Integration of Faradaic electrochemical impedance spectroscopy into a scalable surface plasmon biosensor for in tandem detection

Brandon Hong,^{1,*} Alexander Sun,² Lin Pang,¹ A. G. Venkatesh,² Drew Hall,² and Yeshaiahu Fainman¹

¹Ultrafast and Nanoscale Optics, Department of Electrical Engineering, University of California, San Diego, USA ²Biosensors and Bioelectronics Group, Department of Electrical Engineering, University of California, San Diego, USA

*bjhong@ucsd.edu

Abstract: We present an integrated label-free biosensor based on surface plasmon resonance (SPR) and Faradaic electrochemical impedance spectroscopy (f-EIS) sensing modalities, for the simultaneous detection of biological analytes. Analyte detection is based on the angular spectroscopy of surface plasmon resonance and the extraction of charge transfer resistance values from reduction-oxidation reactions at the gold surface, as responses to functionalized surface binding events. To collocate the measurement areas and fully integrate the modalities, holographically exposed thin-film gold SPR-transducer gratings are patterned into coplanar electrodes for tandem impedance sensing. Mutual non-interference between plasmonic and electrochemical measurement processes is shown, and using our scalable and compact detection system, we experimentally demonstrate biotinylated surface capture of neutravidin concentrations as low as 10 nM detection, with a 5.5 nM limit of detection.

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1. Introduction

Label-free detection systems provide the rapidity of measurement required for the mobile deployment of a biosensor, while maintaining high sensitivity. A great number of label-free biosensors designed for immunoassays and disease detection have technologically matured over recent years. Among them include optical techniques such as surface plasmon resonance and waveguide sensing [1,2], electrical techniques such as electrochemical impedance detection and FET-based gate sensing [3,4], and mechanical techniques such as stress-strain and dynamic resonance shift sensing [5]. Such advances have been bolstered by the rapid progress in optical, electronic, and MEMS miniaturization and integration, and progress in their implementation as biosensors has consistently lowered limits of detection while maintaining a minimalist protocol. The simplicity in these sensors suggest that label-free biosensors are amenable to mobile environments, where training and preparation are not prerequisites for use.

However, what the label-free systems gain in simplicity is lost in specificity. Real or human sampled biological fluids contain a plethora of proteins that interfere with the detection process, often in concentrations significantly higher than the target analyte. Labelfree techniques must find a conjugate molecule specific enough to the target protein to make interferants negligible, or the raw sample must be stringently preprocessed to filter out competing biomarkers. In this manner, significant progress has gone into the careful and complex design of label-free immunoassays and associated protocols [6]. However, the increasing complexity in this direction results in a higher bar to entry for rapid and mobile deployment of label-free biosensors. Even without non-specific interferants, the lack of labeling as an error-check belies the specificity of a biosensor that is sensitive to dynamic changes within the biological sample or instabilities due to environmental noise sources such as temperature (e.g., bulk refractive index changes for SPR). Given the diversity of physical mechanisms currently employed for biosensing, the characteristics of multisensing, the integration of different sensors for the detection of a single analyte, have been investigated [7]. Among these advantages include the reduction of label-free detection error (e.g., false positive/negative) rates by redundancy improved confidence intervals [8] and specificity improvements by physically orthogonal measurements without sacrificing individually characteristic sensitivities. Towards the latter, studies in the label-free multi-sensor integration of surface plasmon resonance and non-Faradaic electrochemical impedance spectroscopy have been conducted, via the plasmonic-EIS detection (p-EIS) scheme [9,10]. In the p-EIS modality, the non-Faradaic electrochemical impedance is coupled to the optical signal as an AC modulated SPR resonance and is extracted from the optical reflection by lock-in detection, while the DC, or drift, response characterizes the SPR resonance.

In this paper, we detail the design and characterization of integrating SPR and Faradaic EIS (f-EIS) onto a single transducer substrate as uncoupled and non-interfering measurements. Given the aim for such a system to impart practicality as a mobile biosensor, we present a design for our integrated system that considers eventual compacting of the device by allowing for scalability. Our concern is in developing a multisensor device platform that can be used both as a multisensor with error-checking redundancy and a platform for the study of physical orthogonalities between SPR and f-EIS measurements, as it applies towards reducing the associated uncertainty of complex biological fluids. We therefore seek to demonstrate that SPR and f-EIS can be integrated as individually functional immunoassay detection systems, without loss of sensitivity in either modality. Towards this end, we experimentally show that in our integrated multisensor, the respective interrogation of SPR or f-EIS does not interfere with the other, and demonstrate the corroboration of multimodal measurements in a neutravidin-capture immunoassay, for low concentrations.

1.1 Surface plasmon resonance

Physically, surface plasmon resonance (SPR) is the resonant excitation of collective electron oscillations, plasmons, along a metal surface. Formally, the resonance is a guided electromagnetic mode confined to and propagating along the dielectric-metal interface, as in Fig. 1. The resonant mode momentum is determined by the electromagnetic response of the interfacial materials, that is, their respective electric permittivities (under the assumption of non-magnetic materials). The dispersion relation describing surface plasmon polariton SPP modes is as follows:

$$k_{spp} = \frac{\omega}{c_o} \sqrt{\frac{\epsilon_1 \epsilon_2}{\epsilon_1 + \epsilon_2}} \tag{1}$$

where ω is the excitation frequency, c_0 is the vacuum speed of light, ε_1 is the dielectric permittivity, and ε_2 is the metal permittivity, which is negative in its real part [11].

Since the electromagnetic mode profile evanescently decays from the interface into both interface materials, the modal effective index is exponentially more sensitive to changes in the permittivity that are closer to the surface. This sensitivity is leveraged in biosensing; by functionalizing the metal surface with ligands, surface captured biomarkers serve to strongly change the effective index of the SPP mode (see Fig. 1), and thus, measurably shift the resonance condition for coupling.



Fig. 1. The angular interrogation of plasmons at the grating surface. The surface is functionalized by a ligand protein for capture of a sample biomarker (green). Note the presence of non-specific biomarkers (red, purple) that can interfere with specific binding. A focused beam contains an angular bandwidth corresponding to the numerical aperture (NA) of the focusing objective. Within the bandwidth, angles which match the grating-modified mode matching condition excite propagating surface plasmons (k_{spp}) which are then sensitive to binding events on the suface (the illustrated colored biomarkers). Measuring the return bandwidth in specular reflection, the excitation angles are manifest as dark fringes corresponding to resonant absorption. As binding events occur, SPR resonance shifts, and the dark angular fringes projected onto the detector array will shift accordingly.

In the context of biosensing, optical excitation of SPPs is arguably the most commonly employed method of excitation [1]. Owing to the boundary conditions of the dielectric-metal interface, SPPs can only be excited by radiation polarized in the plane of incidence, that is, with the magnetic field transverse (TM polarized). Radiative electromagnetic modes lack the intrinsic momentum to directly match the guided mode condition required for SPP excitation; (i.e. schemes with SPP modal interfaces that can be biologically functionalized) momentum matching schemes such as prism coupling or grating coupling must be employed [11]. While evanescent prism coupling is efficient, the total internal reflection condition to be met for modal excitation requires large angles of optical incidence, making sensor geometries bulky and thus difficult, if not impractical, to scale. Grating-excitation of SPP is achieved by coupling evanescent diffraction orders of an incident optical wave into a metal film that is beneath the grating [12], or is conformal to the grating itself [13]. By using gratings of subwavelength periodicity, small incidence angle excitations of SPPs can be achieved, and scalability can be factored into sensor design.

Our own biosensor employs a gold film conformed to a subwavelength grating, with respect to a quasi-monochromatic optical source. To track the SPP resonance shift in response to surface binding events, we employ angular spectroscopy [14], whereby a quasi-monochromatic source excitation is focused onto the functionalized metal grating surface, and projected in reflection onto a detector array (CCD). The appropriate cross section of the detector corresponds to an angular spectrum proportional to the numerical aperture of the focusing element, wherein momentum matching to the SPP mode is evident as a fringe-like resonant absorption of light. This resonant angle is prescribed by the following grating equation:

$$\frac{\omega}{c_o}\sin\theta_i = k_{spp} + m\frac{2\pi}{\Lambda_g}$$
(2)

where *m* is an integer, θ_i the angle of incidence, and Λ_g is the grating periodicity [11].

In particular, this resonant absorption is manifested as a symmetrically separated dark fringes, corresponding to positive and negative angles meeting the SPP mode matching condition for gratings. Our scheme couples to the first backwards propagating SPP order (with respect to the surface projection of incident momentum); an increase in effective index

#249408 © 2015 OSA results in a corresponding decrease of the SPP resonant angles required. Greater detail on the experimental form of angular spectroscopy employed for SPR detection is given in Section 3.

1.2 Faradaic electrochemical impedance spectroscopy

Electrochemical impedance spectroscopy (EIS) is the measurement of the electrical impedance across an electrochemical cell, which is typically composed of an ionic solution in between two metal electrodes. As in Fig. 2(a), small sinusoidal voltage signals, usually with less than 10 mV amplitudes, at multiple frequencies ranging from 0.1 Hz to 100 kHz are applied between the electrodes and the resulting current response for each frequency is used to produce an impedance spectrum. By fitting this spectrum to well-studied circuit models that describe the different electrical mechanisms of the cell, physical and chemical effects that alter these electrical characteristics can be determined (Fig. 2(b)). While this type of modeling of electrochemical cells is employed in many different fields, among which battery design is prominent [15], EIS is well characterized in its use as a sensitive label-free biosensor that does not perturb the biomolecular films that develop on the electrodes with the higher voltage signals typical of other commonly used electrochemical sensing techniques [16].

Two variants are often employed: non-Faradaic and Faradaic EIS. The former method, which ideally has no electron transfer between the electrodes, focuses on measuring the socalled double layer capacitance created by the formation of ions and charged molecules near the electrode surface. Hence, physical displacement of these charges produce a change in the dielectric properties of the electrode-solution interface thereby altering the double layer capacitance. In the context of biosensing, EIS can be used to interrogate the binding of biomarkers to functionalized ligands on the surface of the electrodes by measuring the value of this capacitance. The latter variant, Faradaic-EIS (f-EIS), hosts the analyte in a buffer containing a high concentration of a reversible reduction-oxidation (redox) molecular pair in order to deliberately introduce electron transfer with the electrodes via electrochemical reactions driven by the applied voltage signal. In this case, the electrical component of interest is the resistance created by the redox molecules reacting and transferring electrons, also known as the charge transfer resistance (R_{cl}). Any changes on the surface that impede or discourage the probability of the molecule undergoing redox reactions, such as immunoassay binding events that block the surface of a functionalized electrode, will increase this resistance. Hence, as in Fig. 2(b), detection is measured by the increase in charge transfer resistance extracted from the spectroscopic data, according to the following impedance model:

$$Z(\omega) = R_{s} + \left(\frac{1}{(j\omega)^{m} C_{dl}}\right) \left\| \left(R_{ct} + \frac{R_{W}}{(j\omega)^{0.5}} \right) \right\|$$
(3)

where ω is the frequency in radians per second, R_s is the solution resistance, m is the phase parameter, C_{dl} is the double layer capacitance, R_W is the Warburg resistance, and \parallel indicates the equivalent parallel impedance. While there is still some variation in the double layer capacitance in this version of EIS, the magnitude of the impedance change due to the charge transfer resistance overshadows the signal from dielectric fluctuations.



Fig. 2. EIS works by applying a small voltage signal across the electrodes in an electrochemical cell and measuring the generated current signal as the frequency of the excitation signal is varied to produce an impedance spectrum similar to the ones shown in the Nyquist plots above (a). In f-EIS, the binding events from a label-free immunoassay increase the charge transfer resistance (R_{et}) of the electrochemical cell by blocking the redox molecules from reacting with the surface. R_{et} is calculated by fitting the impedance spectrum to Randles Circuit model shown in (b). Visually, an increase in Rct can be seen in the Nyquist plot as an increase in the radius of the semicircle portion of the curve.

2. Fabrication

Thin film gold gratings with optically sub wavelength periodicity were patterned into coplanar electrodes onto a silicon substrate (see Fig. 3). To electrically isolate the electrodes from the silicon substrate, 1 µm of PECVD grown SiO₂ (Oxford Plasmalab PECVD) was deposited on the bare substrate, then diced to sample size. To pattern the optical grating, 400 nm of AZ1505 resist was spun onto the substrate, then UV holographically exposed and developed to form photoresist gratings with a 1D quasi-sinusoidal profile (owing to the nonlinear dosage curve) with appx, 570 nm periodicity, as measured by SEM (Philips XL30) ESEM). Eight gratings were patterned per sample, for future multispot detection. The sample was then hard-baked at 120C for 30min to confer mechanical stability to the resist, which remains as part of the final structure. A plastic shadow mask was then cut to form 30 mm by 2 mm electrodes (Silhouette Portrait vinyl cutter) with 1 mm coplanar gap, long edge running parallel to the grating wave-vector. The choice in gap reflects a design to maintain low electrode capacitance and reduce electrical modulation in the surface plasmon signal [17]. Finally, in order of deposition, 10nm of SiO₂, 5nm of Ti and 75nm of Au are sputtered (Denton Discovery 18) through the shadow mask to complete both plasmonic transducer and electrode as in Fig. 3. The intermediate 10nm of SiO₂ is sputtered to assist eventual PDMS bonding and protect the underlying resist from mechanical wear and blocking it from fluidic analyte. Thicknesses were measured using a combination of stylus profilometry (Dektak 150 surface profiler) and ellipsometry (Rudolph Auto EL Ellipsometer). For microfluidics, PDMS molds with 0.1mm by 50 mm parallel channels were fabricated according to previously reported methods [18].



Fig. 3. The SPR - f-EIS transducer substrate overlays a gold electrode pattern onto a holographically exposed glass/resist film stack. The periodicity of the plasmonic grating was determined by the grating equation and numerical calculation for a design minimizing the excitation angle for a 785nm source at a gold-water interface (n = 1.33), while avoiding the poor plasmonic coupling efficiency of near-normal incidence excitation brought on by standing wave formation. The thickness of the gold was experimentally swept from 20nm to 200nm to maximize efficiency at the designed excitation angle. Good absorption contrast was found for 75nm of gold, which is necessary for sensitive centroid detection. Each grating area is roughly 1mm in lateral width, and are spaced 2mm apart. Co-planar electrodes are paired length wise (the longer length of the substrate), and form a 1mm gap. The overlap between the electrode and the grating form the interrogation area for SPR and f-EIS. Microfluidic channels from the PDMS chip are mounted parallel to the grating-electrode, and overlap a pair and corresponding gap. The optical beam is cylindrically focused to a vertical line with respect to the image.

Having fabricated the coplanar grating-electrodes, silver conductive epoxy was applied to bond external wire leads to the sample electrode contact pads, and cured at 65C, for 15min (MgChemicals 8331, 17 m Ω -cm). A secondary nonconductive epoxy was overlaid to impart mechanical stability to the conductive bond.

3. Experimental setup

To develop and test a robust system that could be compacted and scaled in size while preserving sensitivity, surface plasmon angular spectroscopy was chosen for its high tolerance for alignment error and simple normal incidence angle interrogation geometry, shown in Fig. 4(a). A 18mW 780nm centered LED (Thorlabs LED780E), bandpassed to 3nm spectral FWHM (Semrock Line filter 785nm) is used as the source to excite surface plasmons on the grating. An LED was chosen over a diode laser in order to remove coherent speckle interference, an artifact of coherence that is severely intolerant of system instability and detrimental to sensitivity [19]. The diverging beam is collimated, passed through a polarizer, and focused by a cylindrical lens onto the grating, through the mounted PDMS. Collimated light fills the cylindrical objective lens, corresponding to a positive angular half-bandwidth of 14.25 degrees (28.5 degrees total bandwidth, centered at 0 degree normal incidence).



Fig. 4. The system was designed for scalability; that is, the optical path geometry is rectangular, with orthogonal incidence and propagation angles, evident in the schematic (a). In the physical implementation (b), the sample (top, orange) is mounted at the end of the optical arm (red), and an impedance potentiometer (bottom, orange) is attached to the sample via leads (top, orange). Two fluidic pumps (black) drive an SPR - f-EIS channel and an SPR-reference channel.

To excite SPPs, both the incident polarization and grating wave vector lie in the plane of incidence spanned by the cylindrically focused beam (i.e. the electric field is parallel to the sample grating wave vector). The reflected signal is collected by the cylindrical interrogation objective, and passed through a beam splitter to the imaging arm. The imaging arm is comprised of two perpendicular cylindrical lenses along the same optical path. The lens with curvature in the plane of incidence re-projects the angular spectrum (i.e. spatial Fourier transform of the objective collected signal), while the lens with curvature out of the plane of incidence images the fluidic channels, with magnification (focal lengths 40mm and 120mm, respectively). A 1.3MP, 6x4.8mm sensor CMOS camera with 3.7µm square pixel size is placed at the imaging plane, and the Fourier transforming lens is placed appx. 10 mm from the imaging plane such that the angular spectrum spans the CMOS camera (eCon See3Cam 12CU NIR).

For the EIS, a commercial benchtop potentiostat (CHI 750E) was used by tying the counter and reference electrodes together and attaching them to one side of the gold electrode via the epoxied wire. The opposite wire was attached to the working electrode treating the sensor as a two electrode electrochemical cell. In future implementations, this device can easily be shrunk to a much smaller size. A PDMS microfluidic chip was then bound to the functionalized grating-electrodes, and two microfluidic channels were overlapped respective grating-electrode pairs and centered on their gaps. Interrogation light through the cylindrical objective lens was focused to a line perpendicular to the running fluidic channel length. With a microfluidic channel length of 5 mm, width of 0.1mm, and an electrode gap of 1mm, the overlap of the fluidic channel over the electrodes for f-EIS was appx. 0.4 mm².

4. Characterization

4.1 Surface plasmon resonance

To calibrate and examine the fidelity of the SPR component of the SPR-EIS system, we performed bulk refractive index detection tests using stepped concentrations of ethylene glycol, diluted with water. A concentration ladder of ethylene glycol was flowed through the microfluidic channel over the grating-electrode surface at 40 μ L/min, stepped from 0% (water) to 6% ethylene glycol. A reference channel of water at the same flow rate is measured over a second channel (not shown). The physical units of sensor response in angular (a 3.75

#249408 © 2015 OSA μ m width pixel) resonance position are reported in response units [RU], in which the unit RU corresponds to the limit of detection 3σ , where σ is the measured signal standard deviation at steady state. This 3σ figure is a measure of noise in centroid position having a strong dependence on intensity contrast and system quality, such as camera shot noise and environmental temperature. A calibration sensogram obtained of the signal channel with reference channel subtraction, is shown in Fig. 5; the 1 RU point indicates a limit of detection of 0.1% glycol, corresponding to a bulk refractive index limit of detection of approximately 9 x 10⁻⁵ RIU. This matches well to previously reported thermal limit for a surface plasmon detector without temperature control [20].



Fig. 5. The full angular spectrum ($\pm 14^{\circ}$) afforded by the objective and projected onto a 3.75um pixel camera is shown (a) with evident SPR excitation fringes for diluted ethylene glycol (second channel from the bottom). The inset shows a length-wise cross section of the channel representing the angular absorption spectrum. The faint fringes outside the channels correspond to partial SPR excitation for the PDMS/gold interface. Since the cylindrical curvature of the lens is in the lateral direction (x), only the vertical (y) direction can be resolved as an image by an imaging cylindrical lens. The SPR excitation angle is tracked (b) for a ladder of ethylene glycol dilutions (right) and the extracted 3σ limit of detection for *bulk refractive index* change is 9×10^{-5} RIU.

The channel is the subimage region of interest, and represents the angular spectrum spanning its length (with normal incidence at the aperture center) and redundant across the channel width, up to noise. This region of interest is a matrix with entries according to single exposure intensities at the corresponding subimage pixel. If we identify the channel length pixels of length L as the ROI columns, and the channel width W as the rows, we can obtain a 1D angular spectrum by computing the intensity sum of the matrix along the ROI rows to obtain a 1 x L angular spectrum vector. The centroid can be calculated by computing the intensity-weighted average of the pixel position, normalized to the total intensity across the vector; this is formally identical to calculation of the center of mass for a distributed body. Thus, as the fringe moves, the centroid will be displaced, providing an experimental sensor measure that is proportional to angle shift.

4.2 Faradaic electrochemical impedance spectroscopy

To verify the functionality of the f-EIS segment of the system, both cyclic voltammetry (CV) and EIS measurements were performed with different concentrations of the redox probe potassium ferro/ferri-cyanide in the same phosphate buffer saline (PBS) solution. For each measurement, the buffer solutions spiked with varying redox probe concentrations from 125 to 1000 μ M were flowed through the microfluidic channel over the gold electrodes at 40 μ L/min. For CV, the voltage was scanned from -0.4 to 0.4 V at a scan rate of 100 mV/s. As shown in Fig. 6(a), the resulting voltammograms appear similar to those of microelectrodes

and show, as expected, that the peak current increases with increasing redox molecule concentration.

For EIS, the frequency is swept from 10 Hz to 10 kHz using an amplitude of 5 mV. The radius of the semicircle of the Nyquist plots (Fig. 6(b)) decrease with increased concentration. Hence, the charge transfer resistance decreases with higher concentration of the redox probe, since a larger presence of the charge carrier at the electrode surface increases the current. Both these trends match with the previously recorded results.



Fig. 6. Voltammograms measured with CV (a) and EIS spectrum (b) made with the Ferro/Ferri solutions in the microfluidic channel.

4.3 SPR – f-EIS calibration

A necessary condition for effectual multisensor redundancy towards detection error reduction is the non-interference or independence between the measurements of each component sensing modalities. Separate calibrations of a multisensor baseline were performed to characterize the effect of one modality on the other. Specifically shown in Fig. 7 are baseline measurements of both SPR resonance angle, without and under f-EIS interrogation, and f-EIS charge transfer resistance, without and under SPR interrogation. In both cases, each measurement exhibits no detectable drift in its baseline, regardless of tandem interrogation by the second modality. Over the f-EIS baseline trend (Fig. 7(a)), the deviation in the mean charge transfer resistance between solo and tandem measurements is 0.11 Ω , within the acquired limit of detection (3 σ) which was measured to be as narrow as 0.59 Ω . Over the SPR baseline (Fig. 7(b)), the maximum deviation in the SPR mean angle between solo and tandem measurements is 0.1 pixel, within the acquired limit of detection (3σ) which was measured to be as narrow as 1.4 pixels. This indicates that both f-EIS and SPR baseline responses during the multisensor measurement process remain well within the limit of detection characterized by respective solo measurements; no false positive/negative can be induced by error propagation through correlated sensor transduction mechanisms. This is a necessary condition for employing multimodal sensors towards redundancy, for detection error reduction. Aside from the variability of binning statistics, we mention that a possible physical cause for fluctiation in the SPR limit of detection (standard deviation) is a previously demonstrated modulation of SPR on a high capacitance electrode surface by AC modulation of the surface charge density [17]. While this scheme (called p-EIS) leverages the high capacitance in order to transduce non-Faradaic electrochemical impedance measurements from the modulated SPR signal, our design intentionally aimed to minimize this effect and decouple the measurement processes by maintaining wide coplanar electrode gaps for minimal electrode capacitance. For our integrated grating-electrode system, SPR signal frames are acquired every 5 seconds, and since the f-EIS modulation exceeds 10Hz, modulatory effects on the SPR would be severely aliased and would be detected as an increase in the SPR 3σ figure. Critically, the detectability

#249408 © 2015 OSA Received 4 Sep 2015; revised 30 Oct 2015; accepted 2 Nov 2015; published 11 Nov 2015 16 Nov 2015 | Vol. 23, No. 23 | DOI:10.1364/OE.23.030237 | OPTICS EXPRESS 30246 of this effect is dependent on the efficiency of surface charge modulation, specifically the effective cell capacitance, which is determined by the electrode geometry, cell and doublelayer film capacitances. As our design employs wide gap coplanar electrodes and the electrochemical modality is the faradaic type with significant charge transfer, the SPR variance does not noticeably change between the absence and application of electrochemical interrogation. We emphasize that the limits of detection (3σ) for both individual and tandem baselines clearly remain well within the same order of magnitude, indicating sensitivity preservation in our multisensor.



Fig. 7. Tandem f-EIS - SPR baseline measurements to calibrate mutual interference. (a) An EIS baseline for charge transfer resistance (black) was obtained with the means and 3σ (limit of detection) for both resistance without (blue) and with (red) SPR interrogation. (b) An SPR baseline for resonance angle position (black, sampled for clarity) was obtained with running means and 3σ for both angle without (blue) and with (red) f-EIS measurement.

5. SPR - f-EIS neutravidin immunoassay

Avidin proteins bind specifically to biotin, are well characterized, and are frequently used to test the surface sensitivity of an immunoassay-based biosensor design. Neutravidin, a deglycosylated complex of avidin, was chosen for its stability and neutrality in the electrochemical buffer used for our experiments [21,22]. Prior to mounting the microfluidic PDMS, the sample was subject to 1 mM KOH / H_2O_2 cleaning, rinsing by deionized (DI) water, and careful drying. To form a functionalized monolayer on the grating-electrode surface, 100 μ M thiolated-biotin reagent solution (ethanol solvent) was incubated over the gold surface, in a closed environment for 1 hour (Sigma Aldrich Biotin-SAM formation reagent). A final phosphate buffer solution wash was performed.

Using two PDMS microfluidic channels for two respective grating-electrodes, the tandem SPR – f-EIS measurements were performed on one grating, while the second channel was employed as a reference channel for SPR. Using a microfluidic pump, all fluidic flow rates through the channel were set at 50 μ L/min. Having previously determined the appropriate redox couple (ferro/ferri-cyanide, K₄[Fe(CN)₆]) / (K₃[Fe(CN)₆) concentration by earlier calibration, 1 mM ferro/ferri-cyanide in PBS buffer was flowed over the biotin functionalized surface for approximately 20 min. The transient rise in the first 10 min is indicative of residual salt removal from the cleaning process and surface stabilization of the biotin monolayer, and the zero response unit (origin) is set to the steady state baseline.



Fig. 8. (a) Neutravidin immunoassay sensogram for tandem SPR - f-EIS measurements. f-EIS sweeps for 3 min with 2 min waiting intervals, and SPR measurements are taken appx. every 5 seconds; SPR (green) and f-EIS (blue) detections of neutravidin capture clearly corroborate by relative signal amplitude change. The initial transience at 10nM is due to residue removal and stabilizes to equilibrium after appx. 10 min. (b) Semi-log equilibrium binding response curve for SPR shows limit of detection (3σ) to be appx. 5.5 nM.

After baseline stabilization, serial injections of decade stepped (10 nM to 1 μ M) neutravidin concentrations in matched buffer (1 mM ferro/ferri-cyanide PBS) were performed. For each concentration, microfluidic pump controllers were programmed to inject 700 μ L at the given flow rate, resulting in approximately 14 min of constant flow throughout each injection step. Given an inlet tubing length of approximately 200 μ L in volume, at the given 50 μ L/min rate, there is an approximately 4 min delay before the given bulk concentration of neutravidin, in microfluidic laminar flow, reaches the grating-electrode surface for binding detection. The dual signal responses, charge transfer conductance in EIS and resonant angle decrease in SPR (normalized to an increase in response units), are shown to sharply change in response to neutravidin capture by the biotin film; both responses reach equilibrium before the flow rate cessation, at appx. 10-12 min after binding begins. Prior to the subsequent injection step, a cessation of flow is briefly maintained to ensure steady state.

The disparity between the number of measurements reflects the physical disparity between both detection processes: f-EIS requires a steady state response for each spectrum comprising its chirped signal to accurately measure the cell impedance, while SPR extracts resonance position per pixel frame, and is limited by the camera frame trigger and image processing rate. This reveals an important aspect behind the integration of f-EIS, which is detailed in the Discussion section. Referring to Fig. 8(a), the 10 nM neutravidin capture is clearly resolved, and estimates for LOD (3σ) based on a logarithmic calibration curve can be estimated (Fig. 8(b)), indicating a limit of detection of 5.5 nM for SPR and 10 nM for EIS. Sensitivity can be improved in the future, with steps taken towards improved temperature stabilization, source power tracking, and appropriate electronic filtering.

6. Discussion

In addition to creating an integrated multi-sensor with practical fabrication techniques and a scalable setup, our goal in this work was to demonstrate tandem detection on the same analyte, without loss of individual signal fidelity or sensitivity in each modality. Our analysis lies in the comparison of individually relative detection responses, and a mapping between charge transfer resistance and surface refractive index change is scaled such that the correspondence between relative amplitudes of f-EIS and SPR neutravidin detection is evident (Fig. 8(a)). Having demonstrated mutual non-interference between f-EIS and SPR detection schemes in our integrated biosensor, close agreement between the two modalities as to the kinetics and equilibrium of neutravidin capture is expected. A semilogarithmic relationship (lin-log) between SPR/f-EIS signals and neutravidin concentration is evident (Fig. 8(b)), which agrees with the characteristic equilibrium concentrations obtained in binding kinetics

#249408 © 2015 OSA Received 4 Sep 2015; revised 30 Oct 2015; accepted 2 Nov 2015; published 11 Nov 2015 16 Nov 2015 | Vol. 23, No. 23 | DOI:10.1364/OE.23.030237 | OPTICS EXPRESS 30248 for a given analyte concentration. Furthermore, the individual sensitivities of f-EIS and SPR are clearly preserved, and agree with previously reported work on the stand-alone modalities [20,23–25]. Tandem non-interfering detection on the same analyte, without loss of individual signal fidelity or sensitivity in each modality, allows for redundancy to be implemented, imparting greater immunity to detection errors that are typically associated with truly label-free methods. Essentially, when the modalities are decoupled, the likelihood of failure in redundant detection due to single modality failure is the significantly lower likelihood of simultaneous failure.

The sparsity of measurements in the f-EIS signal as compared to SPR is a direct consequence of accurately measuring electrical impedance, which requires the chirped signal run long enough to resolve the steady-state impedance at each frequency being measured. In contrast, the SPR signal is acquired at a rate limited only by the exposure and image processsing time for each acquisition of the angular spectrum. However, this draws attention to the depth of f-EIS spectra, which is of particular interest to us. Specifically, the extraction of charge transfer resistance from the impedance spectrum represents a reduction of f-EIS spectrum to a single detection value, that is, a reduction in dimensionality. While this is sufficient for stand-alone f-EIS sensors, a motivation for designing this multisensor platform is to accurately explore the extraction of other electrochemical parameters characterizing the f-EIS spectrum such as solution resistance and diffusion impedance, as they compare to the single dimensional SPR response. Our future studies will investigate this relationship and how specificity might be gained by the SPR - f-EIS platform.

Several prior works in the plasmonic-EIS detection scheme (p-EIS) have thoroughly investigated and leveraged electrical modulation of SPR on an electrode for the simultaneous optical readout of electrochemical current, where the demodulated steady state optical signal is the SPR response. Coupling sensors in this format has the advantage of reducing the detection system to an all-optical format. However, critical sensing advantages are afforded to decoupled sensors, including the separation of sensor-specific noise figures (e.g. drift) and the preservation of individual sensor performances. Such a system provides greater fault tolerance by signal redundancy, reducing the prediction errors from limitations in the receiving operator characteristic (ROC) to which label-free sensors are particularly prone. Moreover, array processing of multisensor signals to improve analytic specificity requires minimal mutual dependence between the sensing modalities, such as electrochemical signal dependence on the physical efficiency of SPR modulation.

7. Conclusion

We have demonstrated the design and fabrication of a compact grating-electrode device for integrated and tandem surface plasmon resonance and Faradaic electrochemical impedance spectroscopic biosensing. Furthermore, by detection of neutravidin capture by a functionalized biotin film on this device, we have experimentally verified that the tandem measurement process is uncoupled, and maintains independent functionality for each of the two modalities while preserving the high sensitivities characteristic to each detection scheme. Our future work aims to use this decoupled biosensor scheme to study how the physically different the sensing mechanisms can be leveraged towards reducing the dimensionality in sensor response to complex fluids and improve the specificity of label-free biosensors. By way of the scalable design of substrate and setup, investigation in the aforementioned direction will enable improvements in the specificity of label-free biosensors.

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