# Novel Nanoplasmonic-Structure-Based Integrated Microfluidic Biosensors for Label-Free in Situ Immune Functional Analysis

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A review of recent progress.

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THE STUDY OF IMMUNE FUNCtional responses is essential to understanding the central role of the immune system in providing immunological host defense and its intercommunication with other systems. The recent development of integrated microfluidic cytokine biosensors has established a new paradigm to identify, isolate, and study immune cell subtypes, cell functions, and intercellular communications that constitute those responses.

In this minireview, we highlight the most recent progress in label-free cytokine detection based on localized surface plasmon resonance optical sensing. We present the applications of newly identified plasmonic nanostructures and the integration with advanced microfluidic devices for novel lab-on-a-chip biosensing systems and discuss the associated challenges and future perspective of such integrative sensing technologies for next-generation immune functional analysis.

## IMMUNE BIOSENSORS OVERVIEW

The immune system protects the body against the invasion of external pathogens via a complex defense mechanism involving dynamic functional interactions between biomolecules, cells, and organs. Cytokines are one of the key biomolecules acting as intercellular mediators and modulators to regulate the diverse functions in the immune response [1]–[3]. Rapid and accurate quantification of cytokine-based immune fingerprints provides critical information for fundamental and clinical-pathological studies in infectious diseases, cancer, autoimmune diseases, and allergy transplantation [4], [5]. Enzyme-linked immunosorbent assay (ELISA), the current gold standard for clinical practice, has been widely used as a whole blood stimulation assay for immune functional analysis [6], [7]. However, this method


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requires time-consuming sample labeling and washing processes, resulting in long assay time, and can only provide snapshot signal readouts at the end of the measurement [8]. Furthermore, such a bulk assay approach measures the overall response from the whole blood, making it difficult to pinpoint the contribution of functional responses from immune cell subpopulations.

Recent advances in nanoplasmonic-material-based biosensors have drawn great attention in the field of biological and biomedical sciences [9], [10]. By exploiting the unique local environment-dependent optical response of the nanomaterials, that is, localized surface plasmon resonance (LSPR), these biosensors allow highly sensitive, label-free, real-time detection of target biomolecules for accurate and timely clinical diagnosis [8], [9], [11]–[16]. LSPR-based detection measures the scattering/absorbance spectrum shift of a nanostructured metal surface upon analyte binding. The sensing characteristics are highly dependent on the size, structure, composition, and morphology of the plasmonic nanoparticles (NPs).

Tremendous efforts have been spent on novel nanoplasmonic structures, inclusive of nanostars (NSRs) [17]– [19], nanorods (NRs) [8], [13], [14], [20], [21], nanobipyramids (NBPs) [12], [22], nanotriangles (NTs) [23], [24], nanohelices [25], nanoshuttles [26], core-shell structures, and bimetallic NPs, showing significantly improved refractive index (RI) sensitivities and sensing figures of merit (FOMs). Owing to the simple optical configuration and extraordinary sensing tunability, these nanostructure-based LSPR biosensors can be readily integrated with microfluidics in a lab-on-a-chip setting (Figure 1).

The incorporation of specially designed microfluidic structures (e.g., micropillars, microwells, grooves, etc.) allows immobilization of target immune cells at designated locations with high stability, purity, and capture efficiency. By coupling the antibody-labeled plasmonic nanostructures in close proximity to the isolated cells of interest, the integrated devices can achieve on-chip isolation of immune cell subsets and simultaneously in situ, label-free, high-throughput detection of cell-secreted cytokines, unlocking the potential in comprehensive immune function analysis.

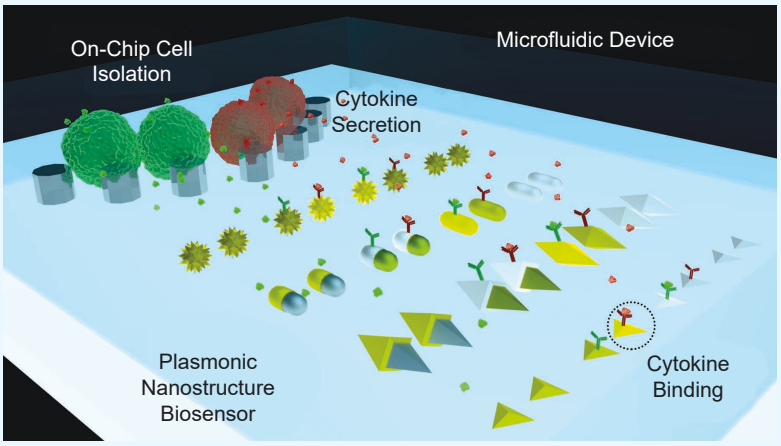


**FIGURE 1** A conceptual illustration of a microfluidic biosensor in a lab-on-a-chip device with different plasmonic nanostructures (AuNSRs, AuNRs, AuNBPs, Ag-Au/Au-Ag core-shell NRs, and NBPs) for in situ detection of cytokines secreted by isolated immune cells.

In this article, we review the state-of-the-art plasmonic NP-based sensing platforms and evaluate the governing factors for plasmonic nanostructures that could potentially improve the sensing performance for cytokine detection. We further discuss the integration of LSPR biosensors with microfluidic systems that can significantly facilitate label-free, high-throughput on-chip cellular functional analysis and project the challenges and future directions in this field toward a new era of immune monitoring and diagnosis.

## NOVEL PLASMONIC NANOMATERIALS FOR LSPR BIOSENSORS

Among all of the noble metal nanomaterials, gold (Au) NPs are the most chemically stable [27] and biocompatible materials [28], with high susceptibilities to RI changes [29], rendering them the most commonly used NPs for plasmonic biosensors. Silver (Ag) NPs exhibit higher susceptibilities in RI changes and thus larger resonance wavelength shifts than AuNPs due to low electromagnetic damping losses. AgNPs are vulnerable in a biological medium and can elicit noticeable cytotoxicity. As a result, surface coating or modification is often required, which limits their direct usage in biomedical applications [30].

Palladium (Pd) as an alternative plasmonic material possesses even higher RI sensitivity than both AuNPs and AgNPs [31]. However, the broad extinction spectra and low scattering efficiency of PdNPs are not ideal in plasmonic sensing. Thus, they have been mostly investigated in combination with other noble metals to optimize the sensing performance [21], [31]. Copper (Cu) is an inexpensive and earth-abundant plasmonic material that has been used in widespread applications. But its plasmonic sensing capability is mainly hindered by the weak and broad LSPR peak and surface chemical instability [32], [33].

Despite the fact that these noble metal nanomaterials exhibit attractive plasmonic properties, their pristine forms (both structure and composition) display relatively low FOMs that still fall short in meeting the surging requirements for rapid, sensitive, label-free biomolecule detection. Because of the aforementioned unique characteristics of Au nanomaterials in biomedical applications, we mostly focused on novel AuNPs and their derivatives in the LSPR sensing scheme.

### *MORPHOLOGICAL MODIFICATION FOR ENHANCED NANOPLASMONIC BIOSENSING*

Au nanospheres (NSs) are the most commonly available Au nanostructures. They

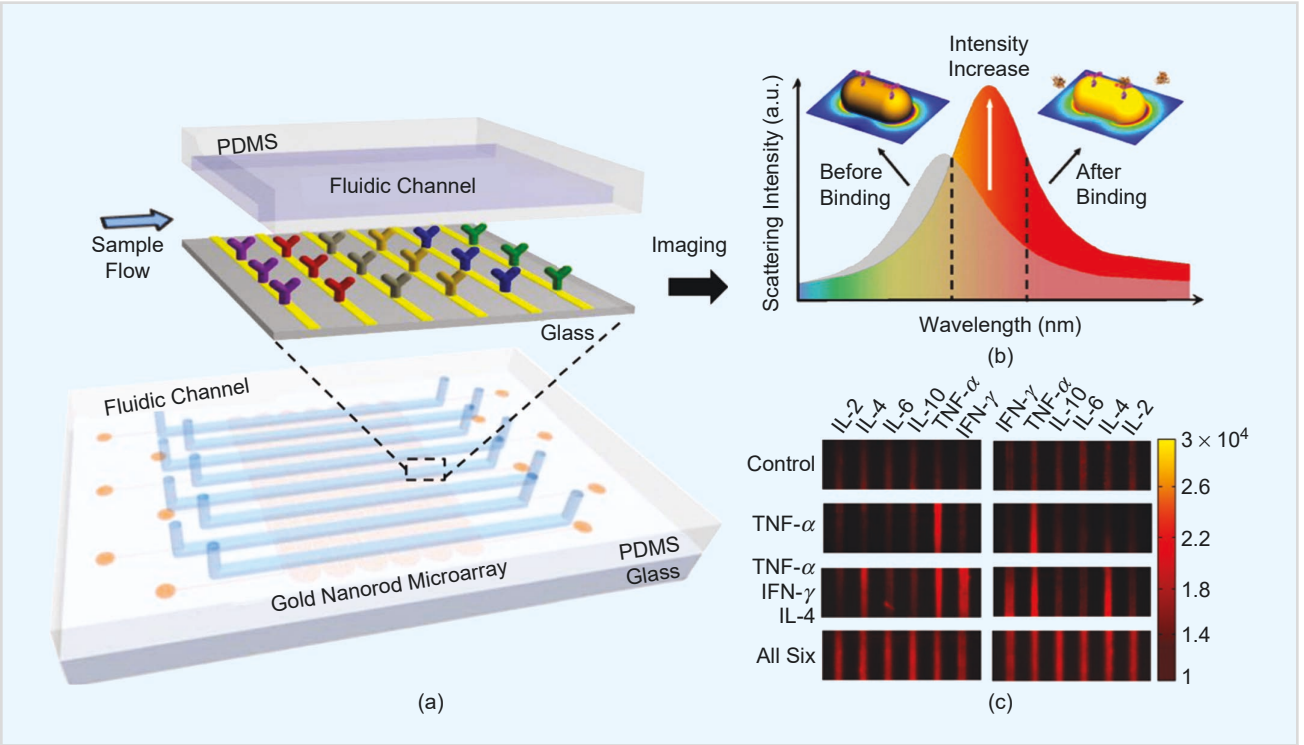


**FIGURE 2** (a) A schematic of an LSPR barcode microfluidic chip with patterned antibody-linked AuNR barcodes on a glass substrate. (b) Scattering spectrum red shifts and intensity increases after AuNRs binding to cytokines. (c) The dark-filed images of an LSPR barcode chip after measuring different cytokine mixture solutions. IFN-$\gamma$: interferon-gamma. (Source: © 2015 American Chemical Society [8]; used with permission.)

can be easily synthesized at a low cost but normally show a low RI sensitivity [44 nm/ RI unit (RIU)] and FOM (0.6) [29]. Morphological modifications have been extensively explored during the past decade to enhance plasmonic characteristics of Au nanostructures for better sensing performance. The modification methods primarily focused on elongation of shapes (i.e., NRs [8], [13], [14] and nanoshuttles [26]) and forming sharp apexes [29] (i.e., NSRs [17]–[19], and NBPs [12], [22]). It has been reported that the modified Au nanostructures possess significantly improved plasmonic RI sensitivities and higher FOMs of 195–288 nm/RIU and 1.7–2.6 (AuNRs), 150–540 nm/RIU and 1.7–4.5 (AuNBPs), and 703 nm/RIU and 0.8 (AuNSRs), respectively [29].

Leveraging on these novel plasmonic nanostructures, a high-throughput, multiplex, label-free cytokine biosensor using AuNRs was demonstrated by Chen et al. [8]. An LSPR microarray device was fabricated by forming self-assembled AuNR stripes on a glass substrate assisted by microfluidic patterning [Figure 2(a)]. The detection of multiple cytokines in a mixed-cytokine solution was performed using dark-field microscopy, and the scattering intensity changes of the corresponding AuNR barcodes were observed upon target cytokine binding [Figure 2(b) and (c)]. As a result, a massive parallel, label-free detection of six different cytokines was achieved with a limit of detection (LOD) down to 5–20 pg/ml. Here, the elongated Au nanostructures generate the longitudinal plasmonic mode on AuNRs, which are highly sensitive to the local RI change. The sharp areas at apexes of AuNRs further enhance the near-field intensity of the electromagnetic field, which collectively contributes to the prominent plasmonic properties of AuNRs for highly sensitive cytokine detection.

Based on this AuNR barcode biosensor, Oh et al. quantitatively determined the dynamic functional response of a human leukemic T-cell line upon exposure to an immunosuppressive agent tacrolimus [13]. More recently, Javed et al. used the same barcode sensing scheme with AuNBPs to simultaneously detect multiple secreted cytokines from T cells in response to pure human islet amyloid polypeptides or human islet amyloid polypeptides with ligand-stabilized AuNP coronas, showing LODs of 34.1 pg/ml for interleukin-6 (IL-6) and 45.6 pg/ml for tumor necrosis factor-alpha (TNF-$\alpha$) [22].

AuNSRs are composed of a number of branches surrounding the core with many plasmonic hot spots at the tip regions, displaying an ultrahigh RI sensitivity [17]–[19]. Park et al. reported an NSR clustering showing an RI of 500 nm/RIU and an FOM of 1.8 for probing streptavidin-avidin binding. Evident plasmonic shifts and strong signal amplification were detected owing to the plasmon coupling of the clustering, indicating potential applications of this material for low-abundance cytokine detection [17].

Moreover, a study on the control of anisotropic structures and optical properties of spiky AuNPs (AuNSRs) was reported by Pallares et al. The spiky AuNPs were formed by core NSs with shell spikes. The dimensions of the shell spikes can be tailored using biocompatible Good's buffer, yielding an optimal RI sensitivity of 533 nm/RIU [18]. The exceptional plasmonic properties of AuNSRs have rendered them a promising

candidate for next-generation LSPR biosensors. However, difficulties in producing uniform spiky shapes, relatively small function/binding sites, and low sensing volume (FOM) remain the major challenges before they can be widely used in practical biosensing applications.

In addition to morphological modifications, the assembly of AuNSs into core-satellite structure is an alternative strategy to improve plasmonic sensitivity. Ode et al. studied core-satellite AuNPs immobilized on a glass substrate with an RI of 350 nm/RIU [34]. This nanostructure consisted of a 50-nm-diameter AuNP core and several 15-nm-diameter smaller AuNPs in the satellite-shell layer. The smaller the diameter of the satellite AuNPs, the higher RI sensitivity was achieved for the core-satellite AuNPs.

Nanoplasmon ruler is another unique structure design by tethering two NPs together with a biomolecule (normally DNA). The dipole–dipole interactions between the NPs elicit exponentially larger scattering intensity as compared to that of single NPs [35]. It was reported by Xie et al. that by using a nanoplasmon ruler with aptamer-linked AuNSs (39 and 13 nm), they obtained a five-order-of magnitude signal enhancement and could detect adenosine triphosphate biomarker with an LOD down to 0.01 μM [36].

Lee et al. demonstrated the detection of matrix metalloproteinase by aptameric Au plasmon rulers. When binding to cytokines, the two AuNSs were brought to close proximity via the aptamer conformation change, resulting in pronounced enhancement of scattering intensity [37]. The remarkable spatiotemporal resolution of this sensing scheme could potentially be used to measure cytokine secretion profiles at single-cell-length scale.

### *BIMETALLIC CORE-SHELL NANOPLASMONIC COMPOSITES*

Composite metal nanomaterials, such as bimetallic core-shell NPs, present unique advantages over monometallic NPs for plasmonic biosensing by using the collectively optical response from both noble metal elements [38]. The nanocore materials can serve as host substrates that facilitate the growth and formation of unconventional shell compositions (i.e., Ag-magnesium fluoride composited NTs [23] and Ag-Cu multilayering NT arrays [24]). The addition of shell materials affords the flexibility to modify the morphology, surface chemistry, and plasmonic properties of the core-shell nanocomplexes [20], [21], [24], [26], [31], [39], [40].

For instance, Dong et al. developed an innovative LSPR biosensor by sequential electrodeposition of Ag and Au onto a transparent indium-tin-oxide-coated glass substrate to form a bimetallic Ag-core and Au-shell NP sensing surface [39]. They reported Ag-core NPs (~67 nm) with an ultrathin (~1.3 nm) Au shell that possess an RI sensitivity of 220 nm/RIU. The high RI change can mainly be attributed to the higher extinction coefficient of LSPR on AgNPs than that for AuNPs of the same size. The coating of AgNPs with Au nanoshells can minimize the cytotoxicity of the AgNPs and allow function of biorecognition molecules on Au nanoshell by simple surface chemistry.

Bai et al. synthesized bimetallic Au–Ag core-shell nanoshuttles and demonstrated excellent RI sensitivity up to 484 nm/RIU and FOM of 3.4 [26]. The sharp tips of the Ag-coated AuNRs provided larger extinction cross-section areas and resulted in an enhanced local electromagnetic field over that on the AuNRs. Further surface modifications would be essential to protect the vulnerable exposed Ag surface to be applicable for biological sample measurements [24]. As an alternative, Pd can be a well-suited shell material because of its tunable extinction resonance wavelengths from ultraviolet to infrared [21] and the readily available surface chemistry [31]. Au–Pd core-shell nanocrystals and NRs were reported to be highly sensitive biosensing materials, where the RI sensitivity of Au–Pd core-shell NRs was measured to be 1,067 nm/RIU, approaching the theoretical limit [21], [40].

### *MULTIFUNCTIONAL MAGNETIC-PLASMONIC CORE-SHELL NPs*

Magnetic NPs have attracted increasing attention in the past few decades because of their distinct magnetic properties, such as superparamagnetism at sub-100-nm scale, showing great potential in bioanalytical applications. With recent developments in the synthesis of noble-metal-decorated/-coated magnetic NPs, multifunctional magnetic-plasmonic core-shell NPs have become a new potential sensing element for LSPR biosensors. The magnetic NP core can allow controllable manipulation of the core-shell NPs with external magnetic fields, which is essential in many bioanalytical processes, such as NP purification, immobilization, and local analyte detection. Because the magnetic NPs do not usually exhibit plasmonic characteristics, the plasmonic NP shells function as optical antennas in response to light illumination [41].

Based on this concept, Cai et al. developed a high-throughput, label-free, multiplex LSPR immunoassay using iron oxide ($Fe_3O_4$)/Au core-shell NPs [Figure 3(a)–(f)]. Assisted by an external magnetic field, the core-shell NPs can be easily assembled onto a glass substrate to form regular and uniform sensing spots over a large area [15]. By exploiting the superparamagnetic property of the $Fe_3O_4$ NPs, the patterned NPs by the external magnetic field remained well dispersed to avoid the unintended plasmonic coupling for optimal sensing performance. The shape, size, and distance between the sensing spots can be controlled by tailoring the design of the polydimethylsiloxane (PDMS) microwell mask. This facile patterning method overcomes the major obstacles of conventional nanofabrication technologies [42]–[45], which do not require dedicated instrumentation and complicated procedures, and thus holds great promise for scalable manufacturing [46]. Combining the LSPR dark-field imaging technique [8], this microarray biosensor can perform 384 tests in parallel to measure four different cytokines in 16 replicates.

## INTEGRATED MICROFLUIDIC LSPR BIOSENSORS FOR ADVANCED IMMUNE ANALYSIS

Microfluidic devices present great advantages in fluid handling, upstream sample preparation, on-chip cell manipulation,

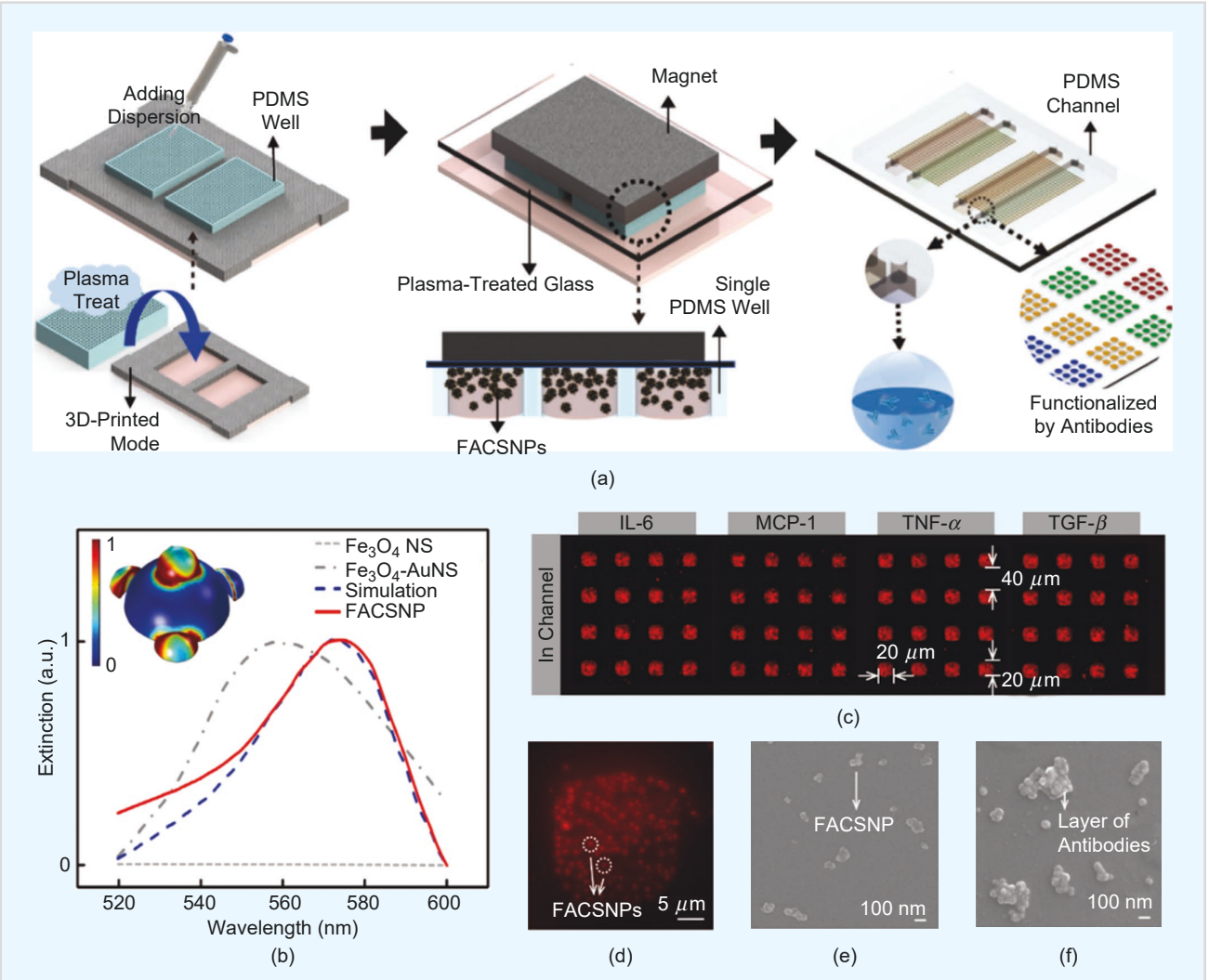


**FIGURE 3** (a) Illustrations of the magnet-assisted patterning process of the $Fe_3O_4$/Au core-shell (FACS)NP microarray. (b) The ultraviolet-visible spectra and optical simulation results of $Fe_3O_4$ NSs, $Fe_3O_4$-AuNSs, and $Fe_3O_4$-Au core-shell NSs. (c) $Fe_3O_4$-Au core-shell NS microarrays functioned with four different antibodies in defined sensing areas for multiplex detection of four cytokines. (d) A dark-field microscopy image of an individual biosensing spot. (e) A scanning electron microscope image of the NPs before antibody function and (f) after successful antibody attachment. MCP-1: monocyte chemoattractant protein-1; TGF-$\beta$: transfroming growth factor $\beta$. (Source: © 2019 John Wiley and Sons [15]; used with permission.)

and sample sparing [47]. Microfluidics overcome the drawbacks of time-consuming and high-requirement fabrication of conventional biosensing platforms in microsystems, mostly involving complex mechanical structures. The flexible design, ease of fabrication, and miniaturization allow the integration of microfluidics with various downstream label-free biosensors for in situ, quantitative, on-chip measurements, which speeds up the transfer process of biosensing platforms from experimental devices to real-life applications [48]. Such integrated sensing platforms offer unique opportunities in 1) sample-sparing, high-throughput, multiplex immunoassay and 2) on-chip cellular functional analysis for rapid, in situ immune monitoring.

## MICROFLUIDIC IMMUNOASSAYS FOR HIGH-THROUGHPUT MULTIPLEX DETECTION

The integration of LSPR biosensors with a sample-sparing microfluidic device can provide rapid, massive parallel analysis of multiple cytokines with high sensitivity and rich statistic information, using only minimal sample volume [4], [49]. The aforementioned AuNR LSPR barcode biosensor reported by Chen et al. [8] and the $Fe_3O_4$/Au core-shell microarray by Cai et al. can perform multiplex cytokine measurements on hundreds of sensing spots simultaneously in real time [15]. The whole assay process, including the loading, incubation, washing of samples and reagents, and multianalyte detection, can be completed within 40 min, using a sample volume down to 1 $\mu$L. Given the shortened assay time, enhanced sample efficiency, and low sample volume requirement as compared to conventional immunoassay (such as ELISA, which would require up to 8 h and a sample volume of 0.5–2 mL per test), these label-free, high-through-

put microfluidic immunoassays can provide an unprecedented discriminating power in immune diagnostic and clinical monitoring.

### *INTEGRATED LSPR MICROFLUIDIC DEVICE FOR CELLULAR FUNCTIONAL ANALYSIS*

A synergistic combination of a cell manipulation microfluidic system with adequate LSPR biosensors into an integrated lab-on-chip device provides a new dimension in immune monitoring by measuring the in situ functional response of immune cells. This is first demonstrated by Oh et al. using a label-free LSPR cytokine secretion assay to detect TNF-$\alpha$ secreted by on-chip isolated immune cells from clinical blood samples [50]. The integrated microfluidic device was composed of a supporting layer, a cell manipulation layer, and a sensing layer. A circular detection pattern of AuNPs was deposited at the center of the LSPR sensing layer, shown as the yellow spot on the black bottom layer in Figure 4(a).

A unique circular structure composed of three arrays of micropillars in the cell manipulation layer was designed to isolate and enrich the target immune cells before the cells were stimulated and incubated for cytokine secretion and detection [Figure 4(a)]. As a result, the microfluidic LSPR immunoassay platform can achieve in situ, quantitative detection of cytokine secretion from a desired subset of immune cells down to a cell population of as few as 1,000 cells.

More recently, a lab-on-chip platform was designed by Zhu et al. that permits a time-course immune cellular functional analysis in a biomimetic microenvironment under inflammatory stimulation [51]. The device was fabricated by integrating LSPR barcode sensor arrays with a microchamber in the center for on-chip culturing of adipocytes and macrophages and subsequent detection [Figure 4(b)]. Such a novel biomimetic microfluidic tissue-on-chip platform could ultimately gear clinicians with the capability to identify stage-specific cytokine secretion profiles for immune diagnostics and prognostics and personalized immunotherapy.

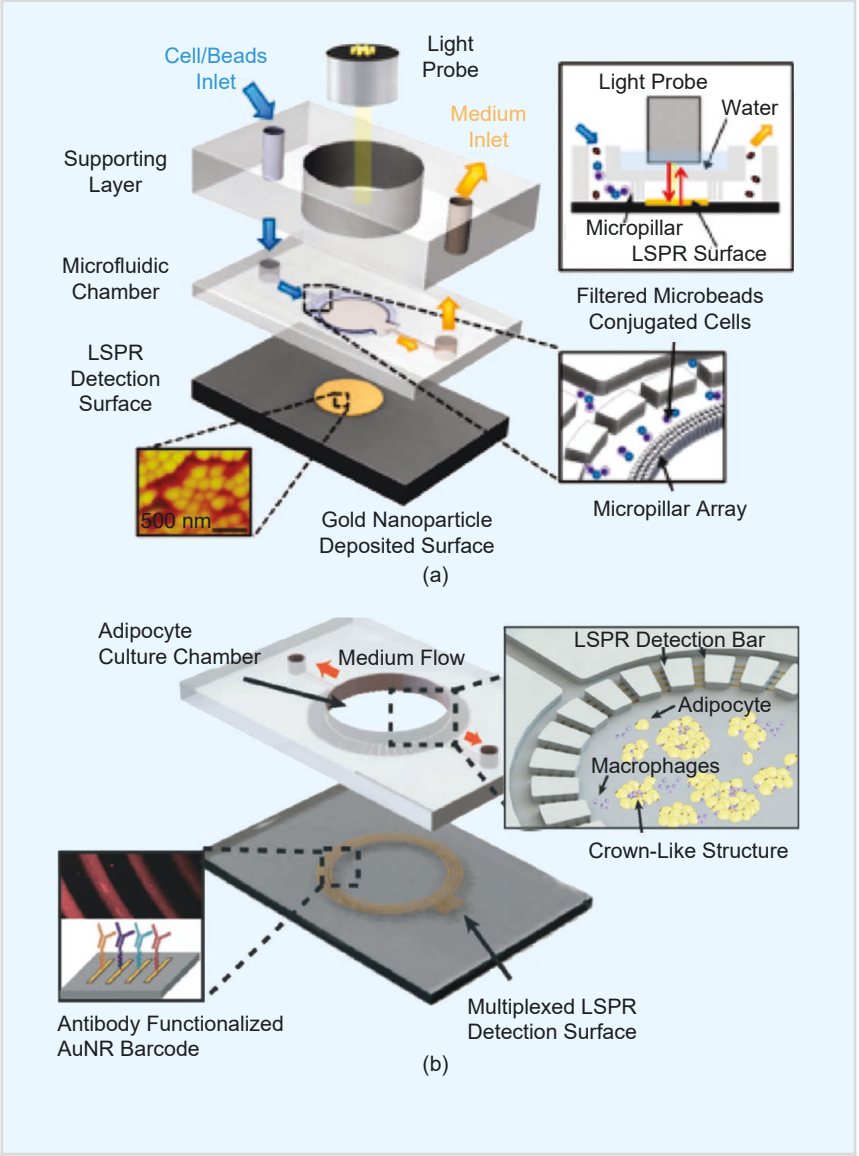


**FIGURE 4** (a) A schematic of the integrated LSPR optofluidic platform device for on-chip cytokine secretion assay. (Source: © 2014 American Chemical Society [50]; used with permission.) (b) A schematic of the lab-on-chip LSPR platform for time-course immune cellular functional analysis. (Source: © 2018 The Royal Society of Chemistry; used with permission.)

## CONCLUSION

We briefly summarized the latest advances in plasmonic sensor platforms for label-free biomolecule detection and highlighted some of the most relevant diagnostic applications targeting immune cytokine biomarkers. We focused on novel plasmonic nanostructures created by morphological and compositional modifications and their applications in LSPR biosensors for real-time, highly sensitive, label-free cytokine detection. Despite the attractive features of nanoplasmonic cytokine biosensors, the route toward effective immune functional analysis involves the development of high-throughput, multiplexed immunoassay and the integration of an advanced microfluidic system for on-chip cell isolation and in situ analysis.

Significant challenges still lie ahead for such LSPR biosensing microfluidic chips, mainly in simultaneously achieving high sensitivity, selectivity, multiplexity, and spatial-temporal resolution. These features are consequential to the accuracy, rapidness, and reproducibility of on-chip cellular secretory analysis, especially at the single-cell level.

In addition, the design, selection, and optimization of cell isolation mechanisms

in microfluidic systems that are optical compatible with label-free nanoplasmonic sensing remain critical issues. We believe, once achieved, these integrated nanoplasmonic microfluidic biosensors could overcome some of the major challenges faced in conventional diagnosis techniques and have immense potential as transformative analytical platforms for accurate and comprehensive analysis of the immune system.

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## REFERENCES

[1] U. Dendorfer, "Molecular biology of cytokines," *Artif. Organs*, vol. 20, no. 6, pp. 437–444, 1996. doi: 10.1111/j.1525-1594.1996.tb04529.x.

[2] C. A. Dinarello, "Historical insights into cytokines," *Eur. J. Immunol.*, vol. 37, no. S1, pp. S34–S45, 2007. doi: 10.1002/eji.200737772.

[3] S. M. Opal and V. A. DePalo, "Anti-inflammatory cytokines," *Chest*, vol. 117, no. 4, pp. 1162–1172, 2000. doi: 10.1378/chest.117.4.1162.

[4] P. Chen, N. T. Huang, M. T. Chung, T. T. Cornell, and K. Kurabayashi, "Label-free cytokine micro- and nano-biosensing towards personalized medicine of systemic inflammatory disorders," *Adv. Drug Deliv. Rev*, vol. 95, pp. 90–103, Dec. 2015. doi: 10.1016/j.addr.2015.09.005.

[5] F. Tian, C. Liu, L. Lin, Q. Chen, and J. Sun, "Microfluidic analysis of circulating tumor cells and tumor-derived extracellular vesicles," *Trends Anal. Chem.*, vol. 117, pp. 128–145, Aug. 2019. doi: 10.1016/j.trac.2019.05.013.

[6] N. Favre, G. Bordmann, and W. Rudin, "Comparison of cytokine measurements using ELISA, ELISPOT and semi-quantitative RT-PCR," *J. Immunol. Methods*, vol. 204, no. 1, pp. 57–66, 1997. doi: 10.1016/S0022-1759(97)00033-1.

[7] F. Scuderi et al., "Effect of pro-inflammatory/anti-inflammatory agents on cytokine secretion by peripheral blood mononuclear cells in rheumatoid arthritis and systemic lupus erythematosus," *Autoimmunity*, vol. 36, no. 2, pp. 71–77, 2003. doi: 10.1080/0891693031000079275.

[8] P. Chen et al., "Multiplex serum cytokine immunoassay using nanoplasmonic biosensor microarrays," *ACS Nano*, vol. 9, no. 4, pp. 4173–4181, 2015. doi: 10.1021/acsnano.5b00396.

[9] J. A. Jackman, A. R. Ferhan, and N. J. Cho, "Nanoplasmonic sensors for biointerfacial science," *Chem. Soc. Rev.*, vol. 46, no. 12, pp. 3615–3660, 2017. doi: 10.1039/C6CS00494F.

[10] G. A. Lopez, M. C. Estevez, M. Soler, and L. M. Lechuga, "Recent advances in nanoplasmonic biosensors: Applications and lab-on-a-chip integration," *Nanophotonics*, vol. 6, no. 1, pp. 123–136, 2017. doi: 10.1515/nanoph-2016-0101.

[11] M. Li, S. K. Cushing, and N. Wu, "Plasmon-enhanced optical sensors: A review," *Analyst*, vol. 140, no. 2, pp. 386–406, 2015. doi: 10.1039/C4AN01079E.

[12] J. M. Rye et al., "Single gold bipyramids on a silanized substrate as robust plasmonic sensors for liquid environments," *Nanoscale*, vol. 10, no. 34, pp. 16,094–16,101, 2018. doi: 10.1039/C8NR03400A.

[13] B. R. Oh et al., "Multiplexed nanoplasmonic temporal profiling of t-cell response under immunomodulatory agent exposure," *ACS Sens.*, vol. 1, no. 7, pp. 941–948, 2016. doi: 10.1021/acssensors.6b00240.

[14] Y. Song et al., "AC electroosmosis-enhanced nanoplasmofluidic detection of ultralow-concentration cytokine," *Nano Lett.*, vol. 17, no. 4, pp. 2374–2380, 2017. doi: 10.1021/acs.nanolett.6b05313.

[15] Y. Cai et al., "Magnet patterned superparamagnetic $Fe_3O_4$/Au core–shell nanoplasmonic sensing array for label-free high throughput cytokine immunoassay," *Adv. Healthc. Mater.*, vol. 8, no. 4, pp. 1–9, 2019. doi: 10.1002/adhm.201801478.

[16] W. Yang, J. He, and P. Chen, "Nanoplasmonic cytokine biosensor towards precision medicine," in Proc. Int. Conf. Sensing Technology (ICST), 2016, pp. 1–4. doi: 10.1109/ICSensT.2016.7796239.

[17] Y. Il Park, H. Im, R. Weissleder, and H. Lee, "Nanostar clustering improves the sensitivity of plasmonic assays," *Bioconjug. Chem.*, vol. 26, no. 8, pp. 1470–1474, 2015. doi: 10.1021/acs.bioconjchem.5b00343.

[18] R. M. Pallares, T. Stilson, P. Choo, J. Hu, and T. W. Odom, "Using Good's buffers to control the anisotropic structure and optical properties of spiky gold nanoparticles for refractive index sensing," *ACS Appl. Nano Mater.*, vol. 2, no. 8, pp. 5266–5271, 2019. doi: 10.1021/acsanm.9b01117.

[19] S. K. Dondapati, T. K. Sau, C. Hrelescu, T. A. Klar, F. D. Stefani, and J. Feldmann, "Label-free biosensing based on single gold nanostars as plasmonic transducers," *ACS Nano*, vol. 4, no. 11, pp. 6318–6322, 2010. doi: 10.1021/nn100760f.

[20] L. Tang et al., "Chirality-based Au@Ag nanorod dimers sensor for ultrasensitive PSA detection," *ACS Appl. Mater. Interfaces*, vol. 7, no. 23, pp. 12,708–12,712, 2015. doi: 10.1021/acsami.5b01259.

[21] S. Rodal-Cedeira et al., "Plasmonic Au@Pd nanorods with boosted refractive index susceptibility and SERS efficiency: A multifunctional platform for hydrogen sensing and monitoring of catalytic reactions," *Chem. Mater.*, vol. 28, no. 24, pp. 9169–9180, 2016. doi: 10.1021/acs.chemmater.6b04941.

[22] I. Javed et al., "Probing the aggregation and immune response of human islet amyloid polypeptides with ligand-stabilized gold nanoparticles," *ACS Appl. Mater. Interfaces*, vol. 11, no. 11, pp. 10,462–10,471, 2019. doi: 10.1021/acsami.8b19506.

[23] S. Larson and Y. Zhao, "Localized surface plasmonic resonance and sensing properties of Ag–$MgF_2$ composite nanotriangles," *J. Phys. Chem. C*, vol. 122, no. 13, pp. 7374–7381, 2018. doi: 10.1021/acs.jpcc.8b00122.

[24] S. Larson, Z. Yang, and Y. Zhao, "Improving LSPR sensing performance using multilayered composition graded Ag–Cu nanotriangle arrays," *Chem. Commun.*, vol. 55, no. 9, pp. 1342–1344, 2019. doi: 10.1039/C8CC08813F.

[25] H. H. Jeong et al., "Dispersion and shape engineered plasmonic nanosensors," *Nature Commun.*, vol. 7, p. 11,331, Apr. 2016. doi: 10.1038/ncomms11331.

[26] T. Bai et al., "Controllable preparation of core-shell Au–Ag nanoshuttles with improved refractive index sensitivity and SERS activity," *ACS Appl. Mater. Interfaces*, vol. 6, no. 5, pp. 3331–3340, 2014. doi: 10.1021/am405357v.

[27] M. Brust, J. Fink, D. Bethell, D. J. Schiffrin, and C. Kiely, "Synthesis and reactions of functionalised gold nanoparticles," *J. Chem. Soc. Chem. Commun.*, vol. 1995, no. 16, pp. 1655–1656, 1995. doi: 10.1039/c39950001655.

[28] T. R. Tshikhudo, Z. Wang, and M. Brust, "Biocompatible gold nanoparticles," *Mater. Sci. Technol.*, vol. 20, no. 8, pp. 980–984, 2004. doi: 10.1179/026708304225019849.

[29] H. Chen, X. Kou, Z. Yang, W. Ni, and J. Wang, "Shape- and size-dependent refractive index sensitivity of gold nanoparticles," *Langmuir*, vol. 24, no. 10, pp. 5233–5237, 2008. doi: 10.1021/la800305j.

[30] P. P. Suthanthiraraj and A. K. Sen, "Localized surface plasmon resonance (LSPR) biosensor based on thermally annealed silver nanostructures with on-chip blood-plasma separation for the detection of dengue non-structural protein NS1 antigen," *Biosens. Bioelectron.*, vol. 132, pp. 38–46, Feb. 2019. doi: 10.1016/j.bios.2019.02.036.

[31] K. Sugawa et al., "Refractive index susceptibility of the plasmonic palladium nanoparticle: Potential as the third plasmonic sensing material," *ACS Nano*, vol. 9, no. 2, pp. 1895–1904, 2015. doi: 10.1021/nn506800a.

[32] G. H. Chan, J. Zhao, E. M. Hicks, G. C. Schatz, and R. P. Van Duyne, "Plasmonic properties of copper nanoparticles fabricated by nanosphere lithography," *Nano Lett.*, vol. 7, no. 7, pp. 1947–1952, 2007. doi: 10.1021/nl070648a.

[33] P. Zheng, H. Tang, B. Liu, S. Kasani, L. Huang, and N. Wu, "Origin of strong and narrow localized surface plasmon resonance of copper nanocubes," *Nano Res.*, vol. 12, no. 1, pp. 63–68, 2019. doi: 10.1007/s12274-018-2178-6.

[34] K. Ode, M. Honjo, Y. Takashima, T. Tsuruoka, and K. Akamatsu, "Highly sensitive plasmonic optical sensors based on gold core-satellite nanostructures immobilized on glass substrates," *ACS Appl. Mater. Interfaces*, vol. 8, no. 32, pp. 20,522–20,526, 2016. doi: 10.1021/acsami.6b06313.

[35] C. A. Tajon, D. Seo, J. Asmussen, N. Shah, Y. W. Jun, and C. S. Craik, "Sensitive and selective plasmon ruler nanosensors for monitoring the apoptotic drug response in Leukemia," *ACS Nano*, vol. 8, no. 9, pp. 9199–9208, 2014. doi: 10.1021/nn502959q.

[36] L. Xie, X. Yan, and Y. Du, "An aptamer based wall-less LSPR array chip for label-free and high throughput detection of biomolecules," *Biosens. Bioelectron.*, vol. 53, pp. 58–64, Sept. 2013. doi: 10.1016/j.bios.2013.09.031.

[37] S. E. Lee et al., "Reversible aptamer-Au plasmon rulers for secreted single molecules," *Nano*

*Lett.*, vol. 15, no. 7, pp. 4564–4570, 2015. doi: 10.1021/acs.nanolett.5b01161.

[38] G. Sharma et al., "Novel development of nanoparticles to bimetallic nanoparticles and their composites: A review," *J. King Saud Univ., Sci.*, vol. 31, no. 2, pp. 257–269, 2019. doi: 10.1016/j.jksus.2017.06.012.

[39] P. Dong, Y. Lin, J. Deng, and J. Di, "Ultrathin gold-shell coated silver nanoparticles onto a glass platform for improvement of plasmonic sensors," *ACS Appl. Mater. Interfaces*, vol. 5, no. 7, pp. 2392–2399, 2013. doi: 10.1021/am4004254.

[40] A. F. Smith, S. M. Harvey, S. E. Skrabalak, and R. G. Weiner, "Engineering high refractive index sensitivity through the internal and external composition of bimetallic nanocrystals," *Nanoscale*, vol. 8, no. 38, pp. 16,841–16,845, 2016. doi: 10.1039/C6NR04085C.

[41] G. Armelles, A. Cebollada, A. García-Martín, and M. U. González, "Magnetoplasmonics: Combining magnetic and plasmonic functionalities," *Adv. Opt. Mater.*, vol. 1, no. 1, pp. 10–35, 2013. doi: 10.1002/adom.201200011.

[42] V. R. Manfrinato et al., "Resolution limits of electron-beam lithography toward the atomic scale," *Nano Lett.*, vol. 13, no. 4, pp. 1555–1558, 2013. doi: 10.1021/nl304715p.

[43] T. Bückmann et al., "Tailored 3D mechanical metamaterials made by dip-in direct-laser-writing optical lithography," *Adv. Mater.*, vol. 24, no. 20, pp. 2710–2714, 2012. doi: 10.1002/adma.201200584.

[44] J. C. Claussen, A. D. Franklin, A. U. Haque, D. Marshall Porterfield, and T. S. Fisher, "Electrochemical biosensor of nanocube-augmented carbon nanotube networks," *ACS Nano*, vol. 3, no. 1, pp. 37–44, 2009. doi: 10.1021/nn800682m.

[45] K. Salaita, Y. Wang, and C. A. Mirkin, "Applications of dip-pen nanolithography," *Nature Nanotechnol.*, vol. 2, no. 3, pp. 145–155, 2007. doi: 10.1038/nnano.2007.39

[46] V. Romanov, S. N. Davidoff, A. R. Miles, D. W. Grainger, B. K. Gale, and B. D. Brooks, "A critical comparison of protein microarray fabrication technologies," *Analyst*, vol. 139, no. 6, pp. 1303–1326, 2014. doi: 10.1039/C3AN01577G.

[47] L. Mazutis, J. Gilbert, W. L. Ung, D. A. Weitz, A. D. Griffiths, and J. A. Heyman, "Single-cell analysis and sorting using droplet-based microfluidics," *Nature Protoc.*, vol. 8, no. 5, pp. 870–891, 2013. doi: 10.1038/nprot.2013.046.

[48] J. F. C. Loo, A. H. P. Ho, A. P. F. Turner, and W. C. Mak, "Integrated printed microfluidic biosensors," *Trends Biotechnol.*, vol. 37, no. 10, pp. 1104–1120, 2019. doi: 10.1016/j.tibtech.2019.03.009.

[49] J. He, A. T. Brimmo, M. A. Qasaimeh, P. Chen, and W. Chen, "Recent advances and perspectives in microfluidics-based single-cell biosensing techniques," *Small Methods*, vol. 1, no. 10, p. 1,700,192, 2017. doi: 10.1002/smtd.201700192.

[50] B.-R. Oh et al., "Integrated nanoplasmonic sensing for cellular functional immunoanalysis using human blood," *ACS Nano*, vol. 8, no. 3, pp. 2667–2676, 2014. doi: 10.1021/nn406370u.

[51] J. Zhu et al., "An integrated adipose-tissue-on-chip nanoplasmonic biosensing platform for investigating obesity-associated inflammation," *Lab Chip*, vol. 18, no. 23, pp. 3550–3560, 2018. doi: 10.1039/C8LC00605A.

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