

High-resolution biosensor based on localized surface plasmons

Marek Piliarik,¹ Hana Šípová,¹ Pavel Kvasnička,¹ Nicolle Galler,² Joachim R. Krenn,² and Jiří Homola^{1,*}

¹*Institute of Photonics and Electronics, Academy of Sciences of the Czech Republic, Chaberská 57, Prague, Czech Republic*

²*Institute of Physics, Karl-Franzens University Graz, Graz, Austria*

* homola@ufe.cz

Abstract: We report on a new biosensor with localized surface plasmons (LSP) based on an array of gold nanorods and the total internal reflection imaging in polarization contrast. The sensitivity of the new biosensor is characterized and a model detection of DNA hybridization is carried out. The results are compared with a reference experiment using a conventional high-resolution surface plasmon resonance (SPR) biosensor. We show that the LSP-based biosensor delivers the same performance as the SPR system while involving significantly lower surface densities of interacting molecules. We demonstrate a limit of detection of 100 pM and a surface density resolution of only 35 fg×mm⁻² that corresponds to less than one DNA molecule per nanoparticle on average.

©2011 Optical Society of America

OCIS codes: (240.6680) Surface plasmons; (280.4788) Optical sensing and sensors; (310.6628) Subwavelength structures, nanostructures.

References and links

1. J. Homola, "Surface plasmon resonance sensors for detection of chemical and biological species," *Chem. Rev.* **108**(2), 462–493 (2008).
2. B. Sepúlveda, P. C. Angelome, L. M. Lechuga, and L. M. Liz-Marzan, "LSPR-based nanobiosensors," *Nano Today* **4**(3), 244–251 (2009).
3. P. Kvasnička and J. Homola, "Optical sensors based on spectroscopy of localized surface plasmons on metallic nanoparticles: sensitivity considerations," *Biointerphases* **3**(3), FD4–FD11 (2008).
4. K. M. Mayer, F. Hao, S. Lee, P. Nordlander, and J. H. Hafner, "A single molecule immunoassay by localized surface plasmon resonance," *Nanotechnology* **21**(25), 255503 (2010).
5. H. W. Huang, C. R. Tang, Y. L. Zeng, X. Y. Yu, B. Liao, X. D. Xia, P. G. Yi, and P. K. Chu, "Label-free optical biosensor based on localized surface plasmon resonance of immobilized gold nanorods," *Colloids Surf., B* **71**(1), 96–101 (2009).
6. J. X. Fu, B. Park, and Y. P. Zhao, "Nanorod-mediated surface plasmon resonance sensor based on effective medium theory," *Appl. Opt.* **48**(23), 4637–4649 (2009).
7. M. D. Arnold, M. G. Blaber, M. J. Ford, and N. Harris, "Universal scaling of local plasmons in chains of metal spheres," *Opt. Express* **18**(7), 7528–7542 (2010).
8. E. Petryayeva and U. J. Krull, "Localized surface plasmon resonance: nanostructures, bioassays and biosensing--a review," *Anal. Chim. Acta* **706**(1), 8–24 (2011).
9. S. Chen, M. Svedendahl, M. Käll, L. Gunnarsson, and A. Dmitriev, "Ultrahigh sensitivity made simple: nanoplasmonic label-free biosensing with an extremely low limit-of-detection for bacterial and cancer diagnostics," *Nanotechnology* **20**(43), 434015 (2009).
10. A. B. Dahlin, J. O. Tegenfeldt, and F. Höök, "Improving the instrumental resolution of sensors based on localized surface plasmon resonance," *Anal. Chem.* **78**(13), 4416–4423 (2006).
11. M. Piliarik, P. Kvasnička, N. Galler, J. R. Krenn, and J. Homola, "Local refractive index sensitivity of plasmonic nanoparticles," *Opt. Express* **19**(10), 9213–9220 (2011).
12. M. Piliarik, L. Párová, and J. Homola, "High-throughput SPR sensor for food safety," *Biosens. Bioelectron.* **24**(5), 1399–1404 (2009).
13. M. Piliarik, H. Vaisocherova, and J. Homola, "Towards parallelized surface plasmon resonance sensor platform for sensitive detection of oligonucleotides," *Sens. Actuators B Chem.* **121**(1), 187–193 (2007).
14. M. Svedendahl, S. Chen, A. Dmitriev, and M. Käll, "Refractometric sensing using propagating versus localized surface plasmons: a direct comparison," *Nano Lett.* **9**(12), 4428–4433 (2009).

15. M. Piliarik and J. Homola, "Surface plasmon resonance (SPR) sensors: approaching their limits?" *Opt. Express* **17**(19), 16505–16517 (2009).
16. B. Lamprecht, G. Schider, R. T. Lechner, H. Ditlbacher, J. R. Krenn, A. Leitner, and F. R. Aussenegg, "Metal nanoparticle gratings: influence of dipolar particle interaction on the plasmon resonance," *Phys. Rev. Lett.* **84**(20), 4721–4724 (2000).
17. J. Homola, *Surface Plasmon Resonance Based Sensors* (Springer-Verlag, Berlin-Heidelberg-New York, 2006).
18. H. Vaisocherová, A. Zítová, M. Lachmanová, J. Stepánek, S. Králíková, R. Liboska, D. Rejman, I. Rosenberg, and J. Homola, "Investigating oligonucleotide hybridization at subnanomolar level by surface plasmon resonance biosensor method," *Biopolymers* **82**(4), 394–398 (2006).
19. K. M. Mayer and J. H. Hafner, "Localized surface plasmon resonance sensors," *Chem. Rev.* **111**(6), 3828–3857 (2011).
20. G. J. Nusz, S. M. Marinakos, A. C. Curry, A. Dahlin, F. Höök, A. Wax, and A. Chilkoti, "Label-free plasmonic detection of biomolecular binding by a single gold nanorod," *Anal. Chem.* **80**(4), 984–989 (2008).
21. S. Techane, D. R. Baer, and D. G. Castner, "Simulation and modeling of self-assembled monolayers of carboxylic acid thiols on flat and nanoparticle gold surfaces," *Anal. Chem.* **83**(17), 6704–6712 (2011).
22. T. Cassier, K. Lowack, and G. Decher, "Layer-by-layer assembled protein/polymer hybrid films: nanoconstruction via specific recognition," *Supramol. Sci.* **5**(3-4), 309–315 (1998).
23. A. Rachkov, S. Patskovsky, A. Soldatkin, and M. Meunier, "Surface plasmon resonance detection of oligonucleotide sequences of the rpoB genes of *Mycobacterium tuberculosis*," *Talanta* **85**(4), 2094–2099 (2011).
24. E. Milkani, S. Morais, C. R. Lambert, and W. G. McGimpsey, "Detection of oligonucleotide systematic mismatches with a surface plasmon resonance sensor," *Biosens. Bioelectron.* **25**(5), 1217–1220 (2010).

1. Introduction

Surface plasmon resonance (SPR) has become the technology of choice in numerous biosensing applications providing a tool for the real-time analysis of molecular interactions and rapid and label-free detection of chemical and biological species [1]. Besides the propagating surface plasmon polaritons (SPP) which have dominated the field of SPR biosensing for nearly thirty years, the last decade has witnessed a growing exploitation of the localized surface plasmons (LSP) generated on metallic nanostructures [2]. In spectroscopic LSP-based biosensors, refractive index changes induced by biomolecular interactions are measured by tracking the resonant feature in the spectrum of scattered or transmitted light [3]. In comparison with SPPs, LSPs generate an electromagnetic field which is more closely tied to the surface of the metal and thus allow for even more localized probing of effects at the interfaces, on the scale comparable with the dimensions of individual biomolecules [4]. Various LSP-based sensor platforms have been developed exploiting LSPs generated on metallic nanopyramids [4], nanorods [5, 6], and chains of coupled nanoparticles [7]. These sensors are typically based on directly illuminating the plasmonic nanostructure with a polychromatic light and measuring changes in the extinction/transmission spectrum [8]. Although LSP-based sensors provide an attractive platform for monitoring interactions of low numbers of biomolecules, their bioanalytical applications have been challenged by the limited number of molecular binding events measured by the sensor and the low concentration of analyte molecules in real-world samples. The results of recent studies suggest that LSP-based biosensors are capable of resolving only a few molecules adsorbed to individual nanoparticles and detecting analytes at low concentrations [9, 10]. However, most of the works demonstrate the detection of biomolecules at nanomolar concentrations and the detection of sub-nM concentrations of biomolecules using LSP-based biosensor has not been convincingly demonstrated.

We report on a new LSP biosensor platform based on the excitation of LSPs on an array of gold nanorods by means of a prism coupler and the total internal reflection. A polarization control scheme (polarization contrast) is employed to take advantage of changes in both the amplitude and phase of the quasi-monochromatic light wave reflected from the array of gold nanorods. It is demonstrated that the presented approach allows for a detection performance comparable with that of the best SPR sensors while requiring a significantly lower number of biomolecular interactions to take place.

2. Surface plasmons in polarization contrast

Surface plasmons are charge density oscillations at a metal-dielectric interface associated with a corresponding electromagnetic field. Localized surface plasmons (LSP) refer to those oscillations confined to sub-wavelength metallic nanoparticles while propagating surface plasmon polaritons (SPP) propagate along a metal-dielectric interface. In general, the coupling of a light wave to a surface plasmon results in a change in the amplitude and phase of the light wave. This phenomenon is illustrated in Fig. 1 which shows the wavelength dependence of light intensity and phase of a light wave coupled to a LSP on a periodic array of gold nanorods on a glass substrate (nanorod dimensions: 40 nm $\times$ 110 nm $\times$ 30 nm, array periodicity 250 nm $\times$ 250 nm). The transmittance and phase of the light wave through the nanorod array was calculated using the finite-difference time-domain (FDTD) method, Fig. 1(a). As follows from Fig. 1, a change in the refractive index of the medium surrounding the plasmonic nanostructure causes a shift in the resonant features in the spectrum of both the light intensity and phase. Similar intensity and phase spectra can be obtained for the light wave coupled to a propagating SPP as shown in Fig. 1(c) and (d) respectively which show light reflectivity as a function of wavelength for a propagating SPP generated via prism coupling on a 50nm thick gold layer.

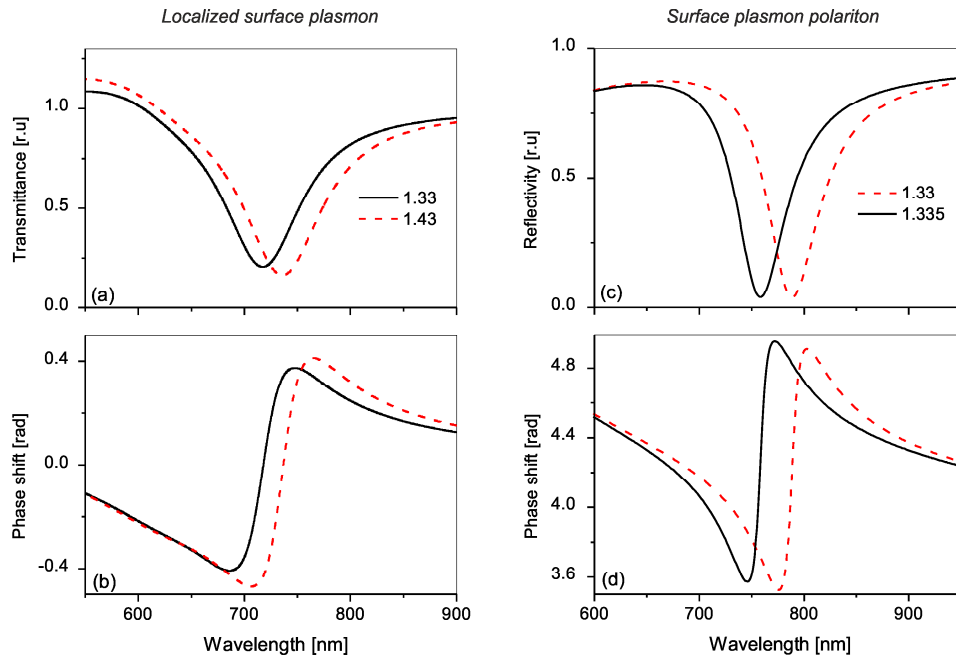


Fig. 1. Wavelength-dependent intensity and phase of a light wave coupled to a LSP on a nanorod array ((a) and (b)) and a propagating SPP ((c) and (d)) calculated for two different refractive indices of adjacent dielectric. a) Transmittance through a nanorod array (ratio of light intensities polarized parallel and transverse to the nanorod axis) and (b) phase-shift (between parallel and transverse polarizations) for a nanorod array. (c) Reflectivity (TM/TE ratio) and (d) phase-shift (TM-TE) for light coupled to a SPP on a 50 nm thick gold film via a prism coupler.

As follows from the comparison of Fig. 1(a) and 1(b) with Fig. 1(c) and 1(d), the sensitivity to the bulk refractive index change is approximately 30 times higher for the propagating SPP than for the LSP. This property is associated with a higher LSP field localization at the metallic nanoparticle [11] and consequently a lower penetration depth of the evanescent field into the dielectric medium. For the considered structure, the penetration

depth of the propagating SPP and LSP was 300 nm and ~9 nm (this is an average figure as the exact field profile of LSP varies along the surface of the nanorod), respectively.

The total internal reflection imaging sensor with polarization contrast takes advantage of both the light intensity changes and phase shift in similar fashion to a previously reported configuration employing a propagating SPP (referred to as SPR imaging) [12]. In this configuration a collimated narrowband light beam passes a prism coupler and is made incident onto a nanorod array attached to the base of the prism (Fig. 2). Upon the incidence on the base of the prism, the light undergoes total reflection and excites LSPs on nanorods via the evanescent field. The amplitude and phase of the reflected light depend on the parameters of the optical system, the parameters of the nanorod array, the polarization of the incident light and the refractive index in the vicinity of the nanorods. When the incident light is linearly polarized and contains both TE and TM polarizations, the light reflected from the array on the nanorods is generally elliptically polarized. The parameters of the polarization ellipse vary with the refractive index in the vicinity of the nanorods and therefore the refractive index changes can be determined by measuring the polarization of the reflected light. By adjusting the phase difference between the TE and TM component (by a waveplate) and selecting an appropriate linearly polarized component (by a polarizer), the sensitivity of the intensity of the light to the refractive index in the vicinity of the nanorods can be maximized. The polarization contrast has been previously used in sensors based on propagating surface plasmons and has been demonstrated to improve the sensitivity and signal to noise ratio by an order of magnitude [13].

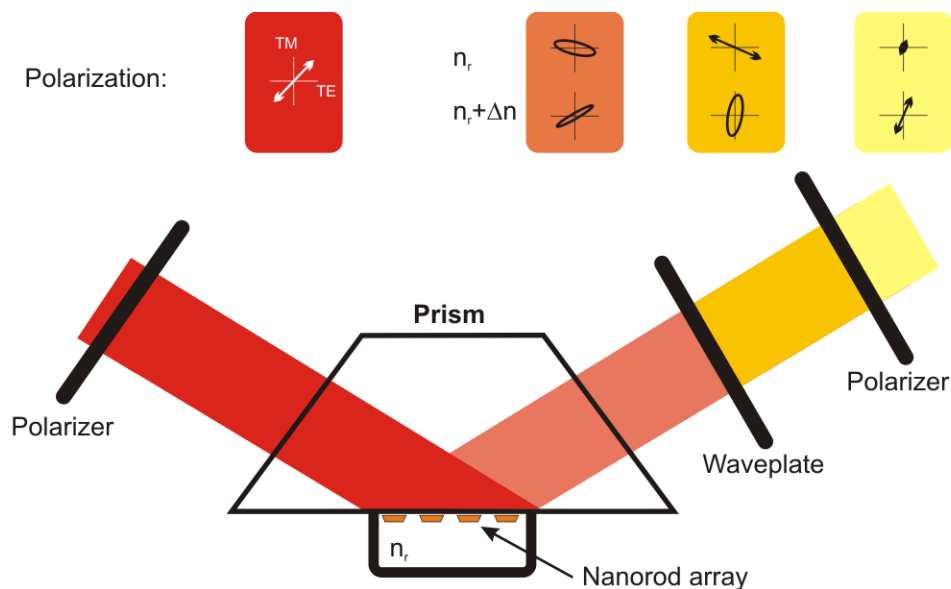


Fig. 2. The concept of an optical sensor based on the excitation of LSPs on an array of gold nanorods by the total internal reflection and polarization contrast (bottom) and the state of polarization of light in different sections of the optical path for two different values of the refractive index in the vicinity of the gold nanorods (top).

The theoretical model of the sensor was developed to predict the response of the sensor and to allow for its performance optimization. The intensity of light transmitted in the polarization contrast was calculated using the previously determined amplitude and phase using the Jones calculus (Fig. 3(a)). As for nanoparticles much smaller than the wavelength, the coupling to LSP has only a weak dependence on the angle of incidence [14], the theoretical model of LSP coupling with a normally incident light wave can be considered to also model the LSP coupling via the evanescent wave in the attenuated total reflection

geometry. The plot in Fig. 3(a) corresponds to the input polarizer set to 53 deg, the waveplate set to -48 deg, and the output polarizer at -39 degrees with respect to the plane of incidence. The simulations suggest a nearly linear dependence of intensity on the change in bulk refractive index and a sensitivity of 700 per cent per RIU (relative intensity change normalized to a refractive index unit). Using the theory described in Ref [15], we compared the performance of the proposed approach and the approach based on spectroscopy of LSPs on the same array of metallic nanorods. Theoretical analysis revealed that the approach based on the total internal reflection and polarization contrast allows for measuring changes in the refractive index at the surface of the nanorods with a resolution twice as good as that provided by spectroscopy of LSPs (the simulations were performed assuming averaging over the area of 400 detector pixels and 100 acquired images, and a shot noise equal to 0.6% of the measured intensity).

The arrays of gold nanorods were fabricated by electron beam lithography using positive resist poly(methyl methacrylate) (PMMA) on glass substrates covered with a 10 nm-layer of indium-tin-oxide (ITO). By incorporating a transparent and conductive layer of ITO, charging of the substrate during e-beam exposure was suppressed. Following exposure, the PMMA layer was developed and the substrates were coated with a gold layer by means of thermal evaporation. A 0.5 nm thick chromium layer was used to promote adhesion of the gold to the substrate. The preparation of the nanorod arrays was completed by lift-off in acetone. The produced nanorod arrays were characterized using scanning electron microscopy (SEM). The inset in Fig. 3(b) shows a part of a resulting nanorod array. The thickness of the gold layer was determined to be 30 nm using a stylus profilometer (Alphastep, Tencor Instruments). The dimensions of the nanorod were measured from SEM image to be 40 nm x 110 nm. The substrate comprising two identical nanorod arrays for sensing and reference channels was interfaced with the prism in such a way that the longer axis of nanorods is parallel to the plane of incidence. In the experiments, the incident light was linearly polarized approximately at 45 deg with respect to the plane of incidence. The waveplate and analyzer were adjusted to maximize the sensitivity of the sensor to changes in the refractive index of the medium adjacent to the nanorods. In order to achieve high performance in which the sensor operates in the shot-noise limited regime, two gold mirrors were prepared on the prism surface. The light blocked and reflected by these mirrors provides dark and bright reference signals for real-time compensation of fluctuations in the intensity of stray light and incident light, respectively. Images acquired from the CCD camera were averaged (100 frames per record) and intensities from pixels within each measurement area were binned. The area of the sensor surface imaged on the CCD camera was 6.4 mm x 9 mm. An acrylic flow cell with gaskets made of vinyl adhesive foil of a total thickness of 50 μ m was pressed against the sensor chip to contain the liquid sample during the experiment. A broadband halogen light source and a spectrometer were used to assist in identifying the optimum polarization contrast. Once the polarization contrast was optimized, a low-coherence narrowband light source (superluminescent diode, central wavelength 780 nm, spectral width 10 nm) and a CCD camera were mounted to image both sensing and reference channels.

As follows from Fig. 3(b), the experimentally obtained spectrum agrees closely with the theoretical model of the LSP in polarization contrast. However, the experimentally obtained spectrum contains a peak corresponding to a polariton at a wavelength different from the LSP resonance. The polariton originates from a dipolar interaction of metallic nanoparticles provided by the grazing diffraction mode of the nanostructure when the light fields corresponding to the -1st order change from evanescent to radiative [16].

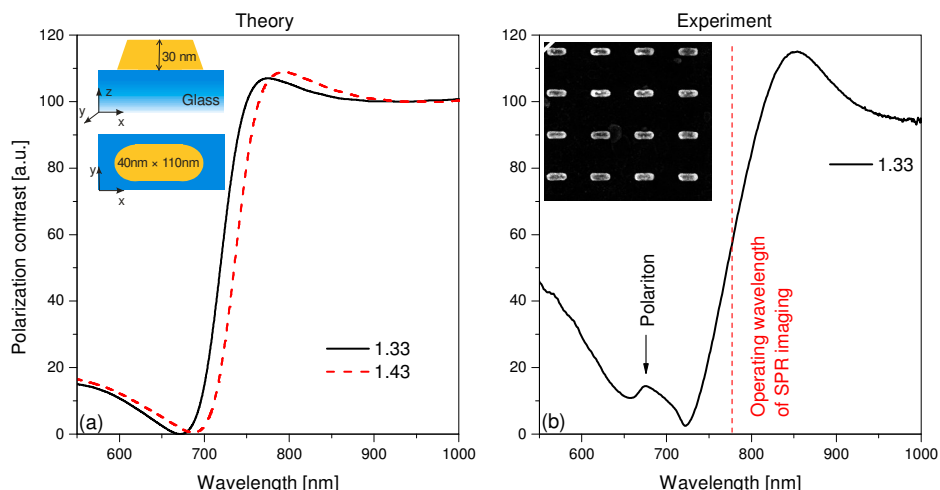


Fig. 3. Wavelength spectrum of the light intensity in polarization contrast configuration. (a) Calculated spectra based on the FDTD model and (b) measured spectra of the fabricated nanorod array.

3. Characterization of the LSP-based biosensor

In general, the sensitivity of LSP-based biosensors depends strongly on the distance from the surface at which the interaction between the biorecognition element and the analyte molecule takes place. In order to account for this feature, the sensitivity of the biosensor at different distances from the surface was characterized by measuring the sensor response to the formation of a multilayer of bovine serum albumin (BSA). Prior to the experiment, the substrate comprised of nanorod arrays was cleaned with UV/O₃ and mounted to the sensor system. Citrate buffer (10 mM sodium citrate, 1 mM sodium hydroxide, pH 4 at 25°C) was flowed across the sensor surface. After 5 minutes in the buffer, the BSA solution (500 µg/mL) was injected in the flow-cell. The solution was flowed through the flow-cell until the sensor response leveled off which corresponds to the surface coated with a monolayer of BSA (after approximately 10 minutes). Then, the sensing surface was washed with CB and solution of dextrane sulfate (DS) (1 mg/ml) was flowed over the BSA monolayer for 10 minutes. Negatively charged DS molecules adsorb electrostatically to the positively charged BSA monolayer creating a foundation layer for adsorption of another layer of BSA. In our experiments, a total of 9 BSA monolayers were formed on the sensor surface by using this approach (for the sensorgram, see Fig. 4(a)).

Considering the thickness of one BSA monolayer to be approximately 5 nm [17], the dependence of the sensor sensitivity on the thickness of the biomolecular layer thickness was reconstructed. The first layer was excluded from this analysis as BSA monolayers adsorbed directly on gold exhibit different properties than those adsorbed on the previously adsorbed BSA/DS molecules. Figure 4 depicts the dependence of the LSP sensor response to one BSA monolayer as a function of the distance from the sensor surface at which the adsorption took place. This dependence exhibits an exponential behavior which can be split into two contributions: (i) the contribution to the sensitivity from the LSP which is dominant in close vicinity to the surface and (ii) a weak contribution originating from the residual polariton which prevails at greater distances from the sensor surface (> 40 nm). Due to the setting of the polarization contrast these two effects influence the sensor response in opposite directions and at a distance of around 30 nm from the sensor surface cancel each other (resulting in zero sensitivity to the formation of the BSA monolayer). Therefore in order to operate the sensor within the LSP sensing mode, the biomolecular interactions under investigation should take place within 30 nm from the surface of the nanorods.

In order to quantify the surface concentration of adsorbed biomolecules and calibrate the biosensor sensitivity, the experiment was repeated using a laboratory sensor based on propagating SPPs (PLASMON IV) developed at the Institute of Photonics and Electronics, Prague, Czech Republic [18]. By comparing results from the two sensing platforms, a surface concentration of $3 \text{ ng} \times \text{mm}^2$ was found to correspond to each monolayer of BSA. Assuming that the adsorption of BSA is driven by its interaction with the BSA/DS layer regardless of the surface geometry on a large scale, a monolayer with the same surface density can be achieved on the nanorod array. This implies that for the nanorod array one monolayer contains about 100 BSA molecules per nanorod. This surface density corresponds approximately to a geometrical consideration of a densely packed monolayer of ellipsoidal molecules with the dimensions of $9 \text{ nm} \times 5 \text{ nm} \times 5 \text{ nm}$.

The standard deviation of the baseline noise obtained during monitoring of the formation of the BSA multilayer was 1.4×10^{-4} (arbitrary units used in the plot) yielding an RMS-based resolution of surface coverage of $35 \text{ fg} \times \text{mm}^{-2}$ (relative to the area including both the Au and ITO surfaces). This minimum detectable surface coverage corresponds to about 0.03 BSA per nanorod, or one BSA molecule per 35 nanorods. Typical sensors based on spectroscopy of LSPs measure changes in the position of the peak in the extinction/transmission spectrum with an accuracy in the order of 0.01-0.1 nm [19, 20]. The accuracy with which the intensity of light is measured in the presented sensor corresponds, in terms of the response of the spectroscopic LSP sensor, to a change in the resonant wavelength in the order of 10^{-4} nm . A similar level of performance has recently been demonstrated by Chen et. al [9] who also achieved the minimum detectable surface coverage of $40 \text{ fg} \times \text{mm}^{-2}$.

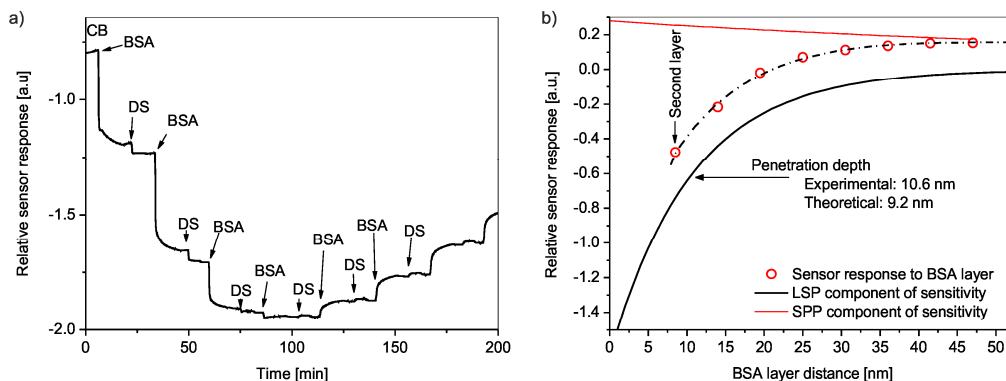


Fig. 4. Calibration of the LSP-based sensor using a BSA multilayer. (a) Temporal sensor response to the formation of the BSA/DS multilayer. (b) Sensor sensitivity as a function of the distance from the surface of the nanorod array (red circles) and the two contributions associated with LSP (black line) and the polariton (red line).

4. Detection of oligonucleotides

To assess the detection capabilities of the LSP-based imaging biosensor a model biodetection experiment was carried out in which short oligonucleotides were detected via complementary oligonucleotides immobilized on the sensor surface. The nanorod arrays were first functionalized with a self-assembled monolayer of ω -carboxyalkylthiols on which streptavidin was attached via the amide bond. Subsequently, biotinylated oligonucleotides were attached to the streptavidin via the streptavidin-biotin link. The immobilization procedure is described below. First, the substrates were immersed overnight in 1 mM solution of (1-mercapto-11-undecyl)hexa(ethylene glycol) carboxylic acid (HSC11-EG6-OCH₂-COOH) and dissolved in absolute ethanol to form a self-assembled monolayer of carboxyl terminated alkanethiols. Then the substrates were mounted into the SPR sensor and the

carboxylic terminal groups of the alkanethiols were activated with a mixture of 0.5M 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and 0.1M N-hydroxysuccinimide (NHS) from GE Healthcare, USA. Subsequently, a 50 μ g/mL solution of streptavidin (Sigma-Aldrich, USA) in 10mM sodium acetate buffer (pH 5.0) was introduced to the flow-cell for 10 min. The non-covalently bound streptavidin was removed with high ionic strength buffer saline. Finally a 100 nM solution of biotinylated DNA probe (Biotin-(TEG)₂—5'-TAT TAA CTT TAC TCC CTT CC-3') in tris buffer with 500mM NaCl was flowed along the sensor surface for 15 min. The sensing channels functionalized only with the self-assembled monolayer of alkanethiols were used as reference.

Freshly functionalized nanostructures were immediately used for experiments. The sensor was exposed to DNA (5'-GGA AGG GAG TAA AGT TAA TA-3') molecules complementary to the probes immobilized in the sensing channel contained in tris buffer with 500mM NaCl. In the detection experiment, the following concentrations of DNA targets were used: 500 pM, 1 nM, 5 nM, 10 nM, and 100nM. The sensor response obtained in the reference channel was subtracted from the one obtained in the sensing channel and recalibrated to the surface coverage using the calibration described in the previous section. For the sensitivity calibration the distance of the hybridization reaction from the nanoparticle surface was estimated based on the thickness of the self-assembled monolayer of alkanethiols of 3.5 nm [21], streptavidin size of 5 nm [22] and the length of the DNA probe of 6 nm. Therefore the DNA interaction was assumed to take place at a distance between 8 nm and 14 nm from the nanoparticle surface (this corresponds to the third BSA monolayer used for the calibration experiment).

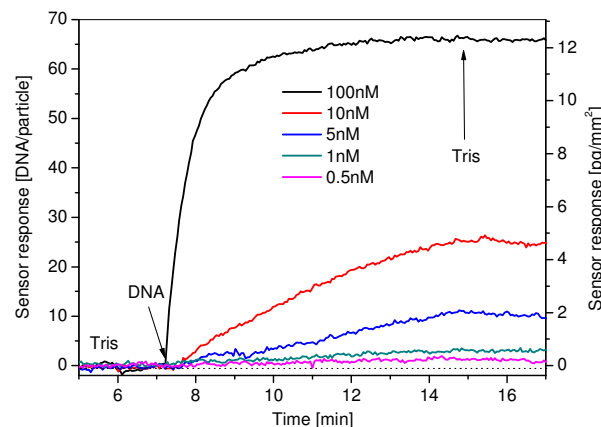


Fig. 5. Temporal response of the LSP-based sensor to five different concentrations of target oligonucleotides.

Figure 5 depicts the temporal sensor response to the hybridization of DNA molecules at the surface of the nanorod array. Clearly, the injection of the DNA sample in the flow-cell results in a strong sensor response, indicating that DNA hybridization takes place. The increase in the sensor response saturates for the highest DNA concentration at the surface coverage of 65 DNA/particle. The lowest concentration of DNA (500 pM) which corresponds to a surface coverage of only 1 DNA molecule per particle results in a sensor response above the baseline noise level. The standard deviation of baseline noise corresponded to only about 0.2 DNA/particle (35 fg $\times$ mm⁻²). A calibration curve was created using the initial binding rate (determined from the initial linear portion of the binding curve) as a sensor output, Fig. 6. The binding rates obtained in the reported experiments are directly proportional to the

concentration which is in agreement with previous studies of DNA hybridization using SPR biosensor technology [18].

The limit of detection (LOD) (defined as the sample concentration which corresponds to the sensor response equal to three standard deviations of the sensor response to a blank sample) was determined to be approximately 200 pM (it should be noted, that this LOD applies to the detection of target DNA in buffer and would be higher in complex samples due to the non-specific interaction between the sensor and complex sample matrix [17]). This LOD is comparable with the LODs obtained using the high-performance SPR biosensors which have detected DNA at nM [23] or 100 pM levels [18, 24]. This result clearly indicates that although the LSP-based biosensor can detect numbers of molecules by orders of magnitude lower than their propagating SPP-based counterparts, the resulting analytical performance of both these approaches is approximately the same. This outcome is mostly due to the kinetics of interacting molecules as the probability of the biomolecular interaction is directly proportional to the number of the interacting molecules.

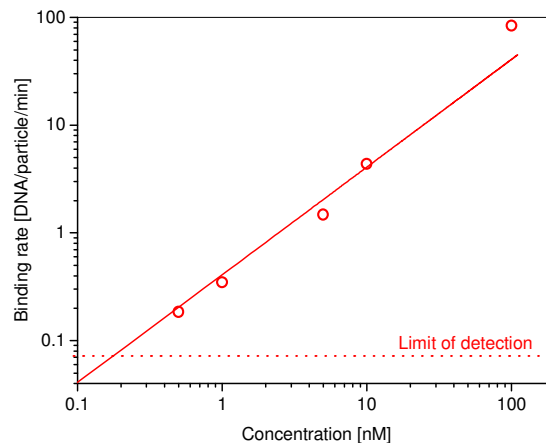


Fig. 6. Calibration curves obtained for the detection of short oligonucleotides. The solid line corresponds to a linear fit through zero.

5. Conclusion

A new approach to the development of high-performance biosensors based on localized surface plasmons (LSPs) is reported. The presented approach is based on the imaging of surface plasmons in polarization contrast and takes advantage of the change in the polarization of light coupled to localized surface plasmons on a gold nanorod array. It is shown that the LSP-based biosensor delivers the same high performance as state-of-the-art SPR biosensors while involving a two orders of magnitude lower number of molecular interactions. We demonstrate that the sensor is capable of detecting only one short DNA molecule per nanoparticle on average and measuring concentrations of short oligonucleotides down to 200 pM. Moreover, the implementation of the LSP-based biosensor is implicitly multichannel and potentially offers high throughput.

Acknowledgments

This research was supported by the Academy of Sciences of the Czech Republic under the contract KAN200670701 and Praemium Academiae, by the Ministry of Education, Youth and Sports under contract OC09058, by COST Action MP0803, and by the European Science Foundation (ESF) under the activity PLASMON-BIONANOSENSE.