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Engineering plasmonic nanorod arrays for colon cancer marker detection

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ABSTRACT

Engineering plasmonic nanomaterials or nanostructures towards ultrasensitive biosensing for disease markers or pathogens is of high importance. Here we demonstrate a systematic approach to tailor effective plasmonic nanorod arrays by combining both comprehensive numerical discrete dipole approximations (DDA) simulation and transmission spectroscopy experiments. The results indicate that 200 × 50 nm nanorod arrays with 300 × 500 nm period provide the highest figure of merit (FOM) of 2.4 and a sensitivity of 310 nm/RIU. Furthermore, we demonstrate the use of nanorod arrays for the detection of single nucleotide polymorphism in codon 12 of the K-*ras* gene that are frequently occurring in early stages of colon cancer, with a sensitivity down to 10 nM in the presence of 100-fold higher concentration of the homozygous genotypes. Our work shows significant potential of nanorod arrays towards point-of-care applications in diagnosis and clinical studies.

1. Introduction

Recently, we have witnessed considerable efforts for the development of plasmonics and photonics-based nanosensors for biochemical and genetic analysis, especially for screening and rapid detection of cancer markers (Anker et al., 2008; Cao et al., 2013; Chao and Guo, 2003; de la Rica and Stevens, 2012; Fan et al., 2010; Hao et al., 2008; Luk'yanchuk et al., 2010; Miroshnichenko et al., 2010; Peng et al., 2013; Rodriguez-Lorenzo et al., 2012; Truong et al., 2011; Verellen et al., 2009; Wen et al., 2013; Xu et al., 2011; Zhang et al., 2013). These systems provide a range of benefits such as shorter analysis time, lower consumption of sample, chemical reagent and energy, lower cost, and portability. Towards this end, the usage of the dielectric sensitivity of localized surface plasmon resonances (LSPR) of nanoparticles has been shown to be particularly robust and very sensitive to changes in the dielectric environment of surrounding (Dahlin et al., 2006; Haes et al., 2005; Willets and Van Duyne, 2007; Yonzon et al., 2005). LSPR sensors have been reported to have sensitivities from 25 to 868 nm/RIU, depending on various substrate parameters (Jeong et al., 2013; Yeom et al., 2013). The effects of periodicity, geometry, polarization, and dielectric environment have been explored for tuning the LSPR responses of nanosphere, triangular nanoprism, nanorods, etc. Highly sensitive LSPR biosensing has been done using the various plasmonic substrates (Chen et al., 2011; Homola, 2008; Mayer and Hafner, 2011; Stewart et al., 2008; Willets and Van Duyne, 2007). LSPR-based sensing of DNA hybridization reached the attomolar scale, antibody-antigen binding has been detected at 2 pg/mL, and thrombin has been detected at concentrations of 1 ng/mL (Fong and Yung, 2013; Guo and Kim, 2012; Jeong et al., 2013; Tang et al., 2013; Yeom et al., 2013). Among these substrates, the nanorods possess outstanding plasmonic and photonic properties, exhibiting intrinsic transverse and longitudinal modes. The longitudinal mode of nanorods has been shown to be sensitive to changes in refractive index and has the potential for high figure of merit (FOM) values. More importantly, bandwidth and the LSPR sensitivity of the AuNRs can be optimized by tuning their aspect ratios. One- and two-dimensional arrays of nanoparticles have been previously studied for their plasmonic resonances and longer wavelength photonic resonances (Ausman et al., 2012; Halas et al., 2011; Liu et al., 2011; Malynych and Chumanov, 2003; Zou et al., 2004; Zou and Schatz, 2004, 2006). Arrays of gold nanorods showed very sharp resonances that shifted significantly with variations in array parameters. Classical mechanics has been found

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to be sufficient to describe a system of nanoscale particles in arrays on the order of a wavelength of visible light, for instance, one of the versatile framework is the discrete dipole approximations (DDA) that has been used to study the classical electrodynamics of such a system (Chen et al., 2010; Lombardi and Birke, 2009; Morton et al., 2011). Previously, DDA simulations of rectangular nanorod arrays showed the appearance of sharp photonic resonances in the near infrared (Ausman et al., 2012), which have not been experimentally identified.

In this work, nanorod arrays were studied for their plasmonic and photonic resonances and their potential as LSPR biosensing substrates. The plasmonic and photonic modes of gold nanorod arrays were studied through rigorous experiments and DDA simulation to observe their extinction efficiency spectra and electric near field. Rigorous periodicity studies were carried out to fully understand the behavior of photonic resonances resulting from the array structure. Upon the engineering and optimization of nanorod array, we have successfully used the AuNR array device as a plasmonic nanosensor for the detection of single nucleotide polymorphisms (SNPs) in codon 12 of the K-*ras* gene that are frequently occurring in early stages of colon cancer – the third most common type of human cancer today and the second most common cause of cancer related deaths (Hadley et al., 2004; Jemal et al., 2007).

2. Results and discussion

2.1. Nanorod resonance engineering

In order to understand the effects of changes in nanorod size and aspect ratio, the discrete dipole approximation (DDA) was used to simulate the extinction spectra of varying nanorod geometries (refer to supplementary information for full detail of simulation). This is an important factor to consider, as small variations in the fabrication procedure can drastically affect the experimental particle shape, which determines the LSPR properties. Fig. 1a shows the variation in resonance of a single nanorod with a constant length and varying width. Spectra for both polarizations are shown, longitudinal (parallel to the nanorod axis) and transverse (perpendicular to the nanorod axis). Fig. 1b shows the extinction spectra of a single nanorod with a constant width but varying length. There is very little variation in transverse resonance position for either variation in aspect ratio, though there is a slight red shift for an increase in nanorod thickness. The variation of aspect ratio, the length over the width of the particle, has a dramatic effect on the longitudinal resonance mode. Tuning the aspect ratio from 2.7 to 8 varies the resonance from 900 to 1400 nm. As the aspect ratio of the rod decreases, it becomes more sphere-like, in geometry and resonance.

To better understand the two modes, the electric near-field was computed. Fig. 1c and d shows the electric near-field contour plots of a single nanorod with longitudinal and transverse polarizations, respectively. The longitudinal mode has hotspots located at the ends of the nanorod. The transverse mode has a more uniform electric near-field with cold spots at both ends of the nanorod. The weak electric field excited in the transverse mode will have weak interactions when particles are arranged in arrays. Changes in transverse periodicity may not make much impact on LSPR. The strong, localized electric field excited in the longitudinal mode, however, will interact strongly and changes in longitudinal periodicity will greatly affect the LSPR. It is noteworthy to comment that the local electric field contour can be directly visualized by near-field technique or indirectly mapped atomic force microscopy approaches, in which specific photosensitive polymers can be used to produce topographic variations due to conformational changes upon light irradiation (Dodson et al., 2013; Girard et al., 2000; Haggui et al., 2012; Hsu 2001; Hubert et al., 2008; Wiederrecht et al., 2005; Wurtz et al., 2003).

2.2. Nanorod array engineering: effects of one dimensional arrays

In order to understand how periodicity affects the LSPR, it is necessary to first understand the effects of the two basic types of one-dimensional arrays. Fig. 2 shows the simulated extinction efficiency spectra for a single 200 × 50 nm nanorod and one-dimensional arrays, for transverse and longitudinal polarizations. Very large period arrays have similar LSPR to a single nanorod, since the particles are far enough apart that coupling is negligible. However, as the period is decreased, and the near field of the particles can interact, the resonance shifts and new features appear. The near field excited in the transverse mode of nanorods does not interact in longitudinal arrays, therefore variation of periodicity in longitudinal arrays did not affect the transverse mode LSPR, as shown in Fig. 2a. The hotspots excited in longitudinal mode of nanorods interact strongly in longitudinal arrays and the longitudinal LSPR was greatly affected by changes in longitudinal periodicity, as shown in Fig. 2b. The appearance of a new peak around 1200 nm in the arrays with 500 and 1000 nm periodicities with longitudinal polarization warranted further studies, and Fig. 2c shows the extinction efficiency of a thorough study of the effect of periodicity in one-dimensional longitudinal arrays with longitudinal polarization. The appearance and subsequent red shift of two peaks is observed. These peaks appear very narrow and red shift and broaden until at their maximum extinction at approximately 830 and 415 nm periods. These values

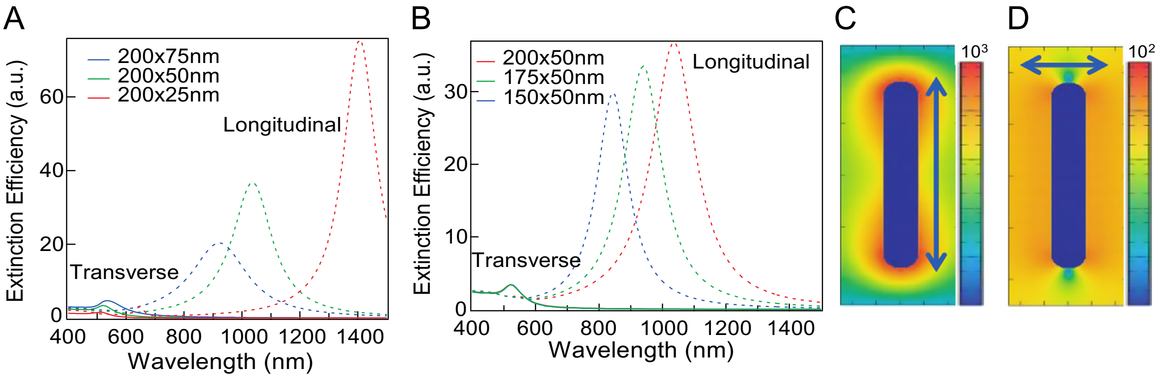

Fig. 1. Simulated extinction and electric field of nanorods. Simulated extinction efficiency of single nanorods with varying (a) widths and (b) lengths. Simulated electric near-field for single 200 × 50 nm nanorods with (c) longitudinal and (d) transverse polarization, demonstrated by the blue arrows. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)