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A microfluidic based differential plasmon resonance sensor

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ABSTRACT

A new type of differential surface plasmon (SPR) sensor integrated with a microfluidic system is presented. The working principle of the microfluidic device is based on hydrodynamic modulation of two laminar streams inside a microchannel to provide periodic changes of the environment on the SPR sensor. The modulated reflectance is then demodulated using a lock-in amplifier. The presented sensor provides sensitivities of index of refraction about 4×10^{-8} RIU together with a 4 orders of magnitude dynamic range. This method demonstrates a sensitive detection scheme which could be used for label-free detection.

1. Introduction

Phase sensitive detection is a remarkably simple technique for recovering weak signals buried in a noisy background. This technique is based on modulation of a signal of interest at a particular frequency by an external parameter and detecting the modulated signal with a phase-sensitive detector (i.e., a lock-in amplifier) [1]. A lock-in amplifier (LIA) amplifies the signal at the frequency of modulation and rejects the uncorrelated signal from noise at other frequencies. Basically LIA demodulates the signal of interest at a particular frequency. Signals with a signal to noise ratio as low as −100 dB can be recovered. Noncontact atomic force microscopy is a good example of this sensitive detection method. The method of phase sensitive detection has been combined with many different modulation techniques such as temperature modulation [2], wavelength modulation [3] and spatial modulation [4]. For example light scattering from a single nanoparticle [4] or a carbon nanotube [5] can be detected by modulation of the position of the particle and phase sensitive detection.

In this work we implement the phase sensitive detection technique to microfluidic systems. The idea is based on the periodic modulation of liquid media flowing with a low Reynolds number inside a microfluidic channel. At low Reynolds number regime, the flow inside microfluidic channels is laminar. Taking the advantage of laminar flow, we generate a rapid periodic hydrodynamic modulation of two streams inside the channel without any turbulent mixing. Here one of the laminar streams provide a reference signal and the difference between the streams is detected as a differential signal. Similar concepts of hydrodynamic modulations have been applied to electrochemical systems to reduce the background electrochemical currents [6] and to study the frequency response of signaling pathways of cells [7].

Here we implement this hydrodynamic modulation technique with surface plasmon resonance (SPR) sensors. Over the last decade, surface plasmon sensors have attracted much interest owing to their high level of sensitivity and ability of surface specific detection. With a suitable surface chemistry, SPR sensors provide unique means of studying interaction of biomolecules on surfaces. The Kretschmann configuration is most commonly used technique to excite surface plasmon on a flat metal surface. The phase matching condition can be achieved at the resonance angle which is written as

$$k_{sp} = k_0 n_{\text{glass}} \sin(\theta_r) \qquad (1)$$

where k_{sp} and k_0 are the wavevector of surface plasmons and excitation photon and θ_r is the resonance angle. The resonance angle depends on the wavevector of the surface plasmon. The dielectric constant of the medium on the metal layer determines the wavevector of the SP. The general sensing mechanism of SPR sensors is based on detecting changes in the intensity of the reflected light as the dielectric constant of the medium changes. The sensitivity of SPR sensors is predicted as high as 10^{-7} RIU [8], however, random fluctuations because of laser noise, thermal drift and vibrations significantly reduce the minimum detectable signal of the sensor. Furthermore, during the measurements, the gradual change of the background level, likely because of thermal effects, reduces the repeatability of the measurements. These problems foster the development of new techniques to eliminate the random and

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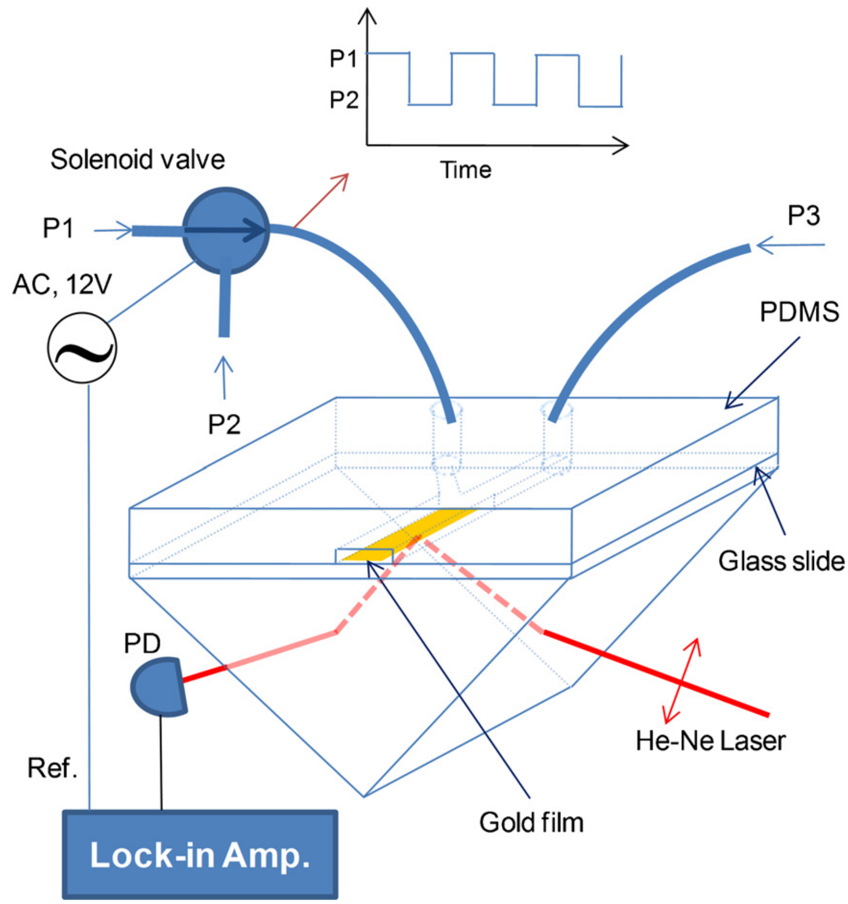

Fig. 1. Schematic representation of the microfluidic device and experimental setup used for hydrodynamic modulation. A thin layer of gold film with a thickness of 50 nm is fabricated inside the microfluidic channels, forming a Y-junction. The flow inside the channel is driven by a gravity induced pressure difference that generates rate of flow about ~10 μL/s. The pressure of the one arm of the Y-junction is modulated by a solenoid valve at a frequency of 2.5 Hz. Surface plasmon-polariton on gold-liquid interface is excited using a high refractive index prism, the Kretschmann configuration. The reflected light from gold film is detected by a photodiode and a lock-in amplifier. The reference signal is used to drive the solenoid valve. The inset graph shows the pressure as a function of time.

systematic errors associated with the experimental setup or the environment. We summaries couple of developed methods to overcome these problems. Wu et al. [9] and Li et al. [10] formed an interferometer which detects the phase change of the reflected light from the metal surface. This method is partially immune of the environmental effects, therefore provides a high level of sensitivity. Zhang et al. [11] introduced a secondary SPR sensor adjacent to the main sensor as a reference. Simultaneously detecting the signal from the reference sensor and the main sensor provides more reliable measurements. More recently, Williams et al. [12] introduced signal-locking SPR sensor which uses a periodic excitation of analyte and a reference SPR sensor with frequency domain signal processing to reduce the uncorrelated signal. These methods and other similar ones [9,13–21] require very tedious alignment for interferometers or a complicated data processing. SPR sensors based on spectroscopy of surface-plasmons integrated with multichannel detectors provide higher sensitivities [22]. Slavík and Homola demonstrated a sensitive SPR sensor based on long-range surface plasmon-polaritons which provides sensitivities of 2.5×10^{-8} RIU [23]. Here, we introduce a simple, yet very sensitive SPR sensor based on a phase sensitive detection scheme using a hydrodynamic modulation inside a microchannel with rates of flow at low Reynolds numbers. The presented method eliminates most of the random fluctuations and background shift during the measurements due to random vibrations, thermal drift and laser noise. The high sensitivity of SPR sensor together with the signal recovery by the phase sensitive detection provide extremely high sensitivities even at very large uncorrelated background noise.

2. Materials and methods

Fig. 1 shows the schematic representation of the fabricated microfluidic device. A three-way solenoid valve (LFAA1201418H, The Lee Corporation), derived with a function generator, controls the pressure on the one arm of the Y-junction microfluidic channel. The other arm is kept at a constant pressure. A gravity driven pressure controller is used to control the input pressures. A TM polarized He–Ne laser is used to excite surface plasmons on the gold layer at bottom of the microfluidic channel. The reflected beam is detected with an unbiased photodiode (Newport 818) connected to the lock-in amplifier. The lock-in amplifier (LIA, Stanford Research, SR830) is operated at current amplification mode with a gain of 10^6. The control signal of the valve is used as the reference signal of the LIA. The amplitude and the phase of the differential signal are recorded using a computer and a data-acquisition software.

Fig. 2 shows the fabrication steps of the sensor. The fabrication starts with a standard UV photolithography to pattern the thin layer of gold film (thickness of 50 nm) and followed by metallization and lift-off process. The microfluidic channels are fabricated by standard rapid prototyping using the soft lithography technique. A photoresist master (SU-8-50 Micro Chem.) fabricated by UV photolithography is used to mold Polydimethylsiloxane (PDMS) elastomer. PDMS microfluidic channel with a Y-junction

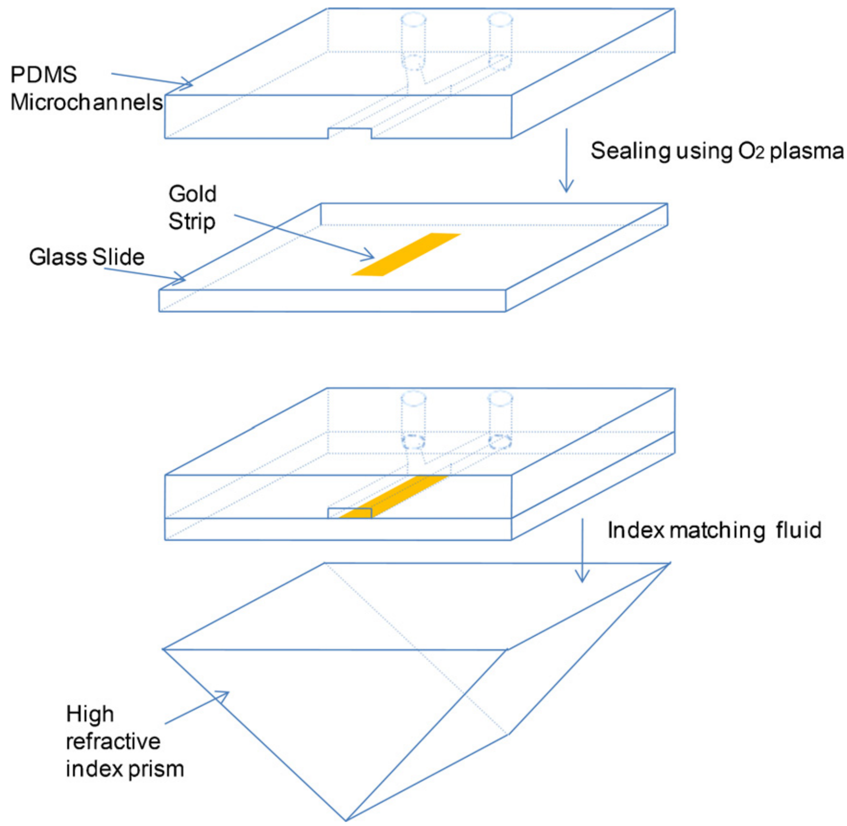

Fig. 2. Preparation of the differential plasmonic sensor. A thin layer of gold with a thickness of 50 nm is patterned on a glass slide by standard UV photolithography. The Y-junction microfluidic channel, molded from a photoresist master, is sealed on the glass slide by microwave plasma treatment. A refractive index matching fluid is used to attach the glass slide to the high refractive index prism with refractive index of 1.78.

geometry is sealed on a glass slide using a 900 W homemade microwave plasma system. During the sealing process, the gold strip is registered underneath the microfluidic channel. The fabricated microfluidic system is attached on a high refractive index prism (Thorlabs, N-SF11, refractive index of 1.78) using an index matching fluid. The surface plasmon-polaritons on the gold strip is exited by a 5 mW He–Ne laser with the Kretschmann configuration. A silicon photodiode (Newport 818) connected to the lock-in amplifier is used to detect the intensity of the reflected beam.

3. Results and discussion

A three-way solenoid valve is used to control rate of flow of one of the arms. Maximum operation frequency of the solenoid valve is around 200 Hz. Fig. 3a shows the fabricated microfluidic chip which has four SPR sensors. To test the device several issues, such as excitation angle and frequency of modulation, must be considered. First, we generate a laminar flow using two liquids, which are named as *liquid A* and *liquid B*, with refractive index difference around 0.01 RIU. Here *liquid A* is DI water ($n_A = 1.33$) and *liquid B* is 10% ethyl glycol (EtG, $n_B = 1.34$) in water. Fig. 3b and c shows the optical micrographs of the channel filled with *liquid A* and *liquid B* for two different pressure levels. The laminar flow boundary between the two streams is seen due to large refractive index difference (0.01 RIU). By switching the pressure of *liquid A* between two different pressure levels (~15 kPa and 13 kPa) while keeping the pressure of *liquid B* constant (~13 kPa), we periodically module the index of refraction of the medium on the SPR sensor. As the index of refraction changes, the surface plasmon resonance angle and reflected power changes accordingly. To find the optimum operation point we measure the reflection of the laser beam from the gold surface as a function of incidence angle. Fig. 3d shows the intensity of the laser beam reflected from the gold strip underneath the microfluidic channel filled with *liquid A* ($R_A(\theta)$) and *liquid B* ($R_B(\theta)$). The reflectivity depends on the incidence angle and the wavelength of the laser. The SPR angle for DI water is 56.76° and for 10% EtG is 57.49°. The resonance angle shifts to larger angles as we increase the index of refraction of the liquid by adding ethyl glycol. Fig. 3e shows the change of the intensity of the reflected beam as function of time for three different concentrations of ethyl glycol (10% (red curve), 2% (green curve), and 0.4% (blue curve)). (For interpretation of the references to color in text, the reader is referred to the web version of this article.) For concentrations less than 0.4%, the change in the reflectivity due to the modulation is buried under noise. The modulation of the reflectance provides the differential signal. As the liquids oscillate on top of the gold layer, the SPR angle modulates at the same frequency as well. The difference of the reflectivity for the two cases generates a periodic signal on the detector. The output of the lock-in amplifier provides the amplitude and the phase of the periodic signal between the liquids.

To understand more inside about the working principle of the device, we drive an expression for the differential signal. The reflectivity from the gold surface is a linear combination of $R_A(\theta)$ *and* $R_B(\theta)$. The total reflectivity can be written as

$$R(\theta) = \frac{x}{w} R_A(\theta) + \frac{w-x}{w} R_B(\theta) \quad (2)$$

where x is the position of the laminar flow boundary which is a periodic function and w is the width of the channel. As the boundary oscillates, the differential signal (S) can be found by taking the

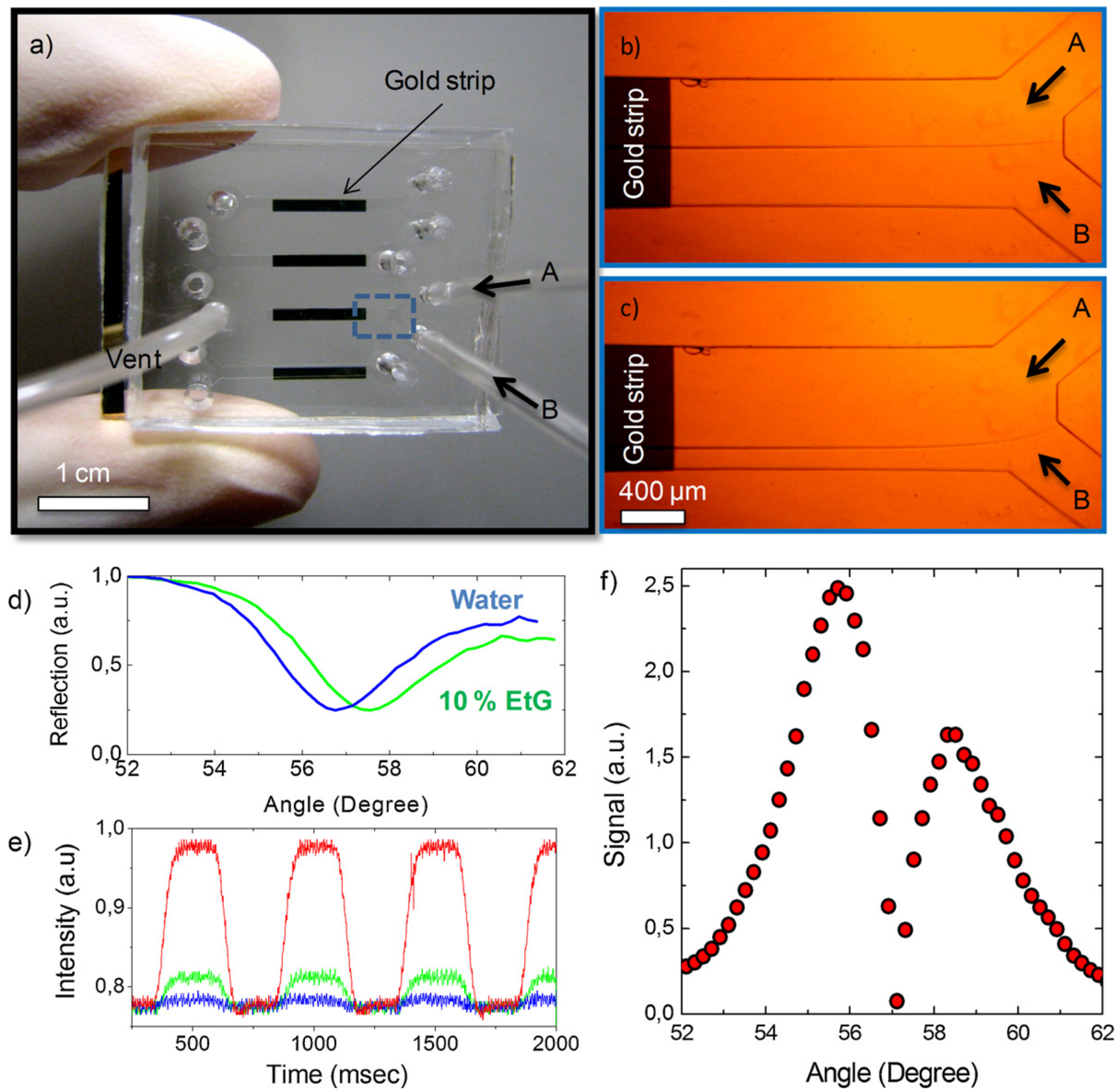

Fig. 3. (a) The image of the fabricated chip which has 4 SPR sensor. The device has a Y-junction geometry with two input and a vent. The gold strip is registered underneath the microchannel. (b and c) Zoomed optical micrographs of the microfluidic channel showing the boundary of the laminar flow for the two pressure levels. The labels 'A' and 'B' show the liquids (water and 10% ethylene glycol in water, respectively) with different index of refraction. (d) Experimental reflectance curves for the gold strip covered by 'A' and 'B', respectively. The SPR angles for water and 10% ethylene glycol in water 56.7 and 57.49 degrees, respectively. (e) Time trace of the reflected beam while the medium in the channel is modulated between 'A' and 'B'. The response time of the boundary is around 100 ms. (f) Differential signal obtained by the lock-in amplifier as a function of the excitation angle. The reference signal of LIA is at 2.5 Hz and the time constant is 3 s.

difference of the reflection. The differential signal can be written as

$$S = \Delta R = \frac{|x|}{w}(R_A(\theta) - R_B(\theta)) \quad (3)$$

where $|x|$ is the oscillation amplitude of the boundary. For small changes in the index of refraction where the difference between $R_A(\theta)$ and $R_B(\theta)$ is very small, we can further simplify the equation as

$$S = C\frac{|x|}{w}\Delta n\frac{dR(\theta)}{d\theta} \quad (4)$$

where Δn is the difference in the index of refraction between the liquids, and C is the proportionality constant which defines the change of the resonance angle with respect to the change in index of refraction of the medium. The differential signal is directly proportional with the oscillation amplitude, the difference of index of refraction and the slope of the reflectivity curve. The differential signal obtained for water and 10% ethyl glycol as a function of the incidence angle is given in Fig. 3f. The signal goes to minima where the reflectivity curves cross and it goes to maxima at an angle which provides the steepest slope in the reflectivity curve.

To understand the mechanism of the hydrodynamic modulation, the frequency dependent measurements are performed. Fig. 4a shows the power spectrum of the signal obtained by changing the internal frequency of the lock-in amplifier. For this measurement the modulation frequency is kept constant at 3 Hz. The time dependent signal shows a nonsinusoidal form which has higher order harmonic components. The intensity of the third harmonic is larger than the second harmonic component because of the square like waveform of the boundary of the two liquids. The frequency dependence of the differential signal is given in Fig. 4a. We obtain the optimum frequency of operation around 2–3 Hz. The optimum frequency of operation depends on the geometry of the channel and the rate of flows.

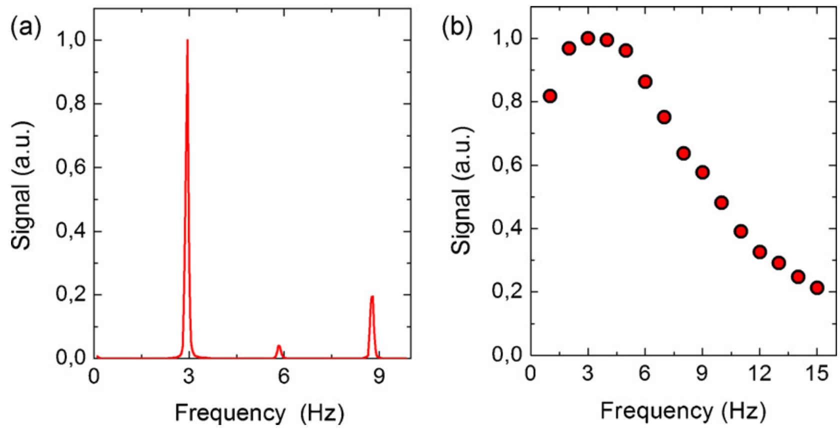

Fig. 4. (a) Power spectrum of the differential signal obtained for the hydrodynamic modulation frequency of 3 Hz. (b) The frequency dependence of amplitude of the signal.

We test the sensitivity of the fabricated sensor by changing the concentration of the *ethylene glycol from* 5.0×10^{-2} M *down to* 4.0×10^{-5} M. For each concentration we take the power spectrum of the photocurrent and record the differential signal from the spectrum. Fig. 5a shows the power spectrum for various concentrations of *EtG*. Fig. 5b shows the amplitude of the differential signal as a function of refractive index difference. The signal shows a linear dependence for a very broad range of refractive index change. The fabricated sensor provides very wide dynamic range covering more than 4 orders of magnitude refractive index change. Usually sensitivity and dynamic range of a sensor provide a tradeoff due to the limited bandwidth. The differential SPR sensor simultaneously provides high sensitivity and wide dynamic range. Fig. 5c shows the real time response of the sensor for very low refractive index changes. We add small amount of EtG in water and record the differential signal. A step like response is observed. The calculated change of refractive index for each step is given on the curve. The stability of the background level is also important for reliable measurements. Another advantage of the presented sensor is that background level is constant over a long period of time. The inset at Fig. 5c shows the time trace of the signal level for 1.5-h time scale. The standard deviation of the signal is around 10 pA which provides a refractive index change of 7×10^{-8} RIU. The origin of this extreme stability is that, the two laminar streams provide a self reference detection scheme for thermal drifts. Therefore the background level shows extreme stability over long time scales. The response of the device for low

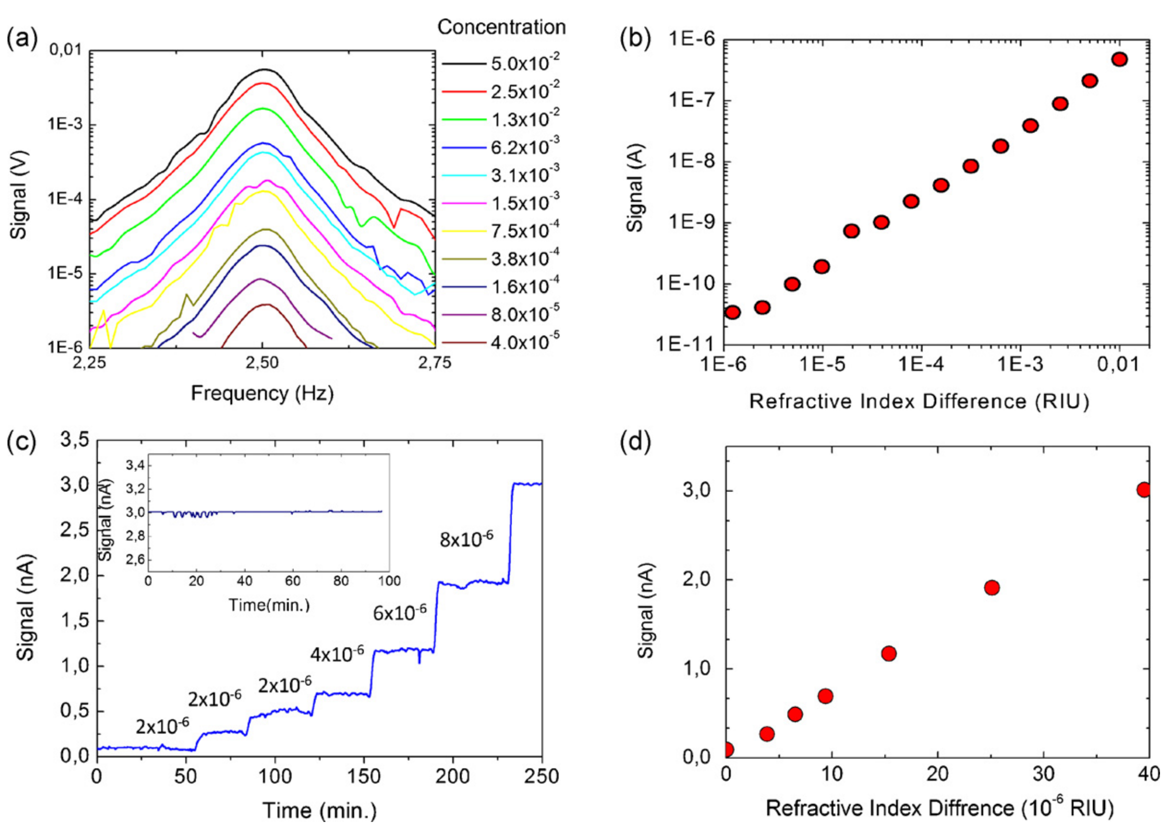

Fig. 5. (a) Power spectrum of the photocurrent for various *ethylene glycol* concentrations. (b) The variation of the intensity of the differential signal versus refractive index difference of the two laminar streams. (c) Real time response curve of the differential signal. The inset shows the time trace of the signal for a long time scale. The numbers on the curve shows the calculated refractive index changes. (d) The change of the differential signal for low refractive index changes. The standard variation of each point is less than the size of the scattered plot. The minimum detectable refractive index difference is around 7×10^{-8} RIU.

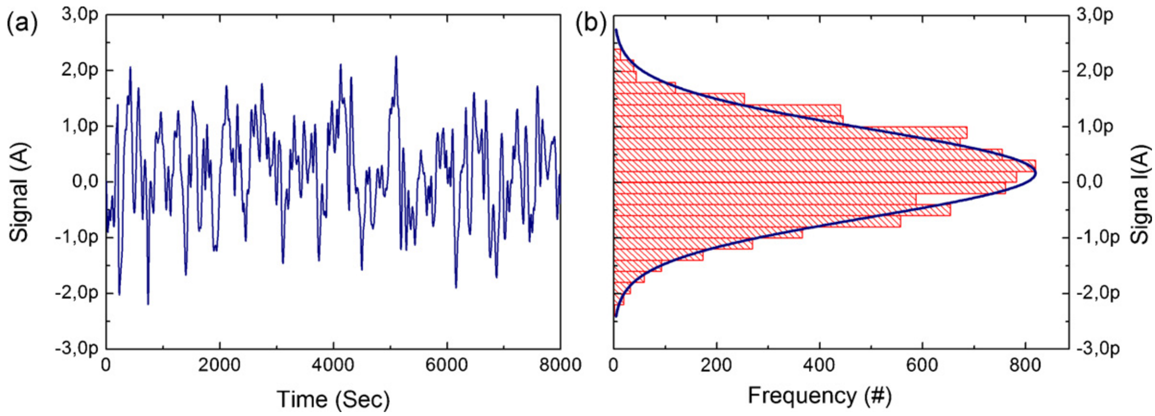

Fig. 6. (a) Variation of the differential signal as a function of time. (b) Histogram of the differential signal.

refractive index changes is given in Fig. 5d. The error in the data points is less than the size of the scattered plot.

To understand the ultimate sensitivity limit of the device we analyze sources of noise. Fig. 6a shows the noise of the differential signal. The histogram in Fig. 6b has a standard variation around 0.3 pA which corresponds 4×10^{-8} RIU. The internal noise of the LIA, shot noise of the detector, and mechanical deformation of the PDMS channel are the main source of noise in the setup. The noise level of the sensor is around 20 pA. The internal electronic noise of the LIA is around 0.12 pA for 3 s time constant at 2.5 Hz. Total noise of the detector and laser is around 2.5 pA. The limiting noise source seems due to mechanical deformation or correlated scattering due to impurities in the liquid. Since the laser beam is reflected from the glass side of the device, the mechanical deformation of PDMS during the hydrodynamic modulation does not affect the recorded signal too much. We speculate that the minimum detectable signal is limited by impurities or inhomogeneities in the liquids which generate a correlated noise at the modulation frequency.

4. Conclusion

As a conclusion, we present a new type of microfluidic device that uses hydrodynamic modulation scheme for a phase sensitive detection. We implement this method to a surface plasmon sensor integrated in a microfluidic device. The fabricated sensor can detect the refractive index difference as small as 4×10^{-8} RIU with an extremely stable signal level and more than 4 orders of magnitude dynamic range. The primary conclusion of this study is that by using a hydrodynamic modulation and phase sensitive detection technique, a simple plasmon sensor with intensity interrogation can provide refractive index sensitivities as high as plasmon sensors with spectral interrogation.

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