Novel bio-nanochip based on localized surface plasmon resonance spectroscopy of rhombic nanoparticles

Shuoli Zhu¹, Fei Li¹, Chunlei Du² & Yongqi Fu²*

¹Author for correspondence
²Institute of Optics & Electronics, Chinese Academy of Sciences, Chengdu 610209, Sichuan Province, People's Republic of China
Tel.: +86 028 8510 0506; Fax: +86 028 8510 0210; E-mail: cldu@ioe.ac.cn
³School of Physical Electronics, University of Electronic Science & Technology of China, Chengdu 610054, Sichuan Province, People's Republic of China
Tel.: +86 028 8320 2590; E-mail: ygfu@uestc.ac.cn

A new silver (Ag) nanostructure with a rectangular distribution array composed of rhombic nanoparticles is described here. The structure has an apparent advantage of strong hot spots that have a much higher signal intensity than that of the previously reported traditional triangular structures. It generates a great enhancement of a localized surface plasmon resonance (LSPR) effect. Moreover, an antigen with longer arm length is applied to strengthen the binding signals of both the antigen and antibody. We performed experiments for the LSPR-induced extinction spectra in each step of the surface modification of the Ag nanoparticles in atmosphere environment. A spectrophotometer was used to measure the extinction spectrum of our proposed nanochip. The results obtained indicate a better sensitivity for our current nanochip than that of the other reported LSPR-based nanochips. Theoretical computational numerical simulation is also carried out with a discrete dipole approximation algorithm. Our computational results are in agreement with the corresponding experimental spectrum. This type of nanochip may have potential utility in many applications, including medical science, biological fields and biochemical analysis.

Localized surface plasmon resonance (LSPR)-based bio-nanochips are of great interest in various applications, such as environmental protection [1,2], biotechnology [3] and food safety [4,5]. As is well-known, LSPR is excited when the incident photon frequency is resonant with the collective oscillation of free electrons of the conductors. The LSPR-based spectrum peaks are sensitive to the electric medium on the surface of metal film, especially for noble metals. The LSPR-based nanosensor is a device that senses variation in effective refractive indices of the biosamples, which relies on the extraordinary optical properties of noble metal (e.g., silver [Ag], gold [Au] and copper [Cu]) nanoparticles [1-3].

The sensing capability of the LSPR sensor can be modified by tuning shape, size and material composition of the metallic nanoparticles.

In this research domain, representative works have been performed by Northwestern University [1-10]. One of their research subjects focused on the measurement of binding signal between the antigen and antibody with the triangular Ag nanoparticles [4-6]. However, a short arm length between the biotin carbon chain and the triangular metallic nanoparticles was found, by which a few hot spots can be used in their works, thus causing poor sensitivity. In this paper, an original rhombic Ag nanoparticle with many more hot spots and a modified biotin with longer carbon chain are proposed to improve detection sensitivity. For our work here, the number of hot spots for each Ag rhombic nanoparticle is double-fold or even more than that of the Ag triangular nanoparticles. It is calculated from the different geometry shapes between the triangular and rhombic structures. Here, the rhombic nanoparticles have very sharp corners in the nanoscale, which do not exist for the previous reported triangle particles. The hot spots can be generated at the apex of the corners whereas light beams illuminate the particle array, normally owing to energy flow and concentration of the surface wave. It apparently gives rise to millions of binding hot spots throughout the whole metal nanoparticle array. Therefore, the Ag rhombic nanoparticle array can enhance signal intensity of the LSPR-based spectrum greatly.

Our study focused partly on optical nanochips based on the LSPR of the rhombic Ag nanoparticles. This nanoparticle-based optical-sensing technique is effective for a quantitative detection of chemical and biological targets [1,2,4-6]. The sensing principle used in these experiments relies on the high sensitivity of the LSPR-based spectrum of the noble metal nanoparticles for sensing the changes in the dielectric constant of the surrounding environment caused by the biosamples. Our Ag rhombic nanoparticles were fabricated by a nanosphere lithography (NSL) method [7-11]. The local environment surrounding the nanoparticles was modified by chemical
disposal and the binding of the biologic molecule. We used a Sciencetech Inc. (Canada) spectrophotometer to measure the extinction spectrum of our proposed nanochip. The results indicate obtained sensitivity for the present nanochip that is better than that of the other LSPR-based nanochips. Theoretical simulation was also carried out using a discrete dipole approximation (DDA) [12,13] algorithm. The results agree well with the experimental spectra. This type of nanochip may have potential utility in many applications, including medical science, biochemical analysis and biological fields.

Theoretical background & design issues
Numerical simulation is performed using the DDA algorithm, which is a powerful numerical method for calculating scattering and absorption for the targets of arbitrary structures. The target is represented as a lattice of polarized cubic elements (N-point dipoles) whose positions and polarizabilities are denoted as \( r_i \) and \( \alpha_i \). The electrodynamics of this array of dipoles in the presence of an applied plane wave field are then solved. To do this, the polarization induced in each dipole as a result of the incident and retarded fields from the other elements can be expressed as [14,15]:

\[
P_i = \alpha_i E_{\text{loc},i}(r_i) \quad i = 1,2,\ldots,N
\]  

(1)

where the local field \( E_{\text{loc},i}(r_i) \) is the sum of the incident and retarded fields of the other N-1 dipoles. For a given wavelength \( \lambda \), the field can be expressed as:

\[
E_{\text{loc},i}(r_i) = E_{\text{inc},i} + E_{\text{ret},i} = E_0 \exp(i k r_i) \sum_{j=1}^{N} \frac{A_{ij} P_j}{r_{ij}}
\]  

(2)

and \( E_0 \) and \( k = 2\pi / \lambda \) are the amplitude and wave number of the incident wave, respectively. The interaction matrix A is then expressed as:

\[
A_{ij} P_j = -\frac{1}{\varepsilon_0} \frac{1}{r_{ij}} \left( \hat{r}_i \times \left( \hat{r}_j \times \frac{1}{r_{ij}} \left( \hat{r}_i \times \left( \hat{r}_j \times \frac{1}{r_{ij}} \left( \hat{r}_i \times \left( \hat{r}_j \times \frac{1}{r_{ij}} \left( \hat{r}_i \times \left( \hat{r}_j \times \frac{1}{r_{ij}} \left( \hat{r}_i \times \left( \hat{r}_j \times \frac{1}{r_{ij}} \left( \hat{r}_i \times \left( \hat{r}_j \times \frac{1}{r_{ij}} \left( \hat{r}_i \times \left( \hat{r}_j \times \frac{1}{r_{ij}} \left( \hat{r}_i \times \left( \hat{r}_j \times \frac{1}{r_{ij}} \left( \hat{r}_i \times (E_0 \exp(i k r_i)) \right) \right) \right) \right) \right) \right) \right) \right) \right) \right) \right) \right) \right) \right) \right) \right) \right) \right) \right) \right)
\]  

(3)

where \( r_{ij} = |r_i - r_j| \) here is the distance vector from dipole i to dipole j. Substituting Equation 2 into Equation 1, we obtain:

\[
\alpha_i P_i = \sum_{j=1}^{N} A_{ij} P_j E_{\text{loc},i} \quad i = 1,2,\ldots,N
\]  

(4)

The polarization vectors and electric fields are then be obtained by solving 3N linear equations of the form:

\[
A P = E
\]  

(5)

where \( E_\text{off} \) is the diagonal element of the matrix, \( A_\text{eq} \), which is the same as \( A_\text{eq} \), and the diagonal element of the matrix, \( A_\text{eq} \), is \( \alpha^{-1} \). After obtaining the polarization vector \( P \), we can calculate cross-section of the extinction as:

\[
C_{\text{ext}} = \frac{4\pi k}{N} \sum \text{Im}(E_{\text{loc},j} P_j)
\]  

(6)

The computer time used in the DDA method is proportional to the number of the dipoles. Depending on the error tolerance in the calculation, the typical cube size required for convergence (for a noble metal particle) is in the range of 0.5 to 2.0 nm and the method is limited to the calculation of a particle or a cluster of particles whose total size is a few hundred nanometers in each dimension. For a periodic array of particles, the local electric field and polarization is a periodic function in two dimensions. So we need to solve the linear equations for a single unit cell only. But for the sum term in Equation 2, it is extended to include periodic replicas of as many cells as the numbers, which is needed to converge the expansion.

The DDA simulation parameters are determined according to the real samples applied in our experiments. That is, as shown in Figure 1A & B, the Ag nano-rectangle has in-plane widths of approximately 140 nm and out-of-plane heights of approximately 40 nm. The angle of the axis from underside is 60°, and the period of the Ag nanorhombus array is 440 nm. The refractive index around the Ag nanorhombus array used in the simulation is chosen by considering the index of air, glass substrates and biology reagent to be a uniform index.

The result of the DDA simulation is presented in Figure 2, in which Figure 2A presents the extinction spectrum of the DDA simulation whose refraction index is equivalent to that of the bare Ag nanoparticles on substrate; Figure 2B corresponds to the extinction spectrum after the self-assembly monolayer (SAM) modification; Figure 2C shows the extinction spectrum for the Ag nanoparticles modified with the biotin, and the extinction spectrum associated with streptavidin is illustrated by Figure 2D. The dielectric constant of the environment for the different
adsorbents was chosen according to the difference of the biosamples. The values are shown later in the discussion and analysis.

Nanofabrication
An extended NSL technique was used to create the surface-confined rhombic Ag nanoparticles supported on a glass substrate, as shown in Figure 3A. First, the glass substrate was cleaned. Then, the self-assembly of size-monodisperse (the sphere size of the chemical solutions that is spin-coated as a monolayer in which the sphere size determines the generated rhombic particle size), polystyrene nanospheres (500 nm, 2%), glass nanospheres (200 nm, 1%) and distilled water were mixed in 5:1:50 and applied to form a monolayer (Figure 3B), followed by hydrofluoric acid to etch off the glass nanospheres of S-layers and glass nanospheres remained on the substrates (Figure 3C). After that, the Ag particles were deposited through the nanosphere masks using a thermal or electron beam-evaporation technique. After removal of the polystyrene nanospheres by sonication in absolute ethanol for 3 min, well-ordered 2D rhombic nanoparticle arrays were finally obtained on the substrates. By changing the nanosphere diameter and the deposited metal thickness, the nanoparticles with different in-plane width, out-of-plane height and interparticle space can be derived. Figure 3 shows the fabrication process of the nanoparticles.

We measured the surface modality of the samples by using JSM-5900LV scanning electron microscope (SEM). The Ag nanorhombus have in-plane widths of approximately 140 nm as measured by SEM and out-of-plane heights of approximately 40 nm as measured by sidestep apparatus. We can see that the size of the Ag rhombic nanoparticles fabricated is not identical, which is a limit of the SAM method, accounting for the errors in our following experiment.
The fabricated Ag rhombic nanoparticles have a larger surface area than that of the Ag triangular nanoparticles when we used the same diameter polystyrene spheres to fabricate them. The surface area of Ag rhombic nanoparticles is 2.5-times larger than the triangular structure. As mentioned earlier, Ag rhombic nanoparticles lead to a stronger enhancement of the localized surface plasmon resonance and thus results in the improvement of the detection sensitivity and efficiency of the binding reaction between the antigen and antibody.

Nanoparticle functionalization
In our LSPR nanochip experiments, the Ag nano-rhombs were first functionalized using the SAM consisting of 3:1 1-octanethiol (1-OT, Figure 4B)/1-mercaptooundecanoic acid (11-MUA, Figure 4A) to produce a surface coverage corresponding to a 0.1 monolayer of carboxylate-binding sites [16]. Carboxylate-binding sites/nanoparticle equivalent to approximately 15,000 were achieved because the maximum number of alkanethiol molecules per nanoparticle is 150,000. Then, with the EDC, we covalently
attached the biotin (Figures 4 & 5) to the carboxylate groups. The number of obtained biotin sites, determined by the EDC coupling reaction yield, was approximately 1–5% coupling efficiency [7] in this experiment. It is expected to be only 150–750 biotin sites/nanoparticles at maximum coverage. The number of biotin sites for each nanoparticle, 130–750, came from [7]. They are calculated by the following method: $6000 \times 2.5 \times 1\% = 150$; $6000 \times 2.5 \times 5\% = 750$. Figure 5 gives an illustration of the LSPR nanobiochip, depicting its exposure to streptavidin.

The third structure has a longer arm, as shown in Figure 4. It enlarges the binding space between the biotin and streptavidin, strengthening the binding effect of the antigen and antibody. This is why we selected biotin in our experiments.

Figure 5 shows a representation of the streptavidin binding to a biotinylated Ag nanobiosensor, which was fabricated by the extended NSL on a glass substrate. Before the specific reaction between the biotin and streptavidin, the surface chemistry of the Ag nanobiochip had been accomplished.

Experimental set-up
To observe the sensor characteristic of this kind of biochip, the extinction spectrum was measured by a Spectrotech Inc. 9055 spectrophotometer. To obtain a visible infrared transmittance spectrum, a white-light source (400–700 nm) was used in the experimental system. Incident light was transmitted through a multimode optical fiber, reaching the collimating lens first, then being illuminated onto the biochip. The light beam transmitted through the sample was collected with an identical focus lens that is attached to the multimode fiber. Then, a monochromator was used to separate the light from the multimode fiber to form a monochromatic light beam. The signal was then sent to a personal computer that is integrated with an analog photomultiplier. The transmittance spectra were displayed directly on the screen of the computer. The measuring process was divided into three steps:

- Measuring of the background light without any input sources and samples;
- Measuring the light source intensity;
- Collecting the transmitted light with the presence of the sample that is placed perpendicular to the incident light.

The transmittance ($T$) can be written as $T = (s - b)/(r - b)$, where $s$, $r$, and $b$ denote the intensity of the sample, reference and background, respectively. The extinction spectra $E$ for each step were achieved by the equation $E = -\log T$. In addition, the transmittance spectra can be plotted and displayed directly in a computer without further data processing.

Results & discussion
The LSPR spectra of the Ag nanochips in each processing step is presented in Figure 6 with incidence wavelength in the range of 400 to 700 nm. The resulting extinction spectrum of the bare Ag nanoparticles is depicted in Figure 6A, where the LSPR $\lambda_{\text{max}}$ was measured to be 538.5 nm. Similarly, Figure 6B shows the extinction spectrum after modification of the Ag nanoparticles with 1 mM 11-MUA-OT. To ensure a well-ordered SAM on the Ag nanoparticles, the sample was incubated in the thiol solution for

---

**Figure 4. Chemical structures of (A) 11-MUA, (B) 1-OT and (C) biotin.**

![Chemical structures of 11-MUA, 1-OT, and biotin](image-url)
24 h. After rinsing carefully and drying thoroughly with N₂ gas, the corresponding peak transmission with LSPR wavelength λ_max was measured as 572 nm. Compared with the bare Ag nanoparticles, λ_max in this surface functionalization step is red-shifted by approximately 13.5 nm. Next, 1 mM biotin was covalently attached by amide bond formation with a two-unit poly(ethylene glycol) linker to the carboxylated surface sites. The obtained LSPR spectrum is indicated in Figure 6C, which shows that the peak transmission occurs at 594.5 nm. It corresponds with an additional 22.5 nm red shift from the second peak (red line). We finally plotted the extinction spectrum in Figure 6 after the reaction between 100 nM streptavidin and 1 mM biotin. The maximum wavelength has a 16.5 nm red shift from 594.5 nm up to 611 nm.

Figure 6. Localized surface plasmon resonance spectra of each step in the surface modification of nanosphere lithography-derived silver nanoparticles to form a biotinylated silver nanobiosensor and the specific binding of SA.

(A) Ag nanoparticles before chemical modification, λ_max = 558.5 nm. (B) Ag nanoparticles after modification with 1 mM 1:3 11-MUA/1-OT, λ_max = 572 nm. (C) Ag nanoparticles after modification with 1 mm biotin, λ_max = 594.5 nm. (D) Ag nanoparticles after modification with 100 nm streptavidin, λ_max = 611 nm. All extinction measurements were collected in air environment.

Ag: Silver; SAM: Self-assembly monolayer.
By contrast, using the same diameter PS spheres, the uniform height of the Ag nanoparticles, under the same metrical condition and processing time, we fabricated the traditional Ag triangular nanosensor and measured the extinction spectrum. However, in our experiments, for the traditional Ag triangular nanosensor, a 7.5-nm red shift in the reaction between 100 nM streptavidin and 1 mM biotin is observed. The shift of 16.5 nm attributed mainly to the hot spot of the rhombic particles mentioned before, which have a dominant role in improving detection sensitivity here.

By analyzing the obtained results, we found that the extinction spectrum varies with each step of the surface functionalization for the Ag nanoparticles. The spectral shifting in these cases can be explained by the change of the local effective refractive index originating from the surface modification in each step.

It should be noted that all the extinction measurements were collected in atmosphere environment, in which the samples were contaminated by dust and other impurities mixed in the air. We expect that the samples should be measured in a sample chamber filling with N₂ gas. The detection sensitivity may then be further improved to a certain extent.

For comparison, calculated and experimental results are plotted together, as shown in Figure 7, in which the square and circle represent the maximum extinction before and after each process for both the experimental and theoretical data, respectively. The values show qualitatively that the calculated data are in accordance with the experimental results. The dielectric constant of the environment was chosen by considering the index of air, glass substrates and biology reagent to be a uniform index in the simulations. Peak shifts of the maximum extinction spectra increase with an increase in the effective refractive indices surrounding the Ag nanoparticles. The discrepancy between the theoretical model and the experimental results can be attributed partially to the contaminations of the samples during the experiments. The advantage of this method is that the substrate can be cleaned thoroughly to improve the fabrication quality of the chips for future experimental use. Compared with the conventional SPR method, the rhombic Ag nanoparticles-based LSPR method has advantages, such as higher spatial resolution, simple system configuration and cost-effectiveness. It has higher sensitivity in comparison to that of the reported triangular Ag nanoparticles. It is because the spatial resolution of the LSPR method is one nanoparticle, whereas the LSPR method, determined by pixel size of the CCD detector used in the system, is approximately 10 μm for normal reported systems. Thus, this method has higher spatial resolution.

Conclusions
We put forth a rhombic Ag nanostructure array fabricated on glass substrate as the biosensors and measured the extinction spectrum of the biosensors. We found that the LSPR-based bio-nanochip has excellent sensitivity and selectivity, because this type of the Ag rhombic nanoparticle-based structure has more hot dots than that of the Ag triangular nanoparticle structures reported before. Moreover, we selected the longer arm length antigen to strengthen the binding reaction between the antigen and antibody. The reason is that when the arm length of the carbon chain of the biotin is increased, the hindrance of the atom-space arrangement can be reduced. This is the main reason for our choice in this experiment. The long PEG spacer arm of the biotin reduces steric hindrance while it binds to avidin molecules. Sciencetech Inc. spectrophotometers had been applied to measure the extinction spectrum of the samples and the corresponding detection sensitivity is 100 nM in atmosphere. We predict that the sensitivity can be further improved by avoiding contamination of the sample.
RESEARCH ARTICLE – Zhu, Li, Du & Fu

For comparison, we used a DDA algorithm to theoretically study extinction properties of our bio-nanochip. The calculated data are in agreement with the experimental results. Thus we confirm the feasibility and validity of our proposed bio-nanochips, which may have applications in many areas, such as medical diagnostics, biotechnology and environmental protection.

Future perspective
This new LSPR-based nano-biosensor with a rhombic Ag nanoparticle array presents a detection approach with higher sensitivity compared with the previously reported triangle Ag particle. In addition, the LSPR-based sensing system is simple and cost effective compared with the SPR-based system. Therefore, it is reasonable to think that the reported modified LSPR-based system will have a potential market in the next 5–10 years. Moreover, it may have significant advantages for a specific bio-sample immunoassay, such as *Staphylococcus aureus* enterotoxin B or amyloid-derived diffusible ligands.

Financial & competing interests disclosure
This research was supported by National Science Foundation of China (No. 60507014 and 60678035). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research
The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

### Executive summary

**Nanoparticle design**
- Calculation of the extinction spectra using the discrete dipole-approximation algorithm is simple and reliable. However, it is difficult to create the geometrical model of nanoparticles with an irregular shape. Moreover, it cannot define the dielectrics with more than one material, such as compound materials and sandwiched structures. By contrast, the finite difference and time-domain (FDTD) algorithm is one possible option for the design owing to its advantages in 3D modeling and flexible dielectric definition. However, running the FDTD is time consuming and occupies much memory space.

**Silver rhombic nanoparticle fabrication**
- Fabrication of the silver (Ag) rhombic nanoparticles using the nanosphere lithography technique has advantages of cost-effectiveness and large-area formation. However, repeatability and uniformity of the fabricated particles are limited by inherent characteristics of the chemical-based approach. Future works will focus on exploring other possible methods, such as laser-interference lithography.

**Applicability of the Ag rhombic nanoparticle-based detection**
- This method can be extended to other biosamples for immunoassay-related detections; for example, amyloid-derived diffusible ligands and staphylococcal enterotoxin B. Our further study regarding these specific biosamples will be published soon.

**Summary**
- The Ag nanoparticle-based localized surface plasmon resonance (LSPR) immunoassay method is inertia to concentration of the biosamples because it senses the interaction between biological molecules and the bio-activated metallic particles. Therefore, it can detect the biosamples in solution at concentration of as low as nm/ml or even pg/ml, thus it can reach high sensitivity.
- Similar to the surface plasmon resonance-based immunoassay method, the LSPR-based immunoassay technique can also be used for the detection of liquid-state biosamples. The molecules attached to the nano-biochips are immersed in chemical solutions for detection in liquid state as long as an optical microscope equipped with objective lenses with long working distance (e.g., 10 mm or longer) are used in the experimental set-up mentioned earlier.
 Novel bio-nanochip – RESEARCH ARTICLE

Bibliography