

Modeling and analysis of discrete particle detection in wide-field surface plasmon resonance microscopy

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ABSTRACT

Wide-field surface plasmon resonance microscopy (WF-SPRM), based on Kretschmann's configuration, is a powerful technique for detecting nano-objects in solution. In this study, key parameters for building a highly sensitive WF-SPRM are optimized, including laser wavelength, prism refractive index, numerical aperture of the objective, and gold layer thickness. A medium model describes the surface plasmon resonance (SPR) sensor principle, treating molecules bound on the sensor surface as an effective medium with an effective refractive index and thickness. On the other hand, the SPR discrete particle model is introduced to describe the detection principle for discrete particles. Theoretical, numerical, and experimental analyses are conducted, and the calculated intensity profiles of single and double nanoparticles from the discrete particle model describe the experimental profiles well. Furthermore, the influence of coating the gold layer with a dielectric layer on the SPR sensitivity is investigated for varying refractive indices.

1. Introduction

Biosensors are essential analytical tools consisting of four main components: analyte, recognition element, transducer, and signal processing. The analyte represents the target molecules being measured, while the recognition element acts as the bio-receptor that specifically interacts with the analyte. The transducer converts the bio-recognition event into measurable signals, and the signal processor prepares and presents the deduced signals for analysis [1]. The optical sensors, including surface plasmon resonances, ring resonators, and interferometers, are known for their high sensitivity. However, detecting and exploring nano-objects, especially at low concentrations, including viruses and extracellular vesicles (ECVs), remain challenging [2,3]. SPR sensors have gained significant attention due to their high sensitivity, real-time measurements, label-free detection, reproducibility, and cost-effectiveness [4,5]. Conventional SPR sensors use monochromatic light directed onto a gold thin film through a prism, which excites surface plasmon-polaritons and generates an evanescent electric field. Objects near the metal surface cause variations in the refractive index, which can be measured by monitoring the incident angle shift to obtain

real-time response curves [6,7].

Wide-field surface plasmon resonance microscopy (WF-SPRM) operates based on the SPR phenomenon using Kretschmann's configuration. However, it offers a distinct detection method and information acquisition, enabling the detection and exploration of discrete nano-objects in solutions bound to the sensor surface. Therefore, WF-SPRM thus allows for the detection of low concentrations of nano-objects. In this technique, a polarized laser beam illuminates a gold layer through a glass prism, and the illuminated region is imaged onto a CCD camera, enabling high-resolution imaging of the binding events. A bright spot appears on the image, indicating the presence of the detected particle bound to the surface [8–14]. A metallic coating is applied to the prism to facilitate attenuated total reflection and support surface plasmon propagation [15].

A traditional medium model describes the principle of the SPR sensor by treating molecules bound to the sensor surface as an effective medium with an effective refractive index and thickness. However, WF-SPRM can detect individual nano-objects in solutions and gases. Therefore, an SPR discrete particle model is introduced to describe the detection principle for discrete particles [16,17].

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This study focuses on the fundamental parameters influencing the imaging performance of WF-SPRM to enhance biosensing sensitivity. Key factors such as laser wavelength, prism refractive index, numerical aperture of the objective, and gold layer thickness are investigated. Validation experiments are performed using polystyrene nanoparticles of varying sizes and nanoparticles made from different materials. The influence on the SPR sensitivity of coating the gold layer with a dielectric layer with varying refractive index is also studied. Furthermore, theoretical analyses are conducted to compare using a gold layer with a dielectric coating to that of a plain gold layer in detecting different types of cancer cells. Our findings shed light on the improved sensitivity and capabilities of WF-SPRM in discrete particle detection for various biomedical applications.

2. Experimental section

2.1. WF-SPRM instrument

This section focuses on optimizing the key parameters for constructing a high-efficiency WF-SPRM system. Initially, red to near-infrared (NIR) light (wavelength range: 600–800 nm) was validated for generating a strong SPR signal. The red light efficiently excites maximum surface plasmon polaritons (SPPs) due to the strong coupling between photons in this wavelength range and the surface electrons of the gold layer under momentum matching. This coupling pulls electrons along the gold surface, producing the SPR effect (Supplementary S1) [18]. Hence, selecting an SPR microscope with a laser wavelength within the 600–800 nm range is ideal, as it provides a smaller SPR angle and greater SPR signal and sensitivity [19]. Additionally, the objective's numerical aperture performance is reduced for wavelengths outside their design range [8].

Fig. 1 presents the schematic diagram of the home-built WF-SPRM experimental setup based on Kretschmann's configuration [20]. The red-light laser diode HL6750MG (Thorlabs GmbH, Germany) with a 685 nm wavelength illuminates the gold layer through a glass prism (SF10, $n=1.725$, Eksma Optics, Lithuania) with four polished sides measuring $20.0 \times 25.0 \times 16.3 \text{ mm}^3$, with an angle of $56^\circ \pm 5^\circ$ at a fixed incidence angle (Figure S1). The choice was made for a glass prism with a high refractive index ($n=1.725$) [21], an objective lens (Canon Compact-Macro Lens EF 50 mm 1:2.5) coupled to a video camera equipped with a CMOS chip (MT9P031) featuring a resolution of 5 Mp and a pixel size of $2.2 \times 2.2 \mu\text{m}^2$, is used for imaging the gold surface. The high numerical aperture (NA) objective ($NA > 1.4$) effectively couples the illumination and imaging optics [22], resulting in a

relatively small field of view, approximately $0.1 \times 0.1 \text{ mm}^2$ [8].

2.2. Gold-sensor substrate preparation

Before depositing the gold layer onto the glass substrate, it is essential to apply a titanium interlayer (Ti) to enhance the adhesion between the gold and the glass surface. The stable titanium layer should have a minimum thickness of approximately 5 nm. Therefore, a Ti layer with a thickness of 5 nm and a complex refractive index of ($n = 2.6112 + 0.1532i$) is considered in the calculations [23]. Additionally, to achieve higher detection accuracy and sensitivity, the optimal thickness range for the gold layer is in the range of 40–45 nm (Supplementary S2), which aligns with the findings of A. Zybin et al. [14]. Accordingly, SF10 polished glass slides with a refractive index matching the glass prism ($n = 1.725$) were procured from Helma Optics (Germany). Subsequently, these glass slides were coated with a titanium adhesive layer, measuring 5 nm in thickness, followed by a 41–45 nm gold layer ($n = 0.15, k = 4.91$) using a magnetron-sputtering technique (Innolume, Dortmund, Germany). To attain the best sensitivity, the transmittance of the gold-sensor substrate at 685 nm is 4%. A meticulous cleaning procedure was performed before employing the gold-sensor substrate in SPR imaging. The substrate is immersed in a piranha solution for 5 minutes. The piranha solution is prepared by slowly adding 30 mL of sulfuric acid (Sigma-Aldrich) to 10 mL of hydrogen peroxide (VWR chemicals) at 4°C due to the exothermic nature of the reaction. Subsequently, the gold-sensor substrates are thoroughly rinsed with distilled water and absolute ethanol (99.85%) to eliminate any residual piranha solution and then dried using argon gas. Following the cleaning process, the gold-sensor substrate is precisely positioned on the base of the glass prism using RI matching immersion liquid (2 μL , Cargille Laboratories via VWR, $n = 1.725$) to ensure there is no air gap between the gold-sensor substrate and the prism. To prevent liquid leakages, a polydimethylsiloxane (PDMS, Sigma-Aldrich) gasket is placed between the gold-sensor substrate and the Teflon flow cell. Subsequently, the 0.51 mm thick Teflon flow cell with an average flow rate of 300 $\mu\text{L}/\text{minute}$ is securely sealed to the gold-sensor substrate (Figure S5). Additionally, the flow rate is directed from down to up to avoid any formation of liquid bubbles.

2.3. Experimental procedure

Polystyrene nanoparticles (PSNPs) are employed as the primary example for WF-SPRM measurements. The PSNPs have an average diameter of 200 nm and a refractive index of $n = 1.59$ [24]. An

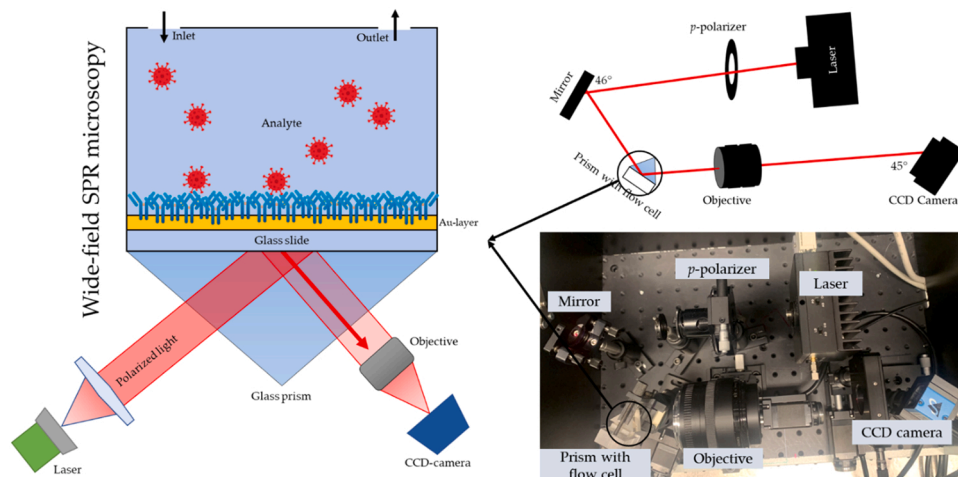


Fig. 1. Schematic diagram of the experimental home-built design of WF-SPRM based on Kretschmann's configuration.

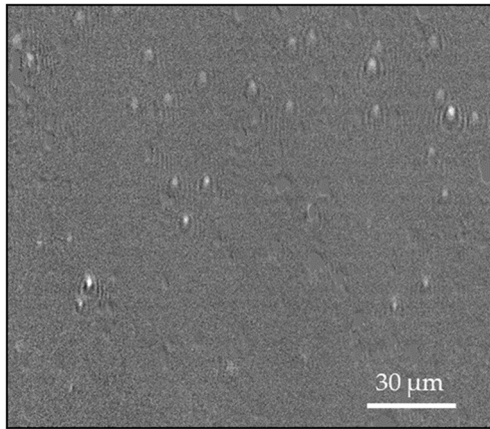


Fig. 2. WF-SPRM image for 200 nm PSNPs. Light spots indicate particle binding to the sensor surface.

aluminum hydroxide chloride layer is deposited on the gold substrate to allow the connection between PSNPs and the sensor surface by applying 150 μL of the solution and allowing it to sit for 10–30 minutes at room temperature. Subsequently, the gold substrate is washed with distilled water and dried using argon gas. To prepare the nanoparticles stock solution, 0.1 g of nanoparticles are mixed with 0.3% sodium chloride in 1.0 L of filtered distilled water at room temperature for 2 hours to achieve a low concentration of nanoparticles. Before introducing the nanoparticle stock solution into the sensor cell, a sodium chloride buffer solution (NaCl, 0.3%) passes through the cell for 1–2 minutes. Subsequently, the nanoparticle stock solution is injected into the sensor cell. After the flow system reaches equilibrium, the prism, along with the mounted flow cell, is rotated until the resonance angle ($4R_{\min}$) is attained. Starting from this minimum, the measuring point is adjusted to $4R_{\min}$ into the linear region of the reflectivity curve by rotating the angle of incidence counter-clockwise [25].

2.4. WF-SPRM data analysis

The data analysis in WF-SPRM is based on observing the perturbation in the propagation conditions of surface plasmons caused by nano-objects adsorption. WF-SPRM allows visualization of optical patterns resulting from the scattering of surface plasmon waves. When a particle binds to the sensor surface, it disturbs the evanescent field of the surface

plasmon wave, leading to local changes in reflectivity, manifesting as a bright spot in the image. ImageJ software is employed to determine the detection signal associated with the nanoparticle binding event [26]. To begin the WF-SPRM data analysis, the image background and noise are suppressed by averaging about 200 frames and subtracting each frame from the background. Following this, the time dependence of the intensity variation is plotted to observe the binding events. An illustrative example of detecting 200 nm polystyrene nanoparticles is presented in Fig. 2 and Fig. 3.

A single particle bound to the sensor surface exhibits an asymmetric shape with concentric surface plasmon waves around it, as discussed in [27,28] (Fig. 3). The WF-SPRM data is collected as a matrix of time points and pixels, allowing for analysis of the time-dependent relative intensity before and after particle binding on the sensor surface (Fig. 3a). The intensity shows low values before PSNPs bind to the sensor surface. However, at approximately 36 seconds, the local intensity increases, indicating the moment when PSNPs are bound to the surface.

2.5. Numerical model

The design and analysis of traditional and proposed models for the SPR sensor are conducted using COMSOL Multiphysics based on the finite element method. A 2D geometry is proposed, consisting of prism/Ti layer (~ 5 nm)/Au layer (~ 50 nm)/water. For the traditional molecular layer model, a 10 nm thick molecular layer of polystyrene is added, while for the proposed particle detection model, a 200 nm polystyrene nanoparticle at 10 nm above the gold layer is placed. The gold layer is illuminated with a light source at a wavelength of 685 nm incident on the top of the SF11 prism. Free triangular meshes were used in the simulated SPR sensor with the element size of 6.09×10^{-5} –0.03 μm . Additionally, the incident light angle varies from 20° to 89° with a 0.1° step. The reflectivity curve as a function of the incident angle is calculated, and the resonance angle is determined at the minimum reflectivity.

3. Results and discussion

Firstly, the traditional mathematical medium model is introduced, wherein molecules bound on the sensor surface are considered an effective medium with an effective refractive index and thickness. Since this model describes the SPR sensor principle by treating molecules bound to the sensor surface as an effective medium, the traditional mathematical medium model cannot describe low concentrations of detected particles. Subsequently, a theoretical model incorporating the

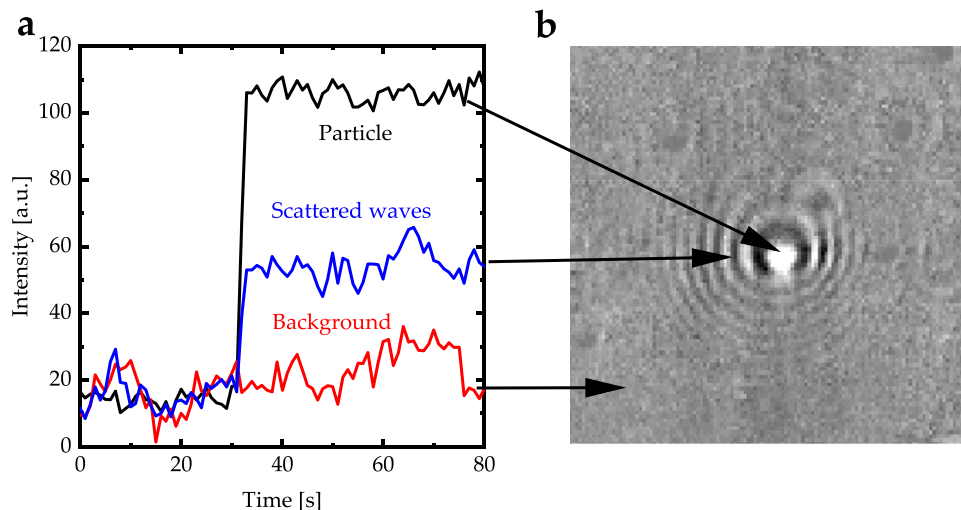


Fig. 3. (a) Temporal evolution of intensity in the central region of the bright spot for PSNPs bound to the Au-layer. (b) Representative processed image depicting polystyrene particles bound to the sensor surface.

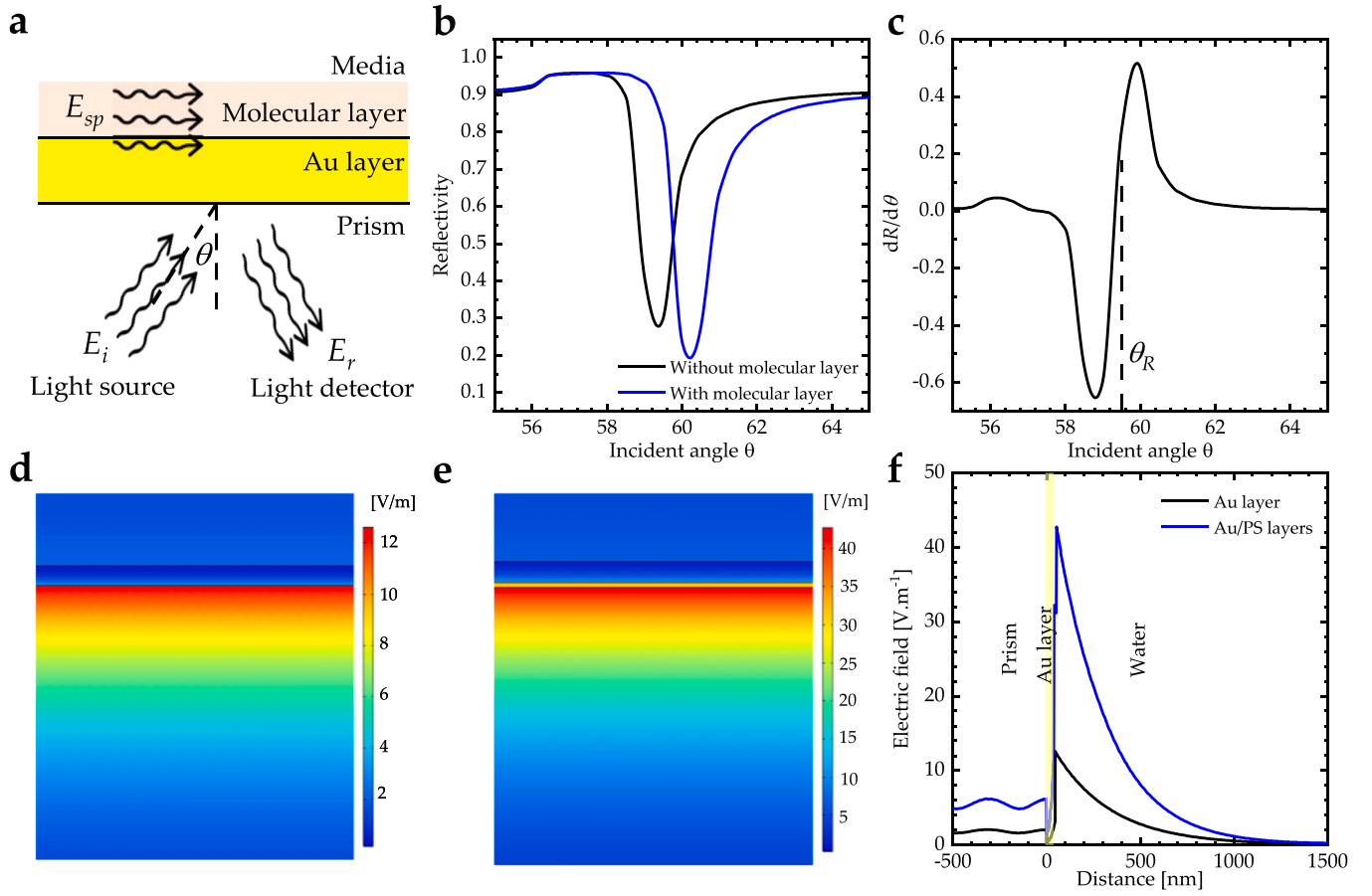


Fig. 4. The traditional medium model of the SPR sensor. (a) Schematic diagram of the traditional medium model showing the setup. (b) SPR reflectivity curves, with the black line representing the curve without a 10 nm thick polystyrene film and the blue line representing the curve with this film, were calculated using Fresnel's equations. (c) The slope of the reflectivity curve indicates the sensitivity. Electric field distributions along the Kretschmann's configuration of the structures, (d) without and (e) with a 10 nm thick polystyrene film at resonance angles. (f) The electric field profile is along the perpendicular direction to the gold layer at the resonance angle.

interaction between discrete particles and the sensor surface (discrete particle theory of SPR), where WF-SPRM can detect individual nano-objects in solutions and gases, is presented. This proposed model is compared with experimental data to validate its accuracy and applicability.

3.1. Traditional mathematical model

The traditional medium model of the SPR sensor treated the detected molecules near the sensor surface as an effective medium with an effective refractive index and thickness (Fig. 4a). When light transmits the Au-layer, *p*-polarized SPR waves are generated at the metal/dielectric media interface. The SPR phenomenon is described using Maxwell's equations in (*x*, 0, *z*) as follows:

$$E_i(\vec{r}, t) = (E_{ix}, 0, E_{iz}) [e^{-|z|k_{iz}} e^{i(xk_{ix} - \omega t)}] \quad (1)$$

$$H_i(\vec{r}, t) = (0, H_{iy}, 0) [e^{-|z|k_{iz}} e^{i(xk_{ix} - \omega t)}] \quad (2)$$

where: ω is the frequency, E is the electric field vector, H is the magnetic field vector, k_{iz} and k_{ix} are the *z* and *x* wave vector components. Applying the continuity conditions,

$$k_{iz} = \sqrt{\epsilon_i \left(\frac{\omega}{c}\right)^2 - k_{ix}^2} \quad (3)$$

and,

$$\frac{k_{z1}}{\epsilon_m} H_{1y} + \frac{k_{z2}}{\epsilon_d} H_{2y} = 0 \quad (4)$$

$$H_{1y} - H_{2y} = 0 \quad (5)$$

When the determinant is equal to zero,

$$k_{z1}\epsilon_d + k_{z2}\epsilon_m = 0 \quad (6)$$

At $K_{x1} = K_{x2} = K_x$, the expression becomes:

$$K_x = \frac{\omega}{c} \sqrt{\frac{\epsilon_m \epsilon_d}{\epsilon_m + \epsilon_d}} \quad (7)$$

Eq. (7) represents the SPW vector $K_x = K_{sp}$, illustrating the surface plasmon generation. ϵ_m and ϵ_d are the dielectric constants of the metal and dielectric layers. The transfer matrix method is used to investigate the reflectivity curve of the *N*-layer Kretschmann configuration model as follows [29],

$$\begin{pmatrix} U_1 \\ V_1 \end{pmatrix} = M \begin{pmatrix} U_{N-1} \\ V_{N-1} \end{pmatrix} \quad (8)$$

where: (U_1, V_1) and (U_{N-1}, V_{N-1}) are the borderline terms of the 1st and *N*th layers of electromagnetic fields. Eq. 9 represents the characteristic matrix [29],

$$\prod_{K=2}^{N-1} M_K = \begin{pmatrix} M_{11} & M_{12} \\ M_{21} & M_{22} \end{pmatrix} \quad (9)$$

where:

$$M_K = \begin{pmatrix} \cos\beta_k & \frac{i\sin\beta_k}{q_k} \\ -iq_k\sin\beta_k & \cos\beta_k \end{pmatrix} \quad (10)$$

$$q_k = \frac{\sqrt{\varepsilon_k - n_1^2 \sin^2 \theta_1}}{\varepsilon_k} \quad (11)$$

$$\beta_k = \frac{2\pi d_k}{\lambda} \left(\sqrt{\varepsilon_k - n_1^2 \sin^2 \theta_1} \right) \quad (12)$$

Where: d_k is the thickness of the k_{th} -layer, θ_k and λ are the incident angle and wavelength of the incident light. Subsequently, the complex reflection coefficient (r_p) is given by,

$$r_p = \left[(M_{11} + M_{12}q_5)q_1 - \frac{(M_{21} + M_{22}q_5)}{(M_{11} + M_{12}q_5)q_1} + (M_{21} + M_{22}q_5) \right] \quad (13)$$

The reflectivity (R_p) is obtained by,

$$R_p = |r_p|^2 \quad (14)$$

When the polarized light illuminates the Au layer through the glass prism, the light reflection varies by changing the incident angle until it reaches the minimum at the resonance angle (θ_R), i.e., for prism/Au

layer/water, the resonance angle is $\sim 59.5^\circ$ (Fig. 4b, black line). The reflectivity reaches the minimum at θ_R , indicating surface plasmon excitation that induces surface plasmon resonance, where the p -polarized light interacts with the free electron of a metal film, resulting in free electron oscillations [30]. Assuming a molecular layer of PS (10 nm) deposited on the Au layer, the resonance angle increases to about 60.2° due to a variation in the refractive index of the media above the Au layer (Fig. 4b, blue line). According to the traditional medium model, the change in the reflectivity (ΔR) is related to the resonance angle shift ($\Delta\theta$) by [31,32],

$$\Delta R(\theta) = -\frac{dR(\theta)}{d\theta} \Delta\theta \quad (15)$$

where: $dR(\theta)/d\theta$ is the slope of the reflectivity as a function of angle shift (Fig. 4c). Additionally, Kretschmann's configuration of the structure (prism/Au/water) is simulated using COMSOL Multiphysics to investigate the electric field distribution across the structure at resonance angle, with and without adding a PS layer of 10 nm (Fig. 4d, e, respectively). Due to the maximum surface plasmon excitations, the electric field is enhanced at the metal/analyte interface for the resonance angle (Fig. 4d). Adding a PS layer on the Au layer increases the electric field intensity at the PS/analyte interface (Fig. 4e). The electric field profile at the resonance angle is illustrated in Fig. 4f. A significant rise in the electric field is generated at the gold/analyte interface, and

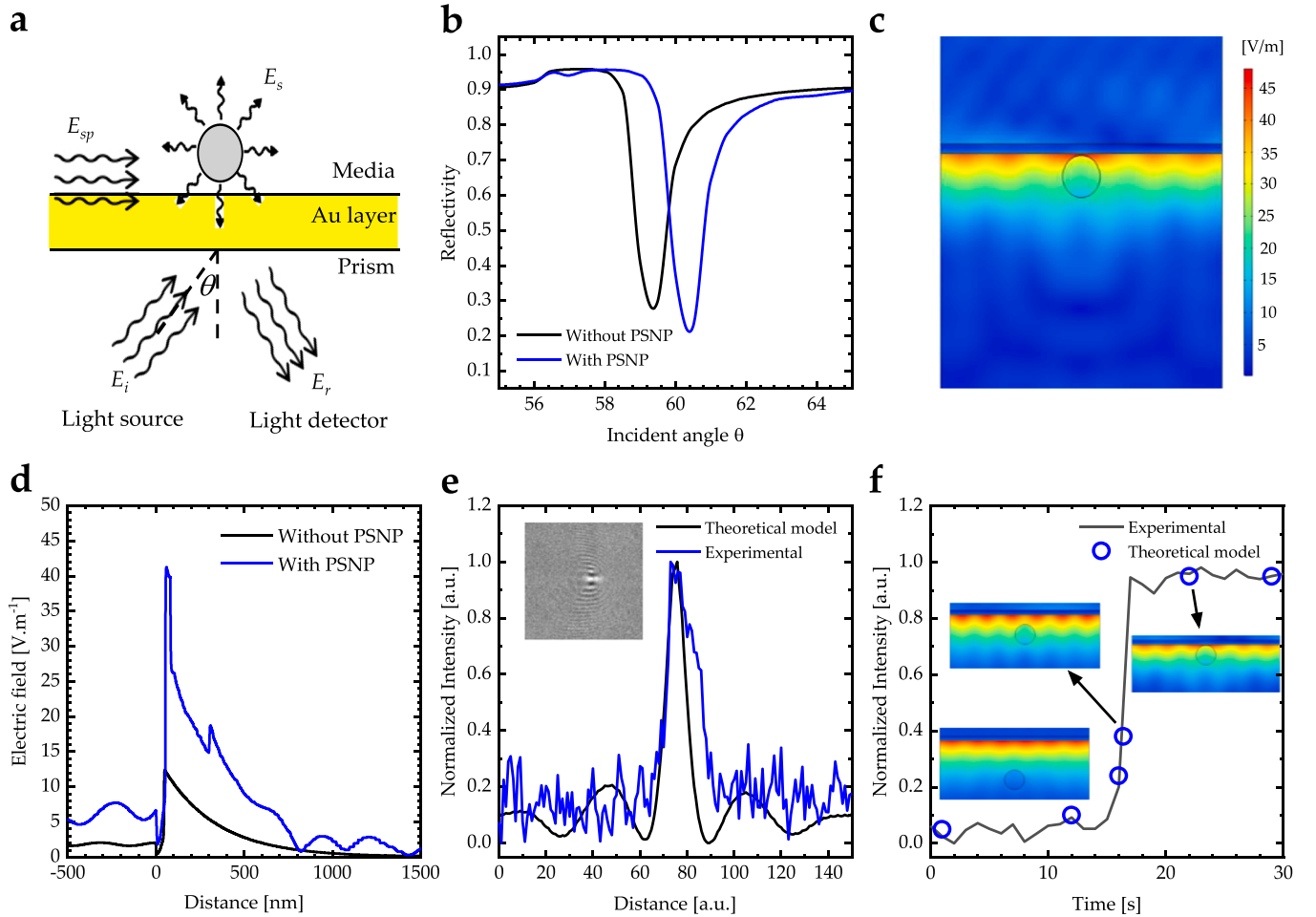


Fig. 5. Discrete particle model of THE SPR sensor. (a) Schematic of the discrete particle model, (b) SPR reflectivity curves without (black line) and with (blue line) PSNP of 200 nm. (c) Electric field distributions along the Kretschmann's configuration of the structure with PSNP of 200 nm. (d) The electric field profile is along the perpendicular direction to the gold layer at resonance angle without/with PSNP of 200 nm. (e) Experimental and calculated intensities vs. distance of PSNP. (f) Time dependence of the intensity in the middle of the bright spot for PSNP bounded to the Au layer compared to calculated intensity for different location distances of PSNP from the Au surface.

the electric field intensity decreases exponentially in the analyte-containing detected biomolecules.

3.2. SPR discrete particle model

WF-SPRM can detect individual nano-objects in solutions and gases. Therefore, an SPR discrete particle model is introduced to describe the detection principle for discrete particles [16,17]. SPR discrete particle model describes the interaction between the surface plasmon polaritons and the detected particle as follows. Fig. 5a illustrates a discrete particle detection using an SPR sensor as well as the electric field generated between the detected particle and the Au-layer. First, the interaction between the incident p-polarized light and the Au-layer is described, where the incident p-polarized light is either reflected from the Au-layer (E_r) or transmitted into Au-layer, exciting surface plasmon waves (E_{sp}) [33]. The total electric field is [34–36],

$$E = E_r \sin(\omega t) + E_{sp} \sin(\omega t + \varphi) \quad (16)$$

where: φ is the phase shift. Additionally, the resulting intensity from the detection process in SPR is given by [14],

$$I = c \cdot \epsilon_0 \cdot n \cdot |E|^2 = c \cdot \epsilon_0 \cdot n \cdot |E_r \sin(\omega t) + E_{sp} \sin(\omega t + \varphi)|^2 \quad (17)$$

or,

$$I = \frac{1}{2} c \cdot \epsilon_0 \cdot n (E_r^2 + E_{sp}^2 + 2E_r E_{sp} \cos \varphi) \quad (18)$$

where: ϵ_0 is the vacuum permittivity. Afterward, the excited surface plasmon waves will scatter from the detected particle with concentric surface plasmon waves around it. Elastic scattering theory describes scattered fields of surface plasmons by a particle as a decaying cylindrical plasmonic wave [28,37,38],

$$E_s(r, r') = E_{sp}^0(r') e^{-\kappa|r-r'|} e^{-ik|r-r'|} \quad (19)$$

where: r' is the particle location, $E_{sp}(r')$ is the surface plasmon field at the particle's location, κ and k are the decaying constant and wavenumber of the SP. Subsequently, according to Born's approximation, the total surface plasmon field (E_{sp}) is [37,39],

$$E_{sp}(r, r') = E_{sp}^0(r) + \alpha E_{sp}^0(r') e^{-\kappa|r-r'|} e^{-ik|r-r'|} = E_{sp}^0(r) + \alpha E_s(r, r') \quad (20)$$

where: α is a scattering coefficient related to the particle's polarizability. $E_{sp}^0(r)$ is the SP field in the absence of the particle. The SPR image contrast of the particle is described by,

$$I(r, r') = \frac{1}{2} c \cdot \epsilon_0 \cdot n \left[\left(E_r^2 + \left(E_{sp}^0(r) + \alpha E_s(r, r') \right)^2 + 2E_r \left(E_{sp}^0(r) + \alpha E_s(r, r') \right) \cos \varphi \right) - \left(E_r^2 + E_{sp}^2 + 2E_r E_{sp} \cos \varphi \right) \right] \quad (21)$$

3.3. Single Particle

When a molecule is bound to the Au surface, it destroys the electric field symmetry on the Au surface that results from the SPR phenomenon. Assuming a polystyrene nanoparticle with a diameter of 200 nm located 5 nm above the Au layer, the resonance angle increases to approximately 60.2°, comparable to a molecular layer of polystyrene (Fig. 5b). The electric field distribution across the structure at the resonance angle

with the PSNP of 200 nm is shown in Fig. 5c. At the resonance angle, the electric field is enhanced at the metal/analyte interface, breaking the electric field symmetry. The electric field profile at the resonance angle with the presence of the PSNP shows a significant increase in the electric field at the gold/analyte interface. At the same time, it decreases exponentially within the detected particles containing the analyte. Additionally, a small peak appears in the middle of the PSNP, indicating the enhancement of the electric field around it (Fig. 5d). On the other hand, when a particle binds to the sensor surface, it disturbs the evanescent field of the surface plasmon wave, leading to local changes in reflectivity, manifesting as a bright spot in the image. The intensity distribution of the binding event between the molecule and the Au surface can be calculated using Eq. 21. The calculated intensity profile describes the experimental profile well, validating our derived model to describe the discrete particle detection by SPR sensors (Fig. 5e). Additionally, the time dependence of relative intensities during the binding of PSNP to the Au surface illustrates the well-matching between the experimental and calculated data (Fig. 5f). From these results, it can be concluded that the SPR discrete particle model can describe the spatiotemporal interactions of the discrete particle detection using WF-SPRM since the model can describe the intensity profile of the detected particle as well as the time dependence of relative intensities during the binding of the detected particle to the Au surface.

The signal intensity of the detected nanoparticles is influenced by two primary parameters, the first being the nanoparticle size [40]. As depicted in Fig. 6a, the signal intensity of polystyrene nanoparticles (PSNPs) shows a linear increase as the particle diameter ranges from 100 to 400 nm. Furthermore, the linear dependence of signal intensity reaffirms that the images correspond to individual particles rather than agglomerates. The second parameter affecting the signal intensity is the refractive index of the nanoparticles. For instance, gold nanoparticles (AuNPs) with a diameter of 80 nm exhibit higher signal intensity compared to silica nanoparticles (with a refractive index of $n = 1.420$ [30]) and PSNPs (Fig. 6b).

3.4. Two-Particle Interference

When double PSNPs are located in proximity to the Au gold surface, at a distance smaller than the SP propagation length, the SP waves around them interact through constructive and destructive interferences, leading to the breakdown of system symmetry in the electric field (Fig. 7a). The electric field profile at the resonance angle, in the presence of the PSNPs, exhibits a pronounced rise in intensity at the gold/analyte interface, followed by an exponential decrease in the electric field intensity within the analyte-containing detected molecules. Additionally, a small peak appears at the midpoint between the two PSNPs, indicating an enhancement of the electric field in this region

(Fig. 7b). The contrast in the surface plasmon resonance (SPR) image of the two PSNPs can be described by the following expression:

$$I(r) = I_1 + I_2 + c \cdot \epsilon_0 \cdot n |E_{s1}| |E_{s2}| \cos(\varphi_1 - \varphi_2) \quad (22)$$

with, E_{sj} is the scattering electric field of particle j, which is given by,

$$E_{sj} = E_{sp}^0(r_j) e^{-\kappa|r-r_j|} e^{-ik|r-r_j|} \quad (23)$$

$$I_j(r, r') = \frac{1}{2} c \quad \varepsilon_0 \quad n \left[\left(E_r^2 + \left(E_{sp}^0(r) + \alpha E_{sj} \right)^2 + 2E_r \left(E_{sp}^0(r) + \alpha E_{sj} \right) \cos \varphi \right) - \left(E_r^2 + E_{sp}^2 + 2E_r E_{sp} \cos \varphi \right) \right] \quad (24)$$

Fig. 7c presents the intensity profile distribution for the experimental case of two PSNPs near the Au surface, compared with the calculated intensity profile. It is evident that the calculated intensity profile described the experimental data well, indicating the validation of our derived model.

3.5. Coating gold layer with a dielectric layer

Coating the gold layer with a dielectric layer is a highly effective modification to enhance the sensitivity of the SPR sensor [41,42]. In this section, the refractive index, extinction coefficient, and thickness of the dielectric layer are optimized to achieve improved sensitivity. Initially, the sensitivity enhancement resulting from the gold coating with a dielectric layer is explained [43]. The propagation constant (β_{ev}) can characterize the propagation of evanescent waves and given by Eq. (25) [44],

$$\beta_{ev} = \frac{2\pi}{\lambda_{light}} n_{prism} \sin \theta \quad (25)$$

Here, n_{prism} and θ represent the refractive index of the prism and the incident light angle at the metal surface, respectively, and λ_{light} is the light wavelength. Additionally, the propagating constant of the surface plasmon wave for the SPR sensor with a single metal layer and sensing medium is given by Eq. (26) [44],

$$\beta_{SPW} = \frac{2\pi}{\lambda_{light}} \sqrt{\frac{n_{metal}^2 n_{sm}^2}{n_{metal}^2 + n_{sm}^2}} \quad (26)$$

Here, n_{metal} and n_{sm} represent the refractive indices for metal and sensing medium, respectively. Eqs. 25 and 26 at resonance can be connected to get the resonance SPR angle as,

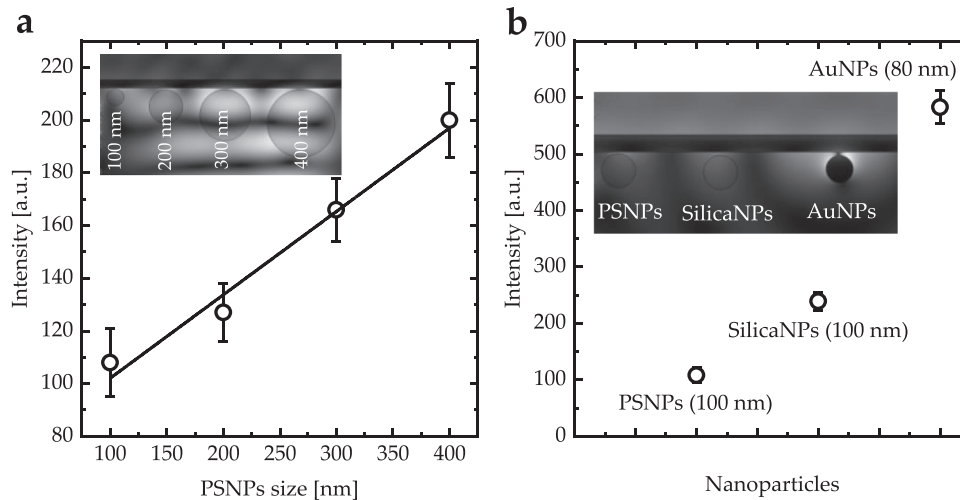


Fig. 6. (a) Response of SPR intensity as a function of a PSNPs size, and (b) Response of SPR intensity for different nanoparticle refractive indices.

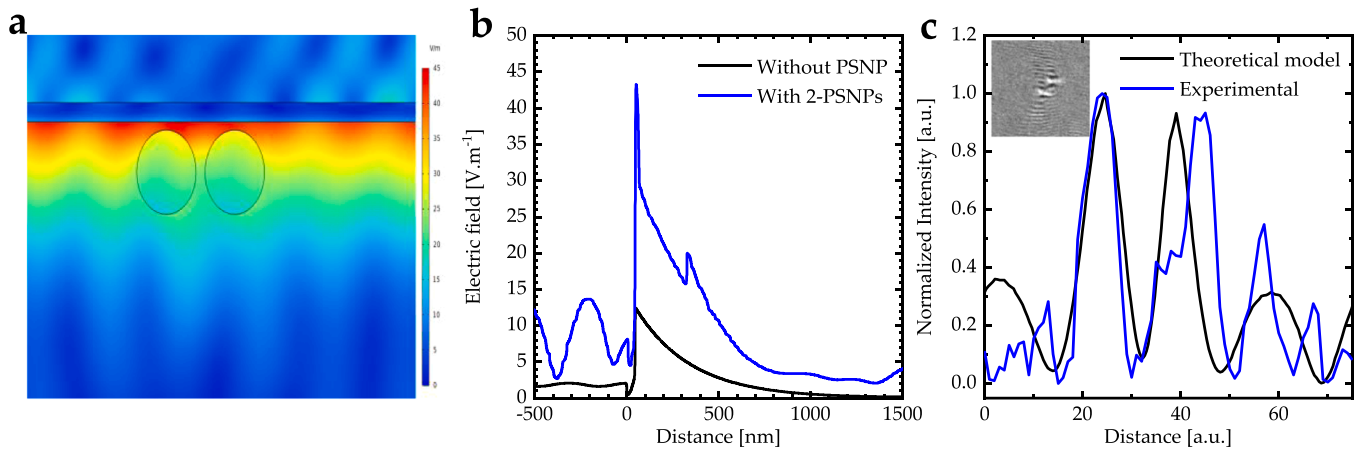


Fig. 7. Two-particle interference model of the SPR sensor. (a) Electric field distributions along the Kretschmann's structure configuration with 2-PSNPs of 200 nm. (b) The electric field profile is along the perpendicular direction to the gold layer at resonance angle without/with 2-PSNPs of 200 nm. (c) Experimental and calculated intensities vs. distance of 2-PSNPs.

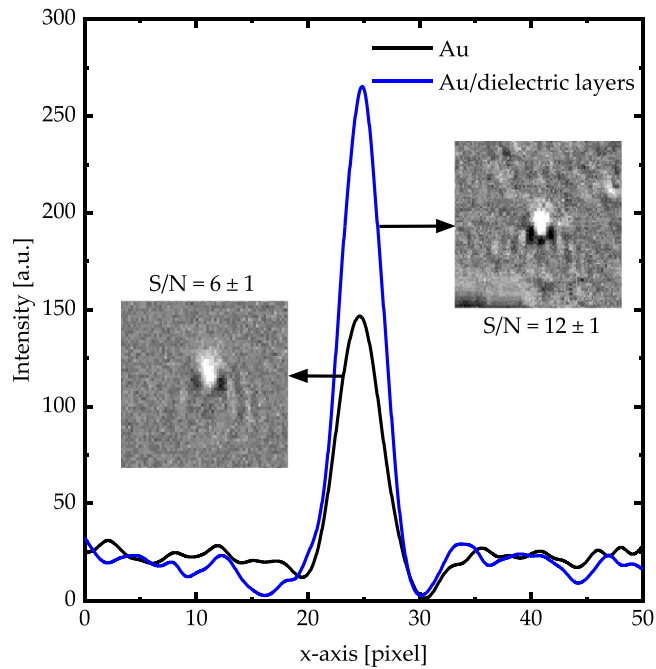


Fig. 8. Line profile plot showing the detection of PSNPs by Au layer and Au/ $n=1.9$ layers.

$$\theta_{SPR} = \sin^{-1} \left(\frac{1}{n_{prism}} \sqrt{\frac{n_{metal}^2 n_{sm}^2}{n_{metal}^2 + n_{sm}^2}} \right) \quad (27)$$

In addition, by coating the metal surface with a dielectric layer of refractive index ($n_{dielectric}$) in the SPR sensor changes the SPR angle equation to [45],

$$\theta_{SPR} = \sin^{-1} \left(\frac{1}{n_{prism}} \sqrt{\frac{n_{metal}^2 n_{dielectric}^2 n_{sm}^2}{n_{metal}^2 + n_{dielectric}^2 + n_{sm}^2}} \right) \quad (28)$$

The sensitivity of SPR sensors (S) is calculated by $S = \Delta\theta/\Delta n$, where: $\Delta\theta$ is the SPR angle shift, and Δn is the refractive index change. As seen in Eq. (28), the SPR angle shift increases with an increase in the effective refractive index of the system. Supplementary S3 demonstrates that coating the gold layer with a dielectric layer having a refractive index above 1.8 significantly increases the sensitivity of the SPR sensor. Furthermore, to achieve high sensitivity with acceptable detection accuracy, the thickness of the dielectric layer should fall within the range of 15–20 nm.

Subsequently, PSNPs were employed as an example for real-time measurements in two systems: prism/Ti(5 nm)/Au(45 nm) and prism/Ti(5 nm)/Au(45 nm)/dielectric($n = 1.9, k = 0.01, 20\text{nm}$). The signal-to-noise ratio (S/N) and the linewidth in the line profile plot for three individually bound PSNPs to the surface are calculated to investigate the efficiency of the SPR sensor (Fig. 8). The signal-to-noise ratio (S/N) of the prism/Ti(5 nm)/Au(45 nm)/dielectric($n = 1.9, k = 0.01, 20\text{nm}$) system outperformed that of the prism/Ti(5 nm)/Au(45 nm) system, thereby confirming our theoretical findings.

Our previously published research investigated the effect of coating the Au-layer with dielectric layers on the SPR sensitivity. For instance, coating the Au-layer by complex composite films based on polyaniline (PANI) doped hydrochloric acid (HCl) incorporated with aluminum nitrate ($\text{Al}(\text{NO}_3)_3$) increases the signal-to-noise ratio for PSNP detecting from 3.95 for bare Au-layer to 8.82 [46]. On the other hand, coating the Au-layer with polyacrylic acid polyelectrolyte brushes (PAA PEBs) increases the signal-to-noise ratio for PSNP detection from 6 for bare Au-layer to 18 [47].

4. Conclusions

A strong SPR signal is generated in the laser wavelength range of 600–800 nm, and to achieve this, a glass prism with a refractive index of 1.725 is selected. A high single numerical aperture objective of 2.5 is used to combine both illumination and imaging optics. The gold thickness range of 40–45 nm is selected to achieve a higher detection accuracy. Polystyrene nanoparticles with an average size of 200 nm serve as an example for real-time WF-SPRM measurements. The first step in the WF-SPRM data analysis is to suppress image background and noise. This is achieved by frame averaging and normalization to the local light intensity. The averaged signals are then subtracted in each frame, and the time dependence of the difference is plotted. Subsequently, the time-dependent relative intensity before and after the binding of particles to the sensor surface is measured. It is observed that increasing particle size leads to an increase in signal intensity. Moreover, AuNPs exhibit a higher signal intensity compared to silica nanoparticles and PSNPs. The traditional mathematical model is utilized to describe the SPR sensor principle, where molecules bound to the sensor surface are treated as an effective medium with an effective refractive index and thickness. Additionally, a discrete particle model of SPR describes the principles of discrete particle detection. Theoretical, numerical, and experimental analyses of the SPR detection principle are performed, considering discrete particle detection. The calculated intensity profiles of single nanoparticles and 2-nanoparticles from the discrete particle model agree well with the experimental data. Furthermore, the effect on the SPR sensitivity of coating the gold layer with a dielectric layer of varying refractive indices is investigated. Finally, polystyrene nanoparticles of size 200 nm have recurred as an example for real-time measurements to validate the results and to present the WF-SPRM data analysis method. In conclusion, an optical imaging technology is developed to detect and quantify discrete particles, such as viruses, proteins, and cancer cells, and monitor the time-dependence interactions between the detected particles and gold sensors. It is anticipated that this technology will complement the traditional technologies and enable the detection of low concentration biological and chemical particles.

CRediT authorship contribution statement

Ahmad Telfah: Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Conceptualization.
Qais Al Bataineh: Writing – original draft, Visualization, Software, Methodology, Formal analysis, Data curation, Conceptualization.
Roland Hergenröder: Writing – review & editing, Validation, Supervision, Resources, Project administration, Investigation, Data curation.
Carlos Tavares: Writing – review & editing, Visualization, Formal analysis.

Declaration of Competing Interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.sna.2024.115266](https://doi.org/10.1016/j.sna.2024.115266).

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