Nanoparticle-Enhanced Diffraction Gratings for Ultrasensitive Surface Plasmon Biosensing

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Ultrasensitive surface bioaffinity sensors are created by the adsorption of gold nanoparticles onto gold diffraction gratings. An enhanced diffraction obtained in a surface plasmon resonance geometry is observed due to the optical coupling of the planar surface plasmons in the grating to the localized surface plasmons in the gold nanoparticles. As a first example, these nanoparticle grating biosensors are employed to detect unmodified DNA at a concentration of 10 fM.

Surface bioaffinity sensing using either planar surface plasmon resonance (SPR) sensors based on gold thin films[1–4] or localized surface plasmon resonance (LSPR) sensors based on metallic nanoparticles[5,6] is currently being applied by numerous research groups for the detection of proteins, antibodies, and nucleic acids in both the diagnostic and discovery application areas. A biosensor that combines the advantages of both the SPR and LSPR methodologies should, in principle, achieve even greater control of the sensor's optical properties and thus higher sensitivity and selectivity. A further advantage is also obtained if the operating wavelength of the biosensor can be readily tuned to a spectral region where the plasmon resonance enhancement generates a maximum optical effect. In this paper, we introduce a new approach where the optical properties of planar surface plasmon polaritons generated on micrometer-scale gold gratings are combined with the optical properties of adsorbed gold nanoparticles to create a diffraction biosensor capable of detecting DNA at concentrations as low as 10 fM. We term these novel surface plasmon biosensors "nanoparticle-enhanced diffraction gratings" (NEDG).

A variety of diffraction and interferometric measurements have been employed previously by researchers for bioaffinity sensing.[7–14] For example, Goh et al. have used changes in diffraction efficiency to detect target binding onto biomolecular surface gratings,[13] and Markov et al. have demonstrated the application of on-chip interferometric sensors for protein quantification.[8] Separately, the group of Knoll has fabricated dielectric gratings on continuous planar gold films to create surface plasmon-coupled sensors whose performance is comparable to that of conventional SPR measurements.[7,9–11] Recently, even higher sensitivities for DNA detection, down to a concentration of 1 pM, have been achieved by Bailey et al. via the measurement of gold nanoparticle adsorption without the excitation of planar surface plasmons.[12] In the work presented here, we demonstrate that by using an SPR excitation geometry we can control the wavelength region of the nanoparticle-enhanced diffraction signal. We show for the first time that the combination of wavelength control via planar surface plasmons on gratings coupled to specific localized surface plasmons on nanoparticles leads to ultrasensitive biosensor surfaces that can be used for the detection of DNA, RNA, and proteins.

EXPERIMENTAL SECTION

Materials. 11-Mercaptoundecylamine (MUAM; Dojindo), sulfosuccinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (SSMCC; Pierce), dithiothreitol (DTT; Aldrich), and triethanolamine (TEA; Sigma) were used as received. The target DNA oligonucleotide (D_T) was HPLC purified and used as received from Integrated DNA Technologies (IDT). Both 5′-thiol (D_1) and 3′-thiol (D_2) modified DNA oligonucleotide probes were also purchased from IDT. The DNA sequences used were as follows: $D_1 = 5'$-S-S-$(CH_2)_6$-$(T)_{15}$ GTG TTA GCC TCA AGT G-3′, $D_2 = 5'$-GTC TAT GCG TGA ACT G$(CH_2)_6$-$(T)_{15}$-S-S-3′, and $D_T = 5'$-CAG TTC ACG CAT AGA CCA CTT GAG GCT AAC AC-3′. A control DNA sequence with 16-base mismatch compared to D_T was 5′-AGA CTC TGA CTC AGT GCA CTA GTC GCA ATG AC-3′. Prior to the surface attachment of thiol-modified DNA to either the gold gratings or nanoparticles, thiol-modified DNA samples were treated with 200 mM DTT to cleave any disulfide bonds followed by binary reversed-phase HPLC purification. The concentrations of thiol and DNA were measured using UV–vis spectroscopy. Further details on DNA purification and concentration measurement can be found elsewhere.[15–18]

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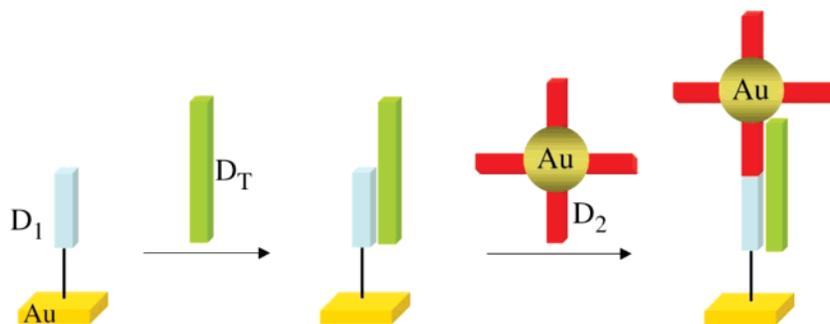
Figure 1. Schematic outlining the nanoparticle surface bioaffinity sandwich assay for the NEDG detection of single-stranded target DNA (D_T). D_T is first hybridized adsorbed onto a gold grating surface functionalized with a monolayer of ssDNA (D_1) that is complementary to half of the D_T sequence. Next, a gold nanoparticle modified with a second DNA sequence (D_2), complementary to the other half of the D_T molecule, is sequence specifically adsorbed onto the surface-immobilized D_1–D_T duplex.

Lift-Off Lithography for Fabricating Gold Diffraction Grating Patterns on Glass Substrates. SF10 glass slides (18 mm × 18 mm, Schott Glass) were soaked in piranha solution (3:1 mixture of 6 M sulfuric acid to 30% hydrogen peroxide) for 30 min (*Caution: Piranha solution is extremely hazardous and must be handled with care*). Subsequently, the slides were thoroughly rinsed with Millipore water and baked in an oven at 120 °C to remove any excess water from the surface of the glass. Microposit S1808 positive photoresist (Rohm and Haas Electronic Materials) was spin coated onto the slides (500 rpm for 10 s, 2500 rpm for 40 s), and the slides were baked at 90 °C for 30 min. Afterward, the slides were photolithographically patterned at 365 nm using a Karl Suss MJB3 mask aligner and a chrome mask with 5-μm Cr lines and 10-μm spacing (Photo Sciences). The exposure time was set at 10 s. Next, the slides were immersed in Microposit MF-319 developer solution (Rohm and Hass Electronic Materials) for 20 s, rinsed with Millipore water, and dried in a gentle stream of nitrogen. This created discrete lines of unexposed photoresist on the glass slide. A thin layer of chromium (1 nm), followed by a layer of gold (45 nm), was deposited onto the surface of the slides using a Denton DV-502A metal evaporator. Next, the slides were kept in acetone for 3 days and then sonicated for 1 min in a 1:1 mixture of acetone and methanol followed by 1 min sonication in Millipore water. This removed the areas of the gold/chromium film that were deposited onto the unexposed photoresist leaving gold lines that were directly deposited onto the glass. Finally, the grating slides were rinsed thoroughly with Millipore water and dried in a nitrogen stream. Gratings with different periods were fabricated by changing the mask design and adjusting the exposure times accordingly.

DNA Attachment Chemistry on Gold Grating Surface and Preparation of DNA-Modified Au Nanoparticles. The gold diffraction gratings were immersed in a 1 mM ethanolic MUAM solution for a minimum of 4 h to form a well-packed, self-assembled monolayer. The MUAM surface was then reacted with a 1 mM solution of the heterobifunctional cross-linker SSMCC in 0.1 M TEA (pH 7) buffer for 30 min to form a thiol-reactive, maleimide-terminated surface. This surface was subsequently reacted overnight with a 1 mM solution of 5′-thiol-modified DNA

(D_1) in 0.1 M TEA (pH 7) buffer.[15,17] The DNA-modified gold grating chip was finally rinsed with Millipore water and dried under a nitrogen stream prior to use. Au nanoparticle solutions with a λ_{max} of 518 nm and an average particle diameter of 13 ± 1.2 nm were prepared using a standard citrate reduction method.[19] Nanoparticle solutions that had a larger average nanoparticle size or larger dispersion resulted in significant nonspecific adsorption and thus were not used. DNA modification of Au particles was carried out following previously described protocols:[15,20,21] A 950-μL aliquot of Au nanoparticle stock solution was first mixed with 50 μL of 100 μM 3′-thiol-modified DNA (D_2) and kept at 37 °C overnight followed by the addition of 350 μL of Millipore water and 150 μL of 1 M NaCl/100 mM phosphate (pH 7.4). This solution was then stored a further 24 h at 37 °C in order to achieve a high loading of DNA onto the nanoparticle surface as described previously.[20,21] The removal of excess DNA was performed by centrifuging the nanoparticle solution at 13 000 rpm for 40 min, removing the supernatant, and resuspending the Au pellet in 0.1 M NaCl/10 mM phosphate buffer (pH 7.4). This wash cycle was repeated three times before a final resuspension in 0.3 M NaCl/ 10 mM phosphate buffer (pH 7.4). The nanoparticle concentration was estimated at 10 nM using UV–vis spectroscopy and an extinction coefficient of 2×10^8 M^{-1} cm^{-1}.[20] Finally, all surface hybridization adsorption experiments involving both target DNA and D_2-modified Au nanoparticles were performed in 0.3 M NaCl/ 10 mM phosphate buffer (pH 7.4). The use of higher salt concentrations was avoided due to possible aggregation of the nanoparticle solutions.

RESULTS AND DISCUSSION

As a first example of ultrasensitive biosensing with the NEDG biosensors, a nanoparticle "sandwich" adsorption assay was employed to detect DNA. Figure 1 shows a schematic diagram of this sandwich assay, which has also been utilized in conjunction with SPRI to detect target DNA at picomolar concentrations.[15,22,23]

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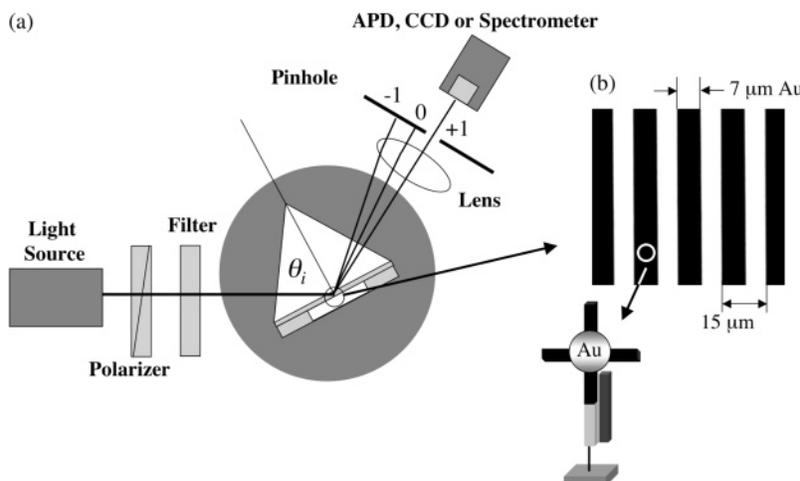
Figure 2. (a) Schematic of the NEDG experimental setup. Collimated white light is directed through a polarizer and filter onto a prism/grating chip/flow cell assembly at an incidence angle (θ_i). The +1 diffraction beam is then collected through a lens and analyzed using one of three detectors: an optical fiber-coupled spectrometer, CCD camera, or avalanche photodiode (APD). (b) A partial image of the gold diffraction grating acquired using a transmission optical microscope. Each gold line is 7-μm wide, 45-nm thick and runs continuously along the 18-mm length of the square SF10 glass substrate with a grating period of 15 μm. The bottom of the figure shows a schematic of D_2-coated nanoparticle adsorption onto D_1-D_T duplexes formed on the gold grating lines.

The gold grating surface is first functionalized with a monolayer of single-stranded DNA (ssDNA) molecules, D_1, which are complementary to half of the sequence of the target ssDNA molecule, D_T. The surface is then exposed to a solution of D_T that adsorbs to the surface by hybridization to D_1 (this process is referred to as "hybridization adsorption"). Next, the surface is exposed to a solution of gold nanoparticles that have been functionalized with a second ssDNA molecule, D_2, whose sequence is complementary to the other half of D_T. Hybridization adsorption of D_2-modified nanoparticles onto the surface D_1-D_T duplex is then monitored using NEDG.

The nanoparticle-enhanced diffraction grating measurements described in this paper detect DNA by monitoring the relative change in the first-order diffraction efficiency that occurs upon hybridization adsorption of DNA-modified nanoparticles onto a functionalized gold grating surface. Figure 2a shows a schematic diagram of our NEDG apparatus. A prism coupled grating geometry (Kretchmann configuration) is used in conjunction with a collimated white light source (model HL-2000, Ocean Optics) that is p-polarized allowing us to excite planar surface plasmons at a selected wavelength. A gold diffraction grating (Figure 2b) was designed with 7-μm wide, 45-nm thick parallel gold lines patterned onto a glass substrate with a grating periodicity of 15 μm. The grating is illuminated with a filtered broadband white light source through the prism at an angle of incidence (θ_i), as depicted in Figure 2a. The gold grating is aligned perpendicular to the plane of incidence of the light source, and a series of diffracted spots are observed in the plane of incidence at angles θ_m related to θ_i by the grating equation:

$$\sin \theta_m = \sin \theta_i + \frac{m\lambda}{nd} \quad m = 0, \pm 1, \pm 2, \ldots \quad (1)$$

where d is the grating period, n is the prism refractive index, λ is the light wavelength, and m is the diffraction order. Upon the hybridization adsorption of DNA-modified gold nanoparticles onto

the gold grating, an increase in intensity is measured at the first order (+1) diffraction spot. The relative change in the first-order diffraction efficiency ($\Delta\%DE$) is calculated using eq 2, where R_0^{+1}

$$\Delta\%DE = \frac{R_{np}^{+1} - R_0^{+1}}{R_0^{+1}} \times 100 \quad (2)$$

and R_{np}^{+1} are the measured reflectivities of the +1 diffraction spot, before and after nanoparticle adsorption, respectively.

The angle of incidence θ_i for these diffraction measurements was selected relative to the specific angle θ_{SP} at which surface plasmons are generated for a given wavelength λ_{SP}. The angle θ_{SP} was first determined experimentally by using a continuous planar 45-nm gold film in place of the grating. Figure 3 shows a series of four differential reflectivity spectra from this film [$\Delta\%R = 100(R_p - R_s)/(R_p + R_s)$, where R_p and R_s are the p- and s-polarized reflectivity spectra, respectively] that were acquired at four different angles of incidence. For each input angle, a strong minimum was observed at a particular wavelength, which we denote as λ_{SP}. This is the wavelength at which we excite surface plasmons on the gold thin film surface. We denote the corresponding input angle as the surface plasmon angle, θ_{SP}. The inset in Figure 3 depicts the relationship between θ_{SP} and λ_{SP} as determined from both theory (three-phase Fresnel calculations)[24] and experiment. For the NEDG experiments in this paper, we have chosen a λ_{SP} of 670 nm corresponding to a θ_{SP} of 56.0°. This wavelength is in the region where a significant enhancement in the grating efficiency is expected from the surface-adsorbed ~13-nm gold nanoparticles.[12]

Once θ_{SP} is determined, we can replace the planar gold film with a diffraction grating and select θ_i and θ_{+1}. Ideally, we would like to set both θ_i and θ_{+1} to θ_{SP}, but that is not possible. Instead, the largest increase in $\Delta\%DE$ was found to occur if we adjusted the incident angle to the value in eq 3, which meant that the first-

(24) http://www.corninfo.ps.uci.edu/calculations.html.

$$\theta_{i} = \theta_{SP} - \Delta/2 \qquad (3)$$

order diffraction occurred at

$$\theta_{+1} = \theta_{SP} + \Delta/2 \qquad (4)$$

where Δ is the angle separation between the zero- and first-order beams. At a wavelength λ_{SP} of 670 nm, we measured Δ to be equal to 1.3°. As shown schematically in Figure 2a, the +1 diffraction spot was separated from the other orders and sent to either an optical fiber-coupled spectrometer (model USB4000, Ocean Optics) for characterization or an avalanche photodiode (C5460−01, Hamamatsu) for the ultrasensitive biosensing measurements.

Figure 4 shows the spectra of the relative change in the first-order diffraction efficiency ($\Delta\%DE$) measured at the +1 diffraction spot due to the adsorption of DNA-functionalized nanoparticles onto a gold grating surface that had been exposed previously to a 500 nM target ssDNA solution. Also shown as an inset in the figure is a CCD camera image of the −1, 0, and +1 diffraction spots. It is clear from both the camera image and the spectrum that a marked increase in the first-order diffraction efficiency is observed for this optical configuration. This is opposite to previous measurements using gold gratings in a transmission geometry where a decrease in grating efficiency was observed.[12] In our case, nanoparticle adsorption resulted in a 100% increase in signal exactly at the targeted resonance wavelength λ_{SP} of 670 nm. This wavelength of maximum $\Delta\%DE$ is denoted as λ_{DE}. In the absence of nanoparticle adsorption, a $\Delta\%DE$ value of only 1.8% was obtained for the same DNA concentration. This experiment was repeated at a number of wavelengths from 600 to 800 nm with the λ_{DE} always tracking λ_{SP} (see Supporting Information for grating efficiency experiments at different wavelengths). These results
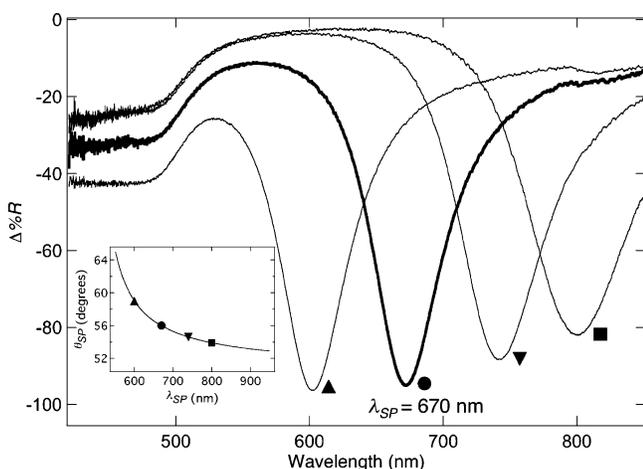
Figure 3. Differential reflectivity ($\Delta\%R$) spectra acquired using an optical fiber-coupled spectrometer at four different incidence angles for a continuous planar 45-nm gold film in contact with water. The figure inset depicts the relationship between the surface plasmon angle, θ_{SP}, and corresponding wavelength, λ_{SP}, where there is a minimum in reflectivity due to the excitation of planar surface plasmons. The solid line in the inset is the theoretically calculated values and the λ_{SP} of each plotted spectra is (▲) 600, (●) 670, (▼) 742, and (■) 800 nm, respectively. A θ_{SP} of 56.0° corresponding to a λ_{SP} of 670 nm (boldface line) was used to determine the incident angle for the diffraction measurements.
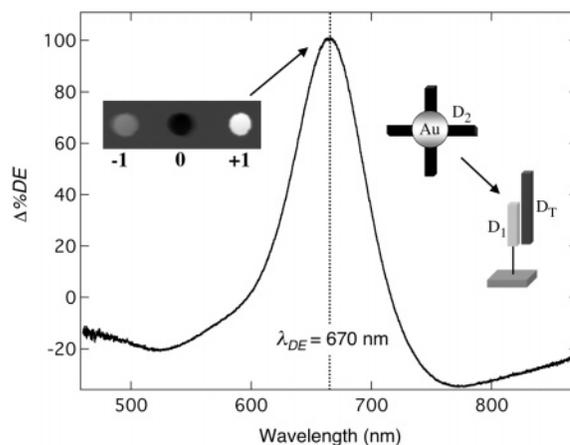
Figure 4. Spectral measurements of the relative change in diffraction efficiency ($\Delta\%DE$) at the +1 diffraction spot due to the adsorption of DNA (D_2)-functionalized nanoparticles onto a gold grating surface that had been previously exposed to a 500 nM target ssDNA solution. The grating was exposed to target ssDNA for 30 min followed by rinsing in buffer. Next, the hybridization adsorption of DNA-coated nanoparticles was performed until no further increase in signal was observed. The NEDG measurement was performed at an incident angle determined from eq 4 with $\theta_{SP} = 56.0°$ and $\Delta = 1.3°$. A maximum increase in diffraction efficiency was observed at the wavelength $\lambda_{DE} = 670$ nm. The right inset is a schematic of D_2-modified nanoparticle adsorption onto surface D_1-D_T duplexes. The left inset shows a difference image obtained by subtracting images acquired before and after the nanoparticle-enhanced detection of a 500 nM solution of D_T.

confirm that we are observing surface plasmon-enhanced diffraction in these experiments.

To demonstrate the high sensitivity of the NEDG biosensor, a series of DNA biosensing measurements were performed to detect femtomolar solutions of a target ssDNA molecule (D_T). For these measurements, a white light source was filtered with a 10-nm band-pass filter centered at 670 nm and modulated with an optical chopper at 1 kHz. The wavelength of 670 nm used in these experiments was chosen as a spectral region where the complex refractive index of the 13-nm gold nanoparticles used in this study produced the largest change in diffraction grating efficiency upon adsorption.[12] The intensity of diffracted light at the +1 order spot was measured with an avalanche photodiode connected to a lock-in amplifier. The light source was stable with drifts in intensity measured at <0.1%/h. For each experiment, hybridization adsorption of D_T was first allowed to proceed for ~4 h in a circulating 100-μL flow cell with a total sample volume of 500 μL. At femtomolar concentrations, ~4 h is the time required to reach a steady-state surface coverage of D_T given a measured hybridization adsorption rate constant of 10^4 M^{-1} s^{-1}.[1] To detect the target DNA, the surface was then exposed to a 10 nM solution of D_2-functionalized nanoparticles, and the increase in $\Delta\%DE$ was obtained from the photodiode measurements as a function of time. The results of these NEDG measurements are shown in Figure 5. Control experiments were performed in the absence of D_T and also using a 16-base mismatch DNA target. In both cases, nonspecific adsorption of DNA-modified nanoparticles was not observed. Using this approach, we were able to measure target DNA concentrations as low as 10 fM with a signal-to-noise ratio of 3:1.
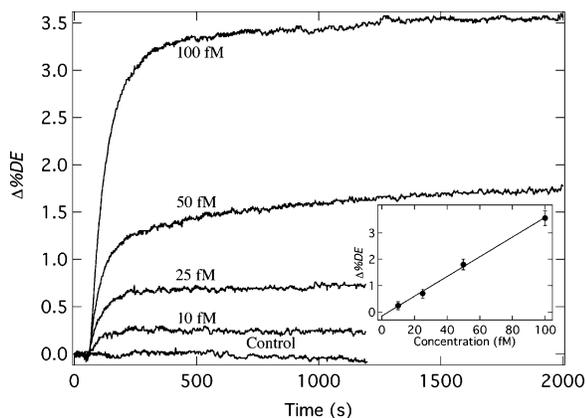
Figure 5. Time-dependent NEDG signal ($\Delta\%DE$) curves for the detection of target DNA at concentrations ranging from 10 to 100 fM. Each DNA solution was continually circulated over a separately prepared grating surface for ~4 h. Changes in the NEDG signal were then monitored upon the introduction of a solution of D_2-modified nanoparticles. The figure inset is a plot of the steady-state $\Delta\%DE$ value measured at each DNA concentration. The control experiment shows that no nonspecific adsorption of nanoparticles was observed in the absence of target DNA on the grating surface.

A plot of the equilibrium $\Delta\%DE$ versus DNA concentration is also shown in an inset in Figure 5. The NEDG signal responded linearly over a DNA concentration range of 10−100 fM with a very high responsivity of +0.035 $\Delta\%DE$/fM. At 1 pM, a $\Delta\%DE$ value of 20% was observed as compared to the expected value of 35%, indicating a saturation of the nanoparticle adsorption signal. At these higher nanoparticle concentrations, the NEDG signal could be returned to a linear response range by reducing the concentration of the gold nanoparticle solution. The linear response combined with the high reproducibility of the NEDG measurements indicates that there is no nanoparticle aggregation either on the grating surface or in solution prior to detection. Furthermore, at femtomolar concentrations, the equilibrium

fractional surface coverage of target DNA on the gold grating lines is extremely low such that the average distance separating each surface-attached nanoparticle will be several orders of magnitude greater than the particle size itself.

CONCLUSIONS

In this paper, we have presented a novel biosensing methodology (NEDG) that combines the concepts of optical diffraction, planar surface plasmon resonance, and nanoparticle-enhanced detection. The direct detection of 10 fM DNA concentrations with a signal-to-noise ratio of 3:1 is over 100 times more sensitive than comparable measurements using either nanoparticle-enhanced SPRI[15,22] or nanoparticle adsorption onto gold transmission gratings.[12] In future, the ability to easily perform NEDG measurements at different resonance wavelengths in the visible−NIR region will be particularly useful when working with various nanoparticle sizes and shapes that have very different surface plasmonic properties. We are also excited at the prospect of combining nanoparticle-enhanced diffraction measurements with various surface enzymatic amplification strategies recently developed in our group.[1,25] This will allow us to perform surface bioaffinity measurements, such as single-nucleotide polymorphism genotyping[15] and microRNA detection,[25] with even higher sensitivity.

SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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