

Plasmonic interferometric sensor arrays for high-performance label-free biomolecular detection†

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A plasmonic interferometric biosensor that consists of arrays of circular aperture–groove nanostructures patterned on a gold film for phase-sensitive biomolecular detection is demonstrated. The phase and amplitude of interfering surface plasmon polaritons (SPPs) in the proposed device can be effectively engineered by structural tuning, providing flexible and efficient control over the plasmon line shape observed through SPP interference. Spectral fringes with high contrast, narrow linewidth, and large amplitude have been experimentally measured and permit the sensitive detection of protein surface coverage as low as 0.4 pg mm^{-2} . This sensor resolution compares favorably with commercial prism-based surface plasmon resonance systems (0.1 pg mm^{-2}) but is achieved here using a significantly simpler collinear transmission geometry, a miniaturized sensor footprint, and a low-cost compact spectrometer. Furthermore, we also demonstrate superior sensor performance using the intensity interrogation method, which can be combined with CCD imaging to upscale our platform to high-throughput array sensing. A novel low-background interferometric sensing scheme yields a high sensing figure of merit (FOM*) of 146 in the visible region, surpassing that of previous plasmonic biosensors and facilitating ultrasensitive high-throughput detection.

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Introduction

Plasmonic nanostructures have attracted increasing attention over the past decade thanks to their unique capabilities to concentrate and manipulate light on subwavelength scales through the excitation of localized surface plasmon resonance (LSPR) or the propagation of surface plasmon polaritons (SPPs).^{1–3} The resonance frequencies of these two modes strongly depend on the shape, size, and surrounding dielectric environment of the nanostructures.³ In particular, the latter dependence enables chemical and biological sensing, which is by far the most common and successful application area in plasmonics.⁴ Nanoplasmonic biosensors can convert small changes in the local refractive index caused by surface biomolecular binding into spectral shifts in the extinction spectra.⁵ This allows real-time label-free detection of the biomolecular interactions using simple and inexpensive transmission spectroscopy. Compared to the prism-based Kretschmann configuration in the conventional surface

plasmon resonance (SPR) technique,⁶ these new biosensors employ a significantly simpler collinear transmission geometry, and offer promising opportunities for system miniaturization and low-cost production. Through further integration on compact microfluidic platforms, nanoplasmonic biosensors hold great promise to develop fast, inexpensive, and portable biomedical devices for point-of-care diagnostics and healthcare applications.^{7–11}

Besides the promise in device miniaturization and cost-effectiveness, another significant advantage of plasmonic sensors is their capacity for massive multiplexing.^{5,7} Such nanostructure-based sensor arrays can have a sensor footprint as small as a few square micrometers and an on-chip packing density as high as 1×10^7 sensors per cm^2 .¹² A system offering such a high multiplexing capacity would tremendously advance research in proteomics, drug discovery, diagnostics, and systems biology, where thousands of biomolecular interactions need to be characterized in parallel to significantly save time and sample consumption.¹¹ Most previously reported work on plasmonic sensors has focused on single-channel spectral interrogation due to its demonstrated superior performance.^{7,14,15} While promising, this sensing scheme requires the sensor array to detect multiple binding events sequentially^{16,17} rather than simultaneously, making it unsuitable for those applications where high temporal resolution is required.¹³ Intensity-based CCD imaging has been suggested as an attractive

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approach to overcome this limitation by simultaneously providing spatial and temporal information.^{12,13,18} Ideally, it is desirable for the plasmonic sensor platform to have both functionalities: a superior spectral sensing capacity comparable to conventional SPR systems and an intensity-based sensing capacity for scalable multichannel sensing applications. However, the development of such a platform poses significant challenges because of the broad resonance linewidths of the existing LSPR and nanohole array sensors, which are associated with strong radiative damping and absorption losses in the metal.^{19,20} These significantly degrade the performance of plasmonic sensors for both spectral sensing and intensity-based imaging.

To optimize plasmonic sensor performance, improvements in several basic plasmon characteristics are required: a very narrow spectral linewidth, a high spectral contrast, and a large spectral shift for modest changes in the refractive index.⁵ Consequently, plasmon line shape engineering is emerging as an important means to optimize sensor performance.^{21–25} The concepts of plasmonic Fano resonance and plasmon-induced transparency have generated considerable interest due to their narrow linewidths associated with subradiant dark modes.^{21–25} Plasmon linewidths as small as 4 nm have been experimentally measured using these approaches.²⁰ However, these efforts suffer from the relatively low spectral contrast and weak resonance intensity of subradiant modes,^{19,20,24} which in fact greatly limit the overall sensor performance. Also, linewidth narrowing in these coupled plasmon systems relies on precise control of the size, shape, and interparticle distance of complex coupled nanoparticle elements, imposing difficulties in fabrication.

In this work, we present a fundamentally new approach exploiting the concepts of phase-sensitive optical interferometry^{26–33} and plasmonic focusing,³⁴ which opens up opportunities for a new class of plasmonic sensors that can easily but significantly tune the plasmon line shape observed through SPP interference. With this approach, we have demonstrated a plasmonic sensor platform that exhibits a spectral sensing performance comparable to state-of-the-art commercial SPR systems, as well as a record high intensity-change sensitivity that is promising for scalable high-throughput sensing applications. The proposed device has a simple structure design, which consists of concentric nanogrooves that function as a plasmonic lens and a central aperture patterned on a gold film. When the whole structure is illuminated by a collimated white light beam, the plasmonic lens excites and focuses the SPPs to the central aperture, where the SPPs interfere with the light that is directly transmitted through the aperture and modulate the far-field transmission. By careful structural tuning, we can effectively control the phase and intensity properties of interfering SPPs and light to generate spectral fringes with high contrast, narrow linewidth, and large amplitude, all key characteristics to achieve optimized spectral sensing. Using this method, we have demonstrated a superior protein surface coverage resolution

of 0.4 pg mm^{−2}. This resolution compares favorably with that of commercial SPR systems (0.1 pg mm^{−2})⁶ but was achieved here using a significantly simpler optical transmission geometry, a sensor footprint smaller by two orders of magnitude, and a low-cost compact fiber-optic spectrometer. Moreover, we further introduced a low-background intensity-based sensing concept, which can be easily combined with CCD imaging to scale up our sensor platform to high-throughput assays. It measures the changes in relative intensity (dI/I_0) induced by a refractive index change dn at a specific wavelength.^{35,36} A widely employed figure of merit, defined as $FOM^* = (dI/I_0)/dn$, is used to evaluate the intensity-based sensor performance.^{10,35,36} Our approach exploits the near-dark reference background ($I_0 \sim 0$) achieved through balanced destructive SPP–light interference and yields a record high FOM^* value of 146 in the visible region, surpassing that of previous intensity-based plasmonic sensors and facilitating sensitive multichannel sensing.

Results and discussion

Working principle of the plasmonic interferometer

The proposed interferometer structure, schematically illustrated in Fig. 1a and b, consists of a gold film containing a nanoaperture surrounded by three nanopatterned concentric grooves. While silver exhibits lower SPP propagation loss and was employed in previous plasmonic interferometers,^{26–29} here we employ gold for its superior stability and biocompatibility for biosensing applications. The multiple circular grooves function as an efficient SPP coupler as well as a focusing lens to scatter the normally incident light into the propagating SPPs and then focus them at the central hole.³⁷ The groove periodicity (P) is carefully chosen so that the SPPs launched at each groove add up approximately in phase in the spectral region of interest, generating a strong propagating SPP wave directed to the central focusing point. The following simple expression may be used to calculate P :³⁸

$$P = \lambda \sqrt{\frac{\epsilon_m + n^2}{\epsilon_m n^2}} \quad (1)$$

where λ is the center wavelength of the spectral region of interest, ϵ_m is the gold permittivity, and n is the refractive index of the medium covering the sensor surface. At the hole position, the focused SPPs interfere with the light that is directly transmitted through the hole and modulate the far-field scattering. The theoretical expression for the transmitted light intensity through the interferometer can be written as:

$$I = E_{\text{light}}^2 + E_{\text{spp}}^2 + 2E_{\text{light}}E_{\text{spp}} \cos\left(\frac{2\pi R}{\lambda} n_{\text{spp}} + \varphi_0\right). \quad (2)$$

Here, E_{spp} and E_{light} are the field amplitudes of SPP-mediated and directly-transmitted light components at the central aperture, respectively. R is the distance between the middle circular groove and the rim of the hole.

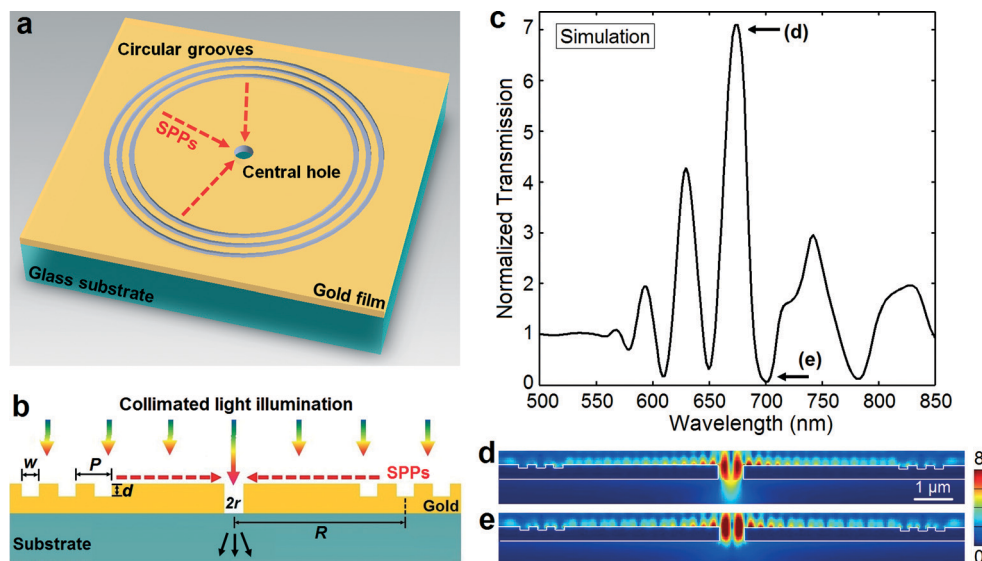


Fig. 1 The working principle of the plasmonic interferometer. (a) Schematic of the proposed plasmonic interferometer. (b) Side view and the operating principle of the device. (c) The simulated transmission spectrum of the proposed interferometer, normalized to that of a reference central aperture. The calculated electric field distributions ($|E|/|E_0|$) at the interference peak and valley wavelengths are plotted in (d) and (e), respectively.

$n_{\text{spp}}(\lambda) = \text{Re}((\epsilon_m n^2 / (\epsilon_m + n^2))^{1/2})$ is the effective refractive index of SPPs propagating along the metal surface between the grooves and the hole, and ϕ_0 is an additional phase shift associated with SPP excitation at the grooves.⁴⁰ The term $(2\pi R n_{\text{spp}} / \lambda + \phi_0)$ represents the phase difference between the two interfering components, which varies with λ and leads to constructive and destructive SPP–light interferences. The spectral positions of the resulting multiple interference peaks and valleys are very sensitive to n_{spp} , which can be modulated by surface biomolecular adsorption and provides the basis of this sensing scheme.

In the context of sensor design, there are several important structural parameters that largely determine the device's performance. Using eqn (2), we can derive the expression for the interference linewidth $\delta\lambda$, defined here as half of the spectral oscillation period:²⁸

$$\delta\lambda \approx \lambda^2 / 2R \left(n_{\text{spp}} - \lambda \frac{dn_{\text{spp}}}{d\lambda} \right). \quad (3)$$

As suggested by this equation and experimentally demonstrated in ref. 28, the interference peaks and valleys can easily be made narrower by increasing the SPP path length R . Note that adjusting R also allows the focused SPP intensity to be tuned through SPP propagation loss. Since the hole radius (r) can also be varied to control the intensity of the directly transmitted light, the geometric parameters can thus be strategically designed to approximately balance the intensities of two interfering components in the spectral range of interest. The resulting spectral fringes with high contrast and relatively narrow linewidths are especially favorable for enhanced spectral sensing. Another important design parameter is the

number of groove couplers. Previous studies on plasmonic interferometry have investigated the use of a single linear nanoslit or nanogroove to excite SPPs.^{26–30} While this approach allows SPP coupling over a broad wavelength range, the generated SPP energy is likely insufficient for further optimization of the device's performance. Our structural design goes beyond previous plasmonic interferometers by introducing multiple circular groove couplers to generate significantly stronger, focused SPPs. This offers more degrees of freedom to optimize and balance the intensities of two interfering components and generates high-contrast interference fringes. Also, this unique design differs from traditional bull's-eye structures that use a periodic Bragg grating for resonant scattering and focus on transmission enhancement only for a single wavelength or a narrow wavelength range.^{37–39} As will be discussed in the next section, here we choose three nanogrooves to balance the trade-off between SPP intensity and sensor working spectral range. The fast spectral oscillation produced over a relatively broad wavelength range serves as a unique advantage of this sensor for optimized spectral sensing.

To examine the interplay between multiple geometric parameters and the device characteristics, we performed three-dimensional finite difference time domain (FDTD) numerical simulations. The dielectric constant of gold used in the simulations was experimentally measured using an ellipsometer (see Fig. S1 in the ESI†). For the parameter optimization process, the preferred groove depth d and width w can be first determined using simulations to maximize the SPP coupling efficiency.⁴¹ Since the white light source used has its maximum power around $\lambda = 625$ nm, the value of P corresponding to this wavelength was calculated using eqn (1) and found to be 430 nm. The parameters R and r were then carefully tuned with the goal of using the largest

possible R , while still balancing the intensities of two interfering components. These simulations yielded an optimal geometric parameter set ($R = 4.3 \mu\text{m}$, $w = 200 \text{ nm}$, $d = 45 \text{ nm}$, $P = 430 \text{ nm}$, and $r = 310 \text{ nm}$) with interfering components perfectly balanced in power and generating broadband, high-contrast, narrow-linewidth spectral interference (see the simulation result in Fig. 1c). Fig. 1d and e show the calculated electric field distributions at the interference peak and valley wavelengths, respectively (see Fig. S2 in the ESI† for the distribution of $|E_x|$ and $|E_y|$ components, respectively). Strong light transmission and perfect light cancellation are clearly visualized at the central hole location, which correspond to the constructive and destructive interferences, respectively.

Device fabrication and spectroscopic characterization

Following the design and optimization of the device geometry, focused ion beam (FIB) milling was used to mill the plasmonic interferometers. The structures were patterned on a 300 nm-thick gold film deposited on a glass substrate that had been previously coated with a 5 nm titanium adhesion layer. Fig. 2a–c show both the scanning electron microscope (SEM) images and the bright-field microscope image of the fabricated interferometers. Here, a large 12×12 array of the proposed interferometers was fabricated to improve the overall light transmission and the spectral signal-to-noise ratio. The device was illuminated from above by a collimated white light beam normally incident on the top sample surface. The blue solid curve in Fig. 2d shows the measured transmission spectrum of the interferometers in a water environment, normalized to that of a reference hole array without the surrounding grooves. This experimental spectrum agrees reasonably well with the simulated result in Fig. 1c, demonstrating the operating principle of the device. The slight discrepancy

likely arises from fabrication imperfections due to difficulties in precise control of groove profile and also from the SPP propagation loss resulting from the gold surface roughness (RMS of 2.9 nm, see Fig. S3 in the ESI†), which is not fully accounted for in the numerical simulations. As one of our major goals is to achieve high modulation contrast for optimized spectral sensing, we now quantify the interference contrast C using the equation $C = (I_{\text{max}} - I_{\text{min}})/(I_{\text{max}} + I_{\text{min}})$.⁴⁰ Here, I_{max} and I_{min} are the adjacent intensity maximum and minimum of the interference fringe, respectively. Because of the balanced intensities of SPPs and free-space light, the experimental contrast of our interferometers (as high as 0.87) greatly exceeds that observed in previous plasmonic interferometers.^{26–30} However, this is still slightly lower than the simulated interference fringe contrast of 0.98, which results from balanced interfering components as well as a perfectly collimated light illumination. In our experiments, the microscope field and aperture diaphragms were both closed to achieve a near-collimated light illumination condition, which, however, still has a 3° light divergence. Higher experimental contrast would be expected by further optimizing the collimating optics.

The red dashed curve in Fig. 2d shows the measured normalized spectrum of an interferometer array with a single circular groove, which clearly exhibits lower interference contrast. From this result, one can see that the use of multiple nanogrooves with properly designed period P increases the generated SPP power and improves the interference amplitude and contrast. Note that while more grooves can be added to further increase the interference peak intensity, the interference contrast decreases when the number of grooves is too high due to resonant Bragg scattering which only efficiently generates SPPs for a specific wavelength.³⁴ Fig. S4 in the ESI† shows the transmission spectra and contrast for a series of interferometers as the number of circular grooves

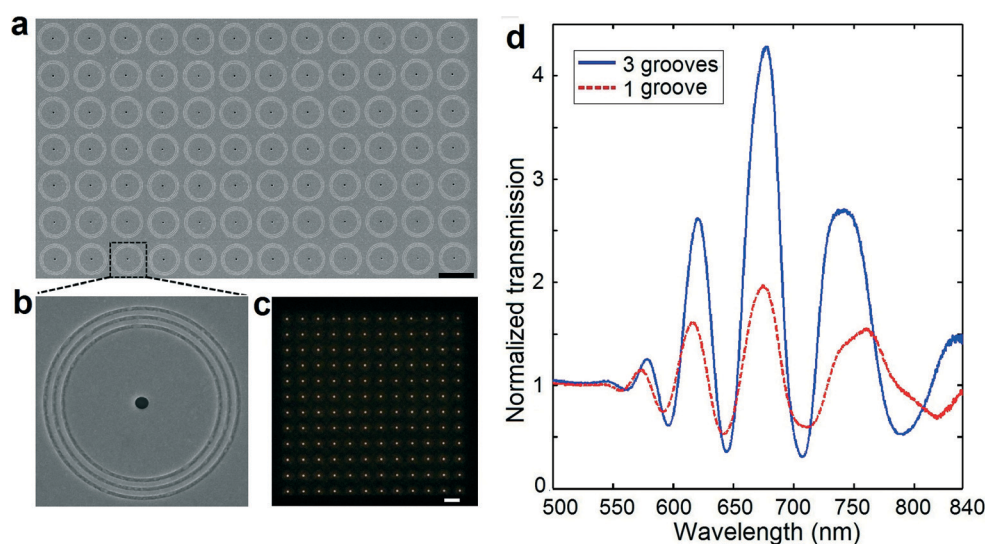


Fig. 2 The fabricated interferometer array and spectroscopy characterization. SEM images of the fabricated interferometer array (a) and one of the interferometers (b). Scale bar: $10 \mu\text{m}$. (c) The bright-field microscopy image of the device. The center-to-center distance between each interferometer is $12.5 \mu\text{m}$, and the sensor array footprint is $150 \times 150 \mu\text{m}^2$. Scale bar: $10 \mu\text{m}$. (d) Experimental spectra for plasmonic interferometer arrays with three circular grooves (blue solid curve) and single circular grooves (red dashed curve).

varies from one to six. In these measurements, the interferometers with three circular grooves exhibit the highest contrast of 0.87. Higher interference contrast is favorable for our multi-spectral sensing measurements and especially for the low-background intensity-based sensing scheme, as will be discussed later. A smaller groove number has the advantage of overall device compactness and ease of fabrication. As a result, in this work, interferometers with three circular grooves were employed for sensing measurements.

Refractometric sensing and multispectral data analysis method

To calibrate the sensor sensitivity and resolution, we integrated the fabricated sensor chip with a polydimethylsiloxane (PDMS) microfluidic channel and injected a series of glycerol–water solutions of different refractive indices. As shown in Fig. 3a, the interference pattern red-shifts upon the increase in the liquid refractive index. The signal changes that occur over a broad spectral range constitute a unique advantage of this interferometric sensor. To exploit this sensor property, we use a multispectral data analysis method,^{42–44} which integrates the magnitude of the relative intensity changes at all wavelengths as the sensor output. This approach improves the detection signal-to-noise ratio and sensor resolution, since more SPP-mediated data are taken into account than for the common single-peak tracking

method. The integrated response (IR) of the sensor can be expressed as:

$$IR = \sum_{\lambda_1}^{\lambda_2} \left(|I(\lambda) - I_0(\lambda)| / I_0(\lambda) \right), \quad (4)$$

where λ_1 and λ_2 define the wavelength range of the integration, and I_0 is the reference transmitted intensity of the sensor in water. Fig. 3b shows the relative intensity changes for liquids of different refractive indices. The signals are most prominent in the spectral range from 570 nm to 800 nm, as indicated by the black dashed lines. The magnitude of the intensity changes was integrated within this spectral range, providing the sensor output IR. As shown in Fig. 3c, the change in IR is approximately proportional to the increase in glycerol concentration, confirming the excellent sensor linearity. Fig. 3d shows the extracted IR values as a function of the refractive index. The linear fit to the data points yields a sensor sensitivity of $2.71 \times 10^6\%$ per RIU. The lower inset of Fig. 3c indicates the noise level of the integrated response, which exhibits a standard deviation of 2.16% on a time scale of 3 minutes. This corresponds therefore to a bulk refractive index resolution of 8.0×10^{-7} RIU (*i.e.*, $2.16\% / 2.71 \times 10^6\%$ per RIU). Note that in this real-time measurement, the time resolution is 10 s (200 spectra are averaged with an integration time of 50 ms for each spectrum). For faster kinetics

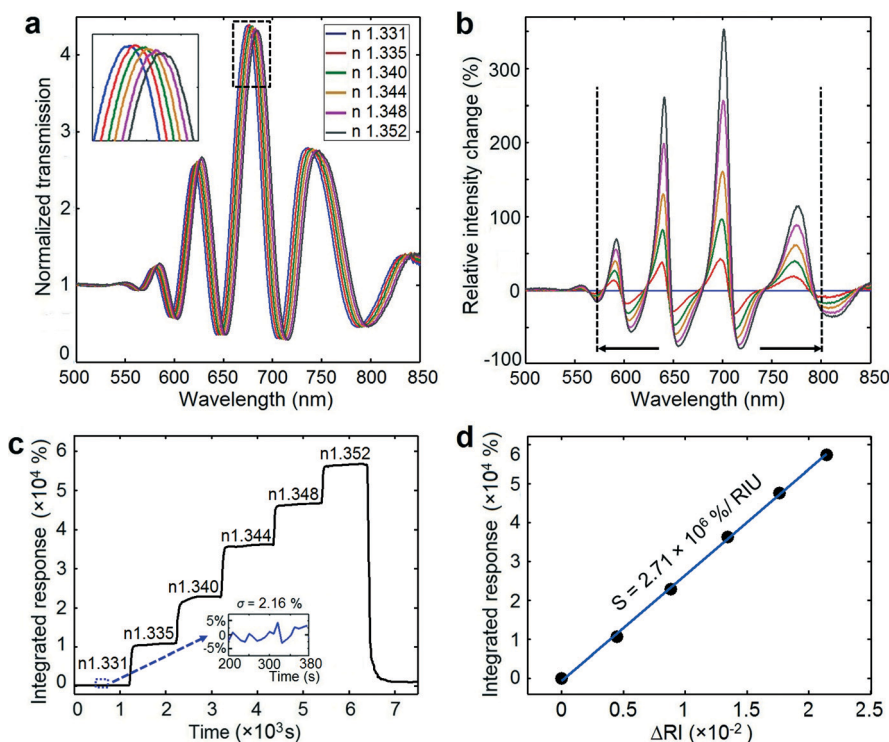


Fig. 3 Refractive index sensing using a plasmonic interferometer array. (a) The normalized transmission spectra of the interferometer array in water and 3, 6, 9, 12, 15% glycerol–water mixtures. (b) The relative intensity changes $(|I(\lambda) - I_0(\lambda)|/I_0(\lambda))$ for liquids with different refractive indices. The dashed lines indicate the spectral region for integration. (c) The integrated sensor response as a function of time. The inset indicates the noise level of the sensor response on a time scale of 3 minutes. (d) The extracted integrated sensor response for liquids with different refractive indices. The blue line is the linear fit to the data points, which shows good sensor linearity.

measurements, a smaller number of spectra can be averaged with a modest increase in sensor noise (see Fig. S5 in the ESI†).

We also measured the sensor resolution by using the common single-peak tracking method. The sensitivity of the interference peak at 674.5 nm is determined to be $470.6 \text{ nm RIU}^{-1}$ (see Fig. S6 in the ESI†), and the standard deviation of the peak position is $7.3 \times 10^{-4} \text{ nm}$, corresponding to a sensor resolution of $1.55 \times 10^{-6} \text{ RIU}$. One can see that this multispectral sensing method improves the sensor resolution by approximately twofold. Our demonstrated sensor resolution surpasses that of state-of-the-art nanohole array sensors ($3.1 \times 10^{-6} \text{ RIU}$)⁴⁵ and is obtained here using a low-cost spectrometer and a simple transmission setup. The improved sensor performance results from the high-contrast, narrow-linewidth interference fringes, the intense transmission peaks from a large interferometer array, and also the broadband sensor response and associated advanced data analysis method. Moreover, several potentially important improvements could further enhance this sensing resolution. First, a larger number of interferometers can be fabricated on a sensor chip using large-area fabrication techniques (*i.e.*, template stripping on a patterned silicon template)⁴⁶ to further increase the spectral signal-to-noise ratio. Second, the interference amplitude is mainly limited by the SPP propagation loss, which could be effectively reduced by employing ultrasmooth metal films.^{19,46} Third, a temperature controller can be integrated with the sensor chip to decrease the sensor noise caused by temperature fluctuations.

Real-time surface biomolecular sensing

While superior bulk sensing resolution was achieved, it does not directly represent the sensor capability for surface biomolecular sensing. In this section, we further demonstrate the feasibility of this platform for biosensing and estimate its detection resolution. We performed label-free real-time monitoring of the specific binding between BSA and anti-BSA molecules. The microfluidic channel was first injected with a PBS buffer for 25 min to rinse the sensor chip and define the baseline of the experiment. A $500 \mu\text{g mL}^{-1}$ BSA solution in PBS buffer was then introduced into the channel and functionalized the surface with a monolayer of BSA molecules. As shown in Fig. 4, a large sensor integrated response IR of $0.91 \times 10^4\%$ was clearly observed (see the first signal change at the time of $1.5 \times 10^3 \text{ s}$). A subsequent 15 min buffer wash has little effect on the sensor response. Then, a $42 \mu\text{g mL}^{-1}$ anti-BSA solution was injected into the channel and followed by a 25 min buffer rinse to wash out the unbound anti-BSA molecules. The small spikes observed at buffer injections are measurement artifacts caused by exchanging syringes. The binding between BSA and anti-BSA leads to an IR change of $0.62 \times 10^4\%$. Note that the saturated BSA monolayer, which has a surface coverage of around 1.7 ng mm^{-2} ,⁴⁷ was detected here with a high signal-to-noise ratio (SNR) of 4213 (*i.e.*, $0.91 \times 10^4\%/2.16\%$). This high SNR, achieved with a low-cost portable spectrometer, exceeds that of

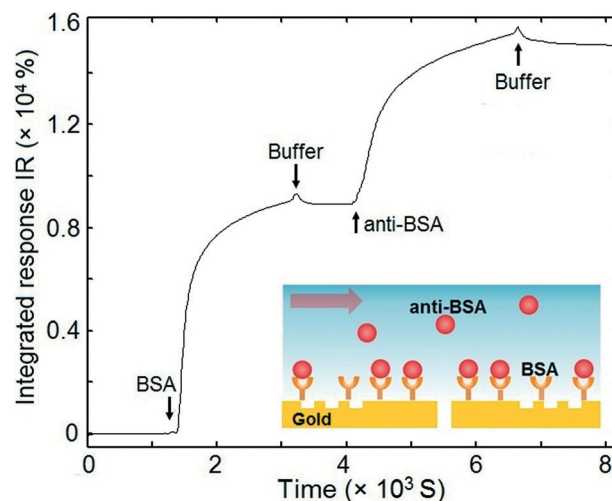


Fig. 4 Real-time integrated sensor response upon BSA adsorption on the sensor surface and subsequent molecular binding between BSA and anti-BSA.

state-of-the-art LSPR biosensors, which exhibit an SNR of ~ 3000 for monolayer protein adsorption and relies on an expensive advanced spectrometer.¹⁴ An SNR of 4213 gives a final sensor resolution of 0.4 pg mm^{-2} (*i.e.*, $1.7 \text{ ng mm}^{-2} / 4213$) for protein surface coverage. This demonstrated that sensor resolution is in fact comparable to that achieved in commercial prism-based SPR systems, which can detect biomolecules down to 0.1 pg mm^{-2} from a sensing area of a few mm^2 .⁶ It is important to note that our sensor footprint ($150 \mu\text{m} \times 150 \mu\text{m}$) is around two orders of magnitude smaller than that of commercial systems. This largely improved sensor resolution per unit area facilitates sensor integration with compact microfluidics and also decreases the sample consumption. Furthermore, the collinear transmission setup of our platform is much simpler than the total internal reflection operational mode of conventional SPR systems and has great potential for system miniaturization, low-cost production, and scaling up to highly-multiplexed sensing measurements. Finally, further improvement in resolution is still possible by employing the optimization methods discussed in the last section. With demonstrated superior performance and possible further improvement, this simple sensor platform may serve as a promising alternative to conventional bulky expensive SPR devices.

Note that in both bulk and bio-sensing experiments, the sensor noise was determined in blank samples (water for bulk sensing and PBS buffer for biosensing). However, for biomolecular detection in complex fluids (serum, urine, *etc.*), other noise terms are present (such as nonspecific biomolecular binding), and the sensor resolution will depend not only on nanostructure design and optical instrumentation but also on sensor surface chemistry and sample complexity.

Low-background intensity-based sensing

In previous sections, we have demonstrated enhanced sensor performance using a spectral sensing method.

However, for applications that require simple and inexpensive instrumentation, a biosensing device based on intensity interrogation is typically preferred.⁴⁸ More importantly, the intensity-based sensing scheme can be easily combined with CCD imaging to advance our sensor platform for scalable high-throughput sensing applications.⁴⁹ In this section, we further investigate the performance of this interferometric platform using intensity interrogation. To better evaluate and compare the sensor performance using this detection scheme, Becker *et al.* have suggested a figure of merit, defined as:³⁶

$$\text{FOM}^* = |dI/I_0|/dn. \quad (5)$$

Here, dI/I_0 is the change in relative intensity at an incident wavelength λ induced by a refractive index change dn . For optimized sensing performance, the sensor operating wavelength λ should be properly chosen to maximize the value of FOM^* . Our interferometric sensor has a fundamental advantage in that balanced destructive interference between SPPs and free-space light leads to extremely low optical transmission through the interferometers ($I_0 \sim 0$). This delicate interference balance can be easily disrupted by a small change in the local refractive index and thus gives rise to a pronounced relative intensity increase in dI/I_0 and a corresponding high FOM^* value. The near-dark reference background I_0 allows the use of stable high-power laser

sources without saturating the detector and produces significantly large intensity-modulated signal change dI for sensitive detection. As shown by the blue curve in Fig. 5a, our experimental FOM^* reaches a maximum value of 146 at a wavelength λ of 700.6 nm, slightly detuned from the interference valley wavelength of 705.1 nm. This observed FOM^* value is higher than that achieved in state-of-the-art plasmonic perfect absorber sensors (87 at 1.7 μm)³⁵ and gold nanorod sensors (24 at 850 nm).³⁶ In addition, our planar single-layer interferometer design is simpler and more easily mass-produced than plasmonic absorbers, which consist of multiple functional layers and rely on reflection measurements.^{35,48} The straightforward transmission geometry of our platform also simplifies the optical design and facilitates system miniaturization. It should be noted that the operational design wavelength of the intensity-based detection scheme must be carefully tailored to match the incident wavelength, which can be accomplished for this platform by appropriately adjusting R . From eqn (2), the relation between the interference valley wavelength λ_v and R can be described as:

$$\frac{\Delta\lambda_v}{\Delta R} = \frac{\lambda_v}{R} = c, \quad (6)$$

where $\Delta\lambda_v$ and ΔR are the changes in λ_v and R , respectively, and c is a constant determined by n_{spp} and ϕ_0 . This equation

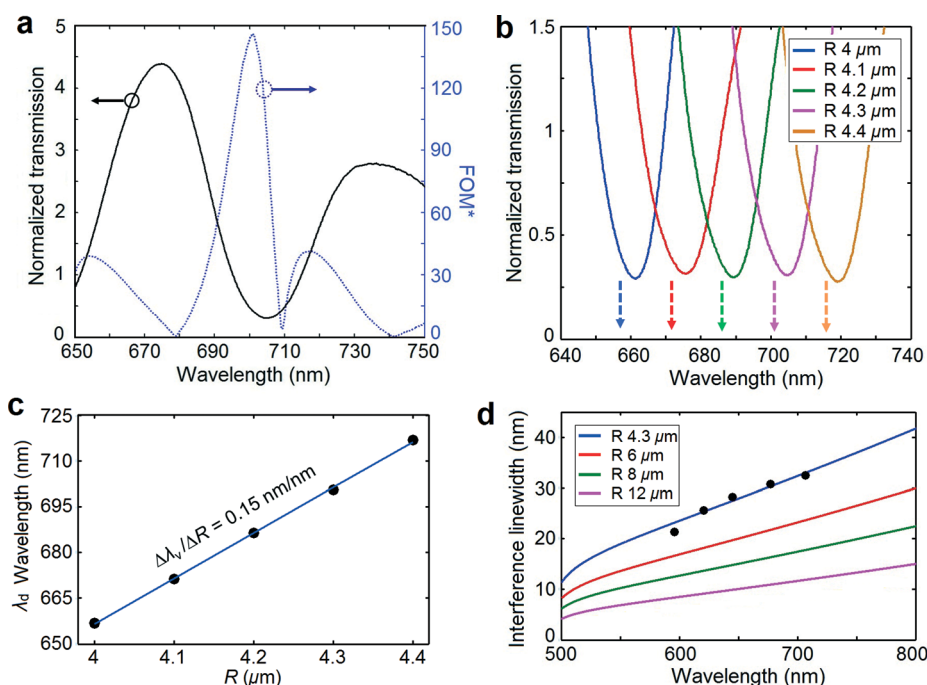


Fig. 5 Low-background interferometric sensing. (a) Experimental FOM^* as a function of wavelength (blue dotted curve). Values of FOM^* were derived using experimental spectra in water and 12% glycerol-water mixture to calculate dI/I_0 at each wavelength. The measured refractive index difference of 0.0177 was used in the denominator to calculate FOM^* . The black solid curve represents the transmission spectrum of interferometers in water. (b) Normalized spectra of interferometers with tailored R values of 4, 4.1, 4.2, 4.3, and 4.4 μm . The sensor operational wavelengths of 656.8, 671.3, 686.4, 700.6, and 716.8 nm (colored dashed arrows) are slightly detuned from their interference valley wavelengths. (c) The black dots represent the sensor operational wavelengths at different R values. The blue line is the linear fit to the data points. (d) The solid curves show the calculated interference linewidths for interferometers with R values of 4.3, 6, 8, and 12 μm . The black dots are experimental linewidths of interferometers with an R value of 4.3 μm , extracted from Fig. 2d.

constitutes a simple design rule, which we can easily use to tune λ_d by suitably varying R . In Fig. 5b, we measured the transmission spectra for a series of interferometers with gradually increasing R from 4 to 4.4 μm . The corresponding operating wavelength clearly red-shifts with the increase in R and is plotted in Fig. 5c. The slope of the linear fit to the data points gives $\Delta\lambda_d/\Delta R = 0.15 \text{ nm nm}^{-1}$, in good agreement with λ_d/R of 0.16 nm nm^{-1} and verifying the proposed design rule.

From the discussion above, one can see that similar to plasmonic perfect absorber sensors, the key issue in designing this interferometric sensor is achieving near-perfect light suppression through destructive SPP–light interference. This can be realized under two conditions. First, all geometric parameters of the interferometers need to be carefully designed to balance the intensities of SPPs and directly transmitted light near the sensor operating wavelength. Second, perfectly collimated illumination is required to eliminate additional SPP–light phase lag caused by different incident angles.⁴⁰ While the first condition can be fulfilled through numerical simulations and fine-tuning of the structure geometry during fabrication, the microscope-based optical setup used in this work has a 3° light divergence. A laser source and related collimating optics are being set up for better light collimation and to further optimize this intensity-based detection scheme. In addition, to achieve perfect light cancellation, another way to optimize the sensor performance is to narrow the interference linewidth and thus to increase the values of dI/dn . As suggested by eqn (3), this can be realized by increasing the SPP path length R . The curves in Fig. 5d show analytically calculated interference linewidths for R values of 4.3, 6, 8, and 12 μm , respectively. The interference linewidths clearly decrease with increasing R . The black dots represent extracted experimental linewidths for interferometers with an R value of 4.3 μm in Fig. 2, which exhibit close agreement with the analytical results and confirm the proposed analytical model. The increasing SPP loss associated with larger R can be easily compensated by fabricating a greater number of circular grooves to increase the generated SPP power. Note that while this method is not suitable for broadband spectral sensing as discussed in Fig. S4,[†] it does not strongly affect single-wavelength intensity-based detection. As a result, the rational design of R and the groove number should lead to further improvement in the sensing FOM* and corresponding sensor performance of this interferometric platform. Since this intensity-based detection can be performed by simply using a single-wavelength light source and a photodetector in a straightforward transmission geometry, it has the potential to significantly reduce the instrumentation cost of the biosensing device. Furthermore, a CCD camera can be integrated into this sensing platform for the simultaneous monitoring of the light transmission from multiple sensor elements, making real-time highly-multiplexed detection possible. Another unique advantage of this sensor design is the minimal crosstalk between sensing pixels (see Fig. S7[†]), which is especially useful to increase the sensor packing density for high-throughput detection. The use

of this novel interferometer platform for sensitive high-throughput biosensing will be reported in another work.

Methods and materials

Fabrication of plasmonic interferometers

E-beam evaporation (Indel system) was used to deposit a 5 nm thick titanium film and subsequently a 300 nm thick gold film onto a pre-cleaned standard glass microscope slide (Fisher Scientific). The deposition rate was 0.1 nm s^{-1} for the titanium layer and the first 50 nm gold film and 1 nm s^{-1} for the remaining 250 nm gold film. FIB (FEI Dual-Beam System 235) milling (Ga^+ ions, 30 kV, 50 pA) was used to fabricate arrays of aperture-grating structures with a center-to-center distance of 12.5 μm between each interferometer. A 12×12 interferometer array thus has a sensor footprint of around $150 \times 150 \mu\text{m}^2$. R , w , r , and P were carefully measured using SEM. Atomic force microscopy (AFM, NT-MDT Solver NEXT) was used to determine the depth of the circular grooves, which was approximately 45 nm. Another identical aperture array without circular grooves was also fabricated as a reference for spectrum normalization. After the FIB milling, the sensor chip was cleaned using oxygen plasma (PX-250, March Instruments) and bound to a microfluidic flow cell using a home-built clamp.

Optical measurement

A 100 W halogen lamp was used to illuminate the sample through the microscope condenser of an Olympus IX 81 inverted microscope. The field and aperture diaphragms of the microscope were both closed to obtain a near-collimated light beam (3° divergence). The transmitted light emanating from the device was collected by a $40\times$ objective lens (with numerical aperture $\text{NA} = 0.6$) and coupled to a fiber-optic linear CCD array spectrometer (Ocean Optics, USB4000) for spectral measurements. A CCD camera (COOKE SensiCam QE) was employed to record the positions of the interferometer arrays. Under identical experimental conditions, the reference spectrum was measured by moving the reference aperture array to the recorded interferometer array position.

Bulk and biomolecular sensing experiments

For sensitivity calibration, the refractive indices of water–glycerol mixtures (0, 3, 6, 9, 12, and 15% glycerol concentration) were measured using a spectroscopic ellipsometer (J. A. Woollam, V-VASE), ranging from 1.331 to 1.352. The PBS 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 $20 \mu\text{L min}^{-1}$. For both spectral measurements, the integration time of the spectrometer is set to 50 ms, and each spectrum shown in this work represents an average of 200 acquisitions.

Numerical calculations

Simulations of the transmission spectra and electric field distributions of the interferometers were carried out using a three-dimensional (3D) finite-difference time-domain (FDTD) commercial software package (Lumerical Solutions Inc.). The permittivity of the Au used in the simulations was measured using a spectroscopic ellipsometer (J. A. Woollam). The perfectly matched layer boundary condition was used in the x, y and z axes in our 3D simulations. A mesh size of 2 nm was utilized.

Conclusions

In summary, we have proposed a novel plasmonic interferometer design that allows flexible control of the phase and amplitude of interfering SPPs, opening a new route to control the plasmon line shape for high-performance plasmonic sensing. We have observed spectral interference fringes with high contrast, large amplitudes, and narrow linewidths and achieved an enhanced spectral sensing resolution of 0.4 pg mm^{-2} within a small ($150 \text{ }\mu\text{m} \times 150 \text{ }\mu\text{m}$) sensing area. This demonstrated superior resolution and simple optical geometry suggest exciting potential for low-cost portable biosensing devices, which would significantly impact point-of-care diagnostics and personal healthcare applications. In addition, we demonstrated sensitive intensity-based detection using a novel low-background interferometric sensing concept and achieved a high FOM* value of 146. This surpasses that of previous plasmonic sensors and still holds potential for further improvement through the optimization of the device's structure. This intensity-based detection scheme can extend the sensing capability of our platform to scalable high-throughput assays through the incorporation of CCD imaging techniques and may serve as a promising tool for future fast drug discovery, screening of novel therapies, and proteomics and basic biology research.

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Notes and references

- 1 C. Genet and T. W. Ebbesen, *Nature*, 2007, **445**, 39.
- 2 J. A. Schuller, E. S. Barnard, W. Cai, Y. C. Jun, J. S. White and M. L. Brongersma, *Nat. Mater.*, 2010, **9**, 193.
- 3 S. A. Maier, *Plasmonics: Fundamental and Applications*, Springer, New York, USA 2007.
- 4 A. G. Brolo, *Nat. Photonics*, 2012, **6**, 709.
- 5 J. N. Anker, W. P. Hall, O. Lyandres, N. C. Shah, J. Zhao and R. P. Van Duyne, *Nat. Mater.*, 2008, **7**, 442.
- 6 J. Homola, *Chem. Rev.*, 2008, **108**, 462.
- 7 K. M. Mayer and J. H. Hafner, *Chem. Rev.*, 2011, **111**, 3828.
- 8 K. A. Tetz, L. Pang and Y. Fainman, *Opt. Lett.*, 2006, **31**, 1528.
- 9 F. B. Myers and L. P. Lee, *Lab Chip*, 2008, **8**, 2015.
- 10 C. Valsecchi and A. G. Brolo, *Langmuir*, 2013, **29**, 5638.
- 11 Y. Gao, Q. Gan and F. J. Bartoli, *IEEE J. Sel. Top. Quantum Electron.*, 2014, **20**, 6900306.
- 12 N. C. Lindquist, A. Lesuffleur, H. Im and S-H. Oh, *Lab Chip*, 2009, **9**, 382.
- 13 J. Ji, G. O'Connell, D. J. D. Carter and D. N. Larson, *Anal. Chem.*, 2008, **80**, 2491.
- 14 A. B. Dahlin, S. Chen, M. P. Jonsson, L. Gunnarsson, M. Käll and F. Höök, *Anal. Chem.*, 2009, **81**, 6572.
- 15 A. B. Dahlin, J. O. Tegenfeldt and F. Höök, *Anal. Chem.*, 2006, **78**, 4416.
- 16 J. Ruemmele, W. P. Hall, L. K. Ruvuna and R. P. Van Duyne, *Anal. Chem.*, 2013, **85**, 4560.
- 17 T. Endo, K. Kerman, N. Nagatani, H. M. Hiepa, D. K. Kim, Y. Yonezawa, K. Nakano and E. Tamiya, *Anal. Chem.*, 2006, **78**, 6465.
- 18 J. C. Yang, J. Ji, J. M. Hogle and D. N. Larson, *Nano Lett.*, 2008, **8**, 2718.
- 19 S. H. Lee, T. W. Johnson, N. C. Lindquist, H. Im, D. J. Norris and S-H. Oh, *Adv. Funct. Mater.*, 2012, **22**, 4439.
- 20 A. A. Yanik, A. E. Cetin, M. Huang, A. Artar, S. H. Mousavi, A. Khanikaev, J. H. Connor, G. Shvets and H. Altug, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 11784.
- 21 N. Liu, T. Weiss, M. Mesch, L. Langguth, U. Eigenthaler, M. Hirscher, C. Sonnichsen and H. Giessen, *Nano Lett.*, 2010, **10**, 1103.
- 22 N. Verellen, P. Van Dorpe, C. J. Huang, K. Lodewijks, G. A. E. Vandenbosch, L. Lagae and V. V. Moshchalkov, *Nano Lett.*, 2011, **11**, 391.
- 23 S. Zhang, K. Bao, N. J. Halas, H. Xu and P. Nordlander, *Nano Lett.*, 2011, **11**, 1657.
- 24 B. Gallinet and O. J. F. Martin, *ACS Nano*, 2011, **5**, 8999.
- 25 M. Rahmani, D. Y. Lei, V. Giannini, B. Lukiyanchuk, M. Ranjbar, T. Y. F. Liew, M. Hong and S. A. Maier, *Nano Lett.*, 2012, **12**, 2101.
- 26 Q. Gan, Y. Gao and F. J. Bartoli, *Opt. Express*, 2009, **17**, 20747.
- 27 Y. Gao, Q. Gan, Z. Xin, X. Cheng and F. J. Bartoli, *ACS Nano*, 2011, **5**, 9836.
- 28 Y. Gao, Z. Xin, Q. Gan, X. Cheng and F. J. Bartoli, *Opt. Express*, 2013, **21**, 5859.
- 29 J. Feng, V. S. Siu, A. Roelke, V. Mehta, S. Y. Rhieu, G. T. R. Palmore and D. Pacifici, *Nano Lett.*, 2012, **12**, 602.
- 30 X. Wu, J. Zhang, J. Chen, C. Zhao and Q. Gong, *Opt. Lett.*, 2009, **34**, 392.
- 31 X. Li, Q. Tan, B. Bai and G. Jin, *Opt. Express*, 2011, **19**, 20691.
- 32 P. Dvořák, T. Neuman, L. Břínek, T. Šamořil, R. Kalousek, P. Dub, P. Varga and T. Šikola, *Nano Lett.*, 2013, **13**, 2558.
- 33 D. Martín-Becerra, G. Armelles, M. U. González and A. García-Martín, *New J. Phys.*, 2013, **15**, 085021.
- 34 Z. Liu, J. M. Steele, W. Srituravanich, Y. Pikus, C. Sun and X. Zhang, *Nano Lett.*, 2005, **5**, 1726.
- 35 N. Liu, M. Mesch, T. Weiss, M. Hentschel and H. Giessen, *Nano Lett.*, 2010, **10**, 2342.
- 36 J. Becker, A. Trugler, A. Jakab, U. Hohenester and C. Sonnichsen, *Plasmonics*, 2010, **5**, 161.

- 37 T. Thio, K. M. Pellerin, R. A. Linke, H. J. Lezec and T. W. Ebbesen, *Opt. Lett.*, 2001, **26**, 1972.
- 38 E. Laux, C. Genet, T. Skauli and T. W. Ebbesen, *Nat. Photonics*, 2008, **2**, 161.
- 39 O. Mahboub, S. C. Palacios, C. Genet, F. J. Garcia-Vidal, S. G. Rodrigo, L. Martin-Moreno and T. W. Ebbesen, *Opt. Express*, 2010, **18**, 11292.
- 40 G. Gay, O. Alloschery, B. V. de Leseqno, J. Weiner and H. J. Lezec, *Phys. Rev. Lett.*, 2006, **96**, 213901.
- 41 Q. Gan, Y. Gao, Q. Wang, L. Zhu and F. J. Bartoli, *Phys. Rev. B: Condens. Matter Mater. Phys.*, 2010, **81**, 085443.
- 42 M. E. Stewart, N. H. Mack, V. Malyarchuk, J. Soares, T. W. Lee, S. K. Gray, R. G. Nuzzo and J. A. Rogers, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 17143.
- 43 K. L. Lee and P. K. Wei, *Small*, 2010, **6**, 1900.
- 44 S.-H. Wu, K.-L. Lee, A. Chiou, X. Cheng and P.-K. Wei, *Small*, 2013, **9**, 3532.
- 45 H. Im, J. N. Sutherland, J. A. Maynard and S.-H. Oh, *Anal. Chem.*, 2012, **84**, 1941.
- 46 N. C. Lindquist, T. W. Johnson, D. J. Norris and S.-H. Oh, *Nano Lett.*, 2011, **11**, 3526.
- 47 L. S. Jung, C. T. Campbell, T. M. Chinowsky, M. N. Mar and S. S. Yee, *Langmuir*, 1998, **14**, 5636.
- 48 A. Cattoni, P. Ghenuche, A. M. Haghiri-Gosnet, D. Decanini, J. Chen, J. L. Pelouard and S. Collin, *Nano Lett.*, 2011, **11**, 3557.
- 49 T. Y. Chang, M. Huang, A. A. Yanik, H. Y. Tsai, P. Shi, S. Aksu, M. F. Yanik and H. Altug, *Lab Chip*, 2011, **11**, 3596.