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Superior Sensitivity of Copper-Based Plasmonic Biosensors

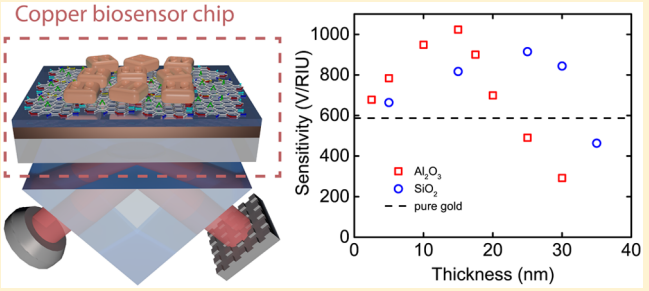
Yury V. Stebunov,*[,†,‡] Dmitry I. Yakubovsky,[†] Dmitry Yu. Fedyanin,[†] Aleksey V. Arsenin,[†,‡] and Valentyn S. Volkov[†,§]

[†]Laboratory of Nanooptics and Plasmonics, Moscow Institute of Physics and Technology, 9 Institutsky Lane, Dolgoprudny 141700, Russia

[‡]GrapheneTek, 7 Nobel Street, Skolkovo Innovation Center, Moscow 143026, Russia

[§]SDU Nano Optics, Mads Clausen Institute, University of Southern Denmark, Campusvej 55, DK-5230, Odense, Denmark

ABSTRACT: Plasmonic biosensing has been demonstrated to be a powerful technique for quantitative determination of molecular analytes and kinetic analysis of biochemical reactions. However, interfaces of most plasmonic biosensors are made of noble metals, such as gold and silver, which are not compatible with industrial production technologies. This greatly limits biosensing applications beyond biochemical and pharmaceutical research. Here, we propose and investigate copper-based biosensor chips fully fabricated with a standard complementary metal–oxide–semiconductor (CMOS) process. The protection of thin copper films from oxidation is achieved with SiO_2 and Al_2O_3 dielectric films deposited onto the metal surface. In addition, the deposition of dielectric films with thicknesses of only several tens of nanometers significantly improves the biosensing sensitivity, owing to better localization of electromagnetic field above the biosensing surface. According to surface plasmon resonance (SPR) measurements, the copper biosensor chips coated with thin films of SiO_2 (25 nm) and Al_2O_3 (15 nm) show 55% and 75% higher sensitivity to refractive index changes, respectively, in comparison to pure gold sensor chips. To test biomolecule immobilization, the copper–dielectric biosensor chips are coated with graphene oxide linking layers and used for the selective analysis of oligonucleotide hybridization. The proposed plasmonic biosensors make SPR technology more affordable for various applications and provide the basis for compact biosensors integrated with modern electronic devices.



Plasmonic biosensors have found numerous applications in the areas of scientific and pharmaceutical research, medical diagnostics, veterinary practice, and food and safety controls.[1−3] SPR biosensing based on the Kretschmann configuration[4,5] has been achieved in many commercial instruments, providing researchers with an indispensable tool for the kinetic analysis of biochemical reactions.[6] In the past years, various compact plasmonic biosensors, which exploits plasmonic effects in systems comprising metal nanoparticles, nanostructured metal films, waveguides, interferometers, optical fibers, and photonic crystals, have also been demonstrated.[7−14] However, the further spread of this approach into personal diagnostics and other areas of in situ biosensing analysis is limited because of the costs and complexity of modern analytical devices. The main obstacle behind the mass production of plasmonic biosensors is the obligate and specific nature of materials for plasmonic structures, which should simultaneously possess plasmonic properties and be compatible with existing manufacturing technologies. Currently, electronic and photonic integrated circuits are mass-produced using low-cost complementary metal–oxide–semiconductor (CMOS) processes, which allow one to fabricate planar multilayered structures and form nanostructured designs. The implementation of these technologies will open up the possibilities for the integration of biosensing components into consumer electronic devises like smartphones and wearables.

Metals with plasmonic properties in the visible and near-infrared ranges include gold, silver, copper, and aluminum as well as various metal alloys.[15−29] The most common material used for plasmonic biosensors is gold, which demonstrates excellent optical properties, resistance to oxidation, and ease in nanopatterning. In addition, the optical properties of gold have been widely investigated and considered regarding the influence of multiple factors such as deposition conditions, the annealing of metal films, the presence of an adhesion layer, grain sizes, and film thicknesses.[15−18] The drawbacks of gold in plasmonics include its high price and incompatibility with microelectronic technological processes. Silver-based plasmonic devices show superior performance due to low optical losses.[16,19,20] However, silver components need to be protected from oxidation when used in biosensors for the detection of biological and chemical agents. An SPR biosensor based on silver films covered with protecting dielectric films demonstrates both the stability of the conducted analyses and an increase in sensitivity due to the

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influence of the dielectric films on the SPR properties.[21−23] Aluminum is one more prospective material for plasmonic biosensors and is compatible with CMOS processes and suitable for devices operating in the UV range.[24−26] Unfortunately, high optical losses in most of the visible range and in IR limit its biosensing applications. In addition, other plasmonic materials have been proposed for infrared applications, such as highly doped semiconductors, transparent conducting oxides, metal nitrides, and 2D materials.[30−33] Because of their compatibility with CMOS processes, some of them could form the basis of compact plasmonic biosensors. Moreover, the ability to tune their optical and structural properties offers many practical advantages for biosensing applications.[33]

In this work, we chose copper as a plasmonic material for designing biosensing interfaces. Besides its excellent optical properties, copper is the most common metal used in CMOS processes. Compared with gold, copper is inexpensive and has lower optical losses in the visible and NIR ranges, which were investigated by ellipsometry and SPR measurements as well as through the analysis of surface plasmon polaritons' propagation along the surface of the copper films.[16,20,34,35] The advantages of copper in plasmonic applications were exploited in ultralow-loss CMOS copper plasmonic waveguides, which could be used for the manipulation of subwavelength optical fields and signal processing.[36] The biosensing applications of copper plasmonics have also been reported; for example, highly sensitive plasmonic biosensors can exploit the localized SPR in copper nanoparticles and the SPR in copper films deposited on a photonic crystal fiber.[37,38] One of the main obstacles for copper-based plasmonic biosensing is the rapid oxidation of the metal. A possible way to overcome this issue is to protect the underlying metal surface with a barrier coating of graphene, which at the same time produces minimal impact on the optical quality of the interface between the sensor surface and the chemical or biological systems to be studied.[20]

Here, we propose SPR sensor chips based on plasmonic copper films covered with different dielectric layers (Figure 1). Thin copper films were deposited on the surfaces of glass substrates by electron beam evaporation, which is an essential part of standard CMOS processing. The additional deposition of dielectric films in the same fabrication cycle prevents the oxidation of the copper and additionally increases its biosensing sensitivity. The performance of the proposed sensor chips was investigated both theoretically and experimentally, with the aim of optimizing the multilayered sensor chip configuration. The analysis of the angular spectra of the light reflectance from the proposed sensor chips provides quantitative data on both the SPR characteristics and the dependence of the sensitivity on the material properties of the protecting films and their thicknesses. Experimentally, the sensitivity of the proposed sensor chips was derived from the SPR signal response upon the injections of different salt solutions with predetermined refractive indices. In addition, the performance of the dielectric-coated sensor chips was validated in the biosensing analysis of oligonucleotide hybridization. For this analysis, the sensor chips were coated with graphene oxide (GO) linking layers and thereafter immobilized with neutravidin protein, which is selective toward biotinylated ligands. The GO substrates provide improved biosensing sensitivity compared to conventional hydrogel-based linking layers.[39] In addition, they can be deposited on various dielectric and metal surfaces using the same procedure.

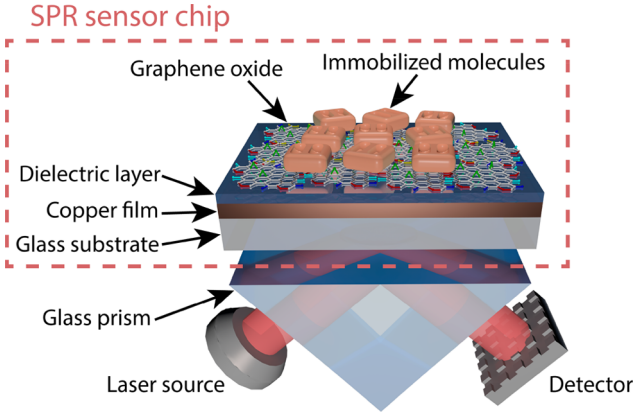

Figure 1. Schematic representation of the SPR biosensor comprising the SPR sensor chip based on plasmonic copper films coated with a dielectric layer to protect against oxidation. The prism and sensor chip substrates are made of the same type of glass, which allows for an efficient optical connection. The immobilization of biomolecules on the biosensor surface can be achieved using a graphene oxide linking layer deposited atop the dielectric layer.

EXPERIMENTAL SECTION

Materials. Unless otherwise stated, all chemicals were purchased from Sigma-Aldrich (Carlsbad, CA). Materials for evaporation, including copper, titanium, silicon dioxide, and aluminum oxide, were purchased from the Kurt J. Lesker Company (Hastings, UK). The glass substrates used were D 263 T Eco-Friendly Thin Glass (SCHOTT AG, Mainz, Germany) and had the dimensions of 14 × 12 × 0.4 mm. An aqueous solution of GO with a concentration of 500 μg/mL was purchased from Graphene Laboratories, Inc. (Calverton, NY), and synthesized by the Hummers method.[40] The following oligonucleotides were used: (1) biotinylated 56 bp single-stranded DNA sequence (D1) (5′-/5Biosg/TCT CTC TGA GTG GCC AAA ATT TCA TCT CTG AAT TCA GGG ATG ATG ATA ACA AAT GC-3′) and (2) the 50 bp single-stranded DNA sequence (D2) (5′-GCA TTT GTT ATC ATC ATC CCT GAA TTC AGA GAT GAA ATT TTG GCC ACT CA-3′). The oligonucleotides were synthesized by Integrated DNA Technologies (Coralville, IA). Neutravidin was purchased from Thermo Fisher Scientific (Waltham, MA). All solutions were prepared in ultrapure water (18.3 Mohm cm).

Fabrication of Copper SPR Chips. SPR sensor chips were fabricated using a NEE-4000 E-Beam Evaporating system produced by NANO-MASTER, Inc. (Austin, TX). Clean SCHOTT's glass substrates were placed in the vacuum chamber of the e-beam evaporator at a pressure of 3×10^{-6} Torr. After that the following films were deposited in a single process: (1) a 1.5 nm thick Ti layer to ensure the adhesion of the copper to the substrate, (2) a 27 nm thick copper film, and (3) SiO_2 or Al_2O_3 protecting layers of different thicknesses (2.5−35 nm). The deposition rate was approximately 2 A/s. Additionally, the thicknesses of the deposited films were confirmed by AFM measurements using an AFM Ntegra Aura produced by NT-MDT (Moscow, Russia). Finally, 5 nm thick graphene oxide linking layers were spray-coated onto the surfaces of the copper SPR chips to perform a biosensing assay.[39]

Ellipsometric Measurements. Ellipsometric measurements were conducted using the VASE Ellipsometer produced by J.A. Woollam Co. (Lincoln, NE). The spectral range was 300−1500 nm, and the angles of incidence of the light beam were 60°, 65°, 70°, and 75°. For the ellipsometric measurements, copper and gold films with thicknesses of 26 and 25 nm, respectively, were deposited using e-beam evaporation on the surfaces of silicon wafers, both capped with a top layer of 2 nm thick SiO_2. The thickness of the metal films was measured by AFM, which allowed for the direct determination of the complex permittivity of the single-layer materials from ellipsometry data. Because of the partial oxidation of the copper, the multilayered

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structure with the copper film (used for ellipsometry analysis) consisted of the layers of copper and copper(II) oxide, with thicknesses of 25.5 and 0.5 nm, respectively. The permittivities of copper and gold in the near-infrared (800−1500 nm) region were approximated by the Drude model:[18]

$$\varepsilon = \varepsilon_\infty - \frac{{\omega_p}^2}{\omega^2 + i\Gamma\omega} \quad (1)$$

where the dielectric function at an infinite frequency ε_∞, the plasma frequency ω_p, and the scattering rate Γ are fitting parameters.

SPR Sensitivity Analysis. For the theoretical and experimental analysis of copper biosensor chips, we reason that the SPR is excited in metal films by means of Kretschman's geometry, which is used in most of the commercial SPR instruments.[4] In resonance, the reflection of p-polarized monochromatic radiation is at its minimum, which results from the direct coupling into the surface plasmon polariton modes that travel along a metal−dielectric interface. Therefore, the parameter characterizing the biosensing performance is the sensitivity to refractive index (RI) changes:

$$S_{RI} = \frac{\Delta P}{\Delta n} \quad (2)$$

where ΔP is the change in biosensor signal induced by the change of RI near the biosensor chip Δn.[41]

Theoretical descriptions of SPR excitation are based on the transfer matrix method (TMM), which uses Fresnel's coefficients to describe the light reflection from the multilayered structure.[42] Using TMM, we investigated the SPR excitation in the copper biosensor chips at 635 nm wavelength (λ = 635 nm), which was also used in the SPR experiments. The multilayered structure with the following layers was used for the simulations: (1) semi-infinite glass substrate with an RI n_0 = 1.521, (2) Ti layer with a thickness t_1 = 1.5 nm and RI n_1 = 2.709 + i3.771, (3) Cu layer with a thickness t_2 = 50 nm and n_2 = 0.0549 + i4.406, (4) different protecting layers of varying thickness made of silicon (n_{Si} = 3.879 + i0.0192), silicon carbide (n_{SiC} = 2.634), zinc sulfide (n_{ZnS} = 2.35), silicon nitride (n_{SiN} = 2.01), aluminum oxide ($n_{Al_2O_3}$ = 1.766), or silicon oxide (n_{SiO_2} = 1.457), and (5) semi-infinite aqueous solution with RI n_4 = 1.33. The minimum angular reflectance from the above-described structures corresponds to the SPR excitation. The change of the RI of the top aqueous solution layer by n leads to the shift of the SPR angle, which was defined as the change of biosensor signal Pin equation (2), which was the calculation of the sensitivity SRI for the particulate biosensor chip. In the calculation, n was taken as 0.005.

The experimental investigation of SPR sensitivity to RI changes was performed using a commercial SPR instrument, the Accolade 404SA produced by the company BiOptix (Boulder, CO). According to the manufacturer, this instrument can detect RI changes of less than 0.5 × 10^{-6}. During the procedure, the SPR chips (fabricated by e-beam evaporation as described above) were inserted into the instrument and consequently rinsed by two solutions: 0.1 M phosphate-buffered saline (PBS) with a pH of 7.4 and 0.5% NaCl solution in the same PBS buffer. The difference between the RIs of these solutions is approximately Δn = 0.88 × 10^{-3}, which resulted in a difference in the absolute SPR signals (measured in volts).[43] Obtained sensorgrams were corrected for linear drift caused by interactions between the sensor chips and the salt solutions.

SPR Biosensing Assay. SPR biosensing measurements were conducted using copper-based biosensor chips comprising a 27 nm thick copper film coated with a 15 nm thick Al_2O_3 protecting layer, which were fabricated according to the above-described procedure. For biomolecule immobilization, a GO linking layer with a thickness of 5 nm was deposited on the surface of a copper SPR chip by spraying it with 1 mL of the GO aqueous solution with a concentration of 25 μg/mL. Afterward, neutravidin protein was immobilized on the surface of the GO layer directly in the flow cell of the SPR instrument using amine coupling.[6] For neutravidin immobilization, the carboxyl groups of the GO sensor chip were activated with a mixture of 0.4 M 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC) and 0.1 M *N*-Hydroxysuccinimide (NHS) solutions in a 4-morpholine-ethanesulfonic acid (MES) buffer with a pH of 6.0 for 7.5 min. Then, a 100 μg/mL neutravidin solution in a 50 mM of sodium acetate buffer with a pH of 4.5 was injected for 30 min. Then, the sensor chip surface was deactivated by exposure to a 1 M tris(hydroxymethyl)-aminomethane hydrochloride (Tris-HCl) solution for 5 min. This procedure created a sensor surface selective toward different biotinylated molecules. To evaluate the selectivity, we analyzed the adsorption of oligonucleotides D_1 and D_2 onto the SPR sensor chip with preimmobilized neutravidin. Oligonucleotides were dissolved in the running PBS buffer (used in all SPR measurements) at a concentration of 50 nM. All SPR responses in the biosensing assay were within the dynamic range (10^5) of the Accolade 404SA.

RESULTS AND DISCUSSION

Optical Properties of Thin Copper Films. The excitation of SPR in the Kretschmann geometry is associated with the optical coupling of laser radiation to a surface electromagnetic wave propagating along the interface between a thin metal film and dielectric medium. The optical properties of the metal film determine the possibility of SPR excitation in a particular system, the resonance characteristics, and the sensitivity of SPR-based biosensing.[44] The permittivity of the copper and gold films used in the proposed SPR biosensor chips was determined by spectroscopic ellipsometry in the wavelength range from 300 to 1500 nm. For this purpose, copper and gold films with thicknesses of 26 and 25 nm, respectively, were deposited on the surfaces of silicon wafers. The thicknesses of the metal films were confirmed by AFM measurements, which were also used to estimate the surface roughness (Figure 2). Electron-beam evaporation allows the deposition of copper films with a root-mean square roughness of 0.5 nm, which is sufficient for SPR applications.

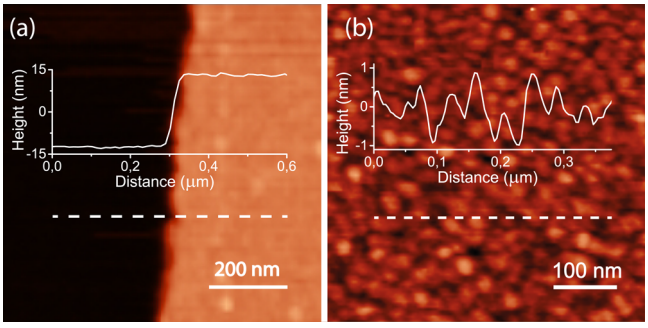

Figure 2. Atomic force microscopy images of (a) the scratch on the surface of a thin copper film deposited by electron beam evaporation and (b) copper crystallites formed during the deposition.

According to the above-described ellipsometric model, the permittivities of the copper and gold films were directly determined from the ellipsometric data (Figure 3). Table 1 shows the parameters of the Drude model (1) for both films. The approximate equality of the plasma frequencies of the copper and gold films results in the same SPR angles. Therefore, SPR sensor chips based on both copper and gold films can be used with the same SPR instruments without any adjustments of their optical configurations. In addition, copper displays a level of optical losses comparable to those of gold, which implies the equality of the biosensing sensitivities of copper- and gold-based sensor chips with the same multilayered configuration. The permittivity of copper was −12.03 + 1.580i at 635 nm, which was the operating wavelength of the laser

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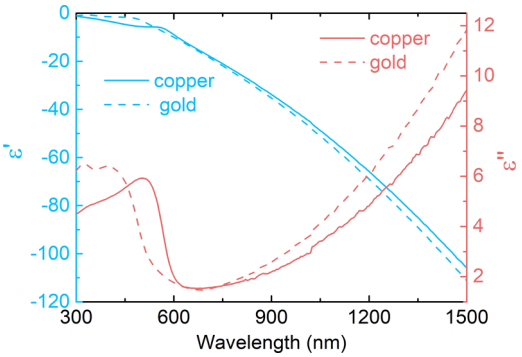

Figure 3. Real (blue lines) and imaginary (red lines) parts of the permittivity of thin copper (solid lines) and gold (dashed lines) films obtained by spectroscopic ellipsometry.

Table 1. Parameters of the Drude Model for Thin Copper and Gold Films Obtained by the Fitting of Their Permittivities

material	ε_∞	ω_p [10^{16}/s]	Γ [10^{13}/s]
Cu	4.68	1.32	10.5
Au	5.08	1.35	12.8

diode used in the SPR instrument. This permittivity value was subsequently used for SRI modeling.

Sensitivity to RI Changes. The sensitivity to the RI changes of copper SPR sensor chips covered with various oxidation-protecting layers was investigated both theoretically and experimentally. The TMM was used for modeling the angular reflection, which gives the angles corresponding to SPR excitation (Figure 4a). The RI change in the upper layers leads to a shift in SPR angular curves, which is the basis of SPR biosensing. Assuming Δn = 0.005, the S_{RI} was calculated for protecting layers composed of Si, SiC, ZnS, Al_2O_3, or SiO_2 thin films with different thicknesses (Figure 4b). For the high-RI protecting layers, the S_{RI} could be improved by nearly 4 times by adjusting the thickness of the protecting layer. The optimal thickness of the protecting layers was determined to be in the range between several nanometers and several tens of nanometers, where a smaller thickness corresponded to the larger RI of the layer. The sensitivity enhancement was a result of a change in the plasmonic field distribution in the way that more electromagnetic energy was concentrated in the dielectric layer above the chip's surface. Further S_{RI} improvement was limited due to the SPR angular shift toward the high angle region, which made it impossible to efficiently excite SPR in structures with thick protecting layers.

Experimentally, S_{RI} was estimated for two types of copper SPR chips covered with thin films of SiO_2 and Al_2O_3 with different thicknesses. SPR measurements were conducted on a commercial SPR instrument, which utilizes a constant excitation wavelength and angle of incidence to detect of phase changes in the reflected beam. S_{RI} was measured by the analysis of the SPR signal response to the injections of salt solutions with a 0.88×10^{-3} difference in RI. The thicknesses of the SiO_2 and Al_2O_3 protecting layers are in the ranges of 5−35 and 2.5−25 nm, respectively (Figure 5).

For all salt injections, the SPR signal demonstrated a stable baseline, which means there was no degradation of the copper plasmonic films. Therefore, the proposed dielectric coatings for the SPR chips provided a sufficient level of oxidation protection for most SPR experiments. However, different thicknesses of

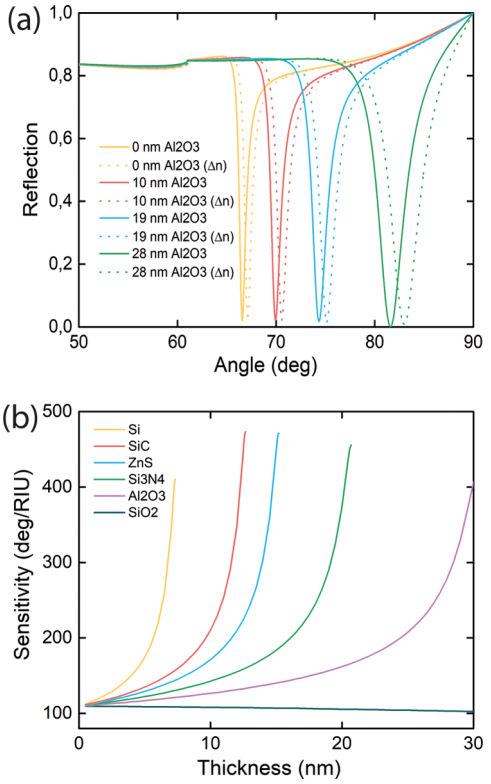

Figure 4. (a) SPR curves of copper sensor chips coated with Al_2O_3 protecting layers of various thicknesses for two sensing media with refractive indices of 1.33 (solid lines) and 1.335 (dashed lines). (b) Sensitivity to refractive index changes of the copper sensor chips coated with various protecting layers depending on their thicknesses.

the protecting layers corresponded to different S_{RI} values, which requires that the final structure of the copper SPR chips will need to be optimized. As shown in Figure 5, the S_{RI} of the SPR chips with thin SiO_2 and Al_2O_3 coatings increased with the thicknesses of these layers, reaching peak values at 25 and 15 nm, respectively. Our results clearly show that the proposed dielectric coatings not only protected the plasmonic films from oxidation but also promoted an overall improvement in the biosensing sensitivity of the SPR chips. Thus, the corresponding peak values of S_{RI} (obtained for Al_2O_3 and SiO_2 coatings) were 75% and 55% higher, respectively, than those of the S_{RI} of the bare gold SPR chip. These results were found to be consistent with the results of the theoretical modeling of the SPR angle curves (see Figure 4).

Graphene Oxide Linking Layers for Measuring Neutravidin−Biotin Binding. The development of a selective interface immobilized with ligands is an important part of the SPR biosensing assay. Traditionally, SPR chips use linking layers based on thiol chemistry due to the ability of sulfur-containing molecules to form strong bonds to metal surfaces.[45] This approach is not applicable to the dielectric-layer-covered copper SPR chips. Instead, each dielectric layer will generally require its own immobilization procedure. However, graphene materials can help overcome this problem. Various SPR interfaces based on graphene and GO have been proposed in recent years. Because of the large surface area of graphene materials and their diverse chemical properties, these interfaces can be applied to the analysis of a wide range of biochemical interactions, providing and even improving the immobilization capacity compared to the thiol-based linking

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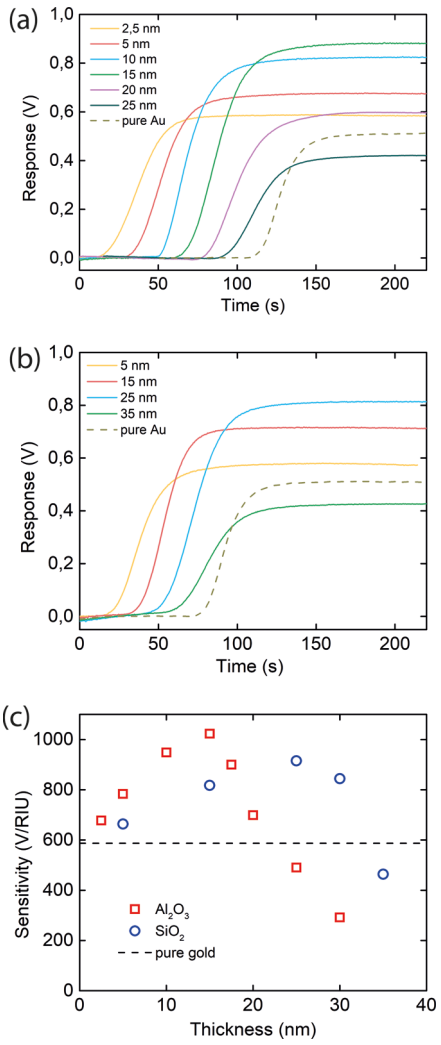

Figure 5. Sensorgrams corresponding to the injections of 0.5% NaCl solution in a running buffer over the copper SPR sensor chips coated with (a) Al_2O_3 and (b) SiO_2 protecting layers of various thicknesses. (c) Sensitivity to refractive index changes of the copper SPR sensor chips coated with Al_2O_3 (red squares) and SiO_2 (blue circles) layers depending on their thicknesses. The dashed line shows the sensitivity level of a bare gold SPR chip.

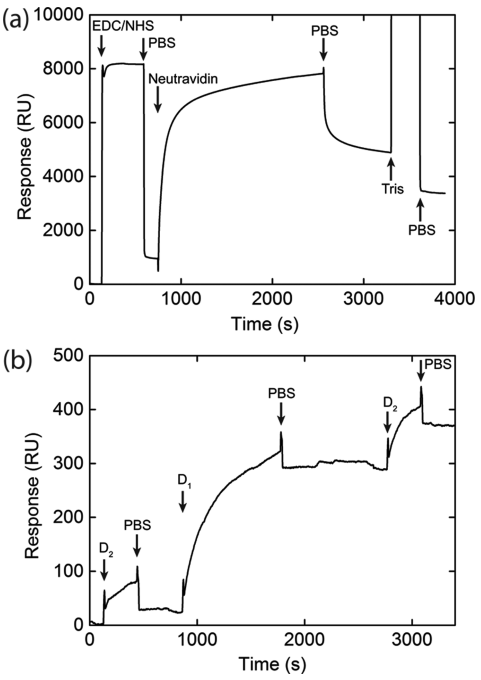

Figure 6. (a) Covalent immobilization of neutravidin on the surface of the graphene oxide (GO) linking layer deposited on the copper surface plasmon resonance (SPR) sensor chip protected by 15 nm thick Al_2O_3 film. Immobilization procedure includes the activation of carboxyl groups of GO by the mixture of 0.4 M 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC) and 0.1 M *N*-Hydroxysuccinimide (NHS) solutions and deactivation of carboxyl groups after neutravidin adsorption by 1 M Tris solution. (b) Adsorption of oligonucleotides D_1 and D_2 on the surface of neutravidin−GO copper SPR chip. D_1 is biotinylated and complementary to non-biotinylated D_2. PBS: phosphate-buffered saline.

layers.[40,46−50] Here, we demonstrated the development of a GO linking layer on the surface of SPR biosensor chips coated with dielectric layers for the first time.

An aqueous solution of GO was spray-coated onto the surface of a copper SPR chip with an Al_2O_3 protecting layer. Thereafter, this SPR chip was used for the SPR analysis of a DNA hybridization reaction. For this analysis, the GO surface was immobilized with a selective layer of neutravidin molecules, which possess four binding sites for biotinylated ligands. The neutravidin immobilization was performed in the flow cell of the SPR instrument using an amine coupling procedure, which includes the activation of carboxyl groups on the surface of the GO and subsequent covalent attachment of the neutravidin to these groups. The SPR signal corresponding to the adsorption of neutravidin was 4000 RU (Figure 6a). For convenience, all SPR signals are given in units of RI, where 1 RU = 10^{-6}.

The next stage of the SPR biosensing assay included the investigation of the selectivity of the developed neutravidin-coated SPR surface as well as the possibility of using this interface to analyze DNA hybridization. For this purpose, three solutions of DNA sequences—D_2, D_1, and D_2—were injected sequentially. D_1 is an oligonucleotide sequence with a biotinylated 5′-end, and D_2 is non-biotinylated and complementary to D_1. The SPR signals corresponding to the three consecutive DNA injections were 25, 270, and 85 RU, respectively (Figure 6B). The amount of D_2 adsorbed via hybridization is more than 3 times higher than the level of nonspecific binding of D_2 to the pure neutravidin surface, which clearly shows the selectivity of the developed SPR biosensor chips. In addition, the successful hybridization of complementary oligonucleotide strands confirms the possibility to use the proposed neutravidin−GO SPR interfaces for the investigation of many other types of biochemical interactions.

CONCLUSIONS

We have proposed SPR biosensor chips based on a copper−dielectric plasmonic interface. Thin copper films support the excitation of surface plasmons, which can efficiently couple with external laser radiation. Moreover, copper biosensor chips can be adapted for any commercial SPR instrument that was developed for gold plasmonic interfaces without any adjustments to the optical configuration. This finding was confirmed both by SPR and ellipsometric measurements, which demonstrate the excitation of SPR in copper- and gold-based optical structures under approximately the same conditions. For SPR biosensing, copper can provide improved biosensing sensitivity compared to gold, owing to lower optical losses. The

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main drawback of copper usage in biosensing is the rapid oxidation of the metal in most biological solutions. However, the deposition of thin dielectric layers on the copper surface can protect biosensor chips from oxidation and, additionally, significantly improve its biosensing sensitivity. According to SPR measurements, the copper SPR biosensor chips coated with 25 nm SiO_2 and 15 nm Al_2O_3 films provided maximum sensitivity to RI changes, which are 55% and 75% higher, respectively, than for gold SPR chips without a dielectric layer on top of them. The subsequent biomolecule adsorption on the surfaces of the proposed SPR chips was achieved using the GO-based linking layers. GO is a promising material for biosensing due to its high surface area, diverse biochemical properties, and cost-effective production, which allows the deposition of GO matrices with high immobilization capacity on any biosensing interface. The selectivity of the SPR biosensing assay was achieved by the immobilization of neutravidin on the surface of the GO. Neutravidin molecules possessing four binding sites for biotin residues were covalently attached to the carboxyl groups of the GO using amine coupling. The SPR corresponding to the neutravidin adsorption was 4000 RU, which provides sufficient sensitivity for the analysis of most biochemical interactions. The performance of the neutravidin−GO surface, which is suitable for the immobilization of various biotinylated ligands, was assessed for the analysis of a DNA hybridization reaction. The SPR signals corresponding to the binding of biotinylated oligonucleotide and oligonucleotide hybridization were 11 and 3 times higher, respectively, than for the nonspecific binding of non-biotinylated oligonucleotide to the pure neutravidin−GO surface. In addition to their use in traditional SPR biosensing devices, copper plasmonic biosensors with GO linking layers can be utilized as integrated components of various analytical devices with applications ranging from medical diagnostics to food and environmental safety controls. Traditional semiconductor-fabrication technologies are suitable for the mass production of the optical transduction part of such biosensors, whereas the GO linking layers can enable the implementation of various qualitative and quantitative biosensing assays.

AUTHOR INFORMATION

Corresponding Author

*E-mail stebunov@phystech.edu; Ph +7-498-744-65-82 (Y.V.S.).

ORCID

Yury V. Stebunov: 0000-0002-9257-9595
Dmitry Yu. Fedyanin: 0000-0001-7264-0726
Aleksey V. Arsenin: 0000-0002-7506-4389

Notes

The authors declare no competing financial interest.

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