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Peak shift measurement of localized surface plasmon resonance by a portable electronic system

Giulia Cappi[a,*], Enrico Accastelli[a], Vera Cantale[b], Maria A. Rampi[b], Luca Benini[c], Carlotta Guiducci[a]

[a] Laboratory of Life Sciences Electronics, Swiss Federal Institute of Technology (EPFL), 1015 Lausanne, Switzerland
[b] Dipartimento di Chimica, Università di Ferrara, via Borsari 46, 44121 Ferrara, Italy
[c] Dipartimento di Elettronica, Informatica e Sistemistica, Università di Bologna, viale Risorgimento 2, 40136 Bologna, Italy

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ABSTRACT

In recent years, the characterization of surface molecular layers by localized surface plasmon resonance (LSPR) has attracted a lot of interest thanks to its ability to provide a higher spatial resolution with respect to standard SPR. LSPR can be observed as a peak in the extinction spectrum of metal nanoparticles such as gold non-connected surface patterns. A plasmon peak red shift is caused both by the presence of molecular layers on the gold surface and by molecular binding events.

The current study presents a portable transmission system to observe the LSPR phenomenon that extracts the peak location employing a discrete number of light sources. The peak location extraction is performed by an algorithm that takes into account the spectral characteristics of all the components. The performance of our LSPR measurement system has been characterized on a set of Fluorinated Tin Oxide-coated slides covered with nanoislands with a diameter of approximately 30 nm. The samples have been modified with a single-stranded DNA layer and the plasmonic peak location has been determined before and after surface treatment. The samples have been characterized in parallel with a high-end spectrophotometer. The results presented demonstrated the performance of our measurement system in determining the peak location with 1 nm precision.

1. Introduction

Nowadays, fast detection and quantification of biomolecules is a critical issue in many sectors, including health-care, from early diagnostics of diseases to personalized medicine. Surface plasmon resonance (SPR) proves to be a very sensitive and powerful method to detect biomolecular binding of different species, such as proteins, oligonucleotides and viruses. Liedberg first employed a SPR setup for biosensing purposes in 1983 [1]. Since then, the field has rapidly developed thanks to advancements in immobilization techniques and optical measurement approaches [2]. In particular, thanks to improved solutions for immobilizing receptors on the metal surface and coating techniques, most early limitations due to adsorption of non-specific molecules and to reduced activity of the receptors have been overcome.

A surface plasmon (SP) is a coherent oscillation of the free conduction electrons that occurs at the interface between a metal and a dielectric. It arises when a light beam is shined at the metal–dielectric interface under a certain angle of incidence greater than the critical angle [3]. SPs are very sensitive to the metal surface and its surroundings. Binding of molecules to the surface causes changes in the local refractive index (RI) and this makes the absorption spectrum of the SP to redshift [4–7]. Hence, this shift, measured as the change in peak wavelength of the absorption curve, gives quantitative information on molecular layers on the surface [8]. SPR enables the observation in real-time of the biomolecular binding, thus allowing the extraction of thermodynamic information.

However, techniques based on SPR detection require rather complex optical systems with limited parallelism. At present, the highest number of different sensing sites on standard SPR has been achieved on the Biacore 4000 [9] with 16 sites and with a portable system developed by Furlong and co-workers featuring 24 parallel sensing areas [10].

To scale up the number of simultaneous sensing areas per experiment, imaging SPR (iSPR) systems have been developed. iSPR measures the spatial refractive index changes on a surface where an array of biomolecules has been immobilized [11–16]. Commercially available systems, such as SPRi-PlexII™ from Horiba [17], or IBIS MX96 [18] provide monitoring of few hundreds of independent biomolecular interactions. Such systems are equipped with sophisticated optics and are thus rather bulky and expensive.

Recently, many studies have been conducted on the *localized* SPs, which are non propagating waves occurring on suspended nano-sized metal particles and on bi-dimensional metallic

* Corresponding author. Tel.: +41 216931172.
E-mail address: giulia.cappi@epfl.ch (G. Cappi).

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nano-patterns [19–23]. The peak wavelength depends on the metal, on the geometry and on the properties of the surrounding dielectric [24–29]. A regular, periodic pattern of nanoislands (NIs) on a surface exhibits an extinction peak (contribution of absorption and scattering) in the visible and near-infrared range that can be narrowed by enhancing the regularity of the patterns [30]. The localized surface plasmon resonance (LSPR) phenomenon can be observed either in reflectance, as for standard propagating SPR, or in transmission configuration (T-LSPR) [31,32]. In particular, the principle of T-LSPR has been extensively studied in the works of the group of I. Rubinstein since 2000 and it has been employed to characterize molecular binding events such as biotin–streptavidin, on discontinued metal layers on inert transparent substrates [33–35]. Moreover, Hutter and Pileni [36] reported the detection of DNA hybridization on a layer of gold nanoparticles of 2.5 nm on glass.

Clearly, a transmission approach is particularly suitable for the development of compact or even integrated measurement setups, which only require alignment among light source, sample and detector. In contrast, in a reflectance-based system, moving elements are needed to observe the changes in the reflectance angle of the light used to excite the sample.

Pushed by the need for point-of-care bio-analytical systems, previous works have reported electronic-based compact implementations of reflectance-based systems for LSPR [37] and classic SPR [10]. Nevertheless, these reflectance-based setups still require specific optical elements that are difficult to realize on an integrated system.

The transmission configuration may greatly facilitate the development of miniaturized, high-parallelism, fully integrated SPR systems. However, the T-LSPR phenomenon has been almost exclusively characterized by conventional UV–Vis spectrophotometers [26,33,34,36,38]. Such systems can reach excellent performance in measuring extinction spectra of liquid samples, though they are not optimized for characterizing quasi bi-dimensional samples such as metal layers on transparent slides.

An example of integration of microfluidics with a T-LSPR structure has been presented by the group of Borghs [39]. This system addresses the detection of transmittance changes at a single wavelength. In this case, the detection principle is based on the changes in absorbance at that wavelength rather than the observation of the plasmon peak shift.

For the first time, here is presented an electronic-based implementation of T-LSPR that avoids bulky equipment such as spectrophotometers and monochromators, while exhibiting excellent repeatability in the peak wavelength determination.

The setup is based on Light Emitting Diodes (LEDs) and on on-board signal amplification. The peak location is extracted by a fast iterative curve-fitting algorithm, which is suitable for implementation on a limited-power computing platform (e.g. a 32 bit microcontroller). The system was tested on DNA-modified gold NIs evaporated on FTO-coated glass slides but in principle it is suitable for any transparent surface exhibiting a LSPR effect.

2. Materials and methods

2.1. Nanoislands fabrication and surface modification

2.1.1. Evaporation of nanoislands

Gold NIs were realized by direct thermal evaporation and by subsequent mild thermal annealing (200 °C overnight). No seed-mediated growth or adhesion layers were employed [38], thus involving a reduced-complexity process. NIs were formed on Fluorine-doped Tin Oxide (FTO) coated glass slides. The usage of FTO coated glass instead of plain glass significantly improves the stability of gold NIs. In fact, gold penetrates in the porous SnO_2 layer during thermal annealing [40].

FTO coated slides are characterized by excellent adhesion properties while showing high optical transmittance and conductivity. The latter is desirable to perform comparative characterization techniques such as electrochemical and Scanning Electron Microscopy.

Samples were derived starting from 5.3 cm × 1.2 cm FTO-coated glass slides and they were prepared by 20 min sonication in a 1:1 solution of 2-propanol and acetone, followed by abundant rinsing with ultra pure water and drying with pure nitrogen.

A layer of 5 nm of gold was evaporated on one surface at RT at 3×10^{-6} mbar at a rate of 0.0016 nm/s. The layer thickness was monitored by a built-in quartz balance. Vacuum evaporation was followed by a thermal annealing overnight at 200 °C.

Fig. 1 shows the evaporated gold layer before (A) and after (B) the annealing process. After mild heating, gold reorganizes in small clusters giving rise to a sharp plasmonic peak (Fig. 2B). Conversely, the extinction spectrum of the FTO-coated slide right after evaporation does not feature a well-defined peak (Fig. 2A) corresponding to a connected pattern.

2.1.2. Surface modification of nanoislands

DNA probes immobilization on gold was achieved by sulfur-gold bond. The NIs FTO-glass slides were incubated at room temperature for 16 h in a PBS buffer solution containing the 5′-thiolated-CAG TGA GGC GTG GCC AGG G-3′-oligonucleotide (ssDNA). After the incubation, the samples were rinsed with PBS buffer and ultra pure water and then gently dried with pure nitrogen. The light extinction of the samples was measured prior and after surface ssDNA immobilization.

A reference slide was prepared to estimate the peak shift due to NIs aging and modification in liquid environment. We immersed NIs FTO-coated glass slides for 24 h in PBS buffer, the same buffer used for the ssDNA immobilization process. The sample was rinsed with ultra pure water and dried with pure nitrogen.

2.2. Transmittance setup

Our low-cost measurement approach is based on the observation that the information content of the complete transmittance curve is very limited and that the key parameter of interest in T-LSPR measurement is the peak shift of the surface plasmon. Thus, we developed an ad hoc technique for measuring the surface plasmon peak shift observed in transmittance using low-cost components and a highly streamlined optical setup. We measure the peak shift by illuminating the sample with a limited number of Light Emitting Diodes (LEDs). Since the intensity of the peak changes as well as the location, at least three LEDs whose spectra cover the peak region are needed to identify the peak shift. The peak location is estimated from the three measured intensities by means of an innovative interpolation technique that will be discussed in Section 2.3.

A typical surface plasmon transmission spectrum of gold NIs evaporated on a transparent slide is plotted in Fig. 3 (dashed curve, left axis); it has been measured with a UV–Vis–NIR spectrophotometer Jasco V-570 in transmittance mode with a scan speed of 200 nm/min and a bandwidth of 1 nm. Transmittance is normalized with respect to bare FTO slides. The peak is located between 550 nm and 595 nm and red-shifts upon modification of the NIs with layers of organic molecules (solid line).

The peak wavelengths of the LEDs spectral distributions are chosen to be across the SP peak and are respectively of 525 nm, 590 nm and 650 nm. Moreover, the NIs layer modifies the LEDs spectra when the light passes through it, resulting in an altered (filtered) spectral distribution (Fig. 3, dotted lines, right axis). The three crosses indicate the transmittance values measured with the three LEDs light sources.