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Patterned Resonance Plasmonic Microarrays for High-Performance SPR Imaging

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Abstract

We report a novel optical platform based on SPR generation and confinement inside a defined 3-dimensional microwell geometry that leads to background resonance-free SPR images. The array shows an exceptionally high signal-to-noise ratio ($S/N > 80$) for imaging analysis and subnanometric thickness resolution. An angular sensitivity of 1 degree/0.01 RIU has been achieved and the signal to background ratio (S/B) improves to 20, one order of magnitude higher than best literature results. The design proves effective for probing supported lipid membrane arrays in real time with a thickness resolution of 0.24 nm and allows for imaging analysis of microfluidic circuits where resonant spots are separated by only one pixel ($\sim 7 \mu\text{m}$). The high image quality and unique chip geometry open up new avenues for array screening and biomicrofluidics using SPRi detection.

Since its conception two decades ago,¹ surface plasmon resonance imaging (SPRi) has gained considerable interest in the biomolecular interaction analysis (BIA) community.² The ability of label-free multi-analyte interrogation and high throughput detection offered by SPRi has led to a wide variety of applications.³⁻⁶ SPRi instruments have also undergone significant technological development, from the optical setup to biochip improvement.⁷⁻¹⁰ Despite the increased interest and broad applications, there are still challenging issues for SPR imaging methods, in particular the acquisition of quality raw data/images that are comparable to fluorescence detection. SPR imaging analysis is generally displayed through differential sensorgrams¹¹⁻¹² or rendered false color images with background subtraction/correction.¹³⁻¹⁵ This is largely due to the fact that the quality and contrast of SPR images are still low.¹⁵⁻¹⁶ However, as the background signal varies in response to surrounding light excitation and solution conditions, the sensitivity and the accuracy of the measurements in the targeted area can be severely compromised with the fixed background analysis.

The image quality, sensitivity and the background effect are not only the result of the optoelectronic components but also depend on the technology used to generate surface patterns or “spots”. The commonly used strategies for realizing spots for SPR microarrays are discharging droplets or based on surface functionalization using different micropatterning techniques including photopatterning,¹⁷ microcontact printing¹⁸ and microfluidics.¹⁹⁻²⁰ Generally, surface functionalization consists of surrounding the spots by an antifouling environment.²¹ This antifouling layer effectively reduces the nonspecific adsorption but has limited effect on the metal surface resonance around the spots. Surface plasmon resonance occurs on the whole surface, giving rise to significant background signals. Some efforts have been made to reduce the background resonance by non-chemical

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SUPPORTING INFORMATION AVAILABLE Supplementary figures S1-S6, videos of FDTD simulations and patterned resonance SPRi microarray during imaging, and video captions are included in the supporting information. This material is available free of charge via the internet at <http://pubs.acs.org>

approaches such as the use of a patterned SPR-carrying layer to obtain metal spots or islands separated by uncoated glass.^{9-10, 22} Although this approach provides bright spots with diminished background, it requires additional instrumental setup (polarizers and waveplates) compared to conventional SPRi,⁹ and the background remains sensitive to RI changes due to the presence of a decaying electric field at the glass-fluid interface. Also, the handling of liquids requires the fabrication of microchannels in a different layer, which is subsequently bonded to the metal-coated substrate.^{10, 20}

Herein, we report the design and fabrication of a novel SPRi microarray based on the spatial variation of the metal thickness and the SPR confinement inside micro-wells. The key innovation is the manipulation of the metal film to attenuate the evanescent field in the background area, while enabling the excitation of surface plasmons in the desired patterns or channels. As a result, a maximum image contrast is obtained, allowing for improved resolution and sensitivity, while offering a convenient and simple format for micro/nanofluidic applications.

EXPERIMENTAL SECTION

Numerical Simulation

Numerical modelling was performed using finite-difference time-domain (FDTD) method-based analysis (EM Explorer). FDTD methods exploit the time and position dependence of Maxwell's equations to model electromagnetic waves in rectangular 3D cells of finite volume.²³ We modelled our structure by using Yee cell size of 0.02 μm , which is about 1/20th of the wavelength, giving an accuracy of 1-2%. As FDTD modeling of large structures is resource and time consuming, arrays of maximum size of $10\mu\text{m} \times 10\mu\text{m}$ were simulated. This limit is large enough (15λ) to provide an accurate image of the electric field distribution in microscale structures. The structure was illuminated with an incident plane wave ($\lambda = 650\text{ nm}$) in Perfectly Matched Layer (PML) absorbing boundary conditions. The optical parameters of gold ($\epsilon = -14.81 + i 0.77$), chromium ($\epsilon = 8.60 + i 8.19$) and silicon dioxide ($\epsilon = 2.12$) were determined by fitting the theoretical reflectivity curves obtained by FDTD calculations with the experimental curves obtained using NanoSPR6. The fitted curves are displayed in Supplemental Figure S1.

Microarray Fabrication

A BK7 glass substrate was used for the fabrication with electron-beam evaporation of 2 nm chromium as the adhesion layer and of $51 \pm 2\text{ nm}$ gold as the SPR-active layer (Supplemental Figure S2). The gold surface was rendered hydrophilic by plasma enhanced chemical vapour deposition (PECVD) of 3-6 nm silicon dioxide using a Plasmatherm 790 system. A photoresist AZ5214E was then spun coated and patterned by photolithography. A second E-beam evaporation was performed to deposit 100 nm metal (Au, Cr or Ni) onto the patterned substrate. The photoresist was then lift-off using acetone for Au-based arrays and AZ400K for Ag-based arrays. The obtained chips were stored under vacuum before use.

Materials

The surface sensitivity to changes in thickness at the metal dielectric interface was studied by the deposition of a lipid bilayer and alkanethiol self-assembled monolayers. L- α -phosphatidylcholine (PC) lipid vesicles are prepared by dissolving 5mg/ml PC solution (from Avanti Polar Lipids, Alabaster, AL) in PBS solution (10 mM, pH 7.4, 150 mM NaCl) after solvent evaporation. PC vesicles were then sonicated, centrifuged and extruded to obtain small unilamellar vesicles of $125 \pm 5\text{ nm}$ diameter.²⁴ The solution was stored at 4°C until use. Self-assembled monolayers of $\sim 1.8\text{ nm}$ thickness were deposited on the gold array by incubation of the substrate overnight in 2 mM 11-mercaptoundecanoic acid (from

Aldrich) solution prepared in ethanol. The sensitivity to changes in refractive index in bulk solution was investigated by using pure liquids (methanol, deionized water, ethanol, isopropanol and n-propanol) rather than using dilutions of the same solution to avoid the errors arising from refractive index determination of mixtures.

SPR Imaging Procedure

The BK7 glass substrate ($n=1.515$) was mounted on an optical stage containing a 300 μ L flow cell. An equilateral SF2 triangular prism ($n=1.616$) was then put in contact with the glass substrate with a matching liquid ($n=1.6$). The optical stage was fixed to a rotating stage that allows the tuning of the incident angle.¹⁸ A red light emitting diode (LED) was used to excite the surface plasmons on the metal surface at a wavelength of 648 nm. The reflected images were captured by a cooled 12-bit CCD camera (Retiga 1300 from QImaging) with a resolution of 1.3 MP (1280 $\times$ 1024 pixels) and 6.7 μ m $\times$ 6.7 μ m pixel size. The injection of different solutions onto the chip surface was monitored in real time by recording changes in the reflectivity every 800 ms inside the wells and on the surroundings. A sensorgram was collected using a home-built LabView program and the image of the array taken every 30 s using p-polarized light and s-polarized light alternatively. Differences images were then obtained by digitally subtracting one image from another. All the experiments were carried out at room temperature (23 $^{\circ}$ C).

RESULTS AND DISCUSSION

Distribution of SPR Evanescent Field

Under attenuated total reflection (ATR) conditions, the incident light on a glass substrate generates a decaying electric field E_{ATR} at the glass-metal or glass-medium interface (Figure 1). The E_{ATR} exponential decay in the direction normal to the interface is characterized by the penetration depth expressed as:²⁵

$$\delta_{\text{ATR}} = \frac{\lambda_0}{2\pi \sqrt{\epsilon_p \sin^2 \theta - \epsilon'_i}} \quad (1)$$

whereas λ_0 is the incident wavelength, θ is the angle of incidence, ϵ_p is the prism or glass permittivity, and ϵ'_i refers to the real part of the dielectric function of the metal layer (ϵ'_m) or the dielectric medium (ϵ'_d), depending on the material contacting the glass substrate. For a glass substrate covered by a gold layer ($\epsilon_i = -14.81 + i0.77$), this equation shows for $\lambda = 648$ nm, the penetration depth is ~ 38 nm. However, the electric field needs more than 100 nm for a complete decay at this wavelength. When the evanescent field E_{ATR} reaches the gold-ambient interface, surface plasmons are excited and a second evanescent field E_{SPP} is generated. Unlike E_{ATR} , the evanescent field associated with SP reaches its maximum at a certain incident angle (i.e., SPR angle) because of the wave coupling. The E_{SPP} intensity is 10~15 fold higher than that of E_{ATR} when the system glass-gold is considered, and its penetration depth is given as:²⁶

$$\delta_{\text{SPP}} = \frac{\lambda_0}{2\pi} \sqrt{\left| \frac{\epsilon'_m + \epsilon_d}{\epsilon_j^2} \right|} \quad (2)$$

whereas ϵ'_m is the real part of the dielectric function of the metal layer, ϵ_d the dielectric function of the contacting dielectric medium, and ϵ_j refers here to ϵ'_m or ϵ_d depending on the direction considered for the decaying electric field.

The spatial distribution of both evanescent fields and the effect of gold layer thickness on this distribution are depicted in Figure 1a. Maximum E_{SPP} intensity is reached for the gold layer close to 54 nm thick. This value can vary from 45 nm to 55 nm following the gold optical properties. Furthermore, the intensity decreases with increasing thickness until it vanishes completely at thicknesses over 150 nm. This attenuation is due to the high imaginary part of the metal permittivity.

Based on these characteristics, we have designed a new SPRi microchip (Figure 1b) in which the variation of the metal thickness is manipulated to enable the confinement of SPR inside the microwells by attenuating the ATR-generated evanescent field in the background area. Additionally, the electric field is enhanced due to SP wave interference resulting from the back reflection at the edge of the wells as shown by the spatially modulated intensity patterns in the right side of the microwell in Figure 1b. The absence of such enhancement in the left side is due to the direction of surface plasmon polaritons propagation and the large size of the well. The enhancement in both sides could be obtained by using micro-wells with a diameter smaller or equal to the propagation length but higher than 2λ (Supporting Information Figure S3). The SPR image obtained with this design consists of a dark dot surrounded by a bright background as shown in Figure 1b. Difference images can be obtained with bright spots in a dark background for the convenience of using relative values of reflectance in analysis. The emergence, propagation and back reflection of SPP waves inside the well can be visualized in supplemental material (Video S1).

Fabrication of the Patterned Microarrays

The fabrication of the micro-patterned resonance SPRi chip is achieved with two main techniques: e-beam evaporation and lift-off (Supplemental Figure S2). Briefly, it involves the deposition of two metal layers on the glass substrate: the first layer is flat and enables formation of SPP while the second layer functions as an electromagnetic barrier, defining the geometry and providing a hydrophobic non-fouling surface. This layer presents a high reflectivity to allow the saturation of the background signal by attenuated total reflection, along with a high imaginary part of the refractive index and sufficient thickness to avoid SPR excitation. This gold layer could be replaced by other metals such as chromium, titanium, nickel or aluminium.

Microarrays of 800 μm (Figure 2) and 100 μm diameter (Supplemental Figure S4) were generated and characterized but only the larger arrays are discussed here. Two types of microarrays of 800 μm diameter have been fabricated with differently desired surface features (Figure 2). The first is characterized by hydrophobic gold wells surrounded by a titanium layer. This surface is intended for modification with self-assembled monolayers (SAM) or thiol-based chemistry. The second presents hydrophilic wells surrounded by a hydrophobic gold layer, suitable for bioadsorption and supported membrane studies. In the latter case, the chip requires an additional treatment by plasma enhanced chemical vapor deposition (PECVD) to obtain a thin hydrophilic film (3-6 nm) of silicon dioxide inside the wells for better wetting. Both chips could be easily functionalized by a simple immersion in the solution of interest, thus avoiding the need for expensive arraying equipment for routine applications. For comparison purposes, chips with gold islands on glass substrates were also fabricated following the same procedure.

SPR Imaging and Array Characterization

The SPR imaging experiments were carried out using a home-built instrument arranged in the Kretschmann configuration.¹⁸ The reflectance from the array was imaged with a CCD camera and the change in reflectance was recorded in real-time. Figure 3 shows raw and difference SPR images obtained by both gold island chips and the patterned resonance

microarray (gold wells). The s-polarized light excitation does not have any effect on the image background of gold well arrays, while for gold island chips the background increased and the gold spots were visible due to the difference in reflection between glass and gold. The high imaginary part of the refractive index (higher energy loss) and lower reflection are the main factor leading to deterioration of the contrast in the difference images in gold islands.

The micropatterned resonance arrays, on the other hand, avoid the coupling of incident light to radiative modes as explained previously. As a result, the signal obtained from SPRi is much higher. Figure 4 shows the characterization of the arrays under different experimental conditions. Gold wells showed 20 times higher signal than the background (Figure 4a), which is at least one order of magnitude better than the microarrays reported in literature including ours using either chemical patterning of planar gold substrates or microfluidics.²⁷⁻²⁹ Compared to gold island chips, which are recently used by several groups,^{9-10, 22} the gold wells are 5 times better (Figure 4a). Another characteristic that improves the array performance is the particularly high signal to noise (S/N) ratio. In fact, the measured signal was ~90 times higher than the noise. This value decreased slightly to 78.2 (–11.5 %) when the wells were filled with water, which is obvious as the noise increases with the Brownian motion and the signal decreases with the absorption.

The patterned resonance microarray was also characterized by high sensitivity to refractive index changes in bulk solution (Figure 4b). A change of 0.05 in R.I. after n-propanol injection induced a shift in the resonant angle by 5 degrees (74 % in reflectivity) for gold-based chips and 7 degrees for silver-based chips. Such high angular shifts are mostly a result of interference-enhanced change of the resonance angle, induced by SPR confinement and multiple back reflections inside the micro-wells, and are advantageous for obtaining high contrast images. For better understanding of the significance of this angular shift, a video based on difference images has been produced and presented in Supporting Information (Video S2). Injection of water (n=1.33) inside the silver-based microarray (filled with ethanol, n=1.36) resulted in the decrease in reflectance from the wells and the coupling of light to SPP at the resonance angle, leading to the illumination of the spots in the difference images.

Another original advantage of the gold wells is demonstrated in Figure 4c,d. The injection of solutions onto the gold island chips induces reflectance changes in the spot but also in the background (Figure 4c). Even with s-polarized light, the background signal remained, in agreement with Figure 3. The same experiment performed with the gold wells, however, demonstrated efficient isolation of the background area from any kind of excitation (Figure 4d). This means that the background area does not support any evanescent field and thus is not sensitive to changes occurring at the interface. This is highly significant for biomolecular interaction analysis as there will be no SPR signal outside the spots even with nonspecific adsorption or contaminations occurring on the well surroundings. A second injection of the same solution induced the same reflectance intensity, demonstrating a high reproducibility. Additionally, after the injection of n-propanol (n=1.38), the reflectance increased until it equaled the background signal leading to the complete disappearance of the spots. This property may enable new applications for real-time monitoring of fluids in a micro-structured environment. Vesicle fusion to form supported membranes in the silicate-coated gold wells has also been studied.

The change in reflectance (ΔR) due to the formation of supported bilayer membrane was 68 % compared to PBS and 17 % compared to the background reflectance (Figure 4d). Taking the thickness of a bilayer membrane (~5 nm) into consideration, the resolved signal of 27 RIU (three fold of the noise level) obtained with this platform corresponds to 0.24 nm,

indicating a sub-nanometer thickness resolution of the method. This is further verified with comparison to a self-assembled monolayer (SAM) deposition where the layer thickness is ~2 nm (Figure 5a).

It should be noted that the change in reflectance as a function of RI is not linear over the range considered here ($n=1.32$ to 1.39) (Supplemental Figure S6), which is in agreement with literature.^{9, 30} In contrast, the shift in the resonance angle shows a linear relationship with the change in RI over the same range (Figure 4b). This suggests that this design is a suitable platform for SPR imagers operating in the angle-resolved mode.³¹ SPR images could be acquired sequentially at different angles of incidence, and changes in mass or RI are then correlated to the resonance angle shifts. An important consequence is that the dynamic range is only limited by the optics.

In conventional SPRi chips, the spatial resolution is limited by optical diffraction in the vertical direction, and by SPP propagation length in the horizontal direction. Considering the optical parameters of the gold film, the propagation length is calculated to be $15.7\text{ }\mu\text{m}$ in air and $9.8\text{ }\mu\text{m}$ in water. Our new chip overcomes these limitations by complete isolation of the resonance inside the wells. With this micropatterned design, there is no possible cross-talk or interference between the adjacent spots. As a result, the distance separating the spots could be lower than the propagation length, thus enabling the fabrication of arrays of considerably high density. SPR difference images of resonant microfluidic circuits have been obtained where micrometric resonant spots were separated by one pixel distance (Figure 5b), which means that the only limitation for imaging is the optical setup. This result also suggests that the isolated resonance inside a microstructure offers the opportunity to fabricate sophisticated fluidic circuits or unique patterns on the same SPR-active substrate. It should be pointed out that the results reported here are obtained using a BK7 glass substrate. Improved performance and lower resonance angles can be conveniently achieved by using high refractive index substrates and different excitation wavelengths.

CONCLUSION

The novel micropatterned biochip we developed for SPR imaging is based on a simple approach to obtain a spatially tuned resonance but the results are vastly attractive. The SPR confinement inside a defined 3-dimensional geometry (wells or channels) has several important outcomes. By eliminating the background, SPR images are obtained with a maximum contrast, and the limitation due to the propagation length can be circumvented, which enables the fabrication of single pixel spots for high density microarrays. Another advantage is the easy functionalization of the SPR active area by a hydrophilic or hydrophobic layer during the fabrication process, thus simplifying the surface chemistry and biomolecule attachment for SPRi users. Furthermore, the large shift in the resonance angle due to RI changes offers a new opportunity for SPRi instruments that operate in scanning angle mode, which can directly record the shift in the resonance angle. Beyond the biological analysis, this new design will likely extend the application of SPR imaging to other studies, including the investigation of fluid dynamics at the nanoscale and diffusion phenomena. Additionally, chemical gradients could be imaged in real-time by monitoring the spatial variation of the refractive index inside the microchannels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Principle of spatial tuning of surface plasmon resonance for background-free SPR imaging.

a) Variation of the electric field amplitude through the multilayer system ($\lambda=648$ nm). E_{ATR} is the ATR-generated evanescent field, E_{SPP} is the SPR-generated evanescent field. The inset represents the variation of the field amplitude as a function of the gold layer thickness. The corresponding points between the graphic and the inset are labeled a, b, c and d. **b)**

Working principle of the designed structure, with a: the simulated design, b: FDTD simulation showing the SPR generation and confinement inside the micro-well, and c: scheme of a raw SPR image.

Figure 2.

Optical images of the micropatterned resonance SPRi chips. The array has a size of 2 cm^2 , containing 12×10 microwells of $800\text{ }\mu\text{m}$ diameter each. a: hydrophobic gold chip, b: hydrophilic silica chip, c: hydrophilic chip after rapid immersion in PBS buffer. The wells are instantaneously filled with the aqueous solution.

Figure 3.

Raw and difference SPR images of the microfabricated arrays. Gold island chips deposited on a glass substrate (left column) are included for comparison. Images are obtained in air by p-polarized (p-pol) and s-polarized (s-pol) lights. The difference image is obtained by subtracting s-pol from p-pol images.

Figure 4.

Figure 4. Array characterization and analytical performance for the microfabricated chips. a) Profile plot of gold island and gold well chips (images given in Figure S5 in Supporting Information). b) Plot of the resonance angle as a function of the refractive index for gold well chips. c) Sensorgram obtained with gold island chips depicting the reflectance changes over time after injection of PBS buffer and isopropanol (i). The image on the right shows that after filling with PBS the system is out of the ATR condition (light is transmitted through the glass substrate leading to a dark area). d) Sensorgram obtained with gold well chips depicting the reflectance changes over time after injection of different solutions: m: methanol, e: ethanol, i: isopropanol, p: n-propanol, and *PC*: L- α -phosphatidylcholine lipid vesicles.

Figure 5.

Assessment of the thickness and spatial resolution of the patterned resonance plasmonic microarrays. **a)** Sensorgram and SPR difference image obtained by the micropatterned resonance array after deposition of a self assembled monolayer and L- α -phosphatidylcholine lipid vesicles (PC). C is the control. **b)** Microfluidic SPR imaging. a: SPR difference image of resonant microfluidic circuits with a zoom on the smallest micro-patterns showing single pixel spots separated by three pixels distance, b: 3D SPR difference image of the highlighted portion of the microchannel. The resonant areas are colored in orange.