anhydride concentration may be chosen as the optimal parameters.

The protocol for antibody immobilization was modified from the standard one [32] so that the carboxy groups were on the solid phase, not in solution. The shelf life of these chips is 1 week.

Optimization of assay protocols for different chips

E-chips

For the assay on E-chips, the following parameters were optimized: (1) duration of PBS pumping, (2) time and concentration during antibody immobilization, and (3) time of surface blocking.

1. PBS pumping at the first stage gives time for stabilization of transient processes such as laminar flow, temperature drifts, and surface–liquid interaction. The moment for finishing of the transition processes was determined by the signal stabilization (drifts no more than 30 pm/min). For more than 90 % of experiments on the E-chips, the signal had met this criterion since the 3rd minute so the duration of PBS washing was chosen to be 2 min. Longer pumping was undesirable due to both increased assay time and hydrolysis of epoxy group on the surface that caused substantial reduction of the specific sorption capacity noticeable in 10–15 min after the experiment start.
2. The dependence of time for antibody immobilization upon antibody concentration in the range from 1 to 100 μg/ml fits the first-order kinetic equation if subtracting the constant time for filling the fluidic system. At 10 μg/ml concentration, in all the experiments, saturation was reached within 5 min.
3. The optimal time of surface blocking was determined as follows. The signal was measured in a series of experiments with different durations of pumping of the blocking buffer starting from 1 h with step-by-step decrease. It was found that 2 min is the minimal duration of blocking that provided unaltered signal while pumping of negative control (BSA and casein at concentrations of 10 μg/ml). There was no reason to increase this time, and its decreasing might result in lower reproducibility because of difficulties in precise time control. If no blocking was applied, pumping of the negative control caused the signal raise of 0.2 nm.

The time until the sensogram saturation during pumping of the sample varied from 5 to 10 min and depended on the HSA concentration. The longest time was observed for HSA concentrations between 0.5 and 1 μg/ml, which agreed well with the theoretical model based on the Langmuir sorption isotherm. Thus, the total assay time was less than 20 min (Fig. 3).

C-chips

No substantial differences were observed in the assays on the C-chips as compared with the E-chips, except for 5 min period required for stabilization of transition processes. The total assay time was 10 to 15 min.

B-chips

For B-chips, the time of streptavidin immobilization was optimized in addition to the parameters optimized for E-chips. Streptavidin immobilization took 1–2 min, and filling of the fluidic system took major part of this time (increase in the sensogram slope near the start of immobilization). Other stages did not differ significantly from those for E-chips, so the assay time for B-chips was less than 30 min.

Calibration curves for the SCI biosensors with different types of chips

The calibration curves have been obtained for the three types of the sensor chips fabricated according to the optimized protocols (Fig. 5).

The LODs determined by the 2σ criterion were 70, 120, and 250 ng/ml for the E-chips, C-chips, and E-chips, respectively. It should be noted that although the E-chips have better LOD, C-chips may be preferable for many applications because of shorter assay time and simpler protocols. The dynamic ranges for measurements of HSA concentration by SCI were 0.07–20, 0.12–30, and 0.25–10 μg/ml for the epoxylated, carboxylated, and biotinylated chips, respectively. The upper limit of the dynamic range was determined as the HSA concentration, at which the signal differed from that observed at 100 μg/ml HSA by two standard deviations. The relative error for HSA determination did not exceed 7 % for the E-chips and C-chips and 10 % for the B-chips.

The obtained experimental results are summarized in Fig. 6.

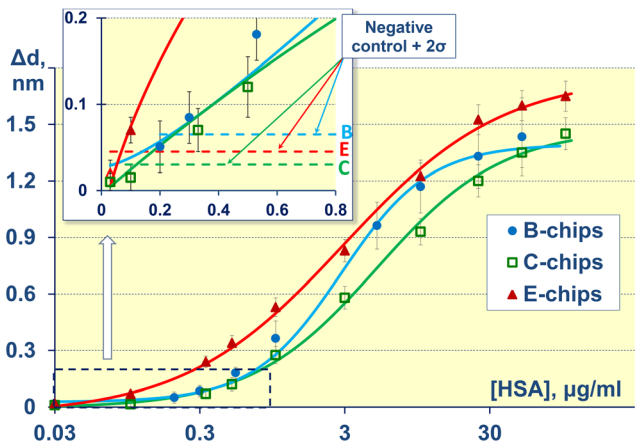

Fig. 5 Calibration curves for measuring HSA concentration with E-chips (*top red curve*), B-chips (*middle blue curve*), and C-chips (*bottom green curve*). Concentrations are in log scale. *Insert*: fragments of the calibration curves at lower concentrations (*linear scale*); *horizontal dashed lines* represent values for all chips that exceed the negative control values by two standard deviations of the negative control in the absence of the antigen

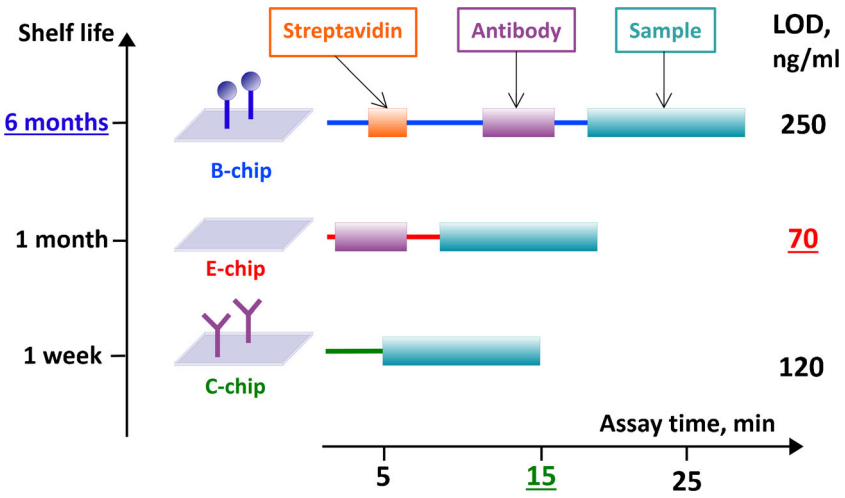

Fig. 6 Assay characteristics for different sensor chips

The B-chips require a preliminary preparation step of streptavidin or avidin immobilization, which prolongs the assay time. However, since biotin is a very stable molecule, such chips have the longest shelf life. In the case of the C-chips, antibody was preliminary immobilized on the chip surface before the assay. This decreases the assay time, but antibody degrades with time, so a shorter shelf life is observed, which could be prolonged by proper chip conservation and sealing in inert atmosphere with low humidity. For E-chips, these parameters have intermediate values, but they show the better LOD, which in our case was defined mainly by variations of single-used chips.

Comparison of the obtained results for HSA detection in buffers with different chips and by other direct optical biosensors [24–26] shows the significance of surface coupling chemistry, which is not less important than the type of the label-free biosensing platform. For testing of the integrated optical Young interferometer immunosensor [25], only one concentration of 50 μg/ml of HSA in PBS was reported. Such concentration of HSA essentially changes the bulk refractive index *n* of PBS buffer, so the authors [25] developed a special procedure to account this effect. For the SCI biosensor, such high concentration of HSA saturated all the chips (Fig. 5), but the related refractive index *n* did not disturb the SCI signals, which is an advantage of the SCI sensors over the refractometric ones. Another advanced commercial SPR system demonstrated HSA detection in buffer in the concentration range 5.3–83 μg/ml [26] with regeneration of the same biochip. The regeneration essentially improves assay reproducibility (by removing the factor of chip-to chip variations) if compared with using of two different SPR chips for each set of measurements [24]. In the latter paper, the competitive assay was developed for HSA detection in urine in the range between 0.1 and 5 μg/ml by application of a differential approach to subtract the matrix effect [24]. The available information about detection of HSA in buffers with SPR biosensors [24, 26] allows one to conclude that the presented here SCI results are on the same level, but importantly, they are obtained using low-cost single-used sensor chips.

It should be noted that the SCI method demonstrated here for label-free immunosensing can also be employed as an effective auxiliary tool for development of various assays including those based on fluorescent, magnetic [22, 23], or plasmonic labels on glass surface. Wide employment of the glass substrates for fluorescent microarrays and biochips is due to low-level autofluorescence. The procedures for monitoring of surface modifications by SCI, as well as the optimized immobilization protocols developed in this work, may also be useful for monitoring production of the glass biochips for the fluorescent assays. Moreover, the SCI biosensors are also compatible with a wide variety of partially transparent interface layers (polymer [37, 38], inorganic materials, etc.) on the glass slips. This allows recording the immobilization of biomolecules on other type of surfaces often used in modern biosensing platforms. This might be tricky for evanescent waves-based biosensors (SPR, grating couplers), which have substantial limitation for additional upper layers with thickness sufficient for mimicking other surfaces, e.g., plastic ones, which are popular in assays for medical diagnostics, as such layers may disturb the resonance much stronger than the studied fine interactions of biomolecules.

Finally, the important advantage of the SCI biosensor as a representative of the reflectometric instruments [6–9] is that it generates signals in well-defined metrological units (pm or nm). This is due to the fact that SCI measures the thickness of biolayer in terms of fraction of the used wavelength, whereas SPR and other evanescent wave biosensors provide raw signals in relative units individual for every type of devices, which have to be calibrated.

Conclusions

Three types of affordable single-used sensor chips that differentiate in surface chemistry have been developed, compared,

and optimized for detection of human serum albumin with a 3-channel biosensor based on the spectral correlation interferometry. The optimization has been carried out toward minimization of chip-to-chip variations to achieve better inter-assay reproducibility and LOD with single-used consumables, shorter assay time, and longer shelf life. The results of detection of HSA in buffers by using low-cost single-used sensor chips are at the same level as the published SPR results. Comparison of the assay characteristics on different sensor chips shows the significance of surface coupling chemistry for assay performance. The immunoassay results on different surface types have revealed the optimal protocols for functionalization of glass surfaces, which can be used for a large variety of other modern immunoassays. Along with direct biosensing, the proposed SCI sensors may become an effective auxiliary tool for monitoring of functionalization of semitransparent sensor interfaces as well as for development of label-based assays including those based on fluorescent, enzyme, and nanoparticle labels.

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