Inflection Point of the Localized Surface Plasmon Resonance Peak: A General Method to Improve the Sensitivity

Peng Chen,§ Nhung Thi Tran,‡ Xinglin Wen,‡ Qihua Xiong,§ and Bo Liedberg*†‡

†Centre for Biomimetic Sensor Science, School of Materials Science and Engineering, Nanyang Technological University, 50 Nanyang Drive, Singapore 637553
‡School of Materials Science and Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798
§School of Physical and Mathematical Sciences, Division of Physics and Applied Physics, Nanyang Technological University, 21 Nanyang Link, Singapore 637371

Supporting Information

ABSTRACT: The shift of the localized surface plasmon resonance (LSPR) spectrum is widely used in bio- and chemical sensing. Traditionally, the shift is monitored at the peak maximum of the extinction spectrum. We demonstrate that the inflection point at the long wavelength side of the peak maximum shows better refractive index sensitivity than the peak maximum. A consistent improvement in bulk refractive index sensitivity of 18−55% is observed for six different nanoparticles such as spherical particles of different sizes, nanostar and nanorods with different aspect ratios. Local refractive index changes induced by molecular adsorption confirm the superior performance of the method. We contribute this improvement in sensitivity to the change in shape of the LSPR peak in response to an increase of the local refractive index. We further illustrate the advantage of using the inflection point method for analyzing DNA adsorption on U-shaped metamaterials, and for using 17 nm spherical gold nanoparticles for detection of matrix metalloproteinase 7 (MMP-7), a biomarker that is heavily up-regulated during certain cancers. With the inflection point, the limit of detection (LOD) for MMP-7 is improved to 0.094 μg/mL from 0.22 μg/mL. This improvement may facilitate early diagnosis of salivary and colorectal cancers. We also envision that this generic method can be employed to track minute optical responses in other analytical areas.

KEYWORDS: inflection point of LSPR peak, plasmonic nanoparticles, metamaterials, mathematic method, improved sensitivity

The LSPR resonance frequency or wavelength of nanosized noble metal particles depends on their size, shape, composition, interparticle distance, and the medium in which they are embedded/dissolved.1 Recently, LSPR of noble metal nanoparticle has attracted tremendous interest in bio- and chemical sensing.2 LSPR sensors for many target molecules including DNAs,3 proteins,4 small biological molecules,5 metal ions,6 enzymes,7 explosives,8 toxins,9 and gases10 have been developed. Sensing with LSPR most often relies on the red shift of the extinction spectrum induced by changes in either refractive index or interparticle distance occurring in response to molecular recognition and/or attachment/bridging. Although useful, the refractive index sensitivity of LSPR sensors is still several orders of magnitude smaller than the sensitivity obtained using propagating SPR sensors.11 As a result, detecting molecules at ultralow concentrations using LSPR is very demanding especially for molecules of low molecular weight. To solve this problem, numerous efforts have been made to improve the sensitivity of LSPR sensors. One approach involves synthesis of novel nanomaterials with higher plasmonarity such as nanorods,12 nanostars,13 nanoshells,14 mushrooms,15 nanorings,15 nanotriangles,16 nanodisks,17 nanoholes,18 alloy nanoparticles,19a,19c closely packed nanoparticles,20 plasmonic composites,21 and plasmonic nanoparticles of other elements19,22 (a chemical/materials approach). Alternatively, physicists also explored the potential of meta-materials23 or Fano resonance24 by fabricating novel submicron plasmonic patterns using lithography techniques (a physical approach). Although both efforts improve the refractive index sensitivity of LSPR sensors to some extent, they also bring many problems such as high cost, low yield, and tedious processing. In addition, with more and more nanostructures being synthesized and tested, further improvements utilizing the aforementioned strategies become very difficult. Therefore, a facile and general method to improve the refractive index sensitivity is of high significance.

In this contribution, we report a simple mathematical approach by tracking shifts at inflection points of the LSPR spectrum, which is completely independent to both aforementioned chemical and physical approaches. The inflection point approach is not an alternative but a complementary technique that can be used to further improve the performance of LSPR assays. A few alternative mathematical methods to evaluate the peaks of SPR/LSPR have been reported before,25 such as centroid, curvature, and A/D ratio, which all

Received: October 12, 2016
Accepted: January 30, 2017
Published: January 30, 2017

DOI: 10.1021/acs.sensors.6b00633
ACS Sens. 2017, 2, 235−242
contributed to an improved sensitivity, signal-to-noise, and LOD in bio- and chemical sensing.

As mentioned before, the traditional way of monitoring changes in the LSPR spectrum relies on the shift of the peak maximum. However, we noticed that the magnitude of the shift is not constant across the spectrum, because of the changes in the shape of the LSPR peak (broadening, asymmetry). Thus, it is critical to reproducibly identify unique and easily tractable locations in the LSPR spectrum that possess maximum shifts in response to refractive index changes/molecular interactions. We recognized two such points in addition to peak maximum, namely, the inflection points around the peak maximum. Mathematically, the inflection points appear as local maxima/minima in the first order derivative spectrum and as zero-crossings in the second order derivative spectrum. Therefore, they are easily located with the help of the first and second order derivative spectra (Figure 1a–c). We demonstrate herein the performance of the method by studying minute shift induced by refractive index changes in the bulk as well as on the surface of gold nanoparticles and gold metamaterials.

**EXPERIMENTAL SECTION**

**Materials.** Sodium citrate tribasic dehydrate (99%), gold(III) chloride trihydrate (99.9%), silver nitrate (99%), ascorbic acid (99%), cetyltrimethylammonium bromide (96%), chitosan (low molecular weight), acetic acid (99.7%), and glycerol (99%) were purchased from Sigma-Aldrich. Hydrochloric acid (37%) was purchased from Merck Millipore, Singapore. Spherical AuNPs with the size of 40 and 60 nm were purchased from BBI solutions. Peptide (CALNN) was purchased from GL Biochem China. Neutravidin was purchased from Thermo Fisher Scientific Inc., USA. Purified oligonucleotide with a thiol modifier (C6 S-S-GGT GGT GGT TGGT GGT GGT GGT GG) was purchased from Integrated DNA Technologies, Singapore.

**Synthesis and Characterization.** The synthesis of polypeptide JR2EC with the sequence of (NAADLEKAIEALEKHLEAKGPC-DAAQLEKQLEQAFEAFERAG) has been describe elsewhere.26 Gold nanoparticles (AuNPs) of size 13 and 17 nm were synthesized by reduction of HAuCl4 with sodium citrate following the procedures published elsewhere.27 Gold nanostar (AuNS) was prepared by the previous protocol in the literature with little modification.28 Briefly, 180 μL HAuCl4 (0.01 M), 50 μL AgNO3 (0.01 M), and 30 μL HCl (1 M) were consecutively added to 3 mL of Milli-Q water in 20 mL glass vial. Subsequently, 100 μL of ascorbic acid (1 wt %) was rapidly added. The color of the mixture immediately changed to a greenish blue color as the sign of the formation of AuNS. The reaction finished within 30 s. To stabilize the as-prepared from truncation of the sharp tip, chitosan solution (1 wt % in 1% v/v acetic acid, dissolved by sonication until getting transparent solution) was added dropwise into the freshly prepared AuNS solution under gentle magnetic stirring. The final concentration of chitosan is 0.3% wt. Centrifugation and wash were repeated twice to remove the unbound chitosan. Gold nanorod (AuNR) of aspect ratio of ∼3.3 (AuNR1) was prepared by the method published in the literature and AuNR with aspect ratio of ∼4.5 (AuNR2) was prepared using the same method with 0.25 wt % AuNR1 instead of AuNS.

![Figure 1.](image)

Figure 1. (a) Extinction, (b) first derivative, and (c) second derivative spectra of 40 nm AuNPs. The three points are indicated with red dots and the red dashed lines serves as a guide for the eye. Points 1 and 3 are two inflection points of the extinction spectrum around the peak maximum, which shows a 0 value in the second order derivative spectrum. (d) Extinction spectra of 40 nm AuNPs exposed in solvents with refractive indices 1.333, 1.362, 1.399, 1.432, and 1.460, respectively. (e) Shift of Point 1, Point 2, and Point 3 with refractive index. (f) Refractive index sensitivity of the three points.
Fabrication of U-Shaped Metamaterials. The U-shaped metamaterials were fabricated by e-beam lithography following the procedure published elsewhere. Briefly, PMMA was spin-coated on the ITO glass and then electron beam was used to define the structure. After exposure, the sample was developed in MIBK solution, followed by the deposition of metal. The metamaterials were obtained after a lift-off process in acetone.

Measurement of Spectra. Bulk refractive index was tuned from 1.333 to 1.46 by changing the fraction of glycerol and water. Local refractive index change was obtained by the adsorption of peptide and neutravidin on the 60 nm AuNPs. The adsorption process was conducted at 100 μM of the molecules dissolved in 10 mM sodium citrate buffer at pH 6 to prevent aggregation of AuNPs. Extinction spectra were recorded in a PMMA cuvette containing 500 μL AuNPs. The transmission spectra of the metamaterials were collected using a microspectrophotometer (Craic 20) in the range 400–2100 nm. The transmission spectra were measured without any polarizer.

Adsorption of Molecules and MMP-7 Sensor. The peptide CALNN and neutravidin were adsorbed to the AuNPs by introducing the molecules of 100 μM concentration into the suspension of AuNPs. The thiolated DNA of 100 μM was first deprotected at the terminal thiol group by mixing with 150 mM dithiothreitol at room temperature for 2 h. Then the thiolated DNA was desalted and purified using a NAP-5 column. After that, 0.4 μL DNA molecule (10 μM) was spotted on clean metamaterials and incubated overnight in a humidity chamber. The samples were subsequently washed with DNA free water to remove any unbound DNA.

Functionalization of the AuNPs with JR2EC peptide was performed by incubation of the AuNPs in the 10 μM peptide solution at pH 6 (10 mM sodium citrate) overnight. The unbound peptide was removed by 5 repeated centrifugations followed by washing with PBS buffer at pH 7.4. The MMP-7 mediated hydrolysis of the immobilized peptides was performed at 25 °C and real-time extinction spectra were recorded with the Lambda 35 spectrometer. The thiolated DNA of 100 μM was first deprotected at the terminal thiol group by mixing with 150 mM dithiothreitol at room temperature for 2 h. Then the thiolated DNA was desalted and purified using a NAP-5 column. After that, 0.4 μL DNA molecule (10 μM) was spotted on clean metamaterials and incubated overnight in a humidity chamber. The samples were subsequently washed with DNA free water to remove any unbound DNA.

Calculation of Derivative Spectrum. First and second order derivative spectrum are calculated from the extinction spectrum using 19 data points. The details of calculation methods are described in the previous work. Point 2 was obtained from the 0 value of the first derivative spectrum while Point 1 and Point 3 were obtained from the 0 value of second order derivative spectrum; see Figure S1. The influence of the number of points on the final noise in the second order derivative spectrum are also shown in Figure S1. Similar calculation was done for the transmittance spectrum for the metamaterials. It is worthwhile to mention that performing the first/second derivative with the 19 points method produces spectra that significantly reduces the propagation of noise.

RESULTS AND DISCUSSION

The extinction spectrum of 40 nm AuNPs and its corresponding first order and second order derivative spectra are shown in Figure 1a,b,c, respectively. The peak maximum (Point 2) and two inflection points (Points 1 and 3) flanking the peak maximum (Point 2) are highlighted in Figure 1a,b,c by red dots. The red dashed lines serve as guidelines for the eye. The two inflection points are not obvious in the extinction spectrum, but appear as local maxima/minima in the first order derivative and have a 0 value in the second order derivative spectrum. Therefore, the inflection points can be easily tracked in the same way as the peak maximum in the extinction spectrum. It is worthwhile to mention that calculating the first/second derivative with the 19 points method produces a spectrum that is almost as smooth as the original one. To illustrate the shift of the three points with changes in bulk refractive index, the extinction spectra of 40 nm AuNPs in different solvents are measured, see Figure 1d. The corresponding first and second order derivative spectra are shown in Figure S1. The shifts of Point 1, Point 2, and Point 3 with refractive index are summarized in Figure 1e. It is clear from Figure 1e that Point 3 shows the highest slope (sensitivity) with bulk refractive index. The refractive index sensitivity of 40 nm AuNPs tracked at the three points is plotted in Figure 1f. The refractive index sensitivity for Point 1, Point 2, and Point 3 are 41.2, 61.8, and 91.4 nm/RIU, respectively. Interestingly, by simply tracking the shift of LSPR spectrum by Point 3, the refractive index sensitivity for 40 nm AuNPs could be improved by 48% with respect to the peak maximum, Point 2.

The LSPR spectra measured in solvents with different refractive indices were carefully evaluated and we found that the LSPR peak becomes broader and more intense, and shifts to longer wavelengths when the refractive index in the surrounding solution increases. The broadening influences the inflection points and the peak maximum to a varying degree. For example, for the point at the right side of the peak maximum (point 3, Scheme 1), the broadening of LSPR peak will induce an additional shift to longer wavelengths, while for the points at the left side of the LSPR peak (Point 1) it will induce a negative shift (to shorter wavelengths; see Scheme 1). Therefore, the reason for the better sensitivity with inflection point 3 is that it accounts for both the shift of the LSPR peak and the broadening, whereas the broadening counteracts the shift of point 1.

To verify the observation of higher sensitivity of Point 3 over Point 2, a few other gold nanoparticles with different sizes and shapes were tested. Figure 2a shows the extinction spectra of spherical AuNPs of 13, 40, and 60 nm diameter, Au nanostar (AuNS), and Au nanorods (AuNR) with aspect ratios of ~3.3 and ~4.7, respectively. The TEM images of these nanostructures are shown in Figure 2b–g, respectively. Extinction spectra of all the nanostructures in Figure 2 were recorded in a mixture of glycerol and water with different refractive indices (see Supporting Information, Figure S2).

The refractive index sensitivity of all the nanostructures tracked at Point 3 (black) and Point 2 (white) are summarized in Figure 3a. Obviously, for all the nanostructures tested, Point
3 demonstrates superior performance in refractive sensitivity with respect to Point 2. The percentage of improvement is plotted in Figure 3b. A consistent improvement in refractive index sensitivity from 18% to 55% was observed for all six different nanostructures. With these observations, we propose that tracking at the inflection point (Point 3) could be used as a general method to enhance the refractive index sensitivity for many different plasmonic nanostructures. It is worthwhile to mention that in biosensing, the performance is determined not only by the sensitivity, but also by the noise of the sensor. A systematic evaluation of the noise using 19 points method for the three points are shown in Supporting Information Table SI, Table SII, and Table SIII. From our study, there is no difference observed in the noise between the three points, which we attribute to the high quality (low noise level) of the original UV–vis data and the 19 points method. Therefore, the improvement in LOD or S/N ratio of our sensor is represented by the improvement in sensitivity.

In biological sensing, the change in local refractive index induced by molecular binding is of significant interest. Therefore, in addition to the bulk refractive index, the response from local refractive index change was studied using two molecules, peptide (CALNN) and neutravidin. The CALNN peptide consists of 5 amino acids and it has a cysteine group at the end, allowing for end-point attachment to AuNPs. The molecular weights of the peptide and neutravidin are 506 g/mol and $\sim 60,000$ g/mol, respectively. Both molecules are commonly used to functionalize AuNPs for biosensing applications. In additional, the $60 \text{ nm}$ AuNPs capped with sodium citrate were used for the experiments with the peptide and neutravidin in solution. Figure 4a shows the normalized extinction spectra of the AuNPs before (red) and after adsorption of peptide (blue) and neutravidin (black). The 60 nm AuNPs capped with sodium citrate were used for the experiments with the peptide and neutravidin in solution. Figure 4a shows the normalized extinction spectra of the AuNPs before (red) and after adsorption of peptide (blue) and neutravidin (black), respectively. The neutravidin molecule induces a larger red shift of the LSPR spectrum than peptide as expected because of its higher molecular weight. The shift upon adsorption of peptide and neutravidin at Point 2 (white) and Point 3 (black) is plotted in Figure 4b. For both molecules, tracking at Point 3 demonstrates a larger shift than that of Point 2 and the
improvement if especially pronounced for the low molecular weight peptide CALNN.

As mentioned before, in addition to the traditional plasmonic nanoparticles synthesized by the bottom-up approach, metamaterials have also attracted tremendous interest in biosensing due to the improved sensitivity.\textsuperscript{33} Metamaterials are virtually well-defined, densely packed periodic structures/patterns, whose optical properties go beyond the limitations of the naturally occurring materials or composites.\textsuperscript{34} To further explore the applicability of this inflection point method, we tested it with gold metamaterials. A SEM image of the U-shaped nanosized metamaterial made of gold is shown in Figure 5a. The transmission spectra of the metamaterials before (black) and after (red) modification with DNA molecules are shown in Figure 5b.

![Figure 5](image)

**Figure 5.** (a) SEM image of the U-shaped metamaterial, scale bar is 1 μm and (b) transmittance spectra of the U-shaped metamaterial before (black) and after (red) exposure to DNA. (c) Shift at different points induced by DNA adsorption calculated from the spectra in (b).

The DNA molecules were chemically adsorbed onto the gold surface via the thiol group at the terminal. The adsorption of DNA shift the transmittance spectrum of metamaterials toward the red direction. The shifts of the spectra were monitored at the two inflection points around the valley and the valley (minimum) (Figure 5c). Interestingly again, with the same DNA adsorption, the amounts of shift at point 1 (left inflection point), point 2 (minimum), and point 3 (right inflection point) are calculated to be 39.9, 47.6, and 54.8 nm, respectively. This observation is consistent with that observed on plasmonic nanoparticles that the inflection point at the right side of the peak of the extinction/valley of transmittance spectrum yields a larger shift. Therefore, we believe that this novel mathematical method opens an avenue to enhance the sensitivity of plasmonic sensors.

From these observations, it is evident that tracking the shift at Point 3 is better than Point 2, regardless of the type of nanoparticles and the cause of the shift (bulk or monolayer refractive index change). Therefore, it is reasonable to expect that the method of tracking shift at Point 3 could further yield a better performance for applications in biological sensing.

An assay for matrix metalloproteinase 7 (MMP-7) previously developed in our laboratory\textsuperscript{7} was employed to evaluate the performance by tracking Point 3. MMP-7 plays a significant role in cell development and in the metastasis of cancers.\textsuperscript{35} It is significantly up-regulated in many cancers, e.g., lung cancer, gastric cancer, colorectal cancer, and bladder cancer.\textsuperscript{36} The concentration of MMP-7 also varies with the stages of cancer development; therefore, potentially it is a useful early biomarker for cancer prognosis.\textsuperscript{37} In addition, it is also an important target for anticancer drugs.\textsuperscript{38} A synthetic polypeptide (JR2EC) with two cleavage sites (A-L and A-Q-L) for MMP-7 had been synthesized and its sequence was modified to enable reliable immobilization to AuNPs through a cysteine (SH-containing) residue at position 22.\textsuperscript{7,36} Digestion of the polypeptide by MMP-7 reduces its net charge from $-5$ to $-1$, which in turn reduces the repulsive force between AuNPs, resulting in massive aggregation of the AuNPs (Figure 6a). The red shift of LSPR spectrum upon aggregation of AuNPs was used to quantify the concentration and activity of MMP-7. Figure 6b shows the extinction spectra of JR2EC functionalized AuNPs incubated with different concentrations of MMP-7 for 4 min. The shift of the extinction spectrum induced by MMP-7 was analyzed with both Point 3 and Point 2. Figure 6c shows the kinetics of the shifts of extinction spectrum at Point 3 (solid squares) and Point 2 (open squares) incubated with MMP-7 at a concentration of 2 μg/mL.

Both response curves were fitted with exponential decay function (red curves). It is clear that Point 3 shows larger responses during the catalytic event. Figure 6d shows the shift of extinction spectrum at Point 3 (solid squares) and Point 2 (open squares) for different concentrations of MMP-7 incubated for 4 min. Both calibration curves were fitted well with exponential decay function (red curves). From the two calibration curves, Point 3 clearly demonstrates a larger response than Point 2 across all concentrations of MMP-7. The LOD (limit of detection) calculated using 3 times standard deviation (0.15 nm) for Point 3 and Point 2 are 0.094 and 0.22 μg/mL, respectively. The noise for peak maximum and inflection point are calculated from the 3 times of the standard variations of the LSPR spectra of blank samples. Both peak maximum and inflection point show a standard deviation of $\sim$0.05 nm. This is an encouraging finding given that the concentration of MMP-7 in cancer patients typically varies between 0.01 and a few μg/mL.\textsuperscript{36,39} Therefore, the lowering of the LOD offers a significant advantage in early diagnosis. Further improvement on LOD can be made by combining this analysis method with better sensing platforms.
CONCLUSIONS

We demonstrate that the inflection point at the red side of the LSPR peak maximum (Point 3) shows higher refractive index sensitivity than the traditionally used peak maximum (Point 2). A consistent improvement in bulk refractive index sensitivity of 18–55% was observed for six different nanoparticles. Local refractive index changes induced by molecular adsorption to gold nanoparticles and gold metamaterials also confirm the superior performance of the inflection point. The better performance of the inflection point at the red side (Point 3) originates from the change in shape of LSPR peak in response to an increase of the local refractive index. We further demonstrate with a colorimetric sensor for Matrix MetalloProteinase 7 that the inflection point approach offers a LOD of 0.094 μg/mL while with the peak maximum shift gives a LOD of 0.22 μg/mL. This improvement in LOD is very encouraging as it offers early diagnosis of certain cancers. We believe that this method also can be used as a general method for tracking minute shifts/responses in other analytical applications.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssens...6b00633.

First and second order derivative spectra of 40 nm AuNPs in different solvents, extinction spectra of all the nanoparticles measured in solvents with different refractive indices, noise analysis (PDF).

AUTHOR INFORMATION

Corresponding Author

*E-mail: bliedberg@ntu.edu.sg. Tel: (+65) 6316 2957.

ORCID

Bo Liedberg: 0000-0003-2883-6953

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the School of Materials Science and Engineering and the Provost office and the Nanomedicine institute and iFood institute, Nanyang Technological University, Singapore and Ministry of Education, Singapore through MOE-2014-T1-001-133.

REFERENCES


