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Multiplexed biosensing: A review of surface plasmon resonance platforms for biomarker analysis

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ABSTRACT

Surface plasmon resonance (SPR) has emerged as an important optical technology for the real-time, label-free analysis of biomolecular interactions. While its utility in detecting single analytes is well-established, the growing complexity of disease diagnostics demands tools capable of simultaneous multi-analyte detection. Many complex diseases, such as cancer, involve multiple biomarkers. Conversely, a single biomarker can be associated with several different diseases. This review article focuses specifically on recent advances in multiplex detection of biomarkers using SPR-based biosensors. We delve into the mechanisms and materials that enable multiplexing across various SPR platforms, including traditional prism-based SPR, localized surface plasmon resonance (LSPR), and SPR imaging (SPRi). The review highlights how these techniques overcome the limitations of single-analyte assays by providing a more comprehensive disease profile through the concurrent measurement of multiple biomarkers and their ratios. Key applications in medical diagnostics, employing immunosensor, aptasensor and protein-based assays, are examined to illustrate the performance of current multiplex SPR systems. This work concludes by discussing the implementation of machine learning in SPR-based detection, comparing various SPR biosensing techniques, and identifying future directions for enhancing sensor performance, analysis in complex matrices, and the expansion of multiplex SPR applications.

1. Introduction

The accurate and early diagnosis of complex diseases, such as cancer, cardiovascular disorders, and infectious diseases, is a cornerstone of modern medicine [1,2]. However, pathological states are rarely governed by a single molecular event but are characterized by intricate networks of biomolecules [3]. In cancer specifically, a single biomarker is usually insufficient to capture the full complexity of the disease stage, patient condition, and the tumor's highly variable microenvironment [4,5]. This demand has driven significant innovation in multiplex biosensing, a powerful paradigm for the parallel quantification of several analytes from a single sample [6]. Consequently, considerable efforts have been devoted to constructing multiplex detection platforms using various technologies [7]. These include electrochemical methods, fluorescence, electrochemiluminescence (ECL), and a range of optical techniques such as fluorescence resonance energy transfer (FRET), surface-enhanced Raman spectroscopy (SERS), and photonic crystals [8–13]. Among these, optical biosensing platforms, which utilize strategies like light absorption, reflectance, fluorescence, and luminescence, have emerged as particularly powerful tools due to their high sensitivity and potential for high-throughput screening [14].

Within this landscape, SPR and its derivatives, including localized surface plasmon resonance (LSPR) and SPR imaging (SPRi), stand out for their label-free, real-time, and highly sensitive detection capabilities [15–18]. The inherent versatility of SPR platforms has paved the way for their successful adaptation into sophisticated multiplexed analytical systems. Techniques such as SPRi, which spatially resolves binding events on a functionalized array, and LSPR, leveraging nanostructured materials, have been instrumental in this evolution, enabling the concurrent monitoring of distinct molecular interactions [19,20]. The evolution of SPR biosensors from a single-channel analytical tool to a versatile multiplexing platform has been driven by key technological

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shifts. The following timeline shows the emergence of the three primary methods (Fig. 1).

This review article is dedicated to exploring the recent advances in multiplex biomarker detection using SPR-based biosensors. We will delve into the fundamental mechanisms that enable multiplexing across these platforms, examining innovative materials, assay designs, and strategies that enhance performance in complex clinical matrices. Also, the work discusses applying machine learning to SPR detection, compares SPR biosensing techniques, and identifies future goals.

2. An overview of SPR-based biosensors

SPR biosensors, with their high sensitivity and ability to quantitatively monitor binding events, are powerful analytical tools that can be used to study biomolecular interactions in real-time without the need for labeling [21,22]. They are widely used in drug discovery, proteomics, immunoassays, and diagnostics. Biosensors are a subset of chemical sensors that utilize a biological recognition process to perform detection with high sensitivity and a low detection limit (LOD) [23]. Hence, analytical devices consist of a biological component (such as enzymes, cells, tissues, receptors, antibodies, and antigens) in close contact with a transducer surface [24]. After the analyte binds, the signal from the biological process is converted into a measurable signal by the transducer. In the SPR technique, polarized light, which can be a laser or LED, is incident on a metal-dielectric interface (usually a thin layer of gold or silver) [25]. The excitation of surface plasmons (collective oscillations of free electrons) at a specific angle to the incident light (the resonance angle) results in the absorption of light energy and a decrease in the intensity of the reflected light [26]. The resonance angle changes as biomolecules bind to the metal surface due to a change in the refractive index of light near the interface. Binding kinetics (rate of binding/dissociation) and affinity (equilibrium constants) can be calculated by measuring the change in angle in real time [27]. In addition, SPR consists of a prism/coupling device to facilitate light-metal interaction, a metal layer, usually made of gold, that supports surface plasmon generation, a flow cell to deliver analyte samples to the sensor surface, a detector to measure the intensity of reflected light, and software to generate sensograms and analyze binding curves [28]. Due to their real-time and label-free detection capabilities, SPR biosensors can be widely used in drug discovery by screening small molecule-protein interactions, measuring binding affinities (K_D, k_{on}, k_{off}) for antibody characterization, and detecting biomarkers of various diseases such as cancer and viral diseases [29]. On the other hand, they can be used in pathogen detection, toxin analysis in the field of food safety and environmental monitoring, as well as in DNA/RNA studies to determine hybridization kinetics and gene regulation [30]. SPR has major advantages over traditional methods such as ELISA, fluorescence assays, and Western blots due to its label-free and real-time measurement, without the need for fluorescent or radioactive labels and kinetic analysis, with minimal analyte amounts (microliters to milliliters) and low molecular weight analytes (approximately 200 Da) [31]. However, SPR, although a powerful technique for analyzing biomolecular interactions, has high costs due to the high cost of the gold chip and other associated equipment. Surface preparation of the chip and ligand binding are challenging, and the ligand can lose its activity over time. On the other hand, non-specific analyte signals on the chip can lead to false-positive or negative signals [22].

2.1. Traditional SPR

Traditional or first-generation SPR is one of the first techniques developed, introduced in the 1980s [32,33]. As shown in Fig. 2, the technology used in traditional systems and all modern systems is based on a Kretschmann prism-coupled configuration, in which a prism with a thin gold film (about 50 nm) is used to excite surface plasmons through total internal reflection [34]. The development of SPR had a great impact on the analysis of biomolecular interactions, as previously the evaluation relied on techniques that required radioactive isotopes or fluorescent labels, which could affect the natural interactions of the molecules [35]. Therefore, the real-time and label-free measurement capability of SPR has provided a huge leap in obtaining information on the binding and separation of biomolecules [25]. The change in resonance angle upon molecular binding is the basis for SPR detection and measurement. This system was first used to detect antibody-antigen binding in real time without fluorescent or radioactive labels [36]. Later modern SPRs, such as SPRi, LSPR, and nanopore SPR, are derived from modifications of traditional SPRs. Traditional SPRs are very sensitive and can detect mass changes as small as 0.1 ng/cm^2 [37,38]. They have expertise in quantifying small quantities of kinetics, precisely determining k_a, k_d, K_D, and are thus regarded as the gold standard for studying biomolecular interactions [39]. However, due to their bulky optics, they require precise prism alignment, and having 1 to 4 flow channels has reduced their throughput. The surface of the gold film is used without nanostructured and smooth enhancement. The limitations of traditional SPR led to the development of modern separation methods based on SPR [40].

2.2. SPR imaging (SPRi)

SPRi is a sophisticated optical biosensing technique that combines the label-free, real-time detection of traditional SPR with the high-throughput analysis of microarrays [25]. Unlike its traditional

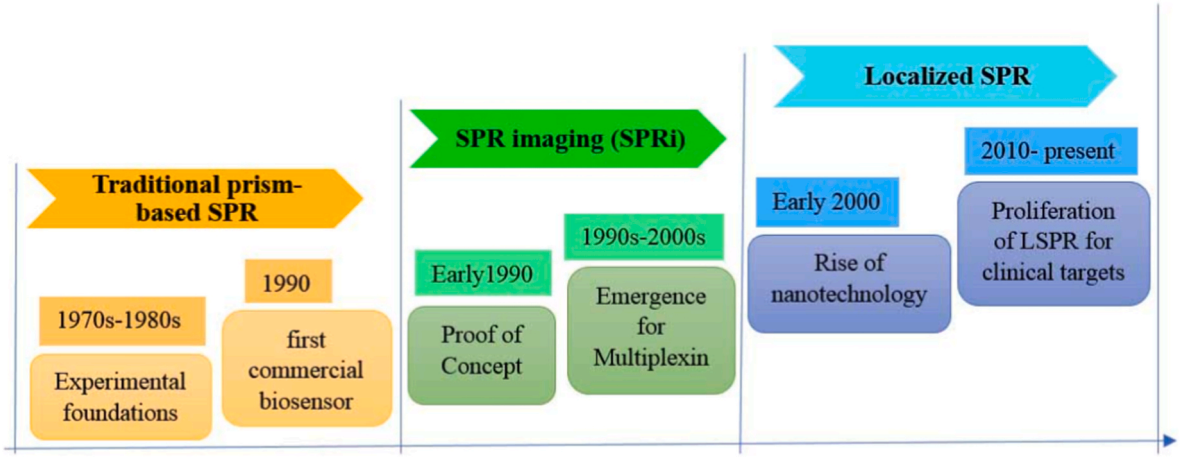

Fig. 1. Timeline chart for the emergence of three SPR-based methods.

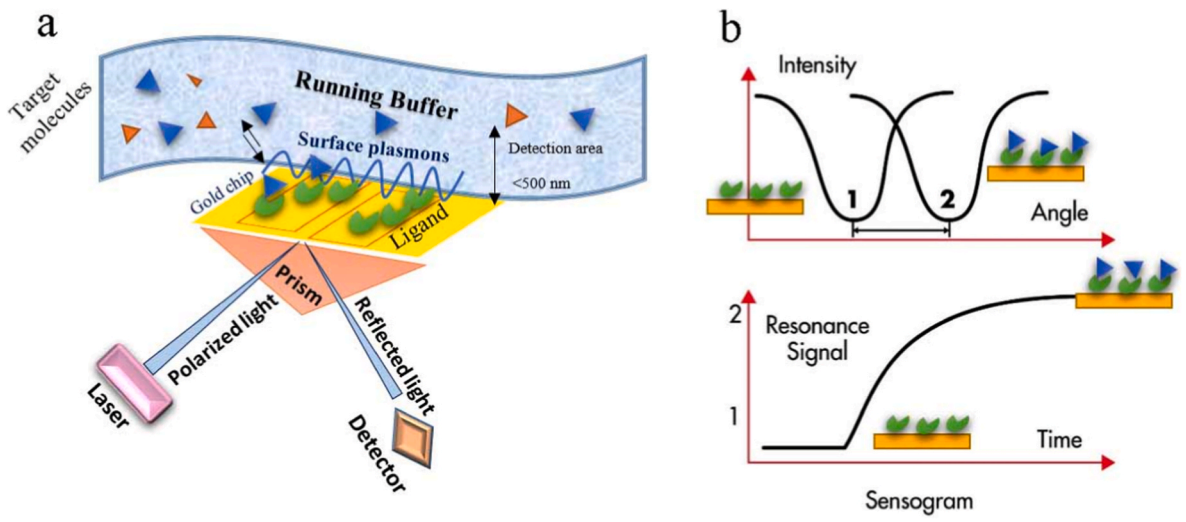

Fig. 2. **a)** Principle of Traditional SPR biosensor with Kretschmann configuration. The target molecules binding to the immobilized ligands causes a change in RI near the surface, **b)** the related SPR curve and sensogram as the shift of the resonance angle (here, measured at a fixed wavelength).

counterpart, which only assesses binding interactions at a single location, SPRi can simultaneously observe hundreds to thousands of molecular interactions across the surface of a modified sensor [41]. This capability allows for rapid screening of multiple biomolecules, such as proteins, antibodies, DNA, and cells. In this system, the reflectance changes in an array with a fixed angle of incident light are measured instead of measuring the change in the resonance angle at a single spot [42]. SPRi optics are similar to traditional prism-based or object-based SPR, except that a charge-coupled device (CCD) camera is used and the sensor chip consists of a gold-coated glass with hundreds to thousands of ligand spots (Fig. 3) [43]. As shown in this figure, the binding of a target molecule to its ligand produces a localized increase in the reflected light intensity, appearing as a bright dot. The absence of binding results in a minimal signal, seen as a dark region [44].

SPRi works by exciting surface plasmons, which result in collective motions of free electrons at the interface between a metal and a dielectric material (usually a gold-coated glass slide). By binding biomolecules to the sensor surface, the refractive index changes, resulting in detectable changes in the intensity, angle, or wavelength of the reflection [25]. SPRi is divided into several categories based on measurement approach, configuration, and application, some of which we describe below. Conventional SPRi (intensity-based SPRi) measures the intensity changes of reflected light illuminated from a fixed angle or wavelength after binding of molecules. This type of SPRi is used for high-throughput screening involving protein-protein, DNA-protein, etc. Interactions [25]. Angle-scanning SPRi is used to measure binding

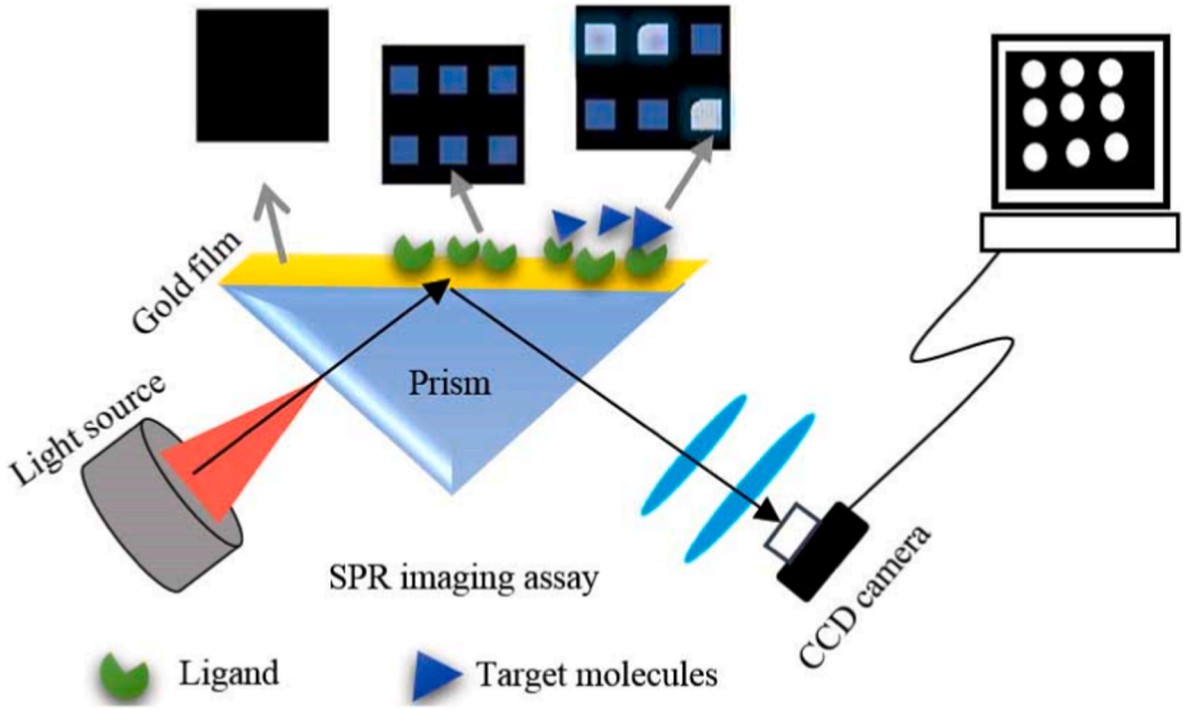

Fig. 3. The instrumentation of a SPRi system (The reflected light from the gold surface is captured by a monochromatic CCD camera and the lenses positioned in front of CCD for higher quality images. Finally, images can be digitally stored using a frame grabber and further analyzed using photography software). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

kinetics quantitatively, which is performed by varying the angle of incident light and measuring its reflectance at different angles, and has better resolution [45]. Wavelength-scanning SPRi, which is used in multi-analyte detection and spectral analysis, changes the wavelength while maintaining the angle of incidence, and binding events are measured along with the resonance wavelength [25].

2.3. Localized surface plasmon resonance (LSPR)

LSPR refers to the phenomenon in which a nanoscale metal (usually gold or silver nanoparticles) interacts with light to generate collective oscillations of conduction electrons (Fig. 4) [46]. In LSPR, surface plasmon propagation occurs in individual nanostructures, resulting in a much-localized enhancement of the electromagnetic field [47]. This is in contrast to conventional SPR, where surface plasmon propagation occurs along a thin metal film [48]. Like conventional SPR, LSPR is label-free and real-time. Therefore, plasmon resonance is associated with individual plasmonic nanoparticles (NPs) or nanostructured surfaces, which change with changes in size, shape, composition of the nanoparticles, and the surrounding environment [25,49]. Strong electric fields near the NP surface lead to an enhancement of the optical signals. The peak position in LSPR depends on factors such as the size of the nanoparticle, its shape, and material, as well as the dielectric constant of the medium [50]. Larger nanoparticles and a dielectric medium with a high refractive index shift the peak to a longer wavelength and red light. In addition, nanoparticles with rod, star, and triangular shapes produce multiple peaks (longitudinal and transverse modes), and gold nanoparticles create higher wavelengths than silver and aluminum (Au (~520-600 nm), Ag (~400-450 nm), Al (~UV range)). The best choice for LSPR is to use gold nanoparticles [51]. Silver nanoparticles, although they have the highest signal enhancement, are less widely used due to their instability. However, studies have shown that the use of bimetallic nanoparticles can bring the advantages of several metals simultaneously [52]. LSPR is miniaturizable and can be used on a smaller scale as point-of-care (POC) devices in the form of low-cost paper-based strips, as well as in the detection of biomarkers of cancer (PSA, HER2), viruses (HIV, SARS-CoV-2) and etc [42].

3. Multiplex detection using SPR-based methods

3.1. Immunosensor

The development of immunosensors for multiplex biomarker detection represents a major advancement in diagnostics [53]. Utilizing SPR-based biosensors, these platforms enable the real-time, label-free, and simultaneous quantification of multiple analytes [54]. By functionalizing the sensor surface with various specific antibodies, SPR immunosensors provide high sensitivity and specificity, revolutionizing early disease detection and personalized medicine through a single, efficient analysis [55,56]. For example, Dai et al. [57] designed a fiber optic-surface plasmon resonance (FO-SPR) biosensor incorporating laser heterodyne feedback interferometry to provide highly sensitive detection of fluoroquinolones (FQs). The biosensor was manufactured by coating 81°-tilted fiber Bragg gratings with a gold film, resulting in a self-assembled monolayer through Au–S bonds, and was functionalized with anti-FQ antibodies and gold nanorods to improve LSPR. Five FQ biomarkers named enrofloxacin, ciprofloxacin, norfloxacin, pefloxacin, and sarafloxacin were detected in tap water and milk samples. The limits of detection (LOD) ranged from 0.31 ng/L (norfloxacin) to 0.97 ng/L (sarafloxacin), significantly outperforming other methods like ELISA and electrochemical sensors. The biosensor exhibited approximately 100-fold signal amplification using AuNRs in contrast to non-enhanced SPR. This label-free, real-time biosensor provides exceptional sensitivity and portability, establishing it as a reliable tool for the environmental monitoring of antibiotic residues [57]. Another study presents a plasmon-enhanced biosensor for multiplexed detection of anti-SARS-CoV-2 antibodies in COVID-19 patients, as shown in Fig. 5a [58]. An array of 400 μm diameter patches of target and control antigens/proteins was fabricated and printed on gold-coated grating-coupled fluorescent plasmonic (GC-FP) biosensor chips. The immune responses across 20 patient samples were assessed by analyzing the biomarkers named anti-SARS-CoV-2 IgG, IgM, and IgA in clinical serum. IgG has a LOD of approximately 0.1 nM, while IgM and IgA have comparable sensitivities. The platform obtains a high level of specificity, allowing for distinguishing of COVID-19-positive samples from negative controls with minimal cross-reactivity. This rapid, label-free SPR system is compatible with plasmonic multiplexing technologies and enables high-throughput serodiagnostics [58].

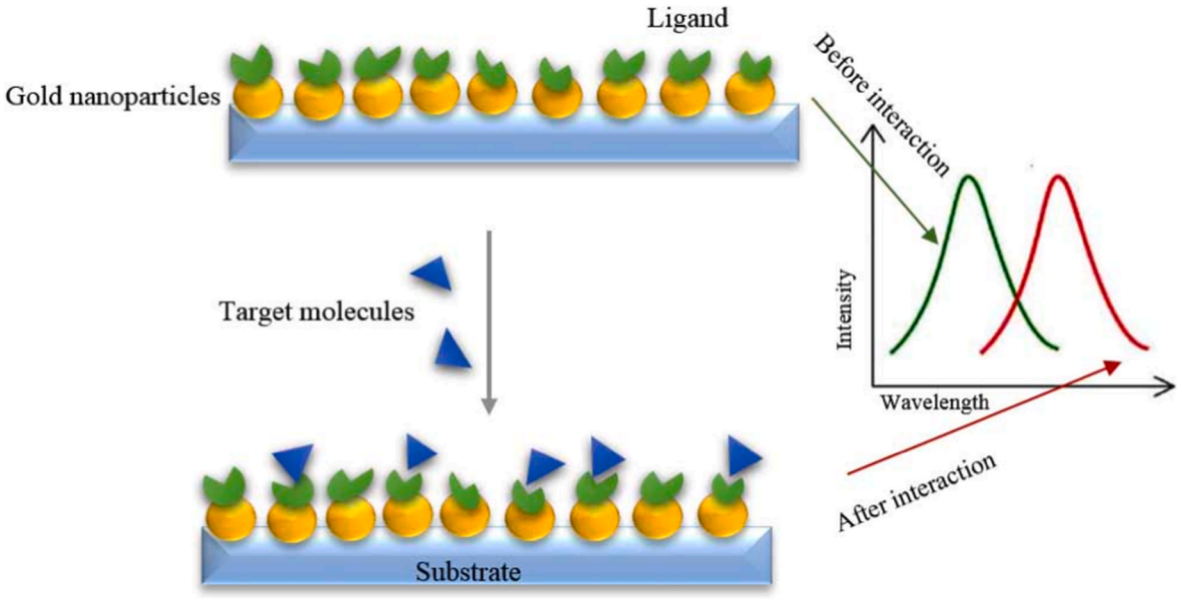

Fig. 4. LSPR biosensing principle. (Biomolecular recognition elements on the surface of metal nanosubstrate produce a local increase in the refractive index at the metal surface, which induces a peak-wavelength shift of the extinction spectra).

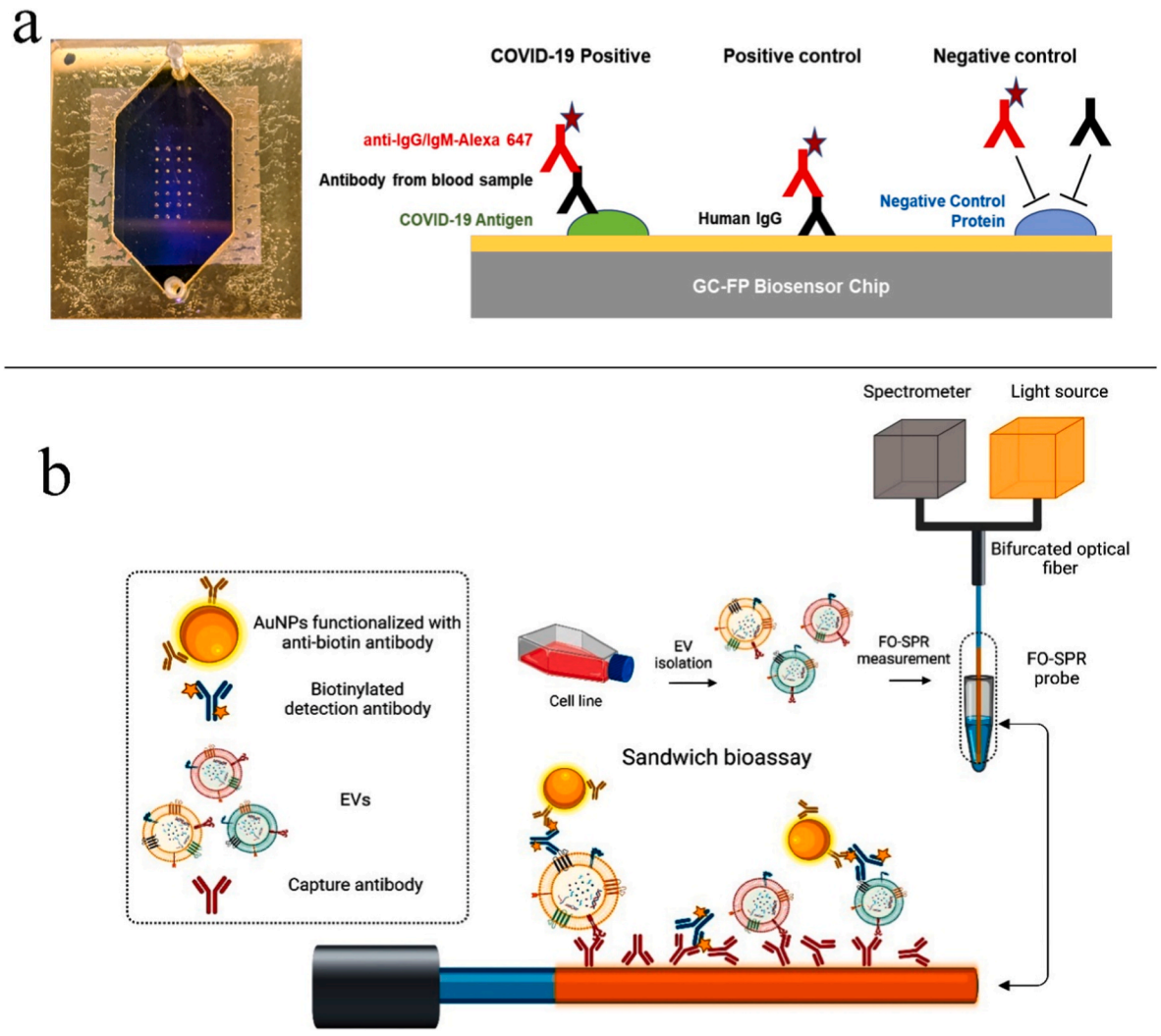

Fig. 5. The application of immuno-based SPR biosensors in multiplex detection. **a)** GC-FP biosensor chip & antigens specific to COVID-19 on chips and evaluated for antibody interactions using human blood samples. Fluorescence detection was enhanced by labeling with Alexa Fluor 647-conjugated anti-human IgG. Reprinted with permission from Ref. [58] **b)** Illustration of the FO-SPR bioassay for capturing EVs from SK-BR-3 or MCF7 cells with anti-HER2/EpCAM antibodies, followed by biotinylated [b]anti-CD9/CD63/CD81 and AuNPs for enhanced detection, displaying EV diversity Reprinted with permission from Ref. [59].

The article by Debnath et al. [60] develops an integrated microfluidic platform integrating a SPR biosensor with a passive plasma separation device (PPS–V2) to detect biomarkers in whole blood. This study incorporates a four-channel SPR microfluidic device functionalized with anti-IgG or anti-IgM antibodies, allowing for the simultaneous detection of human IgG and IgM in unprocessed blood, which are vital for the diagnosis of diseases such as cancer, influenza, HIV, and SARS-CoV-2. The approach comprises the extraction of PPS-V2, which is subsequently followed by SPR analysis on a portable P4SPR instrument with gold chips coated with Afficoat. Standard photolithography techniques were employed to fabricate the PPS-V2 device using poly dimethyl siloxane (PDMS) in conventional microfabrication. In the case of IgG, LOD is 1.3 nM (195 ng/mL), while in the case of IgM, it is 2.4 nM (2280 ng/mL). The validation experiments have confirmed that the SPR units are higher in comparison to the control plasma, indicating that the detection process is robust [60]. A subsequent study involved the fabrication of a miniaturized optical biosensor that integrates organic optoelectronic devices with a nanostructured plasmonic grating (NPG) for multiplexed SPR detection [61]. The NPG, produced using colloidal lithography and plasma etching, features a hexagonal lattice of polymeric pillars within a gold sheet, facilitating LSPR. The biosensor chip encompasses seven channels biofunctionalized with an MCP-2F copolymer covering, enabling the covalent bonding of bioreceptors for targeted analyte detection. The targeted markers were lactoferrin (80 kDa protein) and streptomycin (582 Da antibiotic), identified in milk samples. LOD for bulk refractive index changes was 10^{-4} RIU, corresponding to 3.7 μg/mL for lactoferrin. This label-free, rapid biosensor provides great sensitivity and portability, making it suitable for point-of-need food safety monitoring [61]. Another study conducted by Yildizhan et al. [59] developed a fiber-optic SPR (FO-SPR) biosensor for detecting breast cancer-specific extracellular vehicles (EVs) directly in blood plasma, utilizing a sandwich bioassay calibrated with recombinant EVs, as shown in Fig. 5b. The FO-SPR probes were fabricated by sputtering a gold layer onto multimode optical fibers, subsequently functionalized with COOH self-assembled monolayers (SAMs) and covalently bonded to capture antibodies (anti-HER2 or anti-EpCAM) with EDC/NHS chemistry. The bioassay focused on HER2-positive EVs from SK-BR-3 cells and EpCAM-positive EVs from MCF7 cells, utilizing biotinylated detection antibodies ([b]anti-CD9 or [b]anti-mix) and gold nanoparticles (GNPs) for signal enhancement. LOD was 7.0×10^8 particles/cm^3 for HER2 extracellular vesicles (EVs) and 1.1×10^8 particles/cm^3 for EpCAM EVs in 100-fold diluted plasma, with a LOD of 2.1×10^7 particles/cm^3 for HER2 EVs in buffer. The study indicates that the standardization, minimal sample volume, and plasma compatibility of

the FO-SPR bioassay underscore its potential as a diagnostic instrument for breast cancer [59].

Polonschii et al. [62] fabricated a portable SPR biosensor, MarkerSense, for the prompt diagnosis of peanut and hazelnut allergies. They synthesized sensor chips by coating BK7 glass slides with chromium and gold through thermal evaporation, subsequently functionalizing them with carboxylated cross-linked BSA (cBSA) using EDC/NHS and succinic anhydride. Four peptide epitopes (T12, EO13, EO14, HL19) have been attached to distinct channels by copper-free click chemistry utilizing DBCO-NH_2 linkers, facilitating multiplex detection. Allergen-specific IgE/IgG antibodies against peanut and hazelnut were identified in diluted rabbit serum. The study determined that MarkerSense provides an economical, portable POC platform for individualized allergy diagnosis, yielding results comparable to commercial products [62]. Zhang et al. [63] developed a portable SPRi instrument for the multiplexed detection of urinary proteins. The SPRI instrument functioned by an automatic angle scanning mechanical structure and was designed with a triangular prism coupling optical structure. The SPRI instrument's optical module was composed of a CCD camera (Basler), an SPR sensor chip, a prism with a triangle shape, and a red laser light source. The multiplexing method utilizes a five-channel gold-coated SPR device, with four channels immobilized with antibodies for human serum albumin (HSA), beta-2-microglobulin (B2M), kappa, and lambda light chains, and one reference channel targeting HSA, B2M, kappa, and lambda light chains, which are indicative of multiple myeloma and kidney diseases. LOD for HSA, B2M, kappa, and lambda is 0.36 μg/mL, 0.04 μg/mL, 0.05 μg/mL, and 0.1 μg/mL, respectively, exceeding immunoturbidimetry. Validation against Biacore T200 and clinical samples confirms accuracy, with substantial disparities in HSA and B2M levels between healthy and nephrotic patients [63]. One study developed a SPRi biosensor integrated with ganglioside microarrays for the label-free identification of multiple sclerosis (MS)-related antibodies in serum [64]. The biosensor employs a plasmonically optimized, background-free biochip covered with a perfluorodecyltrichlorosilane (PFDTS) layer to create a self-assembled pseudo-myelin sheath for antigen binding. The microarray was functionalized with gangliosides GA_1, GM_1, and GT_1b, designed to detect anti-GA_1, anti-GM_1, and anti-GT_1b antibodies, associated with multiple sclerosis symptoms such as optic nerve damage and muscle control impairment. LOD for anti-GT_1b was established at 24 ng/mL, enough for identifying MS-related antibody concentrations (3–20 ng/mL) in serum. The biosensor exhibited minimal nonspecific binding in 10% diluted serum, accomplished through the passivation of the PFDTS surface, thus enhancing its applicability in complicated media [64].

The SPRi biosensor was used for the multiplex detection of four cancer metastasis biomarkers, including Fas, HER2, Ang-2, and MMP-9 [65]. The use of an antibody-linked quantum dot chip for signal amplification yielded positive results, improving the signal by 100-fold and achieving an LOD of 25 $pgmL^{-1}$ [65]. For the multiplex detection of mycotoxin biomarkers, another SPRi model was used, which was capable of multiplex detection of deoxynivalenol (DON) and zearalenone (ZEN) via a competitive immunoassay on a single microarray chip. This platform has high potential for rapid and simultaneous analysis of food safety [66]. In addition, nanoparticles were used for the multiplex detection of protein biomarkers (IL-1β, IL-6, IFN-γ, TNF-α) to enhance the SPRi signal [67]. As a result, the developed cascade achieves extremely high sensitivity (LOD 50 fg/mL) and a wide dynamic range in complex biological fluids such as synovial fluid [67].

JS Chen et al. introduced an automated LSPR immunosensor for the multiple detection of inflammatory biomarkers, specifically immunoglobulins (IgG), tumor necrosis factor alpha (TNF-α), and C-reactive protein (CRP) [68]. This label-free biosensor, integrated with microfluidics, was capable of simultaneously measuring a 60 μL sample in 3.5 h [68]. An interesting direct-immunosensor platform has been designed that performs well for the multiplex and real-time detection of airborne viral biomarkers of bacteriophage MS2 and influenza virus [69]. This technology provides detection within minutes even in contaminated environments, which could be the basis for a portable “early warning” bioaerosol monitor for rapid environmental screening of multiple viral pathogens [69].

3.2. Aptasensor

The development of aptasensors for multiplex detection has been propelled by integrating high-affinity aptamers with SPRi and LSPR platforms, enabling the simultaneous, label-free monitoring of multiple biomarkers on a single chip [70,71]. Recent advancements focus on creating sophisticated mixed monolayers and nanostructured surfaces to minimize cross-reactivity and enhance the sensitivity of these multiplexed aptamer-based assays [72]. In this line, Canning et al. [73] constructed an SPR-based biosensor for the amplification-free, multiplexed detection of microRNAs associated with colorectal cancer (CRC). The research provides the fabrication of SERS substrates coated with a dense monolayer of bimetallic gold-silver nanostars (BNS), achieving a uniform surface coverage of 34.75% ± 0.50%. These substrates incorporate spatially distinct regions functionalized with inverse molecular sentinel (iMS) probes, facilitating simultaneous detection of miR-21 and miR-221 in a single assay. The platform achieves a limit of detection (LOD) of 0.84 fM for miR-21 and 0.92 fM for miR-221. The BNS substrates, analyzed by SEM and STEM-EDS, ensure constant signal intensity. This study outlined an approach for synthesizing homogeneous, highly reproducible dense monolayers of bimetallic nanostars essential to assay accuracy and precision [73]. In a further study, a multiplex and regenerable SPR biosensor was developed for the detection of DNA sequences from genetically modified organisms (GMOs). The study employed a Biacore T200 system with a four-flow-cell sensor chip, with three cells functionalized with biotinylated ssDNA probes for the T-nos, CaMV35S, and cry1A genes, all are critical markers of GMOs in crops such as soybean and maize, while one cell functions as a control, facilitating simultaneous detection. LOD is 100 pM (0.1 nM) for all three targets, as established via calibration curves depicting response units (RU) versus concentration. This approach simultaneously analyzes three GMO targets in a single run, as proven by agarose gel electrophoresis of PCR results. This label-free, regenerable SPR platform improves GMO screening efficiency and presents possibilities for POC applications, however it necessitates laboratory-based PCR preprocessing [74]. Another aptamer-based LSPR biosensor was developed for the multiple detection of pathogenic bacterial biomarkers [75]. This sensor was able to successfully detect three different bacterial species on a single chip with high specificity with a detection limit of 30 colony forming units per assay. Interestingly, a rapid multiplex phage-based biosensor for *Pseudomonas putida* or methicillin-resistant *Staphylococcus aureus* was demonstrated using SPRi and phase imaging [76]. Both methods detect bacterial susceptibility in under 2 h, with SPRi providing label-free, real-time monitoring through phage arrays. Phase imaging visualizes lytic replication at the single-cell level without immobilization. Results can be obtained in as little as 30 min, enabling faster personalized phage therapy formulation [76]. Also, for simultaneous detection of foodborne pathogens like Salmonella and *E. coli* in commercial chicken carcass rinse, an SPRi has been developed by B Park et al. [77]. This immunosensor achieved a detection limit of 10^6 CFU/mL, improvable to 1 CFU/mL with enrichment, and successfully identified different bacteria on a single chip. In this work, the SPRi signals revealed complex interference effects among coexisting bacteria species in heterogeneous bacteria solutions [77].

In a study conducted by Wang et al. [78], a multi-parameter SPR biosensor for label-free, amplification-free detection of multiple nucleic acids, utilizing a Kretschmann configuration with a gold-coated sensor chip has been developed. The SPR biosensor specifically targets miRNA-21 and miRNA-141, which are biomarkers for breast and prostate malignancies, with a 30 bp DNA oligonucleotide for comparative analysis, utilizing biotinylated capture probes for hybridization through

four microchannels. An adaptive consistency correction technique was employed for addressing inconsistent channel responsiveness, hence ensuring quantitative comparability across channels. LODs were 33 nM for miRNA-21, 41.2 nM for miRNA-141, and 52.3 nM for DNA, with a minimum detectable quantity of 4 pmol in an 80 μL sample. In comparison to electrochemical and fluorescent techniques, the SPR biosensor provides expedited detection and more straightforward operation [78]. Sun et al. [79] formulated a phase-based SPR biosensor employing one-dimensional (1D) gold gratings for the rapid, sensitive, and multiplexed identification of SARS-CoV-2 nucleic acids, as shown in Fig. 6a. The sensor, constructed by interference lithography, utilizes thiol-modified DNA probes to identify N, E, and ORF1ab gene sequences, reaching a LOD of 40 fM in 5 min without amplification and 1 aM (0.6 copies/μL) in 25 min with recombinase polymerase amplification (RPA). The grating's microscopic differential phase linewidth (~1.2 nm) optimizes sensitivity, with phase shifts assessed by common-path phase quadrature interferometry. A three-channel microfluidic system facilitates the concurrent detection of three gene sequences, enhancing diagnostic precision and efficiency. The sensor's modest, prism-free construction and scalability make it appropriate for POC testing [79].

Also, SPRi was used for development of an aptasensor for the multiplex detection of bacterial 16S rRNA, targeting *Legionella pneumophila*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium* (Fig. 6b) [80]. This biosensor utilizes surface-bound DNA probes for capture. Signal amplification is achieved via gold nanoparticle-grafted detection probes, forming long DNA-RNA sandwich assemblies. This method detected 16S rRNA in total RNA samples with high selectivity, a 10 pg/mL detection limit, and a broad dynamic range (0.01-100 ng/mL), demonstrating a readily adaptable platform for bacterial quantification [80].

In a further research Wu et al. [81] conducted a study presenting a SPRI-based biosensor for the multiplex and extremely accurate detection of non-small cell lung cancer (NSCLC)-associated exosomal miRNAs (miRNA-21, miRNA-378, miRNA-200, miRNA-139) in clinical plasma samples. The biosensor incorporates a gold array chip functionalized with DNA tetrahedral frameworks (DTFs) to detect exosomal miRNAs, subsequently leading to the production of DNA-programmed Au-on-Ag heterostructures (silver nanocubes [AgNCs] coated with gold nanoparticles [AuNPs]) for signal amplification. This approach reaches an extensive dynamic range (2 fM to 20 nM), an exceptionally low LOD of 1.68 fM for miRNA-21, and strong antifouling capabilities in 10% diluted plasma, attributed to DTFs minimizing nonspecific adsorption. The biosensor demonstrates great throughput by concurrently detecting four miRNAs on a single chip with elevated specificity, as validated by unique SPR signals at the respective detection sites [81]. In another study, a low-cost, prism-free SPR imaging biosensor was developed by D. K. Yang et al. using a disposable polymer-based gold nanogrid for multiple biomarker analysis [82]. The present designed aptamer-based sensor, in addition to successfully analyzing multiple kinetics using thrombin as the target biomarker, enabled simultaneous and multi-concentration detection with high temporal resolution with an integrated microfluidic system [82]. Recently, S. Li and colleagues presented an aptamer-based SPR biosensor for multiple detection of cardiac biomarkers in serum [83]. This sensor simultaneously measures

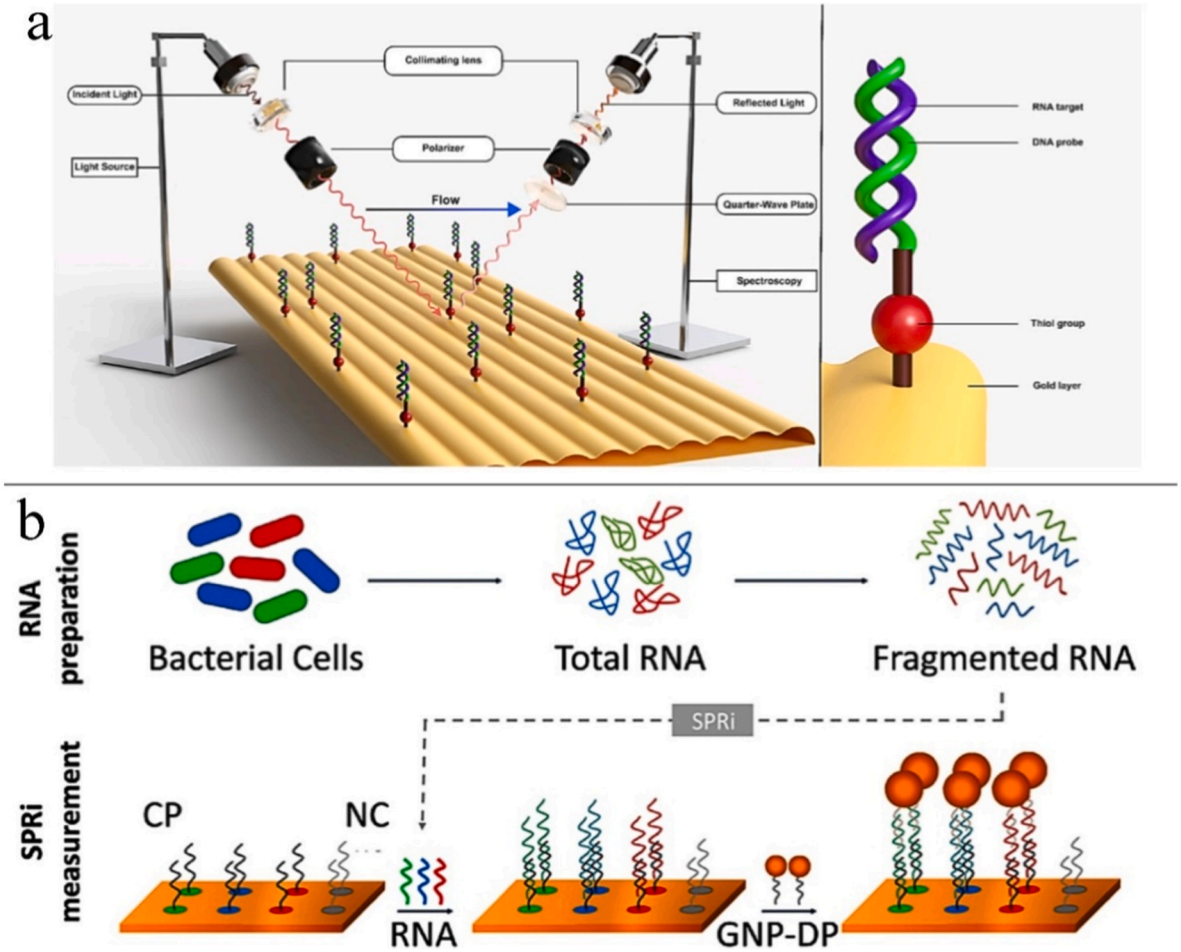

Fig. 6. Illustration of Aptasensor based SPR for simultaneous detection. **a)** schematic of the grating-based SPR sensor, detailing the overall detection mechanism and specific interactions at the sensing interface Reprinted with permission from Ref. [79] **b)** An SPRi biosensor with near-infrared signal amplification enables simultaneous, rapid, and sensitive detection of *L. pneumophila*, *P. aeruginosa*, and S. typhimurium using a simple protocol. Reprinted with permission from Ref. [80].

two key protein biomarkers for acute myocardial infarction, NT-proBNP and TNF-α. , This biosensor uses a "mixed aptamer" chip, in which DNA aptamers specific for each protein are bound to a single gold surface. Subsequent antibody injection forms a surface sandwich complex, increasing selectivity [83]. Distinct and clinically relevant dynamic ranges were established for each biomarker in this platform, and its potential for diagnostic application was confirmed by observing its performance in undiluted patient serum samples.

3.3. Protein based biosensor

Wekalao et al. [84] developed a graphene metasurface-based SPR biosensor for the efficient detection of SARS-CoV-2. The biosensor operated by terahertz (0.6–1.4 THz) electromagnetic simulations conducted with COMSOL Multiphysics and was constructed with four identical graphene-coated circular resonators on a SiO_2 substrate. The optical module of the biosensor consisted of a graphene monolayer, a SiO_2 substrate, and a terahertz light source, with performance optimized by 1D Convolutional Neural Networks (1D-CNNs). This method utilizes a potential sensor array configuration, with the current design targeting SARS-CoV-2 spike glycoproteins, indicative of viable virus particles across strains like Alpha, Delta, and Omicron. LOD is derived from a detection limit (DL) of 0.693 at 1.002–1.004 THz, exhibiting a sensitivity of 400 GHz/RIU and a refractive index resolution of 1.334–1.355 RIU [84].

Sadeghi et al. [85] developed a SPRi biosensor employing a corrodible silver thin film for the detection of hydrogen peroxide (H_2O_2) and glucose, as shown in Fig. 7, an integrated SPRi sensor chip incorporating multiple plexiglass channels on a single substrate has been used, enabling multiple detection of diverse analyte concentrations via intensity changes captured by a CCD camera. This method includes etching a thin silver layer onto a gold-coated glass substrate by H_2O_2 removing the SPR dip and enhancing reflected light intensity at a constant angle. In glucose detection, glucose oxidase (GOx) enzymatically generates H_2O_2, which etches silver, with samples pre-incubated before SPRi analysis. LOD is 40 nM for hydrogen peroxide and 175 μM for glucose. This label-free, real-time SPRi biosensor, characterized by facile fabrication and multiplexed detection, exhibits important prospects for POC glucose monitoring; nevertheless, the issue of chip reusability has yet to be resolved [85].

The study by Wekalao et al. [86] developed a graphene-based metasurface terahertz (THz) SPR biosensor for multiplexed detection of glucose and hemoglobin. The sensor was constructed with a silicon dioxide (SiO_2) substrate incorporating a central graphene-coated square resonator, three copper-coated circular resonators, and two bismuth-coated square resonators, all patterned through electron beam lithography or photolithography, with subsequent chemical vapor deposition (CVD) for graphene and thermal evaporation for metal deposition. The targeted biomarkers were glucose and hemoglobin, essential for the assessment of diabetes and anemia. LOD was established at 0.693 refractive index units (RIU) within the range of 1.002–1.004 THz, exhibiting a sensitivity of 400 GHz/RIU. The sensor's polarization-independent design and elevated sensitivity makes it appropriate for POC diagnostics, with prospects for integration with microfluidic devices for real-time health monitoring [86]. Interestingly, one study introduced a SPRi biosensor for the simultaneous, label-free quantification of insulin, glucagon, and somatostatin [87]. This represents the first biosensor to measure this specific hormonal triad, crucial for understanding paracrine interactions. Key to its success was an antifouling self-assembled monolayer that enabled operation in complex islet secretome. Achieving detection limits down to 1 nM for insulin, this multiplex platform provides a powerful tool to elucidate somatostatin's paracrine effects on insulin secretion, potentially accelerating the development of novel diabetes therapeutics [87]. The reviewed studies underscore the significant advancements in SPR-based biosensors for multiplex biomarker detection. Platforms incorporating SPRi, graphene

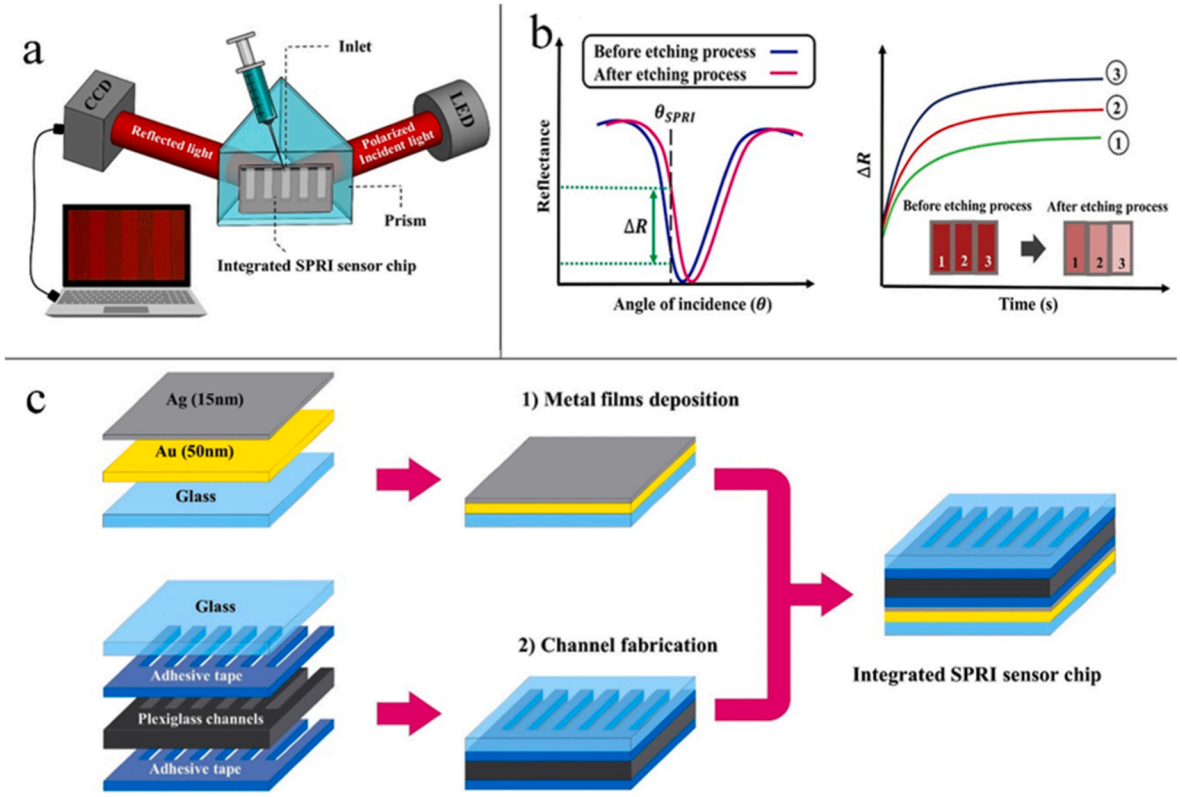

Fig. 7. The application of SPRi biosensors in multiplex detection. **a)** SPRi detection system based on the integrated SPRi sensor chip **b)** SPRi detection principle. Dashed line indicates the SPRi angle. **c)** Fabrication stages of integrated SPRi sensor chip. Reprinted with permission from Ref. [85].

metasurfaces, and innovative nanostructures have demonstrated robust, label-free, and simultaneous quantification of diverse analytes—from viral proteins and hormones to glucose and VOCs. These developments highlight a clear trajectory towards highly sensitive, high-throughput biosensing systems. By enabling comprehensive pathological profiling, these multiplex SPR biosensors hold immense potential to revolutionize POC diagnostics and accelerate therapeutic discovery, paving the way for more precise and personalized medicine. In Table 1 some examples discussed in the manuscript for multiplex detection of biomarkers, using different type of SPR-based methods by socusing on multiplex approach was presented.

4. Machine learning (ML) in SPR multiplex detection

The integration of ML with SPR biosensors is emerging as a powerful strategy to address the increasing complexity of multiplex biomarker detection [88,89]. Conventional SPR data analysis often struggles with spectral noise, overlapping signals, baseline drift, nonspecific binding,

Table 1
Some examples for multiplex detection of biomarkers, using different type of SPR-based methods.

SPR Platform	Biosensor Type	Multiplexing Approach	Biomarkers	LOD	Sample Matrix	Used for	Ref
GC-FP	Immunosensor	Microarray, fluorescence-enhanced	IgG, IgM, IgA (SARS-CoV-2 S1, S1S2, N)	-	Serum, DBS	COVID-19 antibody detection	[58]
FO-SPR with LHFI	Immunosensor	Frequency multiplexing, Au nanorods	EF, CF, NF, PF, SF	0.31–0.97 ng/L	Tap water, milk	Environmental monitoring	[57]
Grating-based SPR	Immunosensor	Seven-channel chip, PCA analysis	Lactoferrin, streptomycin	3.7 μg/mL (lactoferrin), ~200 ng/mL (streptomycin)	Milk	Food safety	[61]
FO-SPR	Immunosensor	Dual antibody-antigen detection	HER2 EVs, EpCAM EVs	7.0×10^8 (HER2), 1.1×10^8 particles/cm^3 (EpCAM)	Blood plasma	Breast cancer detection	[59]
Kretschmann SPR	Immunosensor	Four-channel peptide epitope	Peanut/hazelnut allergen IgE/IgG	-	Diluted serum	Allergy diagnosis	[62]
SPRi	Immunosensor	Microarray of conjugates on a single chip	Mycotoxins: Deoxynivalenol (DON) & Zearalenone (ZEN)	DON: 21/17 ng/mL; ZEN: 16/10 ng/mL	Maize & wheat extracts	Rapid screening for food & feed safety analysis	[66]
Bimetallic Nanostars	Aptasensor	miRNA probe, Au/Ag nanostars	miR-141, miR-375	100 aM (miR-141), 1 fM (miR-375)	Serum	Colorectal cancer diagnostics	[73]
MR-SPR with AuNPs	Aptasensor	Biotinylated DNA probes, AuNP sandwich	T-nos, CaMV35S, cry1A	100 pM (T-nos), 1 nM (others)	Genomic DNA (soybean, maize)	GMO detection	[74]
SPRi	Bacteriophage-based affinity biosensor	Array-based spatial multiplexing	*Pseudomonas putida*, methicillin-resistant *Staphylococcus aureus*	Cell-level detection	Liquid bacterial culture	Phage susceptibility testing	[76]
SPRi	Immunosensor	Antibody arrays immobilized on the same chip	Salmonella spp. and *Escherichia coli*	1 CFU/mL	Chicken carcass rinse	Screening of foodborne pathogens	[77]
Kretschmann SPR	Aptasensor	Four-channel nucleic acid hybridization	miRNA-21, miRNA-141, 30 bp DNA	33–52.3 nM	PBS buffer	Breast/prostate cancer diagnostics	[78]
Grating-based Phase SPR	Aptasensor	Three-channel DNA probe hybridization	SARS-CoV-2 N, E, ORF1ab genes	40 fM (no amp), 1 aM (RPA)	PBS buffer	COVID-19 detection	[79]
Planar gold chip SPR	Aptamer-based/ Sandwich assay	Mixed aptamers on a single surface	NT-proBNP & TNF-α	NT-proBNP: 0.03 nM; TNF-α: 0.06 pM	Human serum	Detection of Myocardial Infarction	[83]
THz SPR (Graphene Metasurfaces)	Protein-based	Sensor array, SPRi microarrays	SARS-CoV-2 spike glycoproteins	0.693 (RI-based)	Saliva, NPS	COVID-19 diagnostics	[84]
THz SPR (Graphene Metasurfaces)	Protein-based	Multi-resonator array, graphene/Cu/Bi	Glucose, hemoglobin	0.693 (RI-based)	-	Diabetic anemia monitoring	[86]
SPRi		Five-channel Au-coated chip, four Ab channels, one reference	HSA, B2M, kappa/ lambda LC	0.36 μg/mL (HSA), 0.04 μg/mL (B2M), 0.05 μg/mL (kappa), 0.1 μg/mL (lambda)	Urine	Renal/tumor diagnostics	[63]
SPRi with Au-on-Ag		Four DTF probes on Au array, Au-on-Ag amplification	miRNA-21, miRNA-378, miRNA-200, miRNA-139	1.68 fM (miRNA-21)	Plasma	NSCLC early diagnosis	[81]
SPRi with Ag Thin Film		2D imaging, Ag film etching, GOx immobilization	H_2O_2, glucose	40 nM (H_2O_2), 175 μM (glucose)	Serum	Diabetes monitoring	[85]
SPRi with Ganglioside MA		Ganglioside surfaces (GA_1, GM_1, GT_1b), ML analysis	Anti-GA_1, anti-GM_1, anti-GT_1b Ab	24 ng/mL (anti-GT_1b)	Diluted/ whole serum	MS diagnostics	[64]

Abbreviations: SPR:Surface Plasmon Resonance**, GC-FP**:Grating-coupled Fluorescent Plasmonics**, FO-SPR**: Fiber Optic Surface Plasmon Resonance**, LHFI**: Localized High-Frequency Induction**, AuNPs**: Gold Nanoparticles**, PCA**: Principal Component Analysis**, DBS**: Dried Blood Spots**, RI**: Refractive Index**, EVs**: Extracellular Vesicles**, HER2**: Human Epidermal Growth Factor Receptor 2**, EpCAM**: Epithelial Cell Adhesion Molecule, **miRNA**: MicroRNA**, T-nos**: Terminator of Nopaline Synthase, **CaMV35S**: Cauliflower Mosaic Virus 35S Promoter, **cry1A**: Cry1A Gene, **RPA**: Recombinase Polymerase Amplification, **VOCs**: Volatile Organic Compounds, **S1, S1S2, N:** SARS-CoV-2 Spike S1, S1S2, Nucleocapsid, **EF, CF, NF, PF, SF**: Enrofloxacin, Ciprofloxacin, Norfloxacin, Pefloxacin, Sarafloxacin, **MR-SPR**: Multiplex Regenerable Surface Plasmon Resonance, **THz**: Terahertz, **NPS**: Nasopharyngeal Swabs; **SPRi**: Surface Plasmon Resonance Imaging, **Au**: Gold, **Ab**: Antibody, **HSA**: Human Serum Albumin, **B2M**: Beta-2-Microglobulin, **LC**: Light Chains, **DTF**: DNA-Templated Fluorophore, **miRNA**: MicroRNA, **NSCLC**: Non-Small Cell Lung Cancer, **Ag**: Silver, **GOx**: Glucose Oxidase, **H_2O_2:** Hydrogen Peroxide, **MA**: Microarrays, **GA_1**, GM_1, GT_1b: Gangliosides GA_1, GM_1, GT_1b, **ML**: Machine Learning, **MS**: Multiple Sclerosis.

and computational complexity in analyzing multi-analyte data from complex biological samples [25,90]. ML techniques, including regression models (e.g., random forest regression), classification algorithms (e.g., neural networks, support vector machines (SVM)), deep learning architectures, and dimensionality reduction methods, enable accurate prediction of resonance parameters, robust classification of binding kinetics, and optimization of sensor designs [91–94].

Fiber-optic SPR systems have benefited from ML in overcoming challenges like wide full width at half maximum in multimode spectra and limited spectrometer resolution. CatBoost and other algorithms (XGBoost, Decision Tree, Linear Regression, K-Nearest Neighbors) predicted resonant wavelengths from normalized transmission spectra, with CatBoost achieving superior accuracy for real-time multi-channel refractive index and temperature sensing [95]. In D-shaped multimode fiber sensors coated with gold and zinc sulfide, neural networks excelled in resonance wavelength prediction and classification, enabling early dengue detection with reduced computation time [96]. In SPRi systems, ML addresses cross-reactivity and nonspecific binding in undiluted serum (Fig. 8a) [97]. Ganglioside-functionalized microarrays on PFDTS-coated gold substrates used PCA, PLS-DA, neural networks, and kNN for autoantibody detection (anti-GA_1, anti-GM_1, anti-GT_1b) with LOD below 7 ng/mL, achieving 88–96% accuracy by analyzing full sensorgram kinetics [97].

Advanced angular and smart SPR configurations incorporate ML for predictive modeling in diverse applications. Five-layer graphene-black phosphorus heterostructures employed KNN regression on reflectance curves for low refractive index prediction [100]. A hybrid multi-material SPR biosensor optimized for non-invasive glucose detection in urine samples, employing a combination of graphene, metal layers, and dielectric materials optimized simulations with ML, reduces simulation time and computational resource demands while accurately predicting resonance behavior, sensitivity, figure of merit, and optimal operating conditions across varying geometric and electrical parameters [101]. Focusing on specific ML applications, a graphene-silver hybrid metasurface biosensor for rapid COVID-19 detection utilized polynomial regression to predict resonance frequency shifts from refractive index variations, delivering a high coefficient of determination with robustness validated through residual analysis and bootstrap iterations [98]. This ML model development is divided into two stages named data processing and ML model evaluation (Fig. 8b). At first, raw data was pre-processed separately for two different models. DNA detection model address 2-class classification problem, focusing on presence or absence of DNA on gold surface; while DNA classification model predicts different types of DNA on gold plate surface [98].

In DNA hybridization detection on gold surfaces, t-SNE feature extraction and min-max normalization were applied to preprocess low-variance data, followed by classifiers including support vector machine (SVM) and multi-layer perceptron [99]. These ML models significantly outperform traditional biosensor analysis by accurately classifying and detecting DNA conformational changes and interactions, enabling reliable dataset generation for disease prognostication linked to over 400 diseases (Fig. 8c). Interestingly, the combining LSPR imaging with machine learning provides a rapid, accurate, low-cost, and point-of-care-compatible method for SARS-CoV-2 detection, with strong

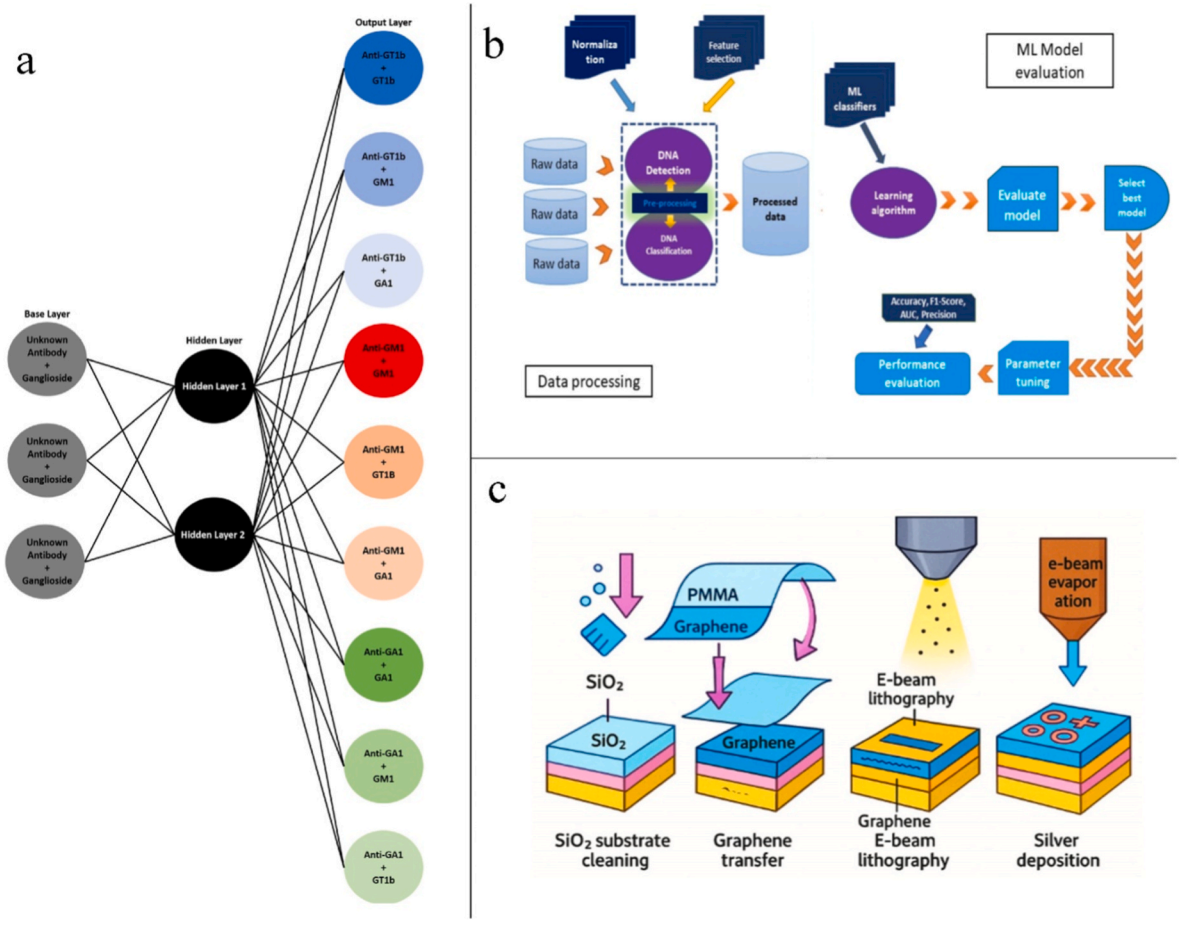

Fig. 8. **a)** Visualization of the machine learning algorithm for a neural network process including base layer, hidden layers, and output layers for all potential analyte antigen interaction of multiple sclerosis biomarkers. Reprinted with permission from Ref. [97] **b)** A flowchart of a data processing and ML model evaluation for detecting DNA attachment on SPR biosensor Reprinted with permission from Ref. [98] **c)** Step-by-step fabrication of the graphene–silver metasurface biosensor. Reprinted with permission from Ref. [99].

potential for broader application in viral diagnostics and pandemic response. In this line, LSPR imaging of intact SARS-CoV-2 particles have been achieved >97% classification accuracy using features extracted from microscopic color images [102]. For cancer biomarker detection, one-dimensional convolutional neural networks (1D-CNNs) can employ to predict and analyze sensor performance across parametric variations, delivering excellent regression accuracy in multiple datasets. The graphene-enhanced multi-material metasurface biosensor, combined with 1D–CNN–based performance prediction, offers a highly sensitive, reliable platform for early-stage cancer detection with strong potential for future biosensing and diagnostic applications [103]. In addition, a 1D-CNN enabled angle-independent prediction of malaria using MXene-graphene conjugated metasurfaces [91]. The MXene–graphene conjugated terahertz metasurface biosensor, enhanced by 1D-CNN machine learning, offers a highly sensitive, angle-robust, and tunable platform for quantitative malaria diagnostics, with strong potential for point-of-care and multiplexed biosensing applications [91].

Terahertz multi-resonator designs for waterborne pathogens used decision tree regression for transmission prediction [104]. Decision tree regression algorithms are applied to model and predict transmission behavior, delivering an outstanding coefficient of determination across multiple parametric conditions and frequency ranges. The graphene-enabled multiresonator metasurface biosensor, enhanced by machine learning optimization, offers superior sensitivity and versatility for real-time monitoring of diverse waterborne pathogens, with strong potential for applications in environmental surveillance and public health infrastructure [104]. Machine learning has been applied to develop VOC- and carbon-binding chimeric peptides using multi-sequence alignment of multiple odorant-binding protein (OBP) structures from protein databases. For example, Nakano-Baker et al. [105] a multiplexed, peptide-sensitized carbon nanotube field-effect transistor (CNT-FET) for detecting volatile organic compounds (VOCs) in exhaled breath associated with COVID-19 was fabricated. OBP-derived peptides, each sensitive to different VOCs, are combined into a small sensor array that convert vapor composition into multi-channel electrical signals. This biomimetic platform, with high reproducibility, supports rapid, non-invasive diagnostics, complementing SPR-based multiplexing [105]. These ML-enhanced approaches significantly enhance the sensitivity, specificity, robustness, and speed of multiplex SPR detection, particularly in challenging biological samples. It enables noise-tolerant prediction, automated classification, kinetic pattern recognition, and reduced reliance on high-resolution instrumentation.

5. Comparative analysis of SPR-based biosensing techniques

To clearly contrast the three core SPR techniques, a summary Table 2 highlights the key distinctions between prism-based SPR, localized SPR (LSPR), and SPR imaging (SPRi). In this table, the main principles, primary advantages (such as high sensitivity for traditional SPR, simpler optics and lower cost for LSPR, and high-throughput multiplexing for SPRi), inherent disadvantages and methodological limitations (including throughput, quantitation, and system complexity), and their typical application domains are compared. In addition, approximate ranges of instrument costs are provided where possible to assist users in assessing the feasibility and accessibility of each platform for specific laboratory needs and research goals.

6. Summary and future prospect

In conclusion, the integration of aptamers, antibodies and proteins with the diverse modalities of SPR technology has profoundly advanced the field of multiplex biomarker detection. Traditional prism-coupled SPR provides a robust, quantitative foundation for kinetic binding studies, while LSPR leverages nanostructured materials for enhanced sensitivity and miniaturization. Furthermore, SPRi is the cornerstone of true multiplexing, enabling the high-throughput, simultaneous screening of dozens of analytes on a single chip. This synergistic combination of specific biorecognition elements and versatile plasmonic transducers has yielded powerful biosensing platforms capable of deconvoluting complex biological signatures with exceptional precision, speed, and minimal sample consumption, thereby holding immense promise for comprehensive clinical diagnostics and point-of-care testing.

Looking forward, the trajectory of this field points toward several key developments. The primary challenge remains the analysis of complex, undiluted clinical samples like blood or serum. Future research must therefore intensify efforts in sophisticated surface chemistry and novel blocking strategies to mitigate non-specific binding, a hurdle more pronounced in multiplexed formats. For aptasensors, the focus will be on the development of high-affinity aptamers against a broader range of clinically relevant targets, including post-translationally modified proteins and microRNAs. A significant trend will be the fusion of different plasmonic techniques; for instance, combining the multiplexing power of SPRi with the ultra-sensitivity of LSPR nanostructures. These systems will leverage artificial intelligence not only for managing the vast, multiparametric data generated but also for predicting disease prognosis by recognizing complex biomarker patterns. In addition, advanced algorithms will enable real-time, high-throughput analysis of complex biomolecular interactions with unprecedented sensitivity and specificity. This synergy will unlock new frontiers in personalized diagnostics, drug discovery, and fundamental life science research, making label-free, multi-analyte sensing more powerful and accessible than ever before.

CRediT authorship contribution statement

Zahra Hashemi: Writing – original draft, Investigation. **Faride Ranjbari:** Writing – original draft, Methodology, Investigation. **Václav Ranc:** Writing – review & editing, Methodology. **Farzaneh Fathi:** Writing – review & editing, Visualization, Validation, Supervision, Investigation, Conceptualization.

Table 2
Comparative analysis of SPR-based biosensing techniques.

Aspect	Traditional SPR	Localized SPR	SPR Imaging
Basic Principle	Measures changes in refractive index near a thin gold layer via prism coupling	Uses plasmonic nanoparticles to generate LSPR	Combines SPR with imaging camera to monitor events
Advantages	High sensitivity; quantitative kinetic data (association/dissociation rates)	Simpler optics; lower instrumentation cost; portable designs possible; sensitive to local environment.	High-throughput; multiplexed detection; spatial mapping of binding events.
Disadvantages	Bulky instrumentation; requires precise alignment	Lower sensitivity compared to traditional SPR.	Lower spatial resolution for kinetic measurements
Approx. Instrumentation Cost	$150,000 – $400,000+ (high-end commercial systems)	$50,000 – $150,000 (benchtop systems); lower for custom setups.	$100,000 – $300,000 (depending on multiplexing and imaging capabilities).
Feasibility & Accessibility	Suitable for well-funded labs focused on detailed biomolecular kinetics.	More accessible for academic/small labs; adaptable for field use.	Ideal for labs requiring parallel screening

Ethical approval

Not applicable.

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Declaration of competing interest

The authors declare no conflict of interest.

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Data availability

Data will be made available on request.

References

[1] N.E. Almansouri, M. Awe, S. Rajavelu, K. Jahnavi, R. Shastry, A. Hasan, H. Hasan, M. Lakkimsetti, R.K. AlAbbasi, B.C. Gutiérrez, Early diagnosis of cardiovascular diseases in the era of artificial intelligence: an in-depth review, Cureus 16 (3) (2024).
[2] F. Ranjbari, Y. Pahlavan, M. Khalifezadeh, M. Fathi, S.H. Khaleghinejad, H. Imanzadeh, M. Saberi, F. Fathi, Molecular recognition of breast cancer biomarkers via localized surface plasmon resonance method, Plasmonics (2025) 1–18.
[3] L. Müller, S. Di Benedetto, V. Müller, From homeostasis to neuroinflammation: insights into cellular and molecular interactions and network dynamics, Cells 14 (1) (2025) 54.
[4] S. Cho, H.C. Yang, W.J.J.B. Rhee, Bioelectronics, Simultaneous Multiplexed Detection of Exosomal Micrornas and Surface Proteins for Prostate Cancer Diagnosis, 146, 2019 111749.
[5] P. Samadi Pakchin, F. Fathi, H. Samadi, K. Adibkia, Recent advances in receptor-based optical biosensors for the detection of multiplex biomarkers, Talanta 281 (2025) 126852.
[6] G. Jarockyte, V. Karabanovas, R. Rotomskis, A.J.S. Mobasheri, Multiplexed nanobiosensors: current trends in early diagnostics 20 (23) (2020) 6890.
[7] H.J.C.c.p. Ahsan, Monoplex and multiplex immunoassays: approval, advancements, and alternatives 31 (2) (2022) 333–345.
[8] Z. Chaloupková, Z. Medříková, M. Král, V. Šedajová, V. Ranc, Label-free determination of PSA and free PSA using MA-SERS, Front. Anal. Sci. 2 (2022) 847730.
[9] F. Fathi, H. Monirinasab, F. Ranjbary, K. Nejati-Koshki, Inverse opal photonic crystals: recent advances in fabrication methods and biological applications, J. Drug Deliv. Sci. Technol. 72 (2022) 103377.
[10] Y. Sun, S. Ge, R. Liu, S. Wang, C. Liu, L. Li, P. Zhao, S. Ge, J. Yu, Potential-resolved electrochemiluminescence biosensor for simultaneous determination of multiplex miRNA, Talanta 266 (2024) 125063.
[11] Y.-K. Yen, G.-W. Huang, R. Shanmugam, Laser-scribing graphene-based electrochemical biosensing devices for simultaneous detection of multiple cancer biomarkers, Talanta 266 (2024) 125096.
[12] X. Tian, P. Wang, G. Fang, X. Lin, J. Gao, Simultaneous quantitative analysis of multiple metabolites using label-free surface-enhanced Raman spectroscopy and explainable deep learning, Spectrochim. Acta Mol. Biomol. Spectrosc. 327 (2025) 125386.
[13] Y. Jahanbani, F. Fathi, P. Bastani, F.J.C.C.A. Ranjbari, Ovarian Cancer Biosensors: Established Glycoproteins to Emerging Molecular Biomarkers, 2025 120759.
[14] A. Bari, S. Aslam, H.U. Khan, S. Shakil, M. Yaseen, S. Shahid, A. Yusaf, N. Afshan, S.S. Shafqat, M.N. Zafar, Next-generation optical biosensors: cutting-edge advances in optical detection methods, Plasmonics (2025) 1–27.
[15] A. Rezabakhsh, F. Fathi, S. Habtemariam, E. Ahmadian, Cardiorenal syndrome: plasmonic biosensors, Clin. Chim. Acta 562 (2024) 119870.
[16] M. Johari-Ahar, M. Abdian, S. Maleki, P. Abbasgolizadeh, F. Fathi, Intercalation of anticancer drug mitoxantrone into DNA: studied by spectral and surface plasmon resonance methods, J. Mol. Struct. 1274 (2023) 134509.
[17] F. Fathi, R. Rahbarghazi, A.A. Movassaghpour, M.-R. Rashidi, Detection of CD133-marked cancer stem cells by surface plasmon resonance: its application in leukemia patients, Biochim. Biophys. Acta Gen. Subj. 1863 (10) (2019) 1575–1582.
[18] F.B. Kamal Eddin, H. Fan, Z. Liu, P. Donati, Y. Amin, Y.W. Fen, J. Liang, P. P. Pompa, S. He, Progress in surface plasmon and other resonance biosensors for biomedical applications, Adv. Mater. Technol. (2025) 2500536.
[19] C.L. Wong, Multiplex spectral-phase surface plasmon resonance 3D imaging biosensor, Opt. Commun. 584 (2025) 131815.
[20] A.J. Wright, H.H. Nasralla, R. Deshmukh, M. Jamalzadeh, M. Hannigan, A. Patera, Y. Li, M. Manzo-Perez, N. Parashar, Z. Huang, Nanoscale-localized multiplexed biological activation of field effect transistors for biosensing applications, Nanoscale 16 (42) (2024) 19620–19632.
[21] S. Das, R. Devireddy, M.R. Gartia, Surface plasmon resonance (SPR) sensor for cancer biomarker detection, Biosensors 13 (3) (2023) 396.
[22] N. Ravindran, S. Kumar, Y. M, R. S, M. CA, N. Thirunavookarasu S, S. CK, Recent advances in Surface Plasmon Resonance (SPR) biosensors for food analysis: a review, Crit. Rev. Food Sci. Nutr. 63 (8) (2023) 1055–1077.
[23] M. Hemdan, M.A. Ali, A.S. Doghish, S.S.A. Mageed, I.M. Elazab, M.M. Khalil, M. Mabrouk, D.B. Das, A.S. Amin, Innovations in biosensor technologies for healthcare diagnostics and therapeutic drug monitoring: applications, recent progress, and future research challenges, Sensors 24 (16) (2024) 5143.
[24] G. Gupta, K. Jha, S. Chaudhary, Biosensor assays types and their roles toward ligand–receptor interactions in drug discovery, Assay Drug Dev. Technol. 21 (5) (2023) 190–201.
[25] M.A. Butt, Surface plasmon resonance-based biodetection systems: principles, progress and applications—a comprehensive review, Biosensors 15 (1) (2025) 35.
[26] M. Aftab, M.S. Mansha, T. Iqbal, M. Farooq, Surface plasmon excitation: theory, configurations, and applications, Plasmonics 19 (4) (2024) 1701–1719.
[27] N.J. Geddes, C.R. Lawrence, Monitoring Immunoreactions with SPR, Handbook of Biosensors and Electronic Noses, CRC Press, 2024, pp. 349–368.
[28] F.J. Tukur, Plasmon-Exciton Coupling for Signal Amplification and Biosensing: Fundamentals and Application, The University of North Carolina at Greensboro, 2023.
[29] I. Steer, Engineering and Characterisation of Optical Nucleic Acid Sensors, Imperial College London, 2021.
[30] A. Dillen, J. Lammertyn, W. Saeys, Duplexed Aptamers and Fiber Optic SPR Sensors to Enable Continuous Biosensing, 2022.
[31] N.-F. Chiu, The current status and future promise of SPR biosensors, Biosensors 12 (11) (2022) 933.
[32] J. Homola, M. Piliarik, Surface plasmon resonance (SPR) sensors, in: Surface Plasmon Resonance Based Sensors, Springer, 2006, pp. 45–67.
[33] R.B. Schasfoort, Handbook of Surface Plasmon Resonance, Royal Society of Chemistry, 2017.
[34] J. Homola, S.S. Yee, G.J.S. Gauglitz, a.B. Chemical, Surface plasmon resonance sensors 54 (1-2) (1999) 3–15.
[35] A. Kumari, A. Yadav, O. Singh, P. Sharan, A review of surface plasmon resonance (SPR) technology in biosensing: innovations, applications and future trends, J. Opt. (2024) 1–9.
[36] M. Drozd, S. Karoń, E. Malinowska, Recent advancements in receptor layer engineering for applications in SPR-based immunodiagnostics, Sensors 21 (11) (2021) 3781.
[37] L.W. Teunissen, Thermoresponsive Polymer Brushes via grafting-to Procedures, Wageningen University and Research, 2023.
[38] F. Fathi, R. Rahbarghazi, M.-R. Rashidi, Label-free biosensors in the field of stem cell biology, Biosens. Bioelectron. 101 (2018) 188–198.
[39] S. Stangherlin, Y. Ding, J. Liu, Dissociation constant (Kd) measurement for small-molecule binding aptamers: homogeneous assay methods and critical evaluations, Small Methods (2024) 2401572.
[40] N.V. Tabiryan, D.E. Roberts, Z. Liao, J.Y. Hwang, M. Moran, O. Ouskova, A. Pshenichnyi, J. Sigley, A. Tabirian, R. Vergara, Advances in transparent planar optics: enabling large aperture, ultrathin lenses, Adv. Opt. Mater. 9 (5) (2021) 2001692.
[41] J. Xu, P. Zhang, Y. Chen, Surface plasmon resonance biosensors: a review of molecular imaging with high spatial resolution, Biosensors 14 (2) (2024) 84.
[42] Y. Hang, J. Boryczka, N. Wu, Visible-light and near-infrared fluorescence and surface-enhanced Raman scattering point-of-care sensing and bio-imaging: a review, Chem. Soc. Rev. 51 (1) (2022) 329–375.
[43] M.F. Jamil, M. Pokharel, K. Park, Light-controlled microbots in biomedical application: a review, Appl. Sci. 12 (21) (2022) 11013.
[44] H. Wang, H. Wang, H. Zhang, Y. Huang, N. Zhang, W. Li, X. Qiu, D. Yu, L. Zhang, Integration of a multichannel surface plasmon resonance sensor chip and refractive index matching film array for protein detection in human urine, Talanta 246 (2022) 123533.
[45] Y. Lee, Y. Choi, S. Hong, Quantitatively sensitive and resolution-enhancing strategies in optical signal conversion of SPR images using extensive angle-scanning and image-reconstruction, Sensor. Actuator. B Chem. 400 (2024) 134897.
[46] A. Raj, R.K. Thomas, Localized surface plasmon resonance (LSPR) applications of gold (Au) and silver (Ag) nanoparticles, in: Optical and Molecular Physics, Apple Academic Press, 2021, pp. 43–69.
[47] B.P. Nanda, P. Rani, P. Paul, S.S. Ganti, R. Bhatia, Recent trends and impact of localized surface plasmon resonance (LSPR) and surface-enhanced Raman spectroscopy (SERS) in modern analysis, J. Pharm. Anal. 14 (11) (2024) 100959.
[48] K. Khurana, N. Jaggi, Localized surface plasmonic properties of Au and Ag nanoparticles for sensors: a review, Plasmonics 16 (4) (2021) 981–999.
[49] F. Fathi, R. Jalili, M. Amjadi, M.-R. Rashidi, SPR signals enhancement by gold nanorods for cell surface marker detection, Bioimpacts: BI 9 (2) (2018) 71.

[50] S. Lv, Y. Du, F. Wu, Y. Cai, T. Zhou, Review on LSPR assisted photocatalysis: effects of physical fields and opportunities in multifield decoupling, Nanoscale Adv. 4 (12) (2022) 2608–2631.
[51] A.D. Kondorskiy, V.S. Lebedev, Size and shape effects in optical spectra of silver and gold nanoparticles, J. Russ. Laser Res. 42 (6) (2021) 697–712.
[52] F. Beck, M. Loessl, A.J. Baeumner, Signaling strategies of silver nanoparticles in optical and electrochemical biosensors: considering their potential for the point-of-care, Microchim. Acta 190 (3) (2023) 91.
[53] C.L. Wong, Spectral-phase 3D surface plasmon resonance imaging biosensor for COVID-19 neutralizing antibody detection, Opt. Express 33 (8) (2025) 18089–18101.
[54] M.F.N. Nashron, S. Karman, W.S.W.K. Zaman, R. Zakaria, S.N.S. Nor, S.W. Harun, Incorporation 2D-TMDC for high performance surface plasmon resonance (SPR) based fiber optic biosensor for early Salmonella detection: a review, Opt. Fiber Technol. 95 (2025) 104464.
[55] H. Wan, H. Cui, H. Liu, G. Zhang, L. He, H. Liu, S. Mahmud, Surface plasmon resonance biosensor chips: fabrications and pharmaceutical applications, J. Pharmaceut. Biomed. Anal. (2025) 117018.
[56] R. D'Agata, N. Bellassai, G. Spoto, Exploiting the design of surface plasmon resonance interfaces for better diagnostics: a perspective review, Talanta 266 (2024) 125033.
[57] Z. Dai, J. Tan, K. Zhou, L. Zhang, X. Zhou, Y. Tan, Optical fiber SPR biosensor with frequency multiplexing compensated laser heterodyne feedback for ultrasensitive detection of fluoroquinolones, Sensor. Actuator. B Chem. 393 (2023) 134335.
[58] N.C. Cady, N. Tokranova, A. Minor, N. Nikvand, K. Strle, W.T. Lee, W. Page, E. Guignon, A. Pilar, G.N. Gibson, Multiplexed detection and quantification of human antibody response to COVID-19 infection using a plasmon enhanced biosensor platform, Biosens. Bioelectron. 171 (2021) 112679.
[59] Y. Yildizhan, K. Driessens, H.S.K. Tsao, R. Boiy, D. Thomas, N. Geukens, A. Hendrix, J. Lammertyn, D. Spasic, Detection of breast cancer-specific extracellular vesicles with fiber-optic SPR biosensor, Int. J. Mol. Sci. 24 (4) (2023) 3764.
[60] N. Debnath, L.S. Live, M. Poudineh, A microfluidic plasma separation device combined with a surface plasmon resonance biosensor for biomarker detection in whole blood, Lab Chip 23 (4) (2023) 572–579.
[61] M. Bolognesi, M. Prosa, M. Toerker, L. Lopez Sanchez, M. Wieczorek, C. Giacomelli, E. Benvenuti, P. Pellacani, A. Elferink, A. Morschhauser, A fully integrated miniaturized optical biosensor for fast and multiplexing plasmonic detection of high-and low-molecular-weight analytes, Adv. Mater. 35 (26) (2023) 2208719.
[62] C. Polonschii, M. Rosu-Hamzescu, S. David, A. Oloumi, V.-D. Ursu, M. Szardenings, K. Kern, A.E. El Salhi, E. Gheorghiu, Point-of-care personalized rapid diagnosis of allergies using peptide epitopes and SPR multiplexed detection, Sensor. Actuator. B Chem. 418 (2024) 136359.
[63] L. Zhang, H. Wang, H. Zhang, N. Zhang, X. Zheng, W. Li, X. Qiu, D. Yu, Development of a portable multiplexed instrument for multi-proteins detection in human urine using surface plasmon resonance, Sensor. Actuator. B Chem. 369 (2022) 132272.
[64] A.S. Malinick, A.S. Lambert, D.D. Stuart, B. Li, E. Puente, Q. Cheng, Detection of multiple sclerosis biomarkers in serum by ganglioside microarrays and surface plasmon resonance imaging, ACS Sens. 5 (11) (2020) 3617–3626.
[65] A. Shabani, M. Tabrizian, Design of a universal biointerface for sensitive, selective, and multiplex detection of biomarkers using surface plasmon resonance imaging, Analyst 138 (20) (2013) 6052–6062.
[66] D. Dorokhin, W. Haasnoot, M.C. Franssen, H. Zuilhof, M.W. Nielen, Imaging surface plasmon resonance for multiplex microassay sensing of mycotoxins, Anal. Bioanal. Chem. 400 (9) (2011) 3005–3011.
[67] J. Hendriks, I. Stojanovic, R.B. Schasfoort, D.l.B. Saris, M. Karperien, Nanoparticle enhancement cascade for sensitive multiplex measurements of biomarkers in complex fluids with surface plasmon resonance imaging, Anal. Chem. 90 (11) (2018) 6563–6571.
[68] J.-S. Chen, P.-F. Chen, H.T.-H. Lin, N.-T. Huang, A localized surface plasmon resonance (LSPR) sensor integrated automated microfluidic system for multiplex inflammatory biomarker detection, Analyst 145 (23) (2020) 7654–7661.
[69] E.V. Usachev, E. Agranovski, O.V. Usacheva, I.E. Agranovski, Multiplexed surface plasmon resonance based real time viral aerosol detection, J. Aerosol Sci. 90 (2015) 136–143.
[70] P.-Y. Lin, Y.-F. Chang, C.-C. Chen, L.-C. Su, I. Willner, J.-a.A. Ho, pH-Responsive Triplex DNA nanoswitches: surface plasmon resonance platform for bladder cancer-associated microRNAs, ACS Nano 19 (7) (2025) 7140–7153.
[71] J.M. Obliosca, O. Vest, D. Patel, T. Ferguson, K. Smith, D. Christy, A. Powers, A. K. Smith, Y. Xu, C.K. Tison, Surface plasmon resonance imaging-based platform enables detection of single, site-specific 5-Methylcytosine associated with post-traumatic stress disorder (PTSD), ACS Pharmacol. Transl. Sci. 8 (2) (2025) 522–532.
[72] F. Khalid-Salako, H. Kurt, M. Yüce, Surface plasmon resonance aptasensors: emerging design and deployment landscape, Biosensors 15 (6) (2025) 359.
[73] A.J. Canning, X. Chen, J.Q. Li, W.R. Jeck, H.-N. Wang, T. Vo-Dinh, miRNA probe integrated biosensor platform using bimetallic nanostars for amplification-free multiplexed detection of circulating colorectal cancer biomarkers in clinical samples, Biosens. Bioelectron. 220 (2023) 114855.
[74] N. An, K. Li, Y. Zhang, T. Wen, W. Liu, G. Liu, L. Li, W. Jin, A multiplex and regenerable surface plasmon resonance (MR-SPR) biosensor for DNA detection of genetically modified organisms, Talanta 231 (2021) 122361.
[75] S.M. Yoo, D.-K. Kim, S.Y. Lee, Aptamer-functionalized localized surface plasmon resonance sensor for the multiplexed detection of different bacterial species, Talanta 132 (2015) 112–117.
[76] L. O'Connell, O. Mandula, L. Leroy, A. Aubert, P.R. Marcoux, Y.J.C. Roupioz, Ultrafast and multiplexed bacteriophage susceptibility testing by surface plasmon resonance and phase imaging of immobilized phage microarrays 10 (5) (2022) 192.
[77] B. Park, B. Wang, J. Chen, Label-free immunoassay for multiplex detections of foodborne bacteria in chicken carcass rinse with surface plasmon resonance imaging, Foodborne Pathog. Dis. 18 (3) (2021) 202–209.
[78] H. Wang, H. Wang, Y. Huang, H. Zhang, Y. Fu, Z. Yang, Y. Chen, X. Qiu, D. Yu, L. Zhang, Multi-parameter surface plasmon resonance instrument for multiple nucleic acid quantitative detection, Biomed. Microdevices 25 (3) (2023) 24.
[79] S. Sun, Y. Zhao, Y. Hao, P. Xue, X. Guo, W. Zhang, C. Zhang, Q. Zhang, W. Zhou, H.C. Ong, X. Yu, Z. Li, J. Li, J. Yao, Rapid, sensitive and multiplexed detection of SARS-CoV-2 viral nucleic acids enabled by phase-based surface plasmon resonance of metallic gratings, Biomed. Opt. Express 15 (9) (2024) 5215–5226.
[80] F. Melaine, M. Saad, S. Faucher, M. Tabrizian, Selective and high dynamic range assay format for multiplex detection of pathogenic Pseudomonas aeruginosa, Salmonella typhimurium, and Legionella pneumophila RNAs using surface plasmon resonance imaging, Anal. Chem. 89 (14) (2017) 7802–7807.
[81] W. Wu, X. Yu, J. Wu, T. Wu, Y. Fan, W. Chen, M. Zhao, H. Wu, X. Li, S. Ding, Surface plasmon resonance imaging-based biosensor for multiplex and ultrasensitive detection of NSCLC-associated exosomal miRNAs using DNA programmed heterostructure of au-on-ag, Biosens. Bioelectron. 175 (2021) 112835.
[82] D.K. Yang, T.T. Chu, S.C. Lo, Y.D. He, J.H. Weng, L.K. Yu, K.L. Lee, P.F. Calmon, P.K. Wei, T. Leïchlé, Polymer-based prism-free nanograting SPR imaging enables multiplexed detection and single-step aptamer binding kinetics, Small (2026) e12092.
[83] S.H. Lee, J.H. Back, H.J. Joo, D.-S. Lim, J.E. Lee, H.J. Lee, Simultaneous detection method for two cardiac disease protein biomarkers on a single chip modified with mixed aptamers using surface plasmon resonance, Talanta 267 (2024) 125232.
[84] J. Wekalao, Graphene metasurface-based surface plasmon resonance biosensor for rapid COVID-19 detection with machine learning optimization, Plasmonics (2025) 1–18.
[85] S. Sadeghi, M. Mohammadimasoudi, H. Mehrzad, A. Goudarzi, Hydrogen peroxide and glucose detection using surface plasmon resonance imaging biosensor with corrodible silver thin film, Talanta 279 (2024) 126593.
[86] J. Wekalao, A.M. Ahmed, H.A. Elsayed, M.A. Abdelkawy, A. Rajakannu, A. Mehaney, S. Bellucci, Gradient boosting-assisted polarization-independent SPR biosensor for multiplexed diagnostics and behavioural prediction, Diam. Relat. Mater. 157 (2025) 112539.
[87] F.R. Castiello, M. Tabrizian, Multiplex surface plasmon resonance imaging-based biosensor for human pancreatic islets hormones quantification, Anal. Chem. 90 (5) (2018) 3132–3139.
[88] D.D. Stuart, W. Van Zant, S. Valiulis, A.S. Malinick, V. Hanson, Q. Cheng, Trends in surface plasmon resonance biosensing: materials, methods, and machine learning, Anal. Bioanal. Chem. 416 (24) (2024) 5221–5232.
[89] M. Kokabi, M.N. Tahir, D. Singh, M. Javanmard, Advancing healthcare: synergizing biosensors and machine learning for early cancer diagnosis, Biosensors 13 (9) (2023) 884.
[90] Y. Liu, X. Liu, X. Wang, H. Jiang, AI-empowered electrochemical sensors for biomedical applications: technological advances and future challenges, Biosensors 15 (8) (2025) 487.
[91] S.S. Birunda, H. Subramani, A.K. U, T. Sheheryar, Machine learning-enhanced terahertz biosensor with mxene-graphene conjugate for high-sensitivity malaria detection, Plasmonics (2025) 1–14.
[92] A.F. Romeiro, C.M. Cavalcante, A.O. Silva, J.C. Costa, M.T. Giraldi, A. Guerreiro, J.L. Santos, A machine learning approach for designing surface plasmon resonance PCF-based sensors, in: 29th International Conference on Optical Fiber Sensors, SPIE, 2025, pp. 845–848.
[93] M. Mohseni-Dargah, Z. Falahati, B. Dabirmanesh, P. Nasrollahi, K. Khajeh, Chapter 12 - machine learning in surface plasmon resonance for environmental monitoring, in: M. Asadnia, A. Razmjou, A. Beheshti (Eds.), Artificial Intelligence and Data Science in Environmental Sensing, Academic Press, 2022, pp. 269–298.
[94] J. Arzola-Flores, A. González, Machine learning for predicting the surface plasmon resonance of perfect and concave gold nanocubes, J. Phys. Chem. C 124 (46) (2020) 25447–25454.
[95] A. Guo, W. Zhao, P. Ding, P. Tang, X. Zeng, Machine learning approach in multi-channel fiber-optic SPR sensors, Opt Laser. Technol. 181 (2025) 111618.
[96] A. Banerjee, J. Thangaraj, SPR-based refractive index sensor design with grated Au-ZnS for dengue detection using machine learning, Spectrochim. Acta Mol. Biomol. Spectrosc. (2025) 126276.
[97] A.S. Malinick, D.D. Stuart, A.S. Lambert, Q.J.B. Cheng, B. X, Surface Plasmon Resonance Imaging (SPRi) in Combination with Machine Learning for Microarray Analysis of Multiple Sclerosis Biomarkers in Whole Serum, 10, 2022 100127.
[98] H.S. Mondal, K.A. Ahmed, N. Birbilis, M.Z.J.S.R. Hossain, Machine learning for detecting DNA attachment on SPR biosensor 13 (1) (2023) 3742.
[99] N. Natraj, A. Mubarakali, M. Alagarsamy, M.Y.H. Al-Shamri, R.J.S.R. Dhivya, Next-generation COVID-19 detection using a metasurface biosensor with machine learning-enhanced refractive index sensing 15 (1) (2025) 33406.
[100] J. Wekalao, H.A. Elsayed, A. Mehaney, A. Rajakannu, H.A. Alqhtani, M. Bin-Jumah, J. Muheki, S. Bellucci, Enhanced surface plasmon resonance biosensor with graphene-black phosphorus heterostructure for ultra-high sensitivity

refractive index detection with machine learning for behaviour prediction, PLoS One 20 (11) (2025) e0332356.
[101] T. Sheheryar, Next-generation hybrid multi-material surface plasmon resonance biosensor for non-invasive glucose detection with machine learning optimization, Plasmonics 20 (12) (2025) 11119–11135.
[102] J. Liang, W. Zhang, Y. Qin, Y. Li, G.L. Liu, W. Hu, Applying machine learning with localized surface plasmon resonance sensors to detect SARS-CoV-2 particles, Biosensors 12 (3) (2022) 173.
[103] J. Wekalao, H.A. Elsayed, A.M. El-Sherbeeny, M.R. Abukhadra, A. Mehaney, Design and optimization of a graphene-enhanced terahertz metasurfaces surface plasmon resonance biosensor with multi-material architecture for cancer detection integrating 1D-CNN machine learning for performance prediction and analysis, Plasmonics (2025) 1–23.
[104] J. Wekalao, S.K. Patel, A. Balamurugan, F.A.J.P. Al-Zahrani, Graphene-enabled multiresonator metasurfaces for ultrasensitive surface plasmon resonance detection of waterborne bacteria across multiple frequencies with machine learning optimization 20 (7) (2025) 4679–4700.
[105] O. Nakano-Baker, H. Fong, S. Shukla, R.V. Lee, L. Cai, D. Godin, T. Hennig, S. Rath, I. Novosselov, S. Dogan, Data-driven design of a multiplexed, peptide-sensitized transistor to detect breath VOC markers of COVID-19, Biosens. Bioelectron. 229 (2023) 115237.