Detection Limit Mediated Advancements in the Fabrication Technologies of SPR Biosensor

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Abstract

Surface plasmon resonance (SPR) biosensors are a key advancement in optical sensing technology for label-free biomolecular detection. Real-time analysis using these sensors is highly sensitive to changes in refractive index. Over the last two decades, in-depth research on nanofabrication and plasmonic engineering has enabled improvements in the detection limits of analytes from micromolar to femtomolar and even attomolar concentrations. However, the physical origins of these enhancements and the factors that ultimately limit sensitivity are frequently examined in isolation. This review brings together recent advances in a noise-sensitivity framework, demonstrating that the limits of detection are set by the balance between electromagnetic-field confinement and measurement stability. Existing studies highlight three main ways to improve the performance of SPR optical sensors: field engineering strategies that enhance resonance response, signal amplification strategies that amplify refractive index perturbations, and hybrid designs that combine both effects to achieve multiple benefits. The next-generation SPR platforms may get closer to detecting single molecules in normal conditions by combining wave physics, materials engineering, and computational optimization. This could change the future of label-free diagnostics with more diversified utilization of SPR-based sensing technologies for (bio)analyte detection.

Keywords: Surface plasmon resonance, Biosensors, Detection limit, Refractive index sensing, Signal amplification, Label-free detection

Introduction

The surface plasmon resonance is an optical effect created by a dielectric medium (usually gold or silver) on the introduction of light. When light hits a metal surface at a certain angle and wavelength, it causes conduction electrons to oscillate together. This creates electromagnetic waves called surface plasmons that move along the metal-dielectric interface. This resonance condition is highly sensitive to changes in the refractive index close to the metal surface, making SPR a great way to transduce signals for biosensing [1–4].

The concept of evanescent waves helps us understand the physical basis of SPR. When light reflects completely inside a high refractive index medium (like a glass prism) and a low refractive index medium (like water or a biological sample), an evanescent electromagnetic field moves into the lower refractive index medium. This evanescent field decreases exponentially as it moves away from the interface, usually going 100 to 300 nanometres into the sample. When a thin layer of metal (usually 40–60 nm of gold or silver) is adhered at this interface, the evanescent wave is capable of interaction with surface plasmons when certain conditions determined by the resonance equation are achieved as shown in fig. 1 [5–11].

The resonance condition is determined by different factors, including the wavelength of the incident light, the angle at which it hits the surface, the optical properties of the metal film, and most importantly, the refractive index of the medium right next to the metal surface. The attachment of biosensing elements (such as antibodies, enzymes, and anti-sense nucleic acids) to the sensor surface changes the local refractive index, which simultaneously changes the resonance condition. This shift can be measured by resonance angle (angular interrogation), wavelength (spectral interrogation), intensity, or phase, giving a quantitative assessment of bioanalyte concentration through interaction with the biosensing element [1,6,12–16].

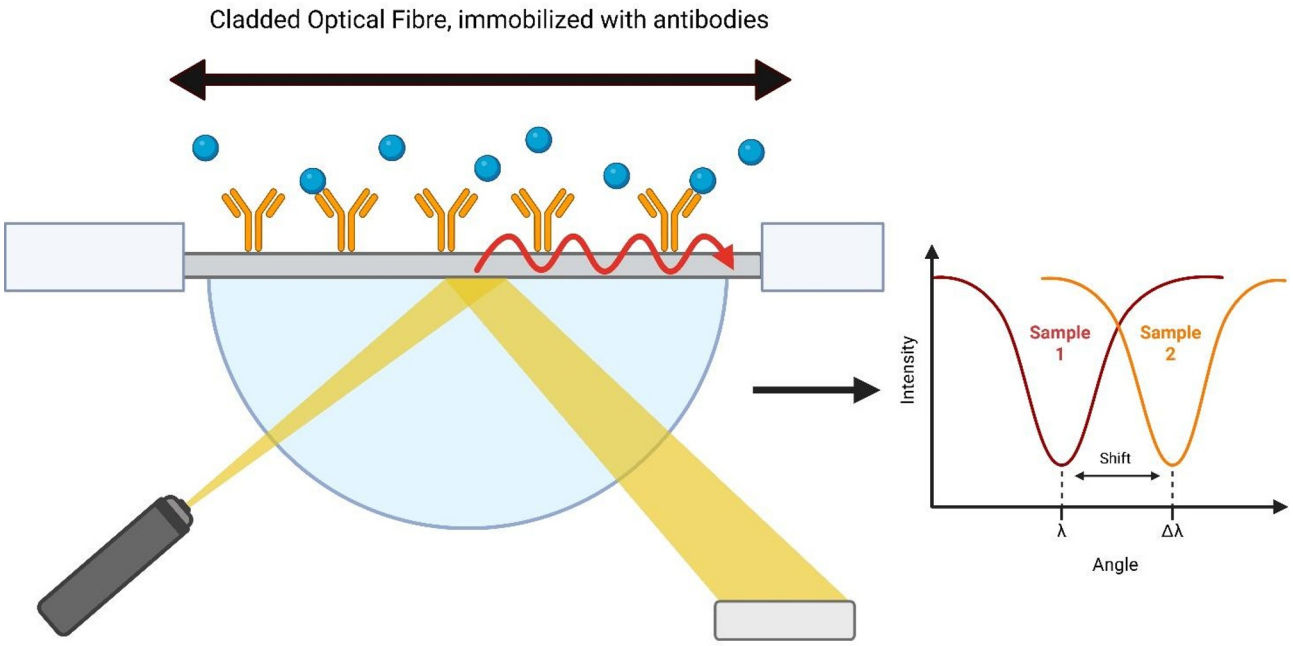

Figure 1: An illustration of the surface plasmon resonance (SPR) sensing principle works

Limit of Detection of Bioanalyte: A Critical Performance Metric

The limit of detection (LOD) is the lowest amount of analyte that can be reliably separated from background noise. The two main parameters that determine the LOD in SPR biosensors are the sensitivity of the sensor and binding efficiency with analyte along with noise intensity. The LOD is the concentration of the analyte that gives a signal that is significantly higher than the standard deviation of the blank measurement [5,17–23].

To improve LOD, the researchers enhance the signal intensity (more signal per unit of analyte concentration) or reduce the noise signal (better measurement precision). Noise in the signal is often reduced by baseline readjustment. It is often seen as a nullification of signals obtained from the control samples, resulting in no measurable output.

Similarly, different ways to improve sensitivity include optimizing the optical setup to get higher field intensity at the sensing surface, adding nanomaterials that increase the change in refractive index, altering the surface chemistry to increase the density and binding efficiency of bioreceptors, and using signal amplification schemes. To reduce noise, it is necessary to make the optics more stable, control the temperature, design the fluidics better, and improve the signal processing algorithms [1,6,7,9,13,16,24–26].

Recent advancements have elevated the detection thresholds of SPR from the micromolar range, achievable by initial devices, to femtomolar and even attomolar levels. This significant gain results in new ideas in sensor architecture, nanomaterial integration, and fabrication precision that regulate design and fabrication strategies in a systematic way, looking at how different choices affect the lowest detection limit that can be reached for disease biomarkers. The subsequent sections will analyze the design and fabrication processes, emphasizing the impact of different methods on the limit of detection attainable for disease biomarkers [19,27–31].

The Role of Design on Quantification of Bioanalytes

Recent progress indicates that LOD is regulated by measurable physical mechanisms intrinsically associated with particular design parameters [32–35]. This facilitates a transition from trial-and-error methodologies to predictive engineering, wherein sensitivity outcomes result from intentional architectural decisions [36–39]. The basic SPR sensing equation states that sensitivity depends on how much the evanescent field overlaps with the analyte layer [40–43]. Every design choice — layer thickness, material refractive index, nanostructure geometry — modulates this overlap according to predictable physical laws. The impedance matching focuses fields at biorecognition interfaces [36,44,45]; phase

singularities increase lateral displacements through zero-reflection conditions [33,46,47]; collective resonances improve quality factors through diffractive coupling [32,37,42]; and catalytic amplification turns binding events into mass accumulation [36,38,41].

This review arranges the development of SPR biosensors into a Design Decision Tree with three branches (paths) illustrated in fig.2: (1) Field Engineering, which changes the distribution of the electromagnetic field to improve intrinsic sensitivity [40,44,46]; (2) Amplification Strategies, which add secondary transduction mechanisms to increase the optical response for each binding event [37,38,41]; and (3) a Hybrid Approach that combines both for very low LOD. Each path has its own needs for stability profiles and LOD ceilings [42,43,45,47].

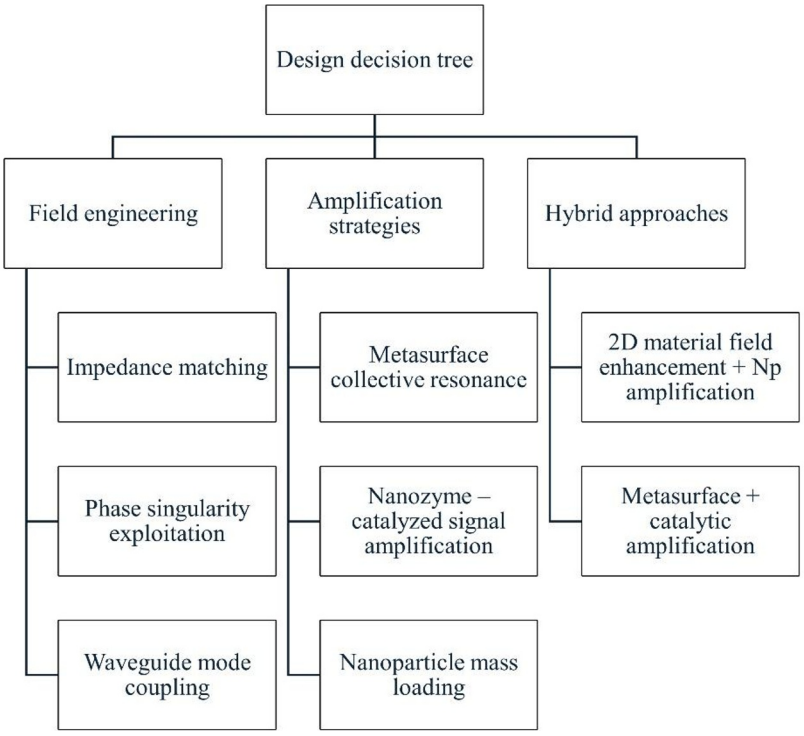

Figure 2: Flowchart illustrating the hierarchical decision-making process for design optimization

Path 1: Field Engineering Strategies

The field engineering strategies control how the evanescent electromagnetic field spreads out or stays in one place and change the phases at the metal-dielectric interface. The sensitivity of SPR increases when the plasmonic field and the analyte region overlap, so moving the field maximum closer to the biorecognition interface directly improves detection performance [40,44]. Representative studies show that field engineering can improve detection sensitivity. Tene et al. demonstrated that the integration of a nanometer-scale dielectric spacer with a 2D overlayer on silver substantially enhanced refractive index sensitivity, enabling sub-picomolar HIV DNA detection [44]. Wang et al. was able to detect femtomolar miRNA-21 by optimizing the design of plasmonic substrates to get near-zero reflection states and using phase-sensitive interrogation to get a stronger response [46]. Liu et al. also showed guided-mode–induced phase mutation in multilayer structures, which led to high-Q resonances and a much higher refractive index resolution than traditional SPR platforms [40].

Impedance Matching via Dielectric Spacers

Adding low-loss dielectric interlayers between the plasmonic metal and the sensing interface changes the conditions for impedance matching and the effective refractive index of the multilayer system [36,44]. From a theoretical standpoint, the spacer: (1) changes the electromagnetic boundary conditions; (2) improves the depth of field penetration; and (3) relocates the maximum of the evanescent field closer to the analyte layer. With the optimized spacer thickness, the overlap integral of the plasmon mode and the sensing region strengthens, which makes the refractive index more sensitive [36,39,44]. The best spacer thickness is about the same as the evanescent decay length (about 100–200 nm), but practical designs use nanometer-thick coatings to fine-tune [35]. This strategy improves the intrinsic electromagnetic sensitivity rather than adding any extra ways to amplify it.

Phase Singularity Exploitation

In SPR, a phase singularity is indicative of the entry of the incident light at an angle leading to no or insignificant reflectance of light. Phase-sensitive SPR detection functions in proximity to zero-reflection (critical coupling) conditions,

characterized by sudden changes in the reflected phase (π-discontinuity) [46,47]. At these unique points, the phase sensitivity denoted by ($S\varphi = d\varphi/dn$) (where, Sφ is the phase of reflected light and dφ/dn is the rate of change of phase with respect to refractive index), increases, surpassing sensitivity based on amplitude. Very small changes in the refractive index can therefore make angular or lateral displacement signals stronger [43,46,47]. The theoretical improvement comes from: (1) steep phase dispersion around critical coupling; (2) the phase slope being steeper than the reflectance slope; and (3) increased sensitivity to changes in the effective refractive index. The accurate adjustment of absorption and effective optical constants, frequently accomplished via ultrathin overlayers, facilitates the ability to enter into these unique regimes [33,46].

Guided Mode Coupling

In multilayered structures, surface plasmon polaritons and waveguide modes interact to create hybrid modes with unique interference patterns. The hybrid modes create Fano resonances, which are resonance curves that are not symmetrical and have much higher quality factors (Q) than standard SPR [40,42]. Given that the sensitivity is directly related to the slope of the resonance curve ($S \propto dR/dn$), a sharper resonance (higher Q) means a higher sensitivity, where S denotes sensitivity, dR denotes change in response of the measured optical parameter, and dN denotes change in refractive index. So, guided-mode resonance sensing raises the detection limit by making the spectrum sharper instead of adding to the signal.

Path 2: Amplification Strategies

Amplification strategies, conversely, enhance the effective refractive index perturbation induced by each binding event more than field engineering does. These methods add secondary transduction mechanisms that make molecular recognition into enhanced optical signals [37,38]. Representative studies elucidate the effectiveness of signal amplification techniques in improving the detection limits of SPR. A study showed that metasurface architectures that use collective plasmonic resonances can detect exosomes with very high sensitivity [32]. Another study showed that nanozyme-catalyzed amplification can detect cancer biomarkers at levels as low as sub-picogram per milliliter. These two studies show how single binding events can be turned into much stronger optical responses that go beyond the limits of electromagnetic fields [38].

Metasurface Collective Resonances

The nanostructured metasurfaces that are periodic show collective resonances because of diffractive coupling and coherent scattering effects [32,37]. The collective modes: (1) lower rates of radiative damping; (2) improve the localization of the electromagnetic field; and (3) produce sharper spectral linewidths. The lattice resonances can give better quality factors ($Q \sim 50–100$) than standard SPR. Multi-mode coupling also makes the spectral response and sensitivity considerably broader. The enhancement mechanism fundamentally relies on resonance sharpening and localized field amplification [32,37,42].

Catalytic (Nanozyme-Based) Amplification

After capturing the analyte, catalytic amplification adds a second mass-loading step. The mechanism can be characterized as a sequential procedure: (1) recognition of specific molecules, followed by (2) catalytically induced accumulation that raises the local refractive index. The effective signal is $\Delta neff = A \cdot \Delta nbinding$, where A (usually between 10^3 and 10^4) is the amplification factor and Δn is the change in refractive indices. This method increases the strength of the signal without changing the electromagnetic sensitivity of the system. The primary effect is amplified mass accumulation at the sensing interface [35,38,41].

Path 3: Hybrid Approaches

Hybrid strategies combine field engineering with amplification mechanisms to reach low levels of detection limits. The theoretical basis is multiplicative enhancement: $Gtotal = Gfield \times Gamplification$, where G is the enhancement factor. Field engineering usually gives a 10–100× intrinsic sensitivity gain, while amplification mechanisms add a 10^2–10^4× signal multiplication. When combined, the overall performance can improve by 10^3 to 10^6 factor. The ultrathin overlayers are used for electromagnetic tuning in successful hybrid systems, followed by controlled nanoparticle or catalytic amplification steps [33,42,43].

A study that used phase-singularity interrogation and 2D nanomaterial enhancement to detect cancer biomarkers at levels below attomolar demonstrated that this method was able to optimize electromagnetic confinement and surface functionalization [43]. Similarly, recent research shows that combining ultrathin dielectric or 2D overlayers with controlled nanoparticle or catalytic amplification leads to performance improvements that are much better than what can be achieved with isolated methods [42]. This shows that synchronized electromagnetic and biochemical design has the potential to push SPR platforms to extreme detection limits that are close to single-molecule sensitivity.

Table 1: Design Decision Matrix for SPR Biosensor LOD Optimization

Strategy	Fabrication Requirement	Key Design Parameter	Best Application	Achievable LOD	Stability	References
Impedance Matching	Thin-film deposition (±1 nm)	Spacer thickness (7–10 nm)	Nucleic acids	10^{-5} RIU, sub-pM	High	[44]
Phase Singularity	2D material transfer	Absorption tuning (2D materials)	miRNA, exosomes	10 fM	Medium	[46]
Guided Mode Coupling	Multilayer uniformity (<5%)	Waveguide thickness (200–500 nm)	Protein biomarkers	10^{-6} RIU	High	[40]
Metasurface Resonance	E-beam lithography	Nanostructure period (300–600 nm)	Exosomes, viruses	0.16 particles/mL	Medium	[37]
Nanozyme Catalysis	Enzyme conjugation	Reaction time (10–30 min)	Cancer biomarkers	0.1 pg/mL	Low	[38]
Hybrid	Combined nanofab + biochem	Multi-parameter optimization	Ultra-rare targets	10^{-18} M	Low	[43]

The existing studies have shown the comparative assessment of different strategies, key design parameters and application of SPR based biosensor (Table 1). This review has shown that the limit of detection is not an enigmatic characteristic arising from trial-and-error but a determinable result regulated by measurable mechanisms [42,44,46]. Understanding the Design Decision Tree — field engineering, amplification, and hybrid strategies — gives a logical way to make sensors that meet the needs of specific clinical applications [34,35,37,38].

Existing studies have shown that LOD is based on mechanisms, not materials. The performance does not stem from utilizing the "best" nanomaterial, but rather from comprehending which physical mechanism (impedance matching, phase singularity, collective resonance, catalytic amplification) effectively tackles the particular detection challenge [37,44,46]. A well-designed structure that matches impedance can work better than a metasurface that is not optimized well, even though it costs less. Also, it is evident that trade-offs are inevitable. The phase singularity approach provides femtomolar LOD but at the expense of bandwidth and stability [46,47]. The nanozyme amplification strategy provides attomolar LOD but with 30–60 min incubation times, which makes real-time analysis impossible [38]. Awareness of trade-offs helps make design choices based on reality rather than aiming for the impossible [45,47].

Conclusion and Future Directions

Surface Plasmon Resonance (SPR) has emerged as one of the key optical sensing techniques, pivotal in diverse biosensing applications. The interaction of the photons in the light with dense medium, resulted in the production of a plasmon, and unique for precise interaction studies between analyte and biosensing elements. The principles of optics are significant in a detailed understanding of the response of biosensing interactions during the SPR based analysis. It is the phase polarized light which often passes through the dense medium, shows changes in the refractive index of the dense or metal coated surface and is responsible for producing plasmons. Thus, the nature of materials to be used for fostering biosensing are significantly important. The existing literature has revealed a strong correlation between the design parameters, fabrication requirements, and stability for catalogues of bio-analytes. The studies will pave the way for the development of more sensitive and accurate SPR-based biosensors for early and rapid diagnosis of diseases.

The coming decade of SPR biosensor research will be driven by an integration of strong fundamental knowledge with a focus on translation. Machine learning will help with design optimization. Nanomaterials will extend the boundaries of sensitivity. Microfluidic integration and scaling will allow point-of-care applications. Multiplexed systems will provide insights into complex disease patterns. The Design Decision Tree framework described in this work offers a foundation — a way to approach the immense design space with knowledge.

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