

Plasmonic interferometers for label-free multiplexed sensing

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Abstract: We report a plasmonic interferometric biosensor based on a simple slit-groove metallic nanostructure that monitors the phase changes of surface plasmon polaritons resulting from biomolecular adsorptions. The proposed sensing scheme integrates the strengths of miniaturized plasmonic architectures with sensitive optical interferometry techniques. Sensing peak linewidths as narrow as 7 nm and refractive index resolutions of 1×10^{-5} RIU were experimentally measured from a miniaturized sensing area of $10 \times 30 \mu\text{m}^2$ using a collinear transmission setup and a low-cost compact spectrometer. A high-density array of such interferometric sensors was also fabricated to demonstrate its potential for real-time multiplexed sensing using a CCD camera for intensity interrogation. A self-referencing method was introduced to increase the sensitivity and reduce sensor noise for multiplexing measurements. The enhanced sensing performance, small sensor footprint, and simple instrumentation and optical alignment suggest promise to integrate this platform into low-cost label-free biosensing devices with high multiplexing capabilities.

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1. Introduction

Following Ebbesen's report on extraordinary optical transmission (EOT) in 1998, metallic nanostructures have been the focus of intense research due to their unique optical properties [1–3]. Surface plasmon polaritons (SPPs) are electromagnetic waves coupled to coherent charge oscillations at a metal-dielectric interface, and the excitation of SPPs can generate large field enhancements within nanoscale volumes [2]. The unprecedented light concentration and resulting strong light-matter interactions in plasmonic nanostructures open up many opportunities in chemical and biological sensing, fluorescence, Raman scattering, lithography, and photovoltaics [3]. Among these applications, plasmonic sensing has been the subject of particularly intense study. Indeed, commercial surface plasmon resonance (SPR) sensors have become the gold standard for label-free biomolecular sensing, and have found utility in wide applications ranging from biomedical diagnostics and immunoassays, to environmental sensing and food safety monitoring [4].

Most commercial SPR systems rely on the prism-based Kretschmann configuration to couple light into SPPs propagating on a flat metal film. While this approach results in relatively high sensitivities, it is not conducive to system miniaturization and low-cost production, because of its optical complexity and bulky instrumentation [5,6]. It is also a significant challenge to extend the sensing capability of current SPR systems to high-throughput multiplexed assays. SPR imaging (SPRi) is the most commonly employed approach to address this need, typically employing a CCD camera to monitor the intensity distribution of light reflected from an SPR surface containing multiple sensing spots [7]. However, the Kretschmann configuration employed in SPRi results in a tilted image plane, leading to image defocusing and optical aberrations [8]. The prism-based coupling scheme also prohibits the use of high numerical aperture (NA) imaging systems to increase spatial resolution [9,10]. The relatively large sensing spot size of commercial SPR imagers (with a typical diameter of 200 μm) [11] limits their effectiveness for probing nanovolumes and single cells and for high-density microarray applications.

Nanoplasmonic biosensors, employing nanostructured metal films to couple incident light directly into SPPs in a simple collinear transmission geometry, are an emerging sensing platform and a promising alternative to conventional SPR sensors [8–10,12–16]. These nanostructure-based plasmonic sensors can have a small footprint, down to a few square micrometers, and can be fabricated on a chip with much higher multiplexing capacity than previous SPRi systems [8,10,17]. The simplicity of the optical alignment also offers greater opportunities for sensor miniaturization, low-cost production, robust operation, and easy integration with compact microfluidics [5]. While these new sensors have significant advantages, their relatively low sensitivities and large detection limits compared to conventional SPR technique [4,14] are drawbacks that must be addressed. To achieve optimal sensing performance in nanoplasmonic sensors, a large resonance shift upon refractive index change and a narrow peak linewidth are required [16,18]. However, existing nanoplasmonic sensors based on localized surface plasmon resonances (LSPR) in nanoparticles or the EOT effect in metal nanoaperture arrays typically exhibit broad peak linewidths because of strong radiative and non-radiative losses [16]. As a result, plasmon line shape engineering is emerging as an important means to develop novel nanoplasmonic sensors with narrow

linewidths [18–21]. For example, plasmonic induced transparency and Fano resonances in coupled plasmon systems [18–20] and diffractive coupling of LSPR in nanoparticle arrays [21] have attracted increasing attention due to their demonstrated narrow resonance linewidths and potentially improved sensor performance.

Surface plasmon interferometry has recently been suggested as a promising new technique to control plasmon line shape for ultrasensitive plasmonic biosensing [22–27]. By tailoring the nanostructure as well as the amplitude and phase of propagating SPPs in two interfering channels, one can effectively engineer the bandwidth and line shapes of interference peaks in plasmonic interferometers, providing new opportunities to improve sensor performance. In our previous report [22], a prototype plasmonic Mach-Zehnder interferometer has been demonstrated that exhibits sensitivity exceeding 3500 nm/RIU and peak linewidth of 16.5 nm. While this nanosensor has superior sensing performance and a compact footprint, its multiplexing capacity is somewhat limited due to its non-collinear transmission geometry [22]. Also, the spectral modulation method that was employed is not suitable for dynamic, highly multiplexed sensing.

In the present work, we propose a new plasmonic interferometric sensing platform, operating in a simple collinear transmission configuration, for real-time, sensitive, and multiplexed sensing applications. This interferometric sensor employs a compact slit-groove nanostructure, and its output is analyzed using a low-cost fiber-optic spectrometer. Sensing peak linewidths as narrow as 7 nm and refractive index resolutions of 1×10^{-5} refractive index units (RIU) were experimentally measured for this miniaturized sensor, which has a sensing area of $30 \times 10 \mu\text{m}^2$. Analytical models were used to predict sensing characteristics and possible ways to further improve the sensor performance. The collinear transmission geometry, narrow sensing peaks, and small sensor footprint suggest promise for sensitive multiplexed sensing using intensity interrogation. As a proof-of-concept demonstration, an array of the proposed interferometers was fabricated on a sensor chip with a packing density of 4×10^4 sensors per cm^2 for real-time multiplexed sensing using a CCD camera and a narrow band light source. A self-referencing method, which will be discussed in detail in Section 5, was also introduced to approximately double the sensitivity and reduce the sensor noise. With demonstrated sensing performance and the possibility for further improvement, this multiplexed sensing platform shows promise for future integration into low-cost, miniaturized, high-throughput biosensing devices.

2. Slit-groove plasmonic interferometer

The proposed plasmonic interferometer, shown schematically in Fig. 1(a), illustrates the compact groove-slit-groove nanostructure design. Our sample consists of a 350 nm silver film deposited onto a previously cleaned microscope cover slide. Focus ion beam (FIB) milling was used to fabricate a series of plasmonic interferometers with varying slit-groove distance L . The 30- μm -long slit is at the center of the two 30- μm -long grooves. The slit and groove widths are 100 and 120 nm, respectively. The groove depth is around 70 nm, measured using atomic force microscopy (AFM). Figure 1(b) shows a scanning electron microscope (SEM) image of a fabricated device with $L = 5.1 \mu\text{m}$. The structure was illuminated through the substrate by a focused white light beam. Under TM-polarized illumination (with the electric field perpendicular to the long axis of the slit), light transmitted through the central slit causes SPPs to be launched on the upper metal surface and propagate toward the two grooves. The SPPs are partially reflected back toward the central slit, where they are then scattered and interfere with the light directly transmitted through the slit (Fig. 1(c)). Here two grooves are employed to double the reflected SPP intensities relative to a single-groove interferometer and improve the interference contrast. The SPP-mediated scattered light was then collected by a $\times 40$ objective for measurement. Illumination of this interferometer platform from the substrate side allows the incident light to be coupled first into a guided mode inside the nanoslit cavity, which has a large acceptance angle for light coupling [28]. As a result, a

focused intense optical beam can be used to increase the signal-to-noise ratio of the transmission spectrum, which is especially useful for a small-footprint sensor with limited light transmission [29]. This is a unique advantage of this design over previous plasmonic interferometers, which typically employ collimated illumination on the front side of the nanostructures [25–27,30]. Moreover, the nanoslit in this device functions as a broadband SPP coupler, which enables sensor operation over a broad spectral range and permits multispectral sensing schemes that have improved sensitivities [15,25,31].

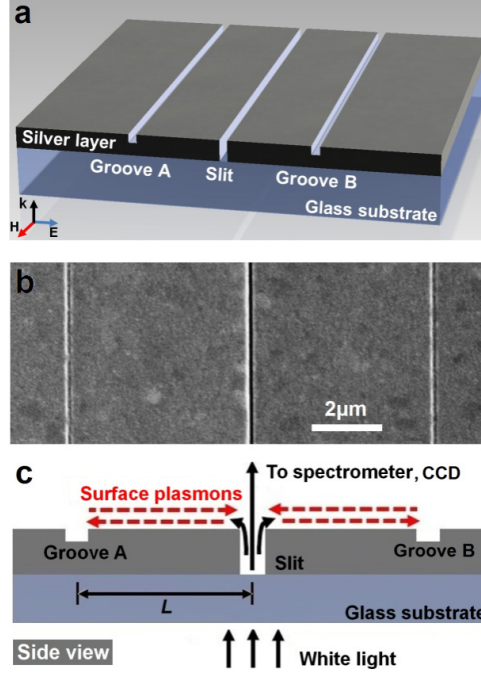


Fig. 1. (a) Schematic of the proposed plasmonic interferometer. (b) An SEM image of a fabricated groove-slit-groove nanostructure with $L = 5.1 \mu\text{m}$. (c) Side view of the proposed interferometer structure.

The solid curves in Fig. 2 are experimental spectra measured in an air environment using two interferometers with slit-groove separations of 5.1 and 9.0 μm . Data were normalized to the transmission spectrum of an identical reference nanoslit milled on the same sample. One can see obvious spectral oscillations with narrow peaks and valleys resulting from the constructive and destructive interference between the light transmitted directly through the slit and SPPs propagating between the grooves and the slit. The interference pattern of the interferometer with the larger L of 9.0 μm exhibits faster spectral oscillations with decreased contrast. To better interpret these measurements, theoretical interference patterns can be expressed as [32]:

$$\frac{I}{I_0} = 1 + \frac{E_{spp}^2}{E_{free}^2} + 2 \frac{E_{spp}}{E_{free}} \cos\left(\frac{4\pi L}{\lambda} n_{spp} + \varphi_0\right). \quad (1)$$

Here E_{free} and E_{spp} are the field amplitudes of directly transmitted light and SPP modes, respectively. $n_{spp}(\lambda) = \text{Re}((\epsilon_m n^2 / (\epsilon_m + n^2))^{1/2})$ is the effective refractive index of SPPs at the metal/dielectric interface, ϵ_m is the metal permittivity [33], n is the refractive index of the dielectric material on top of the metal surface, and φ_0 is an additional phase shift [32] due to SPP reflection at the grooves and scattering at the slit. According to this equation, the intensity of the transmitted light at a specific wavelength depends on the phase difference

between SPPs and free-space light through the term $(4\pi L n_{\text{spp}}/\lambda + \varphi_0)$, which can be modulated by bulk refractive index changes or biomolecule adsorptions at the upper sensor surface between the central slit and two grooves. For broadband illumination, a change in refractive index causes a spectral shift of the interference pattern, providing the basis of the proposed sensing scheme.

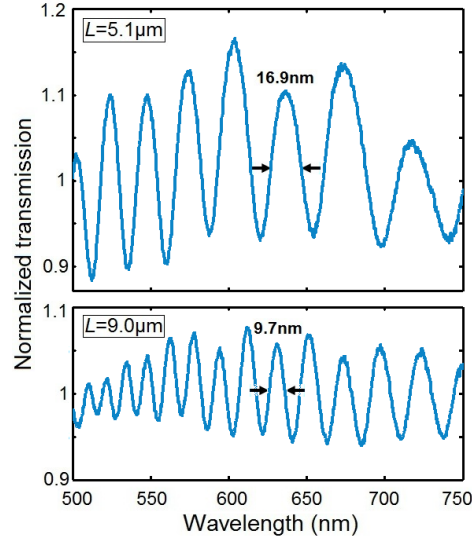


Fig. 2. Experimental interference patterns for slit-groove plasmonic interferometers in an air environment with $L = 5.1$ and $9.0 \mu\text{m}$.

By setting the term n_{spp}/λ to constant in Eq. (1), the refractive index sensitivity of this plasmonic sensor can be derived as

$$S = \left| \frac{\Delta\lambda}{\Delta n} \right| \approx \lambda \left(\frac{n_{\text{spp}}}{n} \right)^3 \left/ \left(n_{\text{sp}} - \lambda \frac{dn_{\text{spp}}}{d\lambda} \right) \right. \quad (2)$$

Equation (2) predicts that S is approximately 481 nm/RIU in a water environment at a wavelength of 650 nm. A unique advantage of this sensing scheme is the extremely narrow linewidth of the interference oscillation. The peak linewidth, $\delta\lambda$, is defined as half of the oscillation period, P , and can be described by the following equation:

$$\delta\lambda = \frac{P}{2} \approx \lambda^2 / 4L \left(n_{\text{spp}} - \lambda \frac{dn_{\text{spp}}}{d\lambda} \right) \quad (3)$$

Using Eq. (3), one can calculate the peak linewidths to be 16.3 nm ($\lambda \sim 636.1$ nm) and 9.1 nm ($\lambda \sim 631.1$ nm) for interferometers with $L = 5.1$ and $9.0 \mu\text{m}$ in air, respectively, agreeing well with the experimental results shown in Fig. 2. The peak linewidths of plasmonic interferometers are determined by the phase properties of propagating SPPs and free-space light, and can be further narrowed down by increasing L according to Eq. (3). This differs from previous nanoplasmonic sensors based on EOT or LSPR, whose resonance linewidths are limited by non-radiative ohmic losses and radiative SPP losses due to scattering. Also note that in this plasmonic interferometer, SPPs travel a round-trip between the slit and groove, which provides half of the peak linewidth observed previously for an interferometer with the same L but using front-side illumination [25,30]. Another widely-used parameter to evaluate the overall performance of nanoplasmonic sensors is the figure of merit $\text{FOM} = S/\delta\lambda$, which is defined as the refractive index sensitivity divided by the sensing peak

linewidth [16,18]. The theoretical FOM of this plasmonic interferometer can be easily derived, yielding

$$FOM = \frac{S}{\delta\lambda} = \frac{4L}{\lambda} \left(\frac{n_{spp}}{n} \right)^3. \quad (4)$$

This simple expression predicts that high FOMs are achievable using the proposed sensing scheme. For example, the calculated FOM value reaches 65 for an interferometer with $L = 9.0 \mu\text{m}$ at $\lambda = 650 \text{ nm}$. This would surpass previous EOT-based sensors, which have a typical FOM value of 23 [34].

3. Refractive index sensing experiment

To experimentally demonstrate the theoretically predicted sensor performance, we integrated our sample with a polydimethylsiloxane (PDMS) microfluidic flow cell and injected a series of glycerol-water solutions with different refractive index. As shown in Fig. 3(a), the interference patterns of interferometers with two different L both red-shift as the liquid refractive index increases. The peak positions were extracted using a Lorentzian fitting method [4] and plotted in Fig. 3(b) as a function of time. For clarity, the sensor response of the interferometer with $L = 5.1 \mu\text{m}$ was vertically displaced by 2 nm in this plot. As seen in Fig. 3(b), both interferometers exhibit stable peak wavelengths at each glycerol concentration and the peak shifts were approximately proportional to the increase in glycerol concentration. The sensing peaks return to their initial spectral positions for both interferometers with the final DI water injection. The lower inset of Fig. 3(b) shows the peak positions as a function of the liquid refractive index. The solid lines are the linear fits to the experimental data, providing sensitivities of the two sensors. For interferometers with $L = 5.1$ and $9.0 \mu\text{m}$, the measured sensitivities are 488.7 and 469.1 nm/RIU, respectively, with peak linewidths of 13.9 and 7.0 nm, and FOMs of 35.2 and 67.0, respectively, all in good agreement with the theoretical predictions (see Table 1).

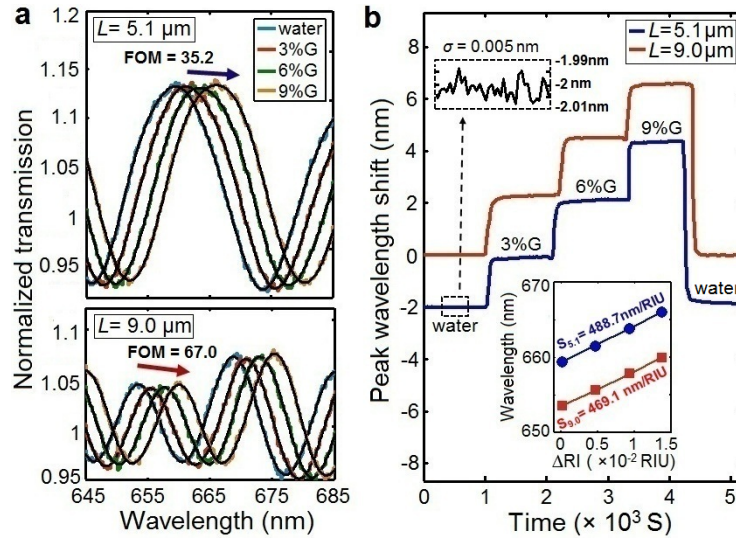


Fig. 3. (a) Measured interference patterns of nanoplasmonic interferometers for water and 3, 6, and 9% glycerol-water solutions. Black curves imposed on the raw data are guides to the eye. The directions of the arrows indicate the red-shifts of the interference patterns. (b) The monitored peak positions for two interferometers as a function of time. The response of the interferometer with $L = 5.1 \mu\text{m}$ was vertically displaced by 2 nm for clarity. The upper inset indicates the sensor noise level and the lower inset shows the spectral positions of the interference peak versus liquid refractive index.

Table 1. Experimental and Calculated Sensing Performances for Interferometers with Two different values of L .^a

$L(\mu\text{m})$	Peak λ (nm)	Sensitivity (nm/RIU)	linewidth (nm)	FOM
5.1	659.4	488.7	13.9	35.2
		<i>484.7</i>	<i>13.4</i>	<i>36.2</i>
9.0	653.6	469.1	7.0	67.0
		<i>482.2</i>	<i>7.4</i>	<i>65.2</i>

^a Numbers in italics are theoretically calculated results.

As shown in the upper inset of Fig. 3(b), the standard deviation (σ) of the monitored peak wavelength of the interferometer ($L = 5.1 \mu\text{m}$) is around $5 \times 10^{-3} \text{ nm}$, corresponding to a sensor refractive index resolution of $1 \times 10^{-5} \text{ RIU}$ [i.e., $5 \times 10^{-3} \text{ nm} / (488.7 \text{ nm/RIU})$]. Importantly, this resolution was measured from a miniaturized sensing area of $30 \times 10 \mu\text{m}^2$ using a compact fiber-optic spectrometer. In previous EOT-based nanoplasmonic sensors, similar spectral resolutions have been achieved but typically require collecting higher light levels from sensing areas at least two orders of magnitude larger [15,31], which limits the multiplexing capacity and prevents the integration with compact microfluidics. The good trade-off achieved between the sensor performance and footprint of this plasmonic interferometer was possible because of the narrow interference sensing peaks, and also from the intense focused beam illumination. It should also be noted that, while the interferometer with L of $9.0 \mu\text{m}$ has a larger FOM of 67, it exhibits very similar sensor resolution as the interferometer with L of $5.1 \mu\text{m}$ (data not shown). This is due to the increased SPP propagation loss for larger L , which results in a lower spectral modulation depth. From this result one can see that while FOM may be a useful metric in comparing the sensing properties of different nanostructures, it is not the only guide for optimizing sensor performance. The sensor footprint, sensing peak intensity, and spectral modulation depth should also be taken into consideration when optimizing the performance of nanoplasmonic biosensors. Due to the smaller footprint, the interferometer with L of $5.1 \mu\text{m}$ will be employed in this work for biosensing and multiplexing experiments in the following sections.

Further optimization of this interferometric sensor is primarily limited by the relatively low interference contrast caused by the unbalanced intensities of interfering SPPs and light. We now discuss reasons for such intensity imbalance and possible ways to optimize it. According to Lalanne's theory [35] and our following experimental validation [30], a large portion of the slit-guided mode (up to ~40%) can be coupled to propagating SPPs (with the rest 60% scattered into free-space light) when the slit width is around 20% of the incident wavelength. As a result, we employed an optimized slit width of 100 nm in this work ($660 \text{ nm} \times 20\% / 1.33 = 99 \text{ nm}$, where the width is scaled by 1.33 as the device is in a water environment). Under this optimized condition, the intensity of the generated SPPs is comparable to interfering free-space light. Therefore, the relatively low modulation depth results mainly from the SPP reflection loss at the nanogrooves and propagation loss at the sensor surface. Here we mention several potentially important improvements that could reduce these losses. First, SPP reflection efficiency at the two grooves can be enhanced by improving the quality of the fabricated grooves (e.g., using Ag-Al double metal layers with the bottom Al layer as a slow etch rate FIB stop to precisely and uniformly control the fabricated groove depth [36]). Second, instead of using single grooves as reflectors, groove or ridge arrays can be designed to function as Bragg mirrors, which have been shown to provide reflection efficiencies larger than 90% after structural optimization [37,38]. Note that a greater number of grooves or ridges make this interferometer less suitable for broadband sensor operation, but do not affect narrow-band intensity-interrogated sensing as will be discussed in Section 5. Third, SPP propagation loss can be reduced by employing ultrasmooth metal films obtained by template stripping [15,39]. Fourth, the sensor noise level could be

further reduced by using a more advanced spectrometer with higher saturation level and lower dark noise [29].

4. Biosensing using plasmonic interferometer

To demonstrate the feasibility of this sensing platform to detect biomolecular binding events, we monitored the specific binding between BSA and anti-BSA molecules. The microfluidic channel was first injected with a 10 mM HEPES buffer for 25 min to rinse the sensor chip and define the baseline of the experiment. A 500 $\mu\text{g/mL}$ BSA solution in HEPES buffer was then introduced into the channel to functionalize the metal surface with a BSA monolayer. This leads to a 0.9 nm shift of the peak wavelength (see the first signal change at the time of 1200 s in Fig. 4). A subsequent 25 min buffer rinse had little effect on the peak wavelength. Then, a 42 $\mu\text{g/mL}$ anti-BSA solution was injected into the channel and followed by a buffer rinse to wash out the unbound anti-BSA molecules. The small spikes observed at time $t = 3000$ s and 4500 s are measurement artifacts caused by exchanging syringes. The specific binding between BSA and anti-BSA corresponds to a peak wavelength shift of 1 nm. We can now calculate the effective protein layer thickness d and the sensor resolution in terms of protein surface concentration by using a well-established quantitative formalism: $\Delta\lambda = S(n_1 - n_b)(1 - e^{-2d/l})$ [40]. Here S is the bulk refractive index sensitivity obtained in the calibration experiment; n_1 and n_b are the refractive indices of protein layer and buffer solution, respectively; and l is the SPP decay length. Assuming the refractive index of pure BSA is 1.57 [40], the observed 0.9 nm peak shift, $\Delta\lambda$, corresponds to an effective thickness of the pure BSA layer, d , of 0.92 nm. By use of the density of BSA (1.3 g/cm^3) [40,41], the surface concentration of this saturated protein monolayer is calculated to be $1.2 \times 10^{-7} \text{ g/cm}^2$ (i.e., $1.3 \text{ g/cm}^3 \times 0.92 \text{ nm}$). As this saturated BSA layer is detected with a signal-to-noise ratio of 180, the resolution of the biosensor can thus be calculated as 6.6 pg/mm^2 in terms of protein surface concentration. This sensor resolution improves when detecting larger biomolecules and can be further optimized through the methods discussed in Section 3.

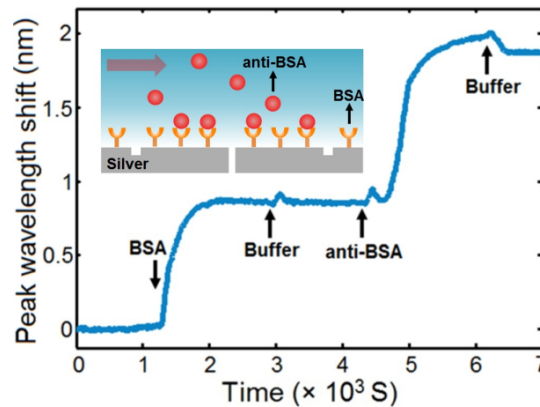


Fig. 4. Real-time sensor response upon BSA adsorption to the sensor surface and subsequent specific protein binding between BSA and anti-BSA. The arrows indicate the injections of analytes and buffer solutions. The upper inset shows a schematic of anti-BSA binding to BSA immobilized on the sensor surface.

5. Multiplexed sensing experiment

While the capability of this interferometer platform for biosensing has been demonstrated, the spectral measurement approach is not suitable for dynamic, highly multiplexed sensing, which requires simultaneous determination of light intensities transmitted through multiple sensing elements. In this section, we further perform real-time, multiplexed sensing experiments using a CCD camera and a narrow band light source for intensity interrogation.

As a proof-of-concept demonstration, we fabricated a 4×3 array of the slit-groove plasmonic interferometer (Fig. 5(a)). Interferometers in the first and third (second and fourth) column of the microarray have a groove-slit distance, L , of 5.1 (5.2) μm . Figure 5(b) and 5(c) show an SEM image of the fabricated sensor array and a CCD image of one of the plasmonic interferometers, respectively. Each interferometer has a footprint of around $300 \mu\text{m}^2$ with the center-to-center distance between each sensing element of $50 \mu\text{m}$, giving a potential packing density of 4×10^4 sensors per cm^2 . The dense packing capability of the proposed sensing scheme illustrates its promise for high-throughput microarray applications. Further improvement in this packing density is still possible by fabricating groove/ridge arrays as Bragg mirrors instead of single grooves to enhance SPP reflection efficiency and decrease crosstalk between sensor elements [8].

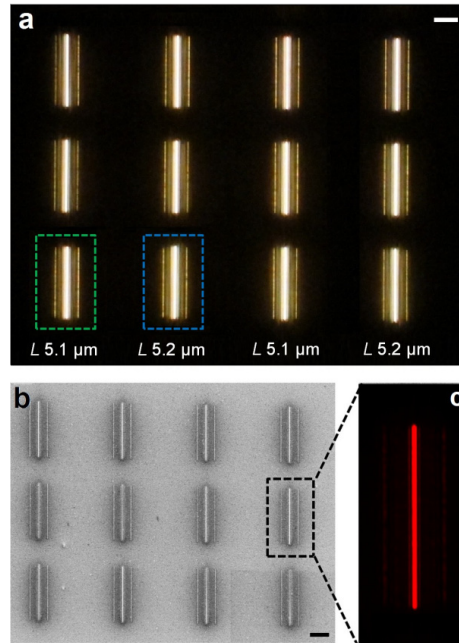


Fig. 5. (a) A bright-field microscope image of the fabricated plasmonic interferometer array. Scale bar: $10 \mu\text{m}$. The interferometers are fabricated with two different L : 5.1 (the first and third column) and 5.2 μm (the second and fourth column) (b) An SEM image of the 4×3 microarray. Scale bar: $10 \mu\text{m}$. (c) A CCD image of one of the interferometers.

For the imaging experiment, the fabricated sensor array was illuminated through the substrate using a white light source passing through an optical band-pass filter centered at 655 nm with a 12 nm bandwidth. The transmitted light from 12 interferometers was then collected simultaneously by a $40 \times$ microscope objective and imaged onto a CCD camera. The intensity change of the transmitted light from each interferometer is determined by two factors: one is the peak shift resulting from refractive index changes and the other is the slope of the spectrum at the illumination wavelength. Accordingly, to achieve optimal sensing performance, the slit-groove distance needs to be carefully designed to locate the high-slope region of the interference pattern at the illumination wavelength. Plasmonic interferometers with L of 5.1 and 5.2 μm are used in this measurement, and their transmission spectra are shown in Fig. 6(a), respectively. The yellow regions indicate the spectral range of the incident light. Both interference patterns red-shift with the increase in the liquid refractive index, and the transmitted light intensities either increase or decrease, depending on the negative or positive slope of the spectrum. Green and blue dots in the inset of Fig. 6(b) present real-time experimental measurements of the transmitted intensities from these two interferometers. As a

series of glycerol-water solutions with increasing refractive index are injected into the channel, the transmission intensity of the interferometer either decrease (for $L = 5.1 \mu\text{m}$) or increase (for $L = 5.2 \mu\text{m}$), in agreement with predictions. Following the 6% glycerol test, DI water was again introduced into the channel, returning the transmitted intensities to their initial levels and validating the reliability of the sensing performance.

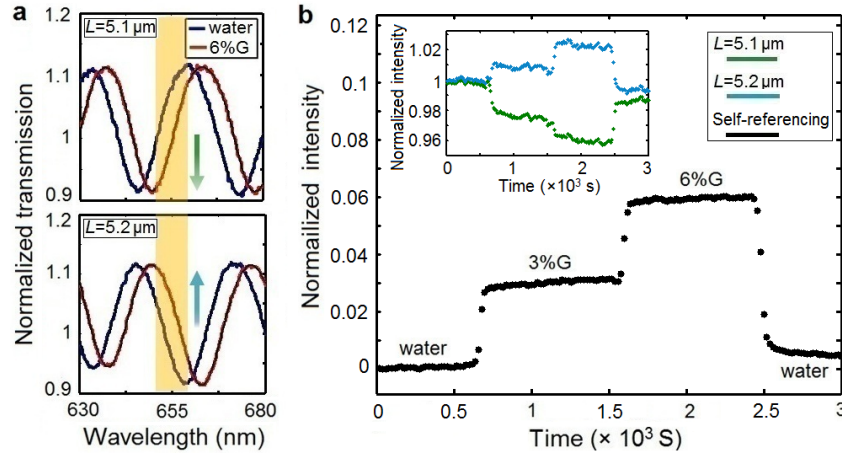


Fig. 6. (a) Transmission spectra of interferometers with two different values of L : 5.1 and 5.2 μm . The yellow regions indicate the spectral range of the filtered white light source. As the dielectric refractive index increases, the transmitted intensity can either decrease ($L = 5.1 \mu\text{m}$) or increase ($L = 5.2 \mu\text{m}$). (b) The blue and green dots shown in the inset indicate the real-time measurements of the normalized transmitted intensities from two interferometers. The black dots are the experimental results obtained after using a self-referencing method.

The standard deviation of the measured light intensity determines the noise level and detection resolution of this intensity-interrogated sensor. To improve the sensor performance, background fluctuations needs to be subtracted, including noise from mechanical vibrations and light intensity fluctuations. Here a self-referencing method was introduced to reduce the influence of these effects and approximately doubles the sensor sensitivity. As shown in Fig. 6(a), interferometers carefully designed with two different L exhibit similar initial transmitted intensities but have positive and negative intensity-change sensitivities. To design such two sensors, the interference pattern of the second interferometer needs to be spectrally shifted by half of an interference period from the first one. The theoretical expression of ΔL (the difference between L of two sensors) can be easily derived from Eq. (1) as: $\Delta L = \lambda / 4n_{\text{spp}}$, where λ is the sensor working wavelength. According to this equation, two interferometers with L of 5.1 and 5.2 μm were designed and fabricated for measurements. When performing the experiment, a signal arising from the refractive index change shifts the transmitted intensities of these two sensors in two different directions (that is transmission increase or decrease), while unwanted signal from light intensity fluctuations and mechanical vibrations change two transmitted intensities in the same direction. Monitoring the intensity difference between the two interferometers results in a near two-fold improvement in sensor sensitivity, and also subtracts the background noises (see the black dots in Fig. 6(b)). The resulting intensity sensitivity is 684%/RIU with a sensor noise level of 0.033%. Here the transmission intensity through single interferometer in water serves as the reference intensity. The sensor resolution is calculated to be 5×10^{-5} RIU (0.033%/ 684%/RIU), 6 times smaller than that of a single interferometer (3×10^{-4} RIU for the interferometer with $L = 5.1 \mu\text{m}$).

This demonstrated sensor resolution is also smaller than previous nanohole array-based multiplexed sensors, with typical refractive index resolutions in the 10^{-4} RIU range [10,42]. Importantly, this demonstrated sensor sensitivity and resolution can be improved by

increasing the interference contrast through the methods discussed in Section 3. Instead of a halogen lamp, an intense and highly stable laser source may also be used to reduce the light source fluctuation and further increase the sensor signal-to-noise ratio. In addition, the dynamic range is also an important sensor characteristic. The dynamic range of this sensor is around 0.01 RIU (the refractive index difference between water and 6% water-glycerol mixture) using the current experimental setup. The dynamic range of this interferometric sensor depends on the bandwidth of the incident light and also the interference linewidth. By setting up a laser to replace the current 12-nm-bandwidth light source, the dynamic range of the device can be improved. Also, by increasing the interference linewidth through tuning L (see Eq. (3)), the dynamic range of the device can be further increased. Note that the increased linewidth might lead to lower intensity sensitivity. Thus, one needs to rationally design L to balance such trade-off between the sensor performance and dynamic range with regard to the target applications. Large-area fabrication of the proposed sensor device is also important for future practical applications. While the FIB fabrication method used in this work is not applicable to large-area patterning, emerging nanofabrication techniques such as template stripping [43] allows mass production of the proposed three-dimensional slit-groove nanostructures.

6. Conclusions

In conclusion, we have demonstrated real-time, multiplexed sensing using plasmonic interferometric sensors. This new group of sensors combines plasmonic architectures with interferometry techniques to monitor the SPP phase changes induced by surface biomolecular adsorptions. The SPP-light interference results in narrow sensing peak linewidths of 7 nm and provides a new route for plasmon line shape engineering by tailoring interferometer structures as well as SPP amplitude and phase properties in two interfering channels. The easy collinear transmission setup and simple, compact slit-groove nanostructure also show promise for future sensor miniaturization and low-cost production. A refractive index resolution of 1×10^{-5} RIU has been measured from a miniaturized sensing area of $30 \times 10 \mu\text{m}^2$ using a low-cost spectrometer. This sensor resolution can be further improved by fabricating large-area interferometer arrays, employing an advanced spectrometer, or using multiple groove/ridges to increase SPP reflection efficiencies at the groove reflectors. We have also demonstrated the real-time, multiplexed sensing capability of this small-footprint sensor by using a CCD camera and a narrow band light source for intensity interrogation. The demonstrated sensing and multiplexing performance and possible further improvements suggest that this novel class of plasmonic interferometric sensors have exciting promise to be integrated into multiplexed, miniaturized sensing devices for label-free biochemical applications.

Appendix: Experimental details

Sample fabrication. Standard glass microscope slides (Fisher Scientific) were first cleaned thoroughly with acetone, isopropyl alcohol (IPA) and deionized (DI) water in an ultrasonic cleaner for 20 min each, and subsequently blow-dried with nitrogen. A 350 nm silver film was then deposited by e-beam evaporation (Indel system) onto glass slides at a deposition rate of 1 Å/s. FIB (FEI Dual-Beam system 235) milling (Ga^+ ions, 30 kV, 30 pA) was used to fabricate a series of groove-slit-groove nanostructures with varying slit-groove distance L . The groove depth is around 70 nm, measured using AFM (NT-MDT Solver NEXT). Another identical single nanoslit was also milled to serve as a reference for spectrum normalization. After the FIB milling, plasma-enhanced chemical vapor deposition was used to deposit a 5 nm thick silicon dioxide film on top of the silver surface. This dielectric film functions as a protection layer to improve biocompatibility and chemical stability of the silver-based device, particularly in aqueous solutions [44]. The prepared sample surface was then cleaned and activated by oxygen plasma and bonded to a microfluidic flow cell.

Optical measurements. White light beam from a 100 W halogen lamp was focused onto the sample from the substrate side through the microscope condenser of an Olympus IX81 inverted microscope. The far-field transmitted light from the interferometer was collected by a 40× microscope objective with numerical aperture NA = 0.6. The collected light could be coupled into a portable fiber-optic spectrometer (Ocean optics USB 4000) for spectral measurements or focused on a CCD camera (Cooke sensicam qe) for multiplexed sensing experiments using intensity interrogation. For spectral measurements, the CCD camera was also employed to record the positions of the slit-groove interferometers. Under identical experimental conditions, the fabricated reference single nanoslit was then moved to the recorded positions of the interferometers and the collected transmission spectrum was used as a reference for spectra normalization.

Refractive index sensing and biosensing measurements. To calibrate the sensor performance for refractive index sensing, a series of glycerol-water solutions with glycerol volume concentrations of 0%, 3%, 6% and 9% was prepared. An ellipsometer (J. A. Wollam, V-VASE) was used to measure their refractive indices at 650 nm wavelength, ranging from $n = 1.3312$ to $n = 1.3450$. The HEPES buffer and anti-BSA used in the biosensing experiment were purchased from Sigma-Aldrich. BSA was purchased from Thermal-Scientific. Solutions were injected into the microfluidic channel (50 μm deep, 4 mm wide) using a syringe pump (Harvard Apparatus) at a flow rate of 20 $\mu\text{L}/\text{min}$. For both real-time refractive index sensing and biosensing experiments, transmission spectra were continuously recorded (100 ms integration time) and averaged over 100 acquisitions as solutions flowed over the sample surface. The temporal resolution for both spectral sensing experiments was 10 s.

Multiplexed sensing experiments using CCD imaging. In multiplexed sensing experiments, the fabricated interferometer array was illuminated through the substrate using a 100 W halogen lamp passing through the microscope condenser and an optical band-pass filter (Semrock) at 655 nm (bandwidth 12 nm). Images were captured every 1 s with an exposure time of 90 ms using a CCD camera (Cooke sensicam qe). A custom made MATLABTM image processing program was used to integrate the transmission intensity over the slit region of each interferometer as the real-time sensor output. The detector dark noise was further removed by subtracting the transmitted intensity integrated over the same area on the sample surface without any nanostructure. CCD images were continuously captured and averaged over 20 images to increase the sensor signal-to-noise ratio. Collecting 20 images with 1 s interval corresponds to a sensor temporal resolution of 20 s.

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