

Long-range surface plasmon Y-junctions for referenced biosensing

Wei Ru Wong,¹ Faisal Rafiq Mahamd Adikan,¹ and Pierre Berini^{2,3,4,*}

¹*Integrated Lightwave Research Group, Department of Electrical Engineering, Faculty of Engineering, University of Malaya, 50603 Kuala Lumpur, Malaysia*

²*School of Electrical Engineering and Computer Science, University of Ottawa, 800 King Edward Ave., Ottawa, K1N6N5, Canada*

³*Department of Physics, University of Ottawa, 150 Louis Pasteur, Ottawa, K1N 6N5, Canada*

⁴*Centre for Research in Photonics, University of Ottawa, Ottawa, ON K1N 6N5, Canada*
berini@eecs.uottawa.ca

Abstract: Long-range surface plasmon Y-junctions are demonstrated as sensors for the detection of bulk refractive index changes in solution and for protein binding. Using a fully-cladded Au stripe waveguide as a reference channel, common drift and noise in the system can be eliminated, relaxing the need for precise optical alignments. The performance of the structure is discussed theoretically, then bulk sensing is carried out experimentally with five solutions of different refractive indices, and protein sensing is demonstrated through physisorption of bovine serum albumin on a carboxyl-terminated Au stripe. The Y-junction biosensor demonstrated a very good ability to perform drift and noise suppression for fast and accurate biosensing.

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1. Introduction

Long-range surface plasmon polaritons (LRSPPs) are optical surface waves excited by transverse magnetic (TM) polarized incident light propagating along a thin metal stripe or slab bounded by symmetric dielectrics [1]. The optical confinement of LRSPPs on metal stripes and their ability to propagate up to centimetres along the waveguides leads to the realization of integrated components, such as S-bends, Y-junctions, couplers and Mach-Zehnder Interferometers (MZIs) [2, 3]. Although the surface sensitivity of single-interface SPPs is higher, the increased propagation length of LRSPPs enables a better overall sensitivity due to increased optical interaction length [4]. Furthermore, the ease of excitation of LRSPPs by butt-coupling a polarisation-maintaining single-mode optical fibre (PM-SMF) to the input waveguide allows miniaturization. LRSP waveguides are sensitive to bulk and surface changes because the mode is bound to the surface of the metal, has fields that peak thereon, and propagates mostly in the background dielectric. Any minor change along the metal surface will affect the mode, changing the waveguide's transmittance. LRSPPs on metal slabs have been demonstrated in sensing experiments using prism-coupled geometries [5–8]. Au is a preferred sensing surface and is most studied [9], but dielectric waveguides in integrated geometries have also been tested for biosensing applications [10–12].

We previously demonstrated the ability of straight LRSP waveguides to detect small changes in the bulk refractive index of solutions and the adsorption of bovine serum albumin (BSA) [13], to perform immunological blood typing of human red blood cells [14], to detect dengue antibody and antigen in infected patient blood plasma [15, 16], to selectively detect gram negative or gram positive bacteria in human urine [17], and to detect leukemic markers in patient blood serum [18]. The surface sensitivity and optimization of straight waveguide biosensors were also discussed [19]. The key challenge in biosensing with straight LRSP

waveguides is achieving a stable baseline signal because the measurand is taken as the output power directly. Thus, before each biosensing experiment, a time-consuming alignment process must be carried out to eliminate any drift in the baseline signal so that the response due to protein binding can be easily identified. Power fluctuations originating from the laser source at the input (coupling) or in the interrogation system can be difficult to remove. To automatically eliminate drift and power fluctuations in the system, a reference channel can be introduced in the design of the biosensor [20].

A simple structure that enables referenced biosensing (one sensing channel and one reference channel) is a Y-junction. A straightforward Y-junction design consists of an input waveguide and two branching waveguides, formed by overlapping and mirrored S-bends (two curved sections bending in opposite directions). Such a design, implemented to operate with LRSPPs, as Au stripes fully embedded in CYTOP [21] (a fluoropolymer with a refractive index close to that of the biologically compatible fluids), was discussed and characterised in terms of its passive operation (insertion, transition, and propagation losses) in previous work [3, 22]. The Y-junction biosensor should have better bulk and surface sensing detection limits than straight waveguides [13, 19] because the common noise and drift in the system are eliminated. In principle, phase-dependent MZI biosensors [4, 23] are better than attenuation-based biosensors, such as straight and Y-junction waveguides. However, attenuation-based biosensors are easier to use, especially the Y-junction biosensor for which a stable baseline is easier to achieve. Prism-coupled LRSPP biosensors [5–8] are used under angular or wavelength interrogation and are comparatively bulkier.

In this paper, we define a sensing channel by etching the top cladding over one arm of a Y-junction to expose the top surface of the Au stripe, and we investigate the performance of the structure as a referenced biosensor. We first derive and model various quantities of interest including the LRSPP bulk and surface sensitivities, and we discuss theoretically drift cancellation. We then investigate experimentally the performance of the biosensor subject to changes in the bulk refractive index of the sensing solution, and to the formation of a protein adlayer through physisorption of BSA on a carboxyl-terminated thiol on the Au sensing stripe. Finally, we demonstrate experimentally the benefits of having a reference channel for the removal of drift and fluctuations in the system.

2. Theoretical

2.1 Y-junction biosensor structure

Figure 1(a) shows a microscope image of a fabricated Y-junction biosensor with one arm etched to define a fluidic channel (and the sensing region), while leaving the other arm fully cladded in CYTOP to define the reference arm. The Y-junction combines two mirrored and overlapped S-bends with a 1 μm inner waveguide separation at the split [22]. The S-bends were designed with a radius of curvature $R = 5.5$ mm to minimize the radiation loss at the curved sections [22]. The separation between the output arms is 160 μm . Straight segments were added to the input and both outputs of the Y-junction. The die is $L_D = 3.5$ mm long.

The Y-junction biosensor is comprised of Au stripes of thickness $t = 35$ nm and width $w = 5$ μm embedded in CYTOP claddings (~ 8 μm thick top and bottom). A microfluidic channel 80 μm wide and $L_F = 1.65$ mm long is selectively etched down to the Au stripe through the top CYTOP cladding. The structure is designed to operate at a wavelength of 1310 nm.

2.2 Y-junction biosensor performance and sensitivities

The Y-junction is non-radiative with loss parameters as labelled in Fig. 1(c). The root-mean-squared electric field phasor at the output of the reference and sensing arms is given by:

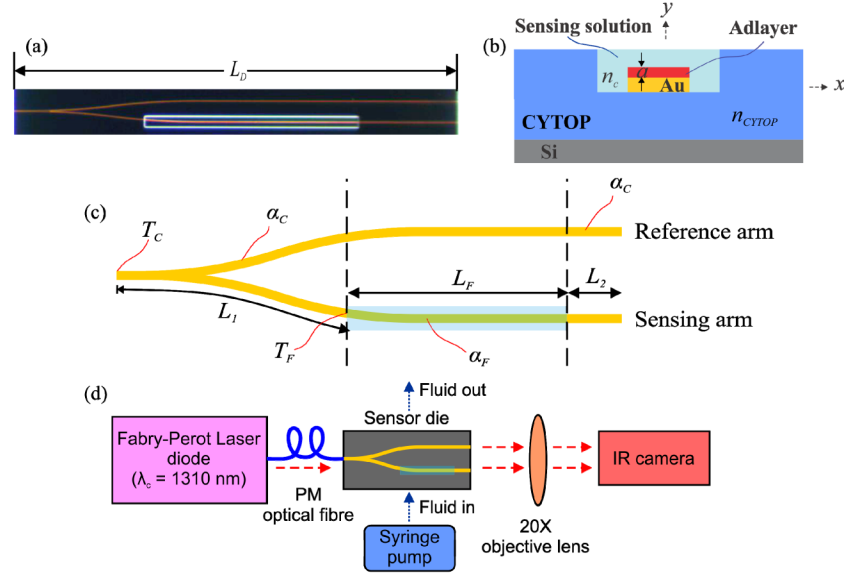


Fig. 1. (a) Microscope image of a fabricated Y-junction biosensor with one arm etched to define a fluidic channel (sensing region); the reference arm remains cladded. (b) Front cross section of the sensing region. (c) Sketch of a Y-junction biosensor highlighting dimensions and loss parameters of relevance. (d) Schematic of the experimental setup.

$$E_{out,C} = E_{inc} \exp(-\alpha_C L_0) \sqrt{T_C} (p \exp(-\alpha_C L_F) \exp(-j\phi_R)) \quad (1)$$

$$E_{out,F} = E_{inc} \exp(-\alpha_C L_0) \sqrt{T_C} (q \sqrt{T_F} \sqrt{T_F} \exp(-\alpha_F L_F) \exp(-j\phi_F)) \quad (2)$$

where $E_{out,C}$ and $E_{out,F}$ pertain to the reference and sensing arms, respectively, E_{inc} is the electric field incident on the waveguide input facet, T_C and T_F are the transmittances at the input facet and at an interface between cladded and fluidic waveguides, α_C and α_F are the mode field attenuation constants of the cladded and fluidic waveguides, L_0 is the optical path length of the input and output sections ($L_0 = L_I + L_2$), L_F is the length of fluidic channel, ϕ_F is the insertion phase through the fluidic channel, and ϕ_R is the insertion phase through the reference arm of the same length (L_F). The parameters p and q form the splitting ratio of the Y-junction where $p^2 + q^2 = 1$. In the ideal case, the Y-junction provides equal split ($p = q = 1/\sqrt{2}$). However, in an actual experiment, fabrication imperfections or a slight misalignment between the optical fiber and the input waveguide can cause the splitting ratio to vary. The transition losses due to the mode mismatch of a straight waveguide and a curved waveguide [3, 22] are small and thus neglected throughout the paper.

The powers output from the Y-junction are:

$$P_{out,C} = E_{out,C} E_{out,C}^* \quad (3)$$

$$= p^2 P_{inc} T_C \exp(-2\alpha_C L_0) \exp(-2\alpha_C L_F) \quad (4)$$

$$P_{out,F} = E_{out,F} E_{out,F}^* \quad (5)$$

$$= q^2 P_{inc} T_C T_F^2 \exp(-2\alpha_C L_0) \exp(-2\alpha_F L_F) \quad (6)$$

where $P_{inc} = E_{inc} E_{inc}^*$. The insertion losses of the Y-junction channels (in dB) are therefore:

$$IL_C = -10\log_{10}(P_{out,C} / P_{inc}) = 20\log_{10}p + C_{dB,C} + L_0MPA_C + L_FMPA_C \quad (7)$$

$$IL_F = -10\log_{10}(P_{out,F} / P_{inc}) = 20\log_{10}q + C_{dB,C} + 2C_{dB,F} + L_0MPA_C + L_FMPA_F \quad (8)$$

where $C_{dB,C} = -10\log_{10}T_C$, $C_{dB,F} = -10\log_{10}T_F$, $MPA_C = 20\alpha_C\log_{10}e$, and $MPA_F = 20\alpha_F\log_{10}e$.

Dividing the output power from the sensing arm by that from the reference arm yields:

$$\frac{P_{out,F}}{P_{out,C}} = \frac{q^2 P_{inc} T_C T_F^2 \exp(-2\alpha_C L_0) \exp(-2\alpha_F L_F)}{p^2 P_{inc} T_C \exp(-2\alpha_C L_0) \exp(-2\alpha_C L_F)} \quad (9)$$

$$= \left(\frac{q}{p}\right)^2 T_F^2 \exp(-2L_F(\alpha_F - \alpha_C)) \quad (10)$$

$$= \left(\frac{q}{p}\right)^2 T_F^2 \exp(-2L_F \Delta\alpha) \quad (11)$$

where $\Delta\alpha = \alpha_F - \alpha_C$ is the difference in the mode field attenuation due to perturbations in the fluidic channel. It is clear from Eq. (11) that forming the power ratio $P_{out,F}/P_{out,C}$ eliminates two major causes of signal instability: the drift in signal due to drift in input coupling T_C and fluctuations inherited from the laser source P_{inc} .

To ease the following derivations, the Y-junction is assumed to have a 50:50 splitting ratio (equal split). Equation (11) then becomes:

$$\frac{P_{out,F}}{P_{out,C}}(n_c, a) = T_F(n_c, a)^2 \exp(-2L_F \Delta\alpha(n_c, a)) \quad (12)$$

where we show explicitly the dependence of parameters on the sensing medium index n_c , and the thickness of an adlayer a (of refractive index $n_a > n_c$) modelling the formation of a thin biochemical adlayer along the sensing stripe, as shown in Fig. 1(b).

The bulk sensitivity of the referenced Y-junction is obtained by taking the derivative of the power ratio with respect to n_c :

$$\frac{\partial}{\partial n_c} \left(\frac{P_{out,F}}{P_{out,C}}(n_c) \right) = \frac{\partial}{\partial n_c} \left(T_F(n_c)^2 \exp(-2L_F \Delta\alpha(n_c)) \right) \quad (13)$$

$$= 2T_F(n_c) \exp(-2L_F \Delta\alpha(n_c)) \left[\frac{\partial T_F(n_c)}{\partial n_c} - L_F T_F(n_c) \frac{\partial \alpha_F(n_c)}{\partial n_c} \right] \quad (14)$$

The surface sensitivity, defined in a similar way, is given by:

$$\frac{\partial}{\partial a} \left(\frac{P_{out,F}}{P_{out,C}}(a) \right) = 2T_F(a) \exp(-2L_F \Delta\alpha(a)) \left[\frac{\partial T_F(a)}{\partial a} - L_F T_F(a) \frac{\partial \alpha_F(a)}{\partial a} \right] \quad (15)$$

The surface sensitivity of the referenced Y-junction is similar to that of a straight waveguide [19], but is independent of the incident power P_{inc} and the input transmittance (coupling) T_C .

The bulk sensitivity of the referenced Y-junction $\partial(P_{out,F}/P_{out,C})/\partial n_c$ is computed by assuming no adlayer on the sensing waveguide ($a = 0$) and approximating the differentials in Eq. (14) via second-order error $O(h^2)$ central finite-difference formulae [24]:

$$\frac{\partial T_F(n_c)}{\partial n_c} = \frac{T_F(n_c + h) - T_F(n_c - h)}{2h} \quad (16)$$

$$\frac{\partial \alpha_F(n_c)}{\partial n_c} = \frac{\alpha_F(n_c + h) - \alpha_F(n_c - h)}{2h} \quad (17)$$

In the computations we set $n_c = 1.3348$ (sensing fluid index equals that of CYTOP, n_{CYTOP}), $h = 10^{-4}$ and we use the finite element method (FEM) to compute the modal properties of the LRSPP on the sensing waveguide (see inset of Fig. 2(a)), yielding T_F and α_F for each n_c , following [19]. The bulk sensitivity of the Y-junction as a function of the waveguide thickness t is plotted in Fig. 2(a) for a fluidic channel length of $L_F = 1.65$ mm. The bulk sensitivity increases over the range of Au thickness considered, however, it is important to note that the attenuation of the waveguides also increases with Au thickness, so the insertion loss should also be considered in order to have output powers that are sufficiently above the noise level of the set-up. The insertion losses of the reference and sensing channels are computed using Eqs. (7) and (8) and plotted in Fig. 2(d).

The surface sensitivity of the referenced Y-junction $\partial(P_{out,F}/P_{out,C})/\partial a$ is computed at a sensing index of $n_c = 1.338$ (the sensing index used for protein sensing in the experimental section), and the differentials in Eq. (15) are approximated via second-order error $O(h^2)$ forward finite-difference formulae [19]:

$$\frac{\partial T_F(a)}{\partial a} = \frac{-3T_F(a) + 4T_F(a + h) - T_F(a + 2h)}{2h} \quad (18)$$

$$\frac{\partial \alpha_F(a)}{\partial a} = \frac{-3\alpha_F(a) + 4\alpha_F(a + h) - \alpha_F(a + 2h)}{2h} \quad (19)$$

We take $h = 1$ nm and $a = 0$ nm for the computations. In Fig. 2(b), the surface sensitivity at $n_c = 1.338$ is noted to first increase with the waveguide thickness to reach a maximum value near $t = 45$ nm and then decrease sharply as t increases further. The surface sensitivity at $n_c = 1.3348$ increases with the Au thickness, and at $n_c = 1.3303$ it generally decreases with t . In Fig. 2(c), we note that at different sensing indices the attenuation sensitivity $\partial\alpha_F(0)/\partial a$ dominates the surface sensitivity of the Y-junction over the range of Au thickness considered.

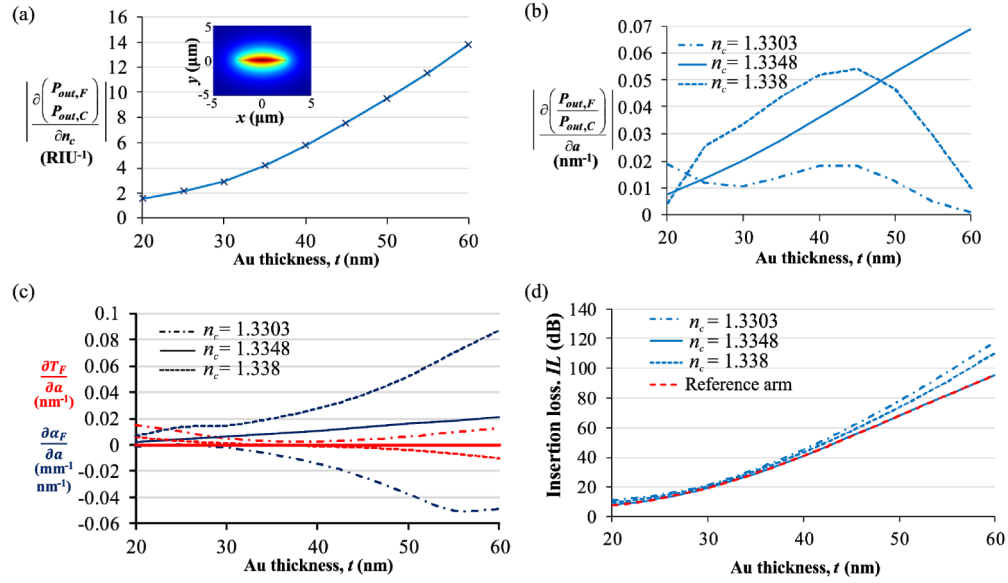


Fig. 2. Computed Y-junction sensitivities. (a) Bulk sensitivity at $n_c = 1.3348$ ($n_c = n_{CYTOP}$); inset shows the field profile (E_y) of the LRSP at $t = 35$ nm and $n_c = 1.3348$. (b) Surface sensitivity $\partial(P_{out,F}/P_{out,C}(0))/\partial a$ at $n_c = 1.3303$, 1.3348 and 1.338. (c) Partial derivatives $\partial T_F(0)/\partial a$ and $\partial \alpha_F(0)/\partial a$ at $n_c = 1.3303$, 1.3348 and 1.338. (d) Insertion loss of the Y-junction channels.

3. Experimental

3.1 Materials and method

3.1.1 Device and setup

Figure 1(a) shows a microscope image of the Y-junction used in the experiments and Fig. 1(d) illustrates a schematic of the experimental setup. The detailed fabrication process of the device was described in [25, 26]. An optical signal provided by a Fabry-Perot laser (FPL1053P, $\lambda_0 = 1310$ nm, Thorlabs) is butt-coupled to the input facet of the waveguide through a polarisation-maintaining (PM) optical fibre (LPF-D1-1300-7/125-P-0.44-1.1-3.2GR, 1.4AS-50-3A-1-1, OZ Optics). The outputs from the Y-junction are collimated by a 20 $\times$ microscope objective (M-20X, Newport) and are then captured by an infrared camera (7290A, MicronViewer). The mode images are recorded every second in real time using frame grabber software (LBA-710PC, Ophir Spiricon), and post-processed using Matlab. The two mode outputs from the Y-junction channels are isolated through the definition of software apertures and the power in each mode is computed by numerical integration of the pixel values within each aperture. The actual power in a mode is obtained through multiplication by a constant which is determined via the calibration of the output power of a straight waveguide using a reference power sensor (8153A, Hewlett Packard).

A custom-made fluidic fixture [13] is used to hold the die under test and provide fluidic inlet and outlets to the microfluidic channel on chip. All solutions were injected over the device by a syringe pump (PicoPlus, Harvard Apparatus) through microfluidic tubing (550 μ m outer diameter, 250 μ m inner diameter, IDEX).

3.1.2 Chemicals and reagents

2-Isopropanol semiconductor grade (IPA), 16-Mercaptohexadecanoic acid (16-MHA), acetone HPLC grade $\geq 99.9\%$, glycerol (electrophoresis grade), bovine serum albumin (BSA), heptane and phosphate buffered saline (PBS) 0.01 M, pH 7.4 were obtained from Sigma-Aldrich. PBS solution was prepared from the package by dissolving containing salts in

1 L of deionized water. Distilled water was deionized using Millipore filtering membranes (Millipore, Milli-Q water system at 16 M Ω ·cm).

3.1.3 Device and solutions preparation

The device cleaning protocols were described in [15]. After cleaning, the device is incubated in 1 mM IPA solution of 16-MHA overnight to allow for the formation of a self-assembled monolayer (SAM). The device is incorporated into the fluidic fixture and integrated into the experimental setup after thorough washing with IPA and drying with nitrogen gas (N₂).

Throughout the experiments, the sensing buffer used is a mixture of phosphate buffered saline (PBS, pH 7.4 - biologically compatible) mixed with glycerol (Gly). The sensing index n_c is varied by altering the weight percentage concentration (w/w) of the standard PBS solution and glycerol. For the bulk sensing experiment, five PBS/Gly mixtures were prepared with an index increment of 2×10^{-3} RIU near the refractive index of CYTOP ($n_{CYTOP} = 1.3348$). The sensing indices were carefully adjusted so that the measured values using a prism-coupler based instrument (Model 2010, Metricon, Prism 200-P1) read $n_c = 1.330, 1.332, 1.334, 1.336$ and 1.338 at $\lambda_0 = 1312$ nm. A PBS/Gly buffer of sensing index $n_c = 1.338$ was used in the subsequent protein sensing experiment. BSA solution was prepared by mixing lyophilized BSA with PBS/Gly buffer to a concentration of 100 μ g/ml.

3.2 Results

3.2.1 Bulk sensing

To investigate the effect of sensing indices n_c on the LRSPP propagation along the sensing channel, the five PBS/Gly solutions with a 2×10^{-3} RIU increment were sequentially injected into the fluidic channel, on a carboxyl-terminated Au stripe waveguide. Unlike previous experiments on straight waveguides [13], a stable baseline while flowing PBS/Gly buffer is not required. Bulk sensing was carried out regardless of the drift in the output signals in order to illustrate the ability of the referenced Y-junction to remove this effect. Two cycles of solution exchange were performed to check the repeatability of the experiment. All solutions were injected over the waveguide at ~ 5 minute intervals at a constant flow rate of 20 μ l/min. An image of the mode outputs for each solution is shown in Fig. 3(b) to visually compare the intensity of the modes relative to the background. The left outputs correspond to the sensing channel and the right outputs to the reference channel.

In Fig. 3(a), it is obvious that both output signals are drifting upward over time. Additionally, during the second cycle of solution exchange, at solution 2 ($n_c = 1.332$), there is a major perturbation to the signals in both arms. As discussed in Section 2.2 (Eq. (9)), we eliminate common perturbations in the system by taking the ratio of the output power from the sensing arm to the output power from the reference arm. A timepoint-by-timepoint power ratio is plotted in Fig. 3(c), from which we observe that the signal drift is eliminated. We also observe reproducibility during the second cycle for solutions 4, 3 and 1.

However, the major disturbance to the signals at 37 min is only partially eliminated. Referring to Eq. (11), additional parameters affecting the performance of the referenced output are p , q and α_c . These normally would not change during a sensing experiment as all pertain to sections of the Y-junction that are fully cladded. However, we suspect here that the signal disturbance was caused by changes in mechanical force from the O-ring in the fluidic fixture to the region of the Y-junction split. During the exchange of solutions, the microfluidic tubes connected to the fluidic fixture are pulled and the force applied to the device changes. The comparably soft CYTOP cladding strains under the force which may alter p , q and α_c . In fact, a mild disturbance to the system is first observed during the second cycle of injection of solution 3 ($n_c = 1.334$) where a slight jump in the power ratio is noticed.

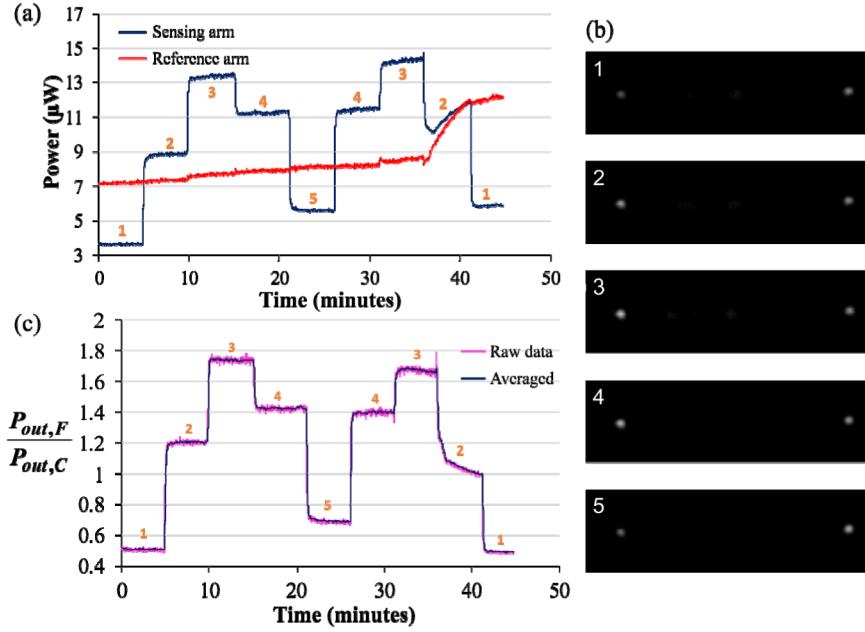


Fig. 3. (a) Response of a Y-junction biosensor to PBS/Gly solutions with different refractive indices in 2×10^{-3} RIU increments at $\lambda_0 = 1310$ nm. The refractive indices of the solutions injected (n_c) are: (1) 1.330, (2) 1.332, (3) 1.334, (4) 1.336 and (5) 1.338. (b) Mode outputs captured using an IR camera for each solution. The mode on the left corresponds to the sensing channel and that on the right to the reference channel. (c) Power ratio of the sensing arm to the reference arm showing the referenced response of a Y-junction biosensor to bulk changes.

As expected from index symmetry [27], the maximum power ratio is observed for the solution with $n_c = 1.334$, which is closest to the refractive index of CYTOP ($n_{CYTOP} = 1.3348$). Similar to straight waveguides [13], the change in the power ratio is not directly proportional to the step change in refractive index. The largest change in power ratio of $\Delta(P_{out,F}/P_{out,C}) = 0.74$ is observed for the step from solution 4 to 5 ($n_c = 1.336$ to 1.338), and the standard deviation of the power ratio in this region is $\sigma = 0.006$, yielding a signal-to-noise (SNR) of $\Delta(P_{out,F}/P_{out,C})/\sigma = 0.74/0.006 = 123$. We carry out time-averaging by computing the average power ratio over blocks of 10 subsequent time points and plot this in Fig. 3(c). The standard deviation of the time-averaged result improves by a factor of 3 to $\sigma = 0.002$ and the corresponding SNR to $\Delta(P_{out,F}/P_{out,C})/\sigma = 0.74/0.002 = 370$. The bulk detection limit of this measurement is thus 5.4×10^{-6} RIU ($\Delta(P_{out,F}/P_{out,C})/\sigma = 1$). This detection limit is close to what has been achieved with a straight waveguide biosensor [13], although further improvements can be expected here by optimizing the interrogation set-up.

3.2.2 Protein sensing

The ability of the Y-junction structure to perform biosensing is demonstrated through BSA adsorption on a carboxyl-terminated surface. A sensing buffer of $n_c = 1.338$ was chosen to obtain sensitive response for protein binding (Fig. 2(b) and [19]). 100 $\mu\text{g/ml}$ of BSA is used to minimize the bulk refractive index change in the buffer [13]. The protein sensing experiment was carried out by ignoring the instability in the baseline signals and not optimising the set-up or input alignment, in order to more effectively demonstrate the benefits of referencing. The BSA solution is injected after flowing PBS/Gly buffer for ~ 7 min. According to previous experience [19], the BSA response reaches saturation after ~ 10 min of flow. The excess BSA was washed by injecting the PBS/Gly buffer.

Figure 4(a) shows the output powers from the sensing and reference arms of the Y-junction during a BSA physisorption experiment. Due to the upward drift in the signals, the binding curve caused by physisorption of BSA cannot be easily discerned in the response of the sensing arm. When the power ratio $P_{out,F}/P_{out,C}$ (computed timepoint-by-timepoint) is plotted in Fig. 4(b), the drift and common fluctuations in the signals are eliminated, and the binding response due to BSA physisorbing on the surface becomes evident.

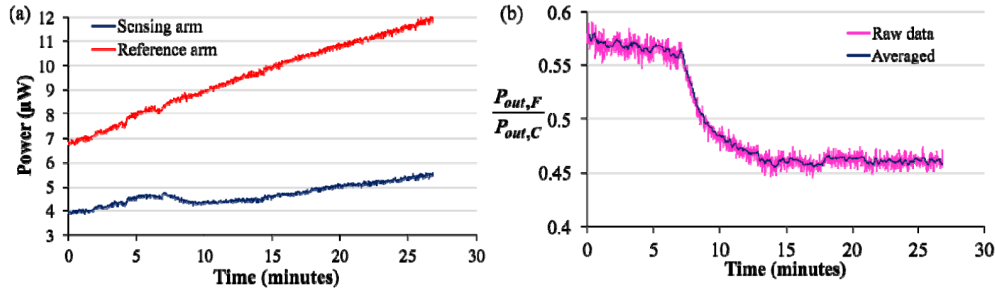


Fig. 4. (a) Response of a Y-junction biosensor to BSA physisorption on a carboxyl-terminated Au stripe. (b) Power ratio of the sensing arm to the reference arm showing the referenced response of a Y-junction biosensor to the formation of a protein adlayer.

The physisorption of BSA on the carboxyl-terminated surface produces a change in power ratio of $\Delta(P_{out,F}/P_{out,C}) = 0.11$ with a signal-to-noise ratio of $\Delta(P_{out,F}/P_{out,C})/\sigma = 16$ for $\sigma = 0.007$ based on the raw data. We carry out time-averaging on the raw data in Fig. 4(b) (similarly to the bulk sensing case - Fig. 3(c)) and plot the time-averaged result in Fig. 4(b). The standard deviation of the time-averaged power ratio is $\sigma = 0.0038$, which is almost half that of the raw power ratio. The signal-to-noise ratio is then improved to 29.

4. Discussion

The performance of the Y-junction biosensor used in the bulk and protein sensing experiments can be predicted by computing the modal properties numerically and substituting them into Eq. (11), thus yielding the theoretical results plotted in Fig. 5. To compare the theoretical and experimental results more accurately, the formation of a self-assembled monolayer (SAM) through incubation in 16-MHA must be considered. We compute the power ratio $P_{out,F}/P_{out,C}$ as a function of the adlayer thickness a at $n_c = 1.338$, as shown in Fig. 5(b). Before the BSA injection during the protein sensing experiment, the value of $P_{out,F}/P_{out,C}$ corresponds to an adlayer of thickness $a = 2.5$ nm. This value agrees well with the typical thickness for 16-MHA SAM which is ~ 2 nm [28] and the length of an MHA molecule which is 2.2 nm [29].

During the bulk sensing experiment, the maximum value of $P_{out,F}/P_{out,C}$ becomes greater than 1 (Fig. 3(b)). This implies that the Y-junction is not splitting equally ($p \neq q$ in Eq. (11)) at $n_c = 1.3348$ (n_{CYTOP}), possibly due to misalignment at the input or a fabrication defect. This hypothesis is supported by the difference in output power levels (Fig. 3(a)) and mode intensities (Fig. 3(b)) of the sensing and reference arms during injection of solution 3 ($n_c = 1.334$, close to n_{CYTOP}). The theoretical values of $P_{out,F}/P_{out,C}$ for each sensing index n_c are computed from Eq. (11) by considering a 16-MHA SAM of thickness $a = 2.5$ nm and $(q/p)^2 = 1.74$, which is the experimental value deduced from $P_{out,F}/P_{out,C}$ at $n_c = 1.334$ (Fig. 3(a)). Figure 5(a) shows the comparison between the theoretical and experimental results for bulk sensing. The trends of the responses agree qualitatively and quantitatively for n_c near 1.334. Note that the bulk sensitivity computed in Fig. 2(a) cannot be used to predict the waveguide performance directly because the 16-MHA SAM was not considered in the computation.

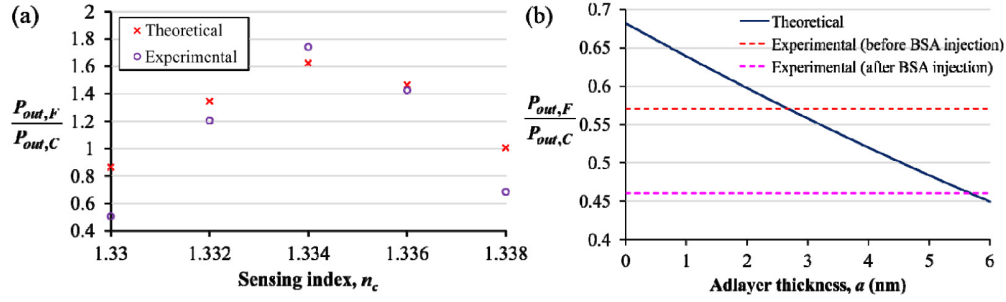


Fig. 5. Comparison between theory and experiment for (a) bulk and (b) protein adlayer sensing.

In the experiment with BSA physisorption, the Y-junction biosensor produced almost equal output power from the sensing and reference arms at $n_c = 1.3348$. Then, the sensing index was changed to $n_c = 1.338$ for the protein sensing experiment. As discussed previously, the value of $P_{out,F}/P_{out,C}$ before BSA injection agrees with the formation of a 16-MHA SAM. After the injection of the BSA solution, the value of $P_{out,F}/P_{out,C}$ corresponds to a total adlayer of thickness $a = 5.5$ nm (Fig. 5(b)), which implies the formation of a BSA adlayer ~ 3 nm thick. The surface mass density of the BSA formed Γ (in g/m^2) can be computed through [30]:

$$\Gamma = \frac{a(n_a - n_c)}{\partial n / \partial c} \quad (20)$$

where $n_c = 1.338$ is the sensing index, $n_a = 1.5$ is the index of a biochemical adlayer [31] and $\partial n / \partial c = 0.185 \text{ mm}^3/\text{mg}$ [30]. Using $a = 3$ nm, we obtain $\Gamma = 2627 \text{ pg/mm}^2$ which is consistent with our previous calculation for a straight waveguide biosensor [13]. Considering the signal-to-noise ratio for the averaged result of $\Delta(P_{out,F}/P_{out,C})/\sigma = 29$, the detection limit of the Y-junction for protein sensing in terms of surface mass density is estimated as $\Delta\Gamma = 90 \text{ pg/mm}^2$ (for $\Delta(P_{out,F}/P_{out,C})/\sigma = 1$). We emphasize that our goal in these experiments was to demonstrate the elimination of drift and common fluctuations using a reference channel, rather than minimising detection limits. Given the similarity in the surface sensitivity of the Y-junction biosensor to straight waveguides, a detection limit at least comparable to the latter [19] ($\sim 100\times$ better) can be achieved by optimizing the interrogation set-up.

5. Conclusion

We demonstrated a new LRSPP structure for biosensing, a referenced Y-junction biosensor, which relaxes the need for a stable baseline signal. The bulk and surface sensitivities of the referenced Y-junction biosensor were predicted theoretically and numerically. The Y-junction biosensor has been shown to successfully respond to five solutions of different refractive indices in 2×10^{-3} RIU increments. Protein sensing was also demonstrated through BSA physisorption on a carboxyl-terminated surface. The experimental results for both bulk and surface sensing are consistent with theoretical modelling. The ability of the structure to eliminate drift and common fluctuations has been successfully demonstrated.

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