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Hybrid localized surface plasmon resonance and quartz crystal microbalance sensor for label free biosensing

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ABSTRACT

We report on the design and fabrication of a hybrid sensor that integrates transmission-mode localized surface plasmonic resonance (LSPR) into a quartz crystal microbalance (QCM) for studying biochemical surface reactions. The coupling of LSPR nanostructures and a QCM allows optical spectra and QCM resonant frequency shifts to be recorded simultaneously and analyzed in real time for a given surface adsorption process. This integration simplifies the conventional combination of SPR and QCM and has the potential to be miniaturized for application in point-of-care (POC) diagnostics. The influence of antibody-antigen recognition effect on both the QCM and LSPR has been analyzed and discussed.

1. Introduction

Optical and mechanically oscillating sensing techniques have applications in in-situ, label free sensing and analysis of chemical and biological binding reactions (Mayer and Hafner, 2011; Arnau, 2008). Optical sensing techniques, such as surface plasmonic resonance (SPR), typically measure the change in the refractive index arising from the molecular adsorption on the metal surface thus measures the molecular mass of thin films (Zong et al., 2008). Quartz crystal microbalance (QCM) devices use the resonance phenomenon of piezoelectric quartz where the frequency of its mechanical oscillation is dependent upon the acoustic mass of the sum of the deposited molecules and the solvent coupled to the adsorbed molecules. Therefore, in a surface adsorption process, the integration of optical and acoustic sensing can not only give information regarding weight but also the film hydration which provides insights into the conformational properties of the molecules in the formed layer and the biomolecule adsorption characteristics.

Owning to the complementary characteristics of SPR and QCM, methods of combining SPR and QCM devices have attracted significant interest. Studies have taken advantage of both modalities to evaluate the thickness of polymer films (Lee et al., 2007) and biomolecule adsorption (Zhou et al., 2004; Malmström et al., 2007). However, the optical and acoustic responses are measured sequentially on different devices, not at the same time on the same film. To eliminate the variations of the experimental conditions, a sensor that can record both the optical and acoustic signal simultaneously is essential. A hybrid sensor using a continuous thin film gold electrode on one side of a bulk-mode acoustic wave (BAW) sensor has been reported (Shinbo et al., 2012). SPR detection was performed by monitoring the variation in the angle of the reflected light from the electrode (Shinbo et al., 2012; Kim et al., 2010; Zong et al., 2008; Laschitsch et al., 2000). In this way it was possible to determine the adsorbed mass. Localized surface plasmon resonance (LSPR) has the advantage that measurements are made by collecting spectra, and suitable equipment is widely available. The sensitivity of LSPR is comparable to SPR in biomolecule binding reactions.(Willets and Van Duyne, 2007) Both LSPR sensor and QCM have great potential regarding to point-of-care (POC) tests. (Aćimović et al., 2014; Yuan and Han, 2015) Hence, in this paper, we report the design of a hybrid sensor that integrates transmission-mode LSPR with a QCM which can be useful for the analysis of immunological reactions, film structures and other biological sorption reactions.

2. Experimental

2.1. Materials

All chemicals were of analytical-reagent grade. Glycerine, (3-Aminopropyl) triethoxysilane (APTES), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), NaOH, bovine serum albumin (BSA), HCl, phosphate buffered saline (PBS) tablets, ethanolamine, rabbit IgG, anti-rabbit IgG, succinic acid and methyl isobutyl ketone (MIBK) were obtained from Sigma-Aldrich. Sulfo-N-hydroxysuccinimide(Sulfo-NHS) was obtained from Abcam. MICROPOSIT MF-CD26 developer was obtained from MicroChem. Isopropyl alcohol (IPA) was obtained from Fisher Scientific. NaOH and

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HCl stock solution was used to adjust the pH value of the buffer solution.

2.2. Equipment

The transmission spectra were obtained using a Foster and Freeman ffTA microspectrometer. The objective has a numerical aperture of 0.1 and magnification of 4 ×. The microspectrometer measurement area is 12.5 μm in diameter. Unpolarized light from a halogen lamp was normally incident on to the backside of the QCM. The transmitted light passed through the spectrometer and was analyzed and recorded by the ffTA software. The characterization of a standard QCM chip and a modified QCM chip was done using an Agilent vector network analyzer (VNA) E5071B. WinCal software was used to collect the return loss S11 spectra. An overview of the combined QCM and LSPR instrument setup can be found in the supplementary material Fig. S1. In addition to the hybrid QCM chip, a second reference QCM was mounted onto the same printed circuit board (PCB). The PCB used in the experiment was made in-house. The oscilloscope used in the experiment was a Tektronix TDS2000C. The QCM resonance frequency was collected by LabVIEW signal express software. A polyethylene terephthalate flow cell was made in-house using a 3D printer. Silicone sealant was applied to seal the flow cell and minimize the mechanical stress in the crystal (Rabe et al., 2003). Finally a glass slide was positioned on top of the flow cell to seal it.

2.3. Procedures

2.3.1. QCM design

A schematic of the hybrid SPR and QCM sensor is shown in Fig. 1. In contrast to traditional QCM devices that have continuous metallic films on both sides of the piezoelectric material our device has a 400 μm diameter window defined in the back electrode and a 400 μm × 400 μm array of Au LSPR nanodiscs patterned in the center of the front electrode. The opening in the back metal electrode is necessary to allow visible light to pass through the device. AT-cut quartz crystal wafers with a diameter of 13.6 mm and a fundamental resonant frequency of-5 MHz were purchased from ICM CO, INC. The LSPR nanostructures and QCM electrodes were fabricated on the quartz substrates using electron beam lithography and photolithography respectively. Fig. 1 shows the experimental results comparing the QCM chip with continuous metallic electrodes and the hybrid LSPR and QCM device. The resonance frequency of the modified QCM device with LSPR structures on it shifts from 5.0058MHz to 5.0115 MHz due to the loss of the metal mass at the center of the electrode. The hybrid LSPR and QCM chip has excellent performance with a Q factor increased by 1×10^4 compared to the standard QCM which has a Q factor of 5.5×10^4.

2.3.2. LSPR design

The spectral transmission characteristics of the LSPR Au nanostructures were simulated using *Lumerical FDTD*. The 60 nm thick Au nanodisc was sandwiched between a semi-infinite SiO_2 substrate and a semi-infinite SiO_2 superstrate. The Au nanodisc was illuminated by a 400 nm to 1 μm plane-wave source, propagating in the z direction, and the transmission spectra were recorded by a frequency-domain field monitor. Symmetric boundary conditions were defined along the x and y axes and perfectly matched layers were set at the top and bottom boundaries to absorb any unwanted reflections. A mesh cell with size of $\Delta x = \Delta y = \Delta z = 5$ nm was set in the region encompassing the Au metal nanodisc layer and the SiO_2 surrounding it. In the simulations, Johnson and Christy's experimental values were used for the complex permittivity data of Au (Johnson and Christry, 1972).

A number of different Au nanostructures were simulated, fabricated and characterized on quartz substrates. Fig. 2 shows the scanning electron micrographs, simulation data and experimental results of several different designs. The resonance wavelength, λ_{peak}, which is the LSPR absorption peak, shows a red shift with increasing periodicity (a) while λ_{peak} blue-shifts as the diameter (d) of the Au nanodiscs decreases. The sensitivity, defined as $\Delta\lambda_{peak}$/RIU, of the LSPR sensor is greater at longer wavelengths. As shown in supplementary Fig. S2, plasmonic sensors with resonances at longer wavelengths display greater sensitivity than those with resonances in the blue region (Willets and Van Duyne, 2007). We tailored the design such that λ_{peak} was in the 800–900 nm (a = 450 nm, d = 200 nm) range as the spectra shows a higher absorption intensity (transmission minimum), and it is close to the upper limit of our spectrometer measurement range.

2.3.3. Fabrication of the integrated chip

The hybrid LSPR and QCM device was fabricated on a 330 μm quartz substrate as follows. First, a 300 nm thick bi-layer of poly(-methyl methacrylate) (PMMA) was spin coated on to the quartz surface. The resist was then baked on a hot-plate at 156 °C for 5 min per coating. To avoid charging effects that can damage the substrate when carrying out electron beam lithography (EBL), a 30 nm Al layer was evaporated on top of the resist. Patterns were written by EBL at a beam voltage of 100 kV. The Al charge dissipation layer was removed by MF-CD26 and the PMMA resist developed in a solution of MIBK: IPA (1:2). Finally, a 3 nm/60 nm Ti/Au layer was evaporated on to the sample (Ti used as an adhesion layer) and the sample then immersed in a beaker of hot acetone for 2 h for lift-off of the unwanted metal. The

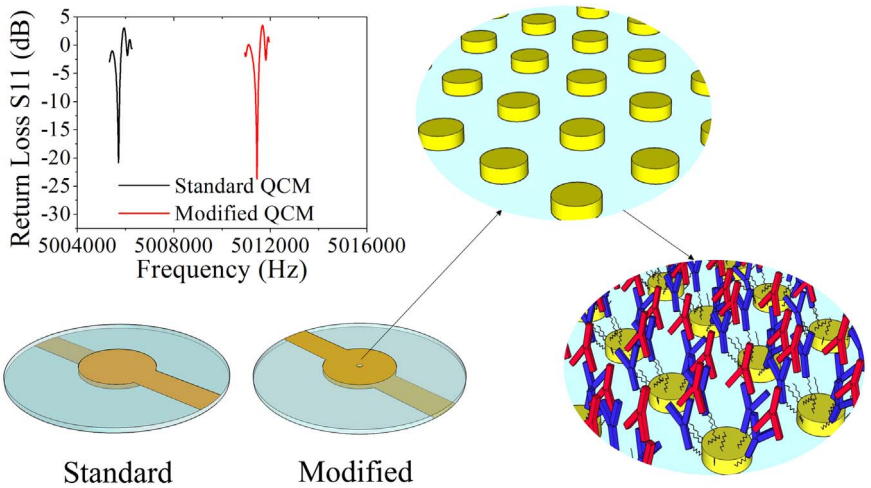

Fig. 1. VNA measured return loss (S11) spectra of the standard QCM and hybrid LSPR and QCM. inset: schematic of the modified QCM showing the antibody/antigen binding reaction attached to the Au LSPR nanostructures.