LSPRi biosensing – high-throughput label-free diagnostics of the future?

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Abstract

In the past couple of decades, the development of plasmonic nanosensors underwent rapid progress, both in the fields of fabrication, integration, and realized applications. *Localized surface plasmon resonance imaging* (LSPRi) promises the possibility of high-throughput biomolecule sensing integrated into miniaturized point-of-care (PoC) devices. This article provides an overview of the working principle of LSPRi devices and discusses the key requirements for nanoplasmonic sensors elements to realize these goals. The possibilities and limitations of available nanofabrication technologies are also discussed.

1. Introduction to biosensing

Biosensors are analytical devices that utilize biologically active molecules (such as enzymes, nucleotides, antigens-antibodies) as sensing elements and can detect the presence or measure the concentration of target molecules in complex biological samples (blood, saliva, urine etc.). Structurally they consist of a *bioreceptor* (or *biorecognition*) *layer* – which is composed of the bioreceptor molecules interacting with the tested sample and specifically binding the target molecules (analyte) – and a *transducer* that converts the receptor-target molecular scale binding event into a measurable physical quantity, most commonly into an electrical or optical signal, subsequently processed by electronics to display the concentration data. In *label-free biosensing*, the presence and quantity of the target molecule alone generate a signal in the transducer through an inherent property of the target molecule (such as mass, charge, optical density, etc.) Depending on the interaction, we can distinguish electrochemical, optical, calorimetric, semiconductor-based, and acoustic wave-based transducers. While each type has its own merits, today, we can consider the electrochemical and optical biosensors the most widespread, and surface plasmon resonance (SPR) based sensors are one of the most promising members of the latter.

2. SPR, LSPR, and the concept of imaging

Surface plasmon polaritons (SPPs) are the collective oscillation of delocalized electrons at a metallic surface in response to an external electric field. Since their first application for sensing purposes in the early 80s SPR based instruments became one of the most widely used tools of our time for the label-free characterization of biomolecular interactions [1]. There are three distinct advantages of SPR based chemical and biosensors compared to alternative sensing methods. They have excellent sensitivity (expressed in refractive index unit, around 10^{-7} RIU), and they are only sensitive to the changes in the refractive index of the medium in the hundred-nanometer vicinity of the metal-dielectric interface. Besides, SPR yields real-time kinetics information about molecular interactions, which is a great advantage compared to the end-point systems [2].

The third strength of SPR is its possible extension into a high-throughput multi-analyte/multi-biosensor concept, called SPR imaging [3], which is achieved by using a defocused laser illumination and a CCD (charge-coupled device) camera as a detector that measures the intensity distribution in a large surface area with multiple sensing spots. Considering recent advances in microarray fabrication methods (e.g., for DNA molecules) and CCD resolutions, the number of parallel measurements in a cm^2 area can be in the several 1000 range [4]. Besides the apparent success and the widespread distribution of SPRi instruments, a disadvantage of the configuration is that the classical Kretschmann-type reflective optical setup is hard to be integrated into small, handheld point-of-care (PoC) devices. This might be the main reason for the comparatively limited success of integrated SPR constructions, and for the lack of handheld SPRi devices on the market [2].

Plasmons incited in nanoparticles with a size comparable to or smaller than the wavelength of the illuminating light are called localized plasmons, since compared to the propagating plasmons in the classic thin film-based SPR, they are confined in the nanoparticle. The most significant difference between LSPR and classic SPR is that localized surface plasmon resonance on nanoparticles is more easily excitable, and thus more straightforward measurement configurations can be used [2,5].

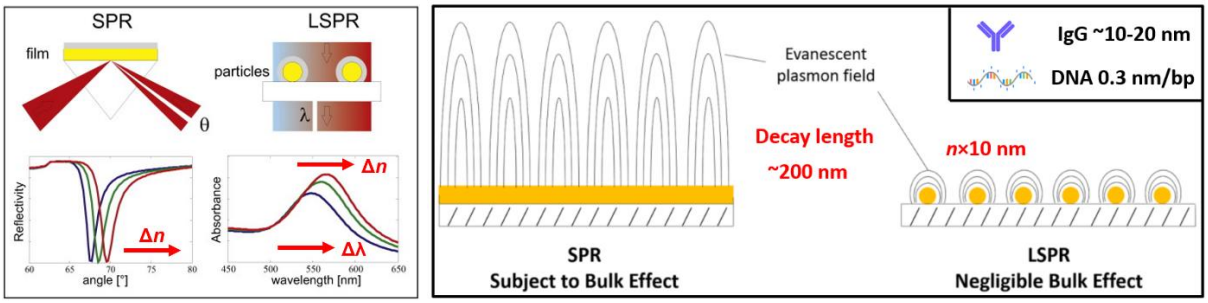

Fig. 1. Left: Illustration of the SPR and LSPR measurement configurations and resulting spectra. In the classical Kretschmann SPR setup, the intensity of a beam reflected from a gold thin film (usually around 50 nm thickness) is measured in the function of the incidence angle. In the chip-based LSPS setup, the absorbance of gold nanoparticles is measured with transmission optical spectroscopy. **Right**: Comparison of the decay lengths of classic SPR (on thin films) and LSPR (on nanoparticles) with respect to the size of typical biomolecules used in biosensing. Reproduced based on [6] under a Creative Commons license.

There are three main measurement configurations in which nanoparticles can be used as sensor transducers: colloidal (homogenous) form, surface-confined (heterogenous) single nanoparticle form, and surface-confined (heterogenous) nanoparticle array form [7]. In heterogenous setups, the nanoparticles can be used as coatings on optical fibers [8] or fixed to the surface of a usually transparent substrate [9], which is often referred to as a chip-based LSPR setup (as illustrated in Fig. 1). Although both the colloidal and single-particle configurations have distinct advantages (e.g., colloid-based colorimetric assays are usually fast, simple and cheap, while single-particle spectroscopy-based methods demonstrated exceptional limit of detections (LODs) in attomolar concentrations or even single molecular sensitivity [10]), focusing on integrated-LSPRi as the target application, only the chip-based setups can be considered as valid alternatives. By using simple transmissive optical setups with chip-based, surface-confined nanoparticle arrays (on a sufficiently large surface area), the integration of the LSPR principle into small, handheld, point-of-care LSPR imaging devices could finally be possible [2,11,12].

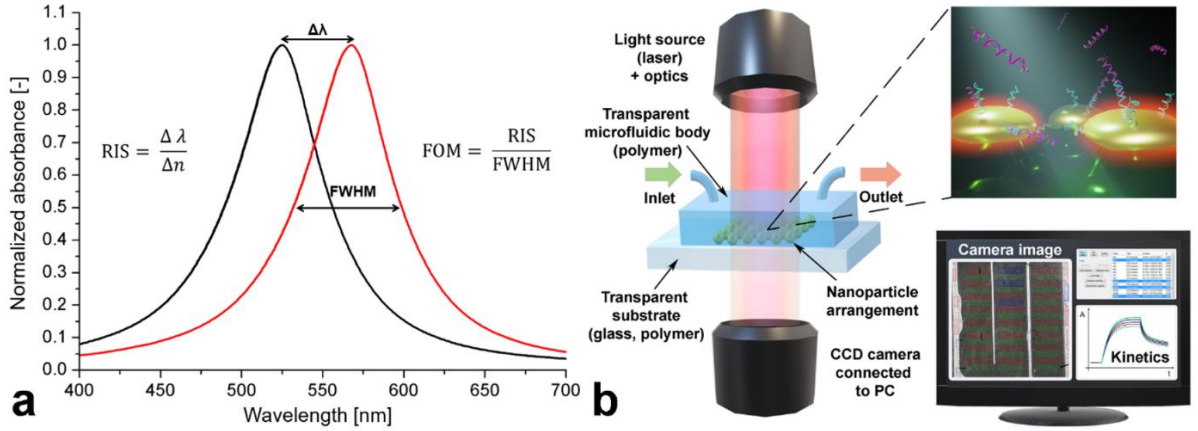

Fig. 2. a): Illustration of the refractive index sensitivity (*RIS*) and figure of merit (*FOM*) of an LSPR sensor. (Δn: refractive index change, Δλ: absorbance peak shift, FWHM: full width at half maximum of the absorbance peak). During biosensing applications, binding biomolecules on the surface of the nanoparticles (e.g., receptor and target molecules) increases the effective refractive index of the surrounding medium, which causes a similar redshift in the measured absorbance spectrum. **b)** Schematic illustration of a typical transmission-based optical setup for LSPRi, consisting of a preferably monochromatic light source and a CCD camera as the detector. The camera monitors a large surface area (e.g., up to a few cm^2) of the chip with the nanoparticle arrangement. Image processing is used to measure the intensity change in selected areas and monitor the kinetics of molecular binding events (the shift in the absorbance spectrum translates to intensity shift when monitored at a fixed wavelength). Reproduced based on [2] under a Creative Commons license. The insert shows epoxy-gold nanomushroom formations fabricated by T. Lednický (reproduced from [13]).

Here, LSPR / LSPRi distinction is intended to be used analogously to SPR / SPRi. Contrary to *one-spot LSPR,* which has spectroscopic information from only one focused illuminated spot, *LSPRi* uses a defocused illumination on a large sensor area (cm^2) and a CCD camera for imaging. As illustrated in Fig. 2b, similarly to SPRi constructions, the broad-spectrum light source (e.g., halogen lamp) and the spectrometer are replaced with a narrow band laser (tuned to an appropriate wavelength), and a CCD camera as the wavelength shift is transformed into intensity change in the detector. In this setup the whole illuminated sensor area is measured real-time; the evaluation of the designated areas (can be even several thousand parallel spots) is done via image processing without the need for hardware multiplexing (opposed to LSPR constructions where one illuminated spot is scanned along an area). In LSPRi the spatial and temporal resolution is defined by the CCD camera's size and frame rate [2].

It has to be mentioned that LSPRi can be extended into *LSPR imaging spectroscopy*, where spectroscopy information is obtained in every imaged pixel of the sensor area. Such concepts can be realized by using either a tunable light source or tunable filters and correlating the measured light intensity with the varied illumination wavelength [11,14].

To characterize and quantify the physical sensing performance of LSPR based sensors, the two most commonly used parameters are the bulk refractive index sensitivity (*RIS*) and the figure of merit (*FOM*), as illustrated in Fig. 2a. *RIS* is defined as the change in the plasmon resonance wavelength (e.g., extinction spectrum peak shift) caused by the bulk refractive index change in the surrounding medium. *FOM* is the sensitivity divided by the bandwidth of the resonance peak (or full width at half maximum, *FWHM*). Since a higher physical RI sensitivity naturally means a higher molecular sensitivity as well, recent efforts in LSPR sensor development generally aim to increase and maximize *RIS* and *FOM* to reach an ultralow limit of detection (*LOD*) [15]. This is done by controlling the size, shape, composition (e.g.,

bimetallic or core-shell structures), and arrangement of the particles. These factors elaborately interact with each other; thus, there are many strategies to improve the *RIS* of a plasmonic sensor (for a review on the topic, see [16]). However, the relationship between the physical *RIS* of an LSPR sensor and their molecular sensitivity or achievable *LOD* is not trivial. It is long known that although the *RIS* of nanoparticle-based LSPR is usually around one order of magnitude smaller compared to the classic, thin-film-based SPR (the majority of LSPR sensors are in the couple of 100 $nmRIU^{-1}$ range, compared to the ~3300 $nmRIU^{-1}$ of SPR), for bio-sensing applications, LSPR can match the molecular sensitivity of SPR [5]. This is attributed to the shorter plasmon field decay length of the nanoparticles, which focused field makes them exceptionably well applicable for detecting molecular interactions on their surface (as illustrated in Figure 1). [17] Generally, the near field intensity and its decay around the particles depend on the size, shape, and material properties of the nanostructures in an elaborate manner. Anisotropy in the nanoparticles usually comes with an increased refractive index sensitivity, so exotic shapes (nanocages, nanostars) are favored compared to simple nanospheres [8]. Considering nanoparticle arrangements (e.g., lattices, arrays etc.), coupling and interparticle distances also play a significant role in near field intensity and, thus, sensitivity enhancement. These interparticle effects and their necessary optimization for 2D nanoparticle lattices are discussed by several in-depth reviews [2,18].

3. Nanofabrication – possibilities and limitations

Due to all of these requirements, selecting a nanofabrication method for LSPRi sensor construction demands compromises. A technology with control over the particles' size, shape, distribution, uniformity, and stability in a sufficiently large surface area (several cm^2), preferably also cheap and scalable, could be considered optimal. Usually, not all of these conditions can be met simultaneously, and our decision must take into account the potential advantages and disadvantages of each different approach.

Colloidal (homogenous) synthesis and surface chemistry

Perhaps the most precise control over the size and shape could be achieved through the colloidal synthesis of the nanoparticles. Both gold and silver nanoparticles in diverse shapes are widely employed in plasmonic sensors. Here, the challenge is the subsequent binding of the nanoparticles to a substrate, which is most commonly achieved by silanization (to glass or silicon substrates) or through thiol chemistry (for gold surfaces). In both of these cases, the control over the resulting distribution of the nanoparticle array (density, uniformity) is limited. For LSPRi, the surface density of the particles should be increased since uncoupled nanoparticles usually have lower molecular sensitivities, but particle aggregation has to be avoided [2].

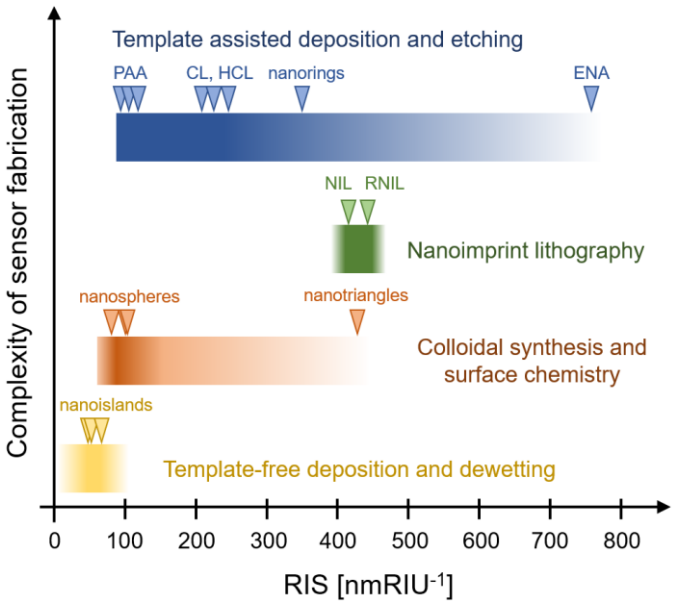

Figure 3. The subjective complexity of the LSPRi sensor fabrication approaches and their reported refractive index sensitivity (*RIS*). The arrowheads mark the reported sensitivities. Reproduced based on [2] under a Creative Commons license.

Heterogenous chemical synthesis

By using metal precursors (e.g., the aqueous solutions of $HAuCl_4$ for gold, $AgNO_3$ for silver), it is possible to directly synthesize nanoparticles on the surface of (mostly polymer) substrates by reducing the metal cations on suitable functional groups. The resulting polymer-Au/Ag surface nanocomposites can be successfully employed as plasmonic biosensors [19]. Although the technology could be implemented on a chip-based substrate as well, a drawback of the direct chemical synthesis is the lack of control over the distribution of the particles [2].

Template-free thin film deposition and dewetting

Vacuum deposition of gold/silver thin films on glass/silicon substrates and their subsequent thermal annealing – also called solid-state dewetting – is a simple template-free technology to prepare plasmonic nanostructures on a large surface area. The resulting size and distribution of the nanoislands can be controlled with the technological parameters (deposited layer thickness, annealing time and temperature), thus tuning the plasmonic properties to an extent. As the main tradeoff of simplicity, nanoisland-based LSPR sensors generally have poor *RIS* and *FOM* and, consequently, small molecular sensitivity. Another common problem is the instability of the nanoislands in fluidic environments, especially the aggregation of particles that can interfere with the detection, causing redshift and possible false signals. [2]

Template assisted thin film deposition

Perhaps the most widely used templates for nanopatterning large surface areas (in the cm^2 range) are prepared by colloidal lithography (CL) and hole-mask colloidal lithography (HCL). By using polystyrene (PS) monolayer colloidal crystals (MCC) as sacrificial polymer masks, fabrication of nanoarrays with different features are possible, such as nanoislands (Fig. 4a [21]), nanodiscs, spherical/elliptical nanoholes, nanorings (Fig. 4c [23]) or bow-tie structures [2]. Although the size, shape, and distribution of the nanoparticles are uniform on a large surface area, a drawback of the technology is that the size/spacing (or interparticle distance)

ratio is limited by the nature of the sacrificial patterning – a large fragment of the surface is shadowed by the polymer mask. This results in uncoupled particles with low surface densities (number of nanostructures per unit area), probably accounting for the low/mediocre reported molecular sensitivities with this method [2].

Another versatile template for nanoparticle synthesis is the porous anodic alumina (PAA) (or anodized aluminum oxide (AAO)) grown on aluminum sheets. By controlling the anodizing conditions in given electrolytes, the pore size of the hexagonally self-ordered PAA structure can be controlled, which can be used either as nanobowl or tubular pore templates for nanoparticle or nanowire array fabrication, respectively. The tubular PAA structures are usually combined with electrodeposition to fill the pores with gold, forming nanorods/nanowires. Klinghammer used such nanowire arrays, the tightly packed nanoantennas can be seen in Fig. 4b [21]. The nanobowled templates were successfully applied by Lednický to create epoxy-gold nanocomposite arrays on a large surface area (illustrated in the insert of Fig. 2b), with similarly promising performance [2,13].

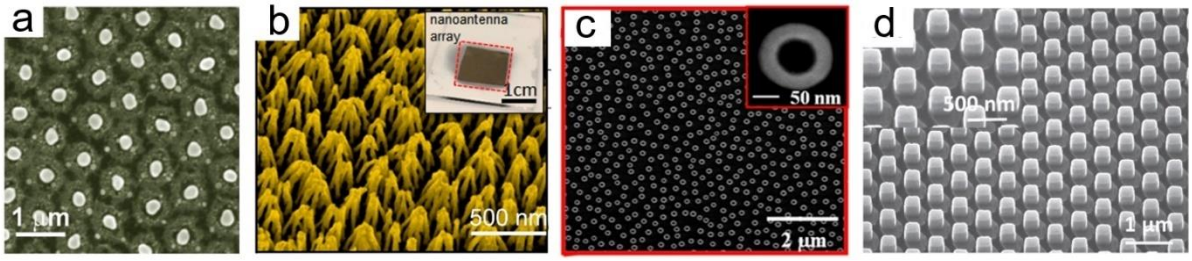

Figure 4. a) Ordered gold nanoisland array on glass prepared by colloidal lithography [21] b) SEM image of vertically aligned Au nanoantennas prepared by filling tubular PAA structures [22] c) images of an Au nanoring array on glass [23] d) SEM images of an ordered 3D multilayered nanostructure consisting of Au films on SU-8 nanopillars [24]. Reproduced based on [2] under a Creative Commons license.

Direct writing lithography

With electron beam or ion beam lithography it is possible to control the size and distribution of the nanostructures (with around 10 nm resolution) but patterning large surface areas (cm^2) is expensive and time-consuming with this method. Since the active sensor area of reported solutions is usually in the 10-100 μm^2 range [20], this construction is only limitedly suitable for LSPRi applications [2].

Nanoimprint lithography (NIL)

Nanoimprint lithography is a family of nanofabrication methods where a pattern is transferred by pressing a designed master mold into a softer resist. Several variants are distinguished based on combinations with other processes (e.g., lift-off), template stripping, copolymer lithography, or reversal nanoimprint lithography. Although the generally complimented advantages of NIL compared to other methods are its low cost, high throughput, and high resolution, these are strongly depending on the master mold. There are commercially available polymer-based molds with sufficiently large surface areas (cm^2). Kawasaki et al. [24] used a moth-eye COP mold (cyclic olefin polymer) combined with transfer technology to fabricate a core-shell nanocone array (NCA) with remarkable sensitivity (418 $nmRIU^{-1}$).

As another outstanding example in Fig. 4d, Zhu et, al. fabricated a three-dimensional multilayered plasmonic sensor consisting of Au nanosquares on top of aligned SU-8 pillars

with reversal nanoimprint lithography (RNIL), by using an intermediate polymer stamp (IPS) [25]. Their complex structures have three distinct resonance peaks around 580 nm, 800 nm, and 1050 nm, and the peak at 800 nm has a sensitivity of 442 nmRIU^{-1} [2].

4. Outlook

The feasibility of the vision offered by the LSPRi principle, high-throughput biomolecule sensing integrated into miniaturized point-of-care (PoC) devices, depends on the nanoplasmonic sensor element. For this, an optimal fabrication technology should yield large sensor areas (cm^2 range) with uniform and stable nanoparticle arrangements, where the size, shape, and distribution of the particles are all controlled to maximize sensor performance. Due to the complex requirement system, selecting a nanofabrication method for LSPRi sensor construction usually demands compromises. Considering scalability, nanoimprint lithography and template-based deposition technologies stand out among their peers.

This article is based on the author's in-depth review of the topic [2]. For interested readers, the references section contains other recent related reviews [1,3,4,9,10].

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