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Microfluidics for biochemical analysis: advances and perspectives

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Highlights:

- Overview of microfluidic methods for biochemical analysis with different readout modalities.
- Microfluidic biochemical applications for molecules, extracellular vesicles, and cells.
- Perspectives on AI-enhanced and wearable microfluidic biochemical devices.

Abstract: Biochemical analysis by converting biological information into measurable signals is fundamental to life science research, clinical diagnosis, and environmental monitoring. Yet conventional techniques suffer from bulky instrumentation, labor-intensive procedures, and suboptimal sensitivity for low-abundance targets. Microfluidic techniques that precisely manipulate nanoliter-to-picoliter fluids within microscale channels offer a powerful tool for biochemical analysis. By exploiting high surface-to-volume ratio, integration of multiple steps, and enhanced mass and heat transfer, microfluidic devices enable sample-to-answer biochemical assays with improved sensitivity, reduced sample and reagent consumption, and shortened reaction time. This review systematically summarizes recent advances in microfluidic methods for biochemical analysis, with a focus on five major readout modalities including colorimetry, fluorescence, surface-enhanced Raman scattering (SERS), electrochemistry, and mass spectrometry (MS). For each microfluidic method, we overview the underlying working principle and highlight representative applications in biochemical analysis, covering diverse biological targets such as small molecules, proteins, nucleic acids, extracellular vesicles, and cells. The challenges and opportunities in the field of microfluidics for biochemical analysis are also discussed.

Keywords: biochemical analysis; microfluidics; biomarkers; detection modalities; integration

1. Introduction

Biochemical analysis refers to the qualitative and quantitative detection of specific targets (e.g., small molecules [1], proteins [2], nucleic acids [3], extracellular vesicles (EVs) [4], and cells [5]) in biological samples, as well as their structures, functions, interactions, and dynamic behaviors [6,7]. By converting biological information into measurable optical, electrochemical, or mass spectrometric signals, biochemical analysis serves as an important tool in many fields, including life science research, food safety, environmental monitoring, and clinical disease diagnosis [8,9]. Conventional techniques such as enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), and flow cytometry have been widely used for biochemical analysis [10]. Despite being reliable, these approaches suffer from complicated and time-consuming procedures, hindering their application in resource-limited settings where simple, affordable, and portable diagnostic tools are urgently needed [11–14]. Moreover, some traditional methods with suboptimal sensitivity are unsuitable for the clinical detection of low-abundance targets.

Microfluidics with its inherent advantages in integration and miniaturization offers a versatile tool for biochemical analysis [15–17]. The high surface-to-volume ratio in microchannels enhances mass and heat transfer, which accelerates reaction kinetics and increases the binding efficiency between targets and capture probes. This feature improves the detection of low-abundance biomarkers (e.g., circulating tumor cells or tumor-derived EVs), whereas conventional assays often lack sufficient sensitivity [18–20]. In addition, microfluidic platforms facilitate seamless integration of multiple functional units, including sample purification, preconcentration, target amplification, and signal readout, onto a single microchip, enabling "sample-in-answer-out" assays with minimal reagent consumption and low cost [21,22]. These microfluidic devices have proven effective for high-resolution single-molecule or single-cell analysis to advance precise biochemical profiling and clinical precision medicine [23]. By addressing the limitations of bulk methods, microfluidics paves the way for next-generation biochemical analysis [24–26].

Given the considerable progress of microfluidic biochemical analysis, this review systematically summarizes key advances in the field. Unlike previous reviews that focus on specific microfluidic platforms [27,28] or applications [29,30], this review provides a comparative overview of microfluidic biochemical analysis categorized by five major readout modalities, including colorimetry, fluorescence, surface-enhanced Raman scattering (SERS), electrochemistry, and mass spectrometry (Figure 1). For each modality, we introduce the underlying working principle and highlight representative applications for detecting diverse biological targets, ranging from small molecules and proteins to nucleic acids, EVs, and cells. This readout-based framework facilitates side-by-side comparison of the strengths and limitations of different detection strategies, enabling informed selection of appropriate methods for given analytical scenarios. Finally, we discuss the future directions and opportunities for microfluidics-based biochemical analysis.

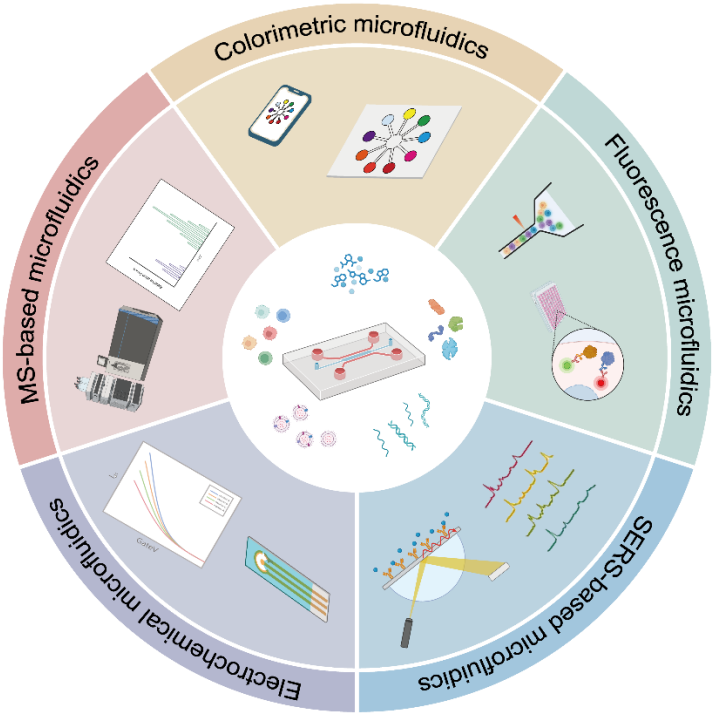

Figure 1. Overview of microfluidic biochemical analysis platforms classified by five major readout modalities.

2. Overview of microfluidics-based biochemical analysis

Microfluidics originated from microelectromechanical systems (MEMS) [31] and was initially conceptualized as miniaturized total analysis systems (μTAS) for chemical sensing [32,33]. Early μTAS devices were fabricated in silicon or glass using photolithography, a procedure that remained costly and inflexible. The introduction of soft lithography with polydimethylsiloxane (PDMS) soon revolutionized the field by enabling rapid prototyping of microchannels [16,34]. Several microfluidic platforms have since been developed to advance biochemical analysis. Centrifugal microfluidics (lab-on-a-disc) uses centrifugal force to drive liquids through microchannels on a rotating disc, facilitating parallel processing of multiple samples with precise liquid control for high-throughput immunoassays and nucleic acid testing [35]. Droplet microfluidics generates water-in-oil droplets as independent microreactors, allowing high-throughput single-cell analysis, digital polymerase chain reaction (dPCR), and rare target isolation with minimal cross-contamination [36]. Digital microfluidics (DMF) manipulates discrete droplets via electrowetting-on-dielectric without pumps or complex channels, delivering reconfigurable automation for immunoassays and nucleic acid testing [37–41]. Paper microfluidics, which creates hydrophilic channels on hydrophobic paper substrates, offers low-cost, disposable devices ideally suited for point-of-care diagnostics in resource-limited settings [42].

Collectively, these microfluidic platforms each contribute distinct advantages to biochemical analysis (Table 1). When integrated with diverse readout modalities including colorimetry, fluorescence, SERS, electrochemistry, and mass spectrometry, microfluidics offers a powerful and versatile toolkit for next-generation biochemical analysis. This review will systematically discuss the principles, applications, and future prospects of these integrated systems in the following sections.

Table 1. Microfluidic platforms for biochemical detection.

Readout modality	Chip type	Analyte	LoD	Reference
Colorimetry	Paper microfluidics	Glyphosate	75 ng mL^{-1}	[43]
	Paper microfluidics	Cre, Alb	Cre: 60 ng mL^{-1} Alb: 230 ng mL^{-1}	[44]
	Paper microfluidics	ALP, BChE	ALP: 4.03×10^{-3} U mL^{-1} BChE: 14.19×10^{-3} U mL^{-1}	[45]
	Paper microfluidics	EVs (PSMA, PSA, PTK7, EpCAM, EGFR)	3×10^3 particles mL^{-1}	[46]
	Paper microfluidics	SCN^-, BSA, Glucose, NO_2^-	BSA: 1.817×10^5 ng mL^{-1}	[47]
	Continuous-flow microfluidics	ALT, AST, ALP, ALB, TP, Cl^-	ALT: 7.9×10^{-3} U mL^{-1}; AST: 8.8×10^{-3} U mL^{-1}; ALP: 5.3×10^{-3} U mL^{-1}; ALB: 6.8×10^6 ng mL^{-1}; TP: 1.16×10^7 ng mL^{-1}; Cl^-: 18.3 mM	[48]
	Continuous-flow microfluidics	SARS-CoV-2 RNA, H1N1 RNA, *Escherichia coli* (*E. coli)* DNA, MRSA DNA	SARS-CoV-2: 5×10^3 copies mL^{-1}; H1N1: 4×10^3 copies mL^{-1}; *E. coli*: 14.6 copies mL^{-1}; MRSA: 11 copies mL^{-1}	[49]
	Continuous-flow microfluidics	*E. coli* DNA, *E. faecalis* DNA	< 50 CFU mL^{-1}	[50]
	Centrifugal microfluidics	MCF-7 EVs	1×10^6 particles mL^{-1}	[51]
Fluorescence	Continuous-flow microfluidic	CRP	1.7×10^{-4} ng mL^{-1} (saliva); 2×10^{-4} ng mL^{-1} (serum)	[52]
	Continuous-flow microfluidic	EVs (PD-L1)	9.95×10^3 ng mL^{-1}	[53]
	Centrifugal microfluidics	MRSA-mecA DNA, H1N1-HA RNA	MRSA DNA: 7×10^2 copies mL^{-1} H1N1 RNA: 1.2×10^3 copies mL^{-1}	[54]
	Centrifugal microfluidics	SARS-CoV-2 RNA	2 copies per reaction	[55]
	Centrifugal microfluidics	Circulating small EVs	8.4×10^5 particles mL^{-1}	[56]
	Droplet microfluidics	EVs (GPC-1)	10 particles mL^{-1}	[57]
	Droplet microfluidics	CTCs	5 CTCs mL^{-1}	[58]
	Droplet microfluidics	VEGF	N/A	[59]
	Digital microfluidics	Influenza A virus and influenza B virus nucleic acids	1.69 copies per reaction	[60]
	Digital microfluidics	NT-proBNP, IL-6, TNF-α	NT-proBNP: 1 aM IL-6: 1.5 aM TNF-α: 2.5 aM	[61]
SERS	Continuous-flow microfluidic	ctDNA	10 fM (buffer) 100 fM (whole blood)	[62]
	Continuous-flow microfluidic	Normal human lung epithelial cells (BEAS-2B) and non-small cell lung cancer subtypes (NCI-H460, NCI-H226) EVs	BEAS-2B EVs: 1.64×10^6 particles mL^{-1}; NCI-H460 EVs: 2.66×10^5 particles mL^{-1}; NCI-H226 EVs: 5.08×10^5 particles mL^{-1};	[63]
	Centrifugal microfluidics	CRP	7.7×10^{-4} ng mL^{-1}	[64]
Electrochemistry	Continuous-flow microfluidic	Three pesticides: FIP, THI, ACE	FIP: 0.17 ng mL^{-1}; THI: 0.14 ng mL^{-1}; ACE: 0.18 ng mL^{-1}	[65]
	Continuous-flow microfluidic	PSA, PSMA, IL-6, PF-4	PSA: 1.5×10^{-4} ng mL^{-1}; IL-6: 5×10^{-5} ng mL^{-1}; PF-4: 1×10^{-4} ng mL^{-1}; PSMA: 1.5×10^{-4} ng mL^{-1}	[66]
	Continuous-flow microfluidic	MCF-7 EVs	239 particles mL^{-1}	[67]
	Digital microfluidics	HPV16 DNA	1 fM	[68]
	Digital microfluidics	PD-L1-positive EVs	3.84×10^5 particles mL^{-1}	[69]
Mass spectrometry	Continuous-flow microfluidic	sEVs protein	1×10^{-5}–9.661×10^{-2} ng mL^{-1}	[70]
	Continuous-flow microfluidic	CTCs	28 cells mL^{-1}	[71]
	Digital microfluidics	EV lipids	N/A	[72]
	Digital microfluidics	Uracil, Indoline, Arginine	N/A	[73]

3. Microfluidic colorimetric assays

Colorimetric detection quantifies analytes by measuring the intensity of color change from specific biochemical reactions, typically with chromogenic reagents or enzyme-catalyzed reactions [74]. Compared with other readout modalities, colorimetric detection can be visualized directly by the naked eye without sophisticated instrumentation, making it suitable for point-of-care testing and resource-limited settings [75]. Integration with microfluidics furthers the utility of colorimetric assays. The miniaturized nature of microfluidic chips drastically reduces sample and reagent consumption, lowering the cost per test. Multiple assays can also be performed on a single device by designing parallel channels or arrays, which allow high-throughput detection with minimal user intervention.

Particularly, paper-based microfluidic devices stand out as the most representative platforms, owing to their ultra-low cost, disposability, and excellent compatibility with colorimetric readout. These microfluidic paper-based analytical devices (μPADs) use paper patterned with hydrophobic barriers to define microchannel networks, where fluids are driven spontaneously by capillary forces without external pumps. In addition, the porous structure of paper provides a high surface area for immobilizing chromogenic reagents or enzymes, and the white background offers an ideal contrast for colorimetric signal readout. For instance, Zhang *et al*. [43] developed a paper-based microfluidic colorimetric platform using ZnCo-ZIFs@MIL-101(Fe) nanozyme as the signal reporter for rapid on-site detection of trace glyphosate. Microchannels were fabricated on filter paper via wax printing, allowing ordered delivery of sample solutions for the whole detection process. The presence of glyphosate led to a change in the catalytic ability of the nanozyme, which was reflected by the colorimetric signals at the detection zone. Using smartphone-based image analysis, this platform achieved visual quantitative detection of glyphosate, with a limit of detection (LoD) as low as 75 ng mL^{-1}. Goman *et al*. [44] reported an origami μPAD for the simultaneous colorimetric detection of two chronic kidney disease biomarkers, albumin and creatinine. Mn-doped ZnS quantum dots were integrated with molecularly imprinted polymers to prepare core-shell nanozymes capable of specifically recognizing albumin and creatinine. These nanozymes were immobilized onto the paper preprinted with hydrophobic barriers and hydrophilic channels. Based on the colorimetric signals from the catalytic reaction upon target recognition, the device achieved sensitive detection of albumin and creatinine, with LoD of 230 ng mL^{-1} and 60 ng mL^{-1}, respectively.

In another work, Li *et al*. [45] engineered a double-layer origami μPAD for the simultaneous and precise visual quantification of alkaline phosphatase and butyrylcholinesterase activities in serum (Figure 2A). The chip was sequentially folded to control a cascaded two-stage process: enzymatic reactions on the first layer to generate reducing species, and colorimetric reaction on the second layer to yield synchronized colorimetric and photothermal signals. This dual-mode self-calibration design substantially enhanced anti-interference performance and detection accuracy, with the LoD for butyrylcholinesterase approximately one order of magnitude lower than that of conventional paper-based assays. Wang *et al*. [46] developed a sliding-folded three-dimensional μPAD for multiplexed digital quantification of surface protein profiles on tumor-derived EVs in plasma. The device pre-stored capture antibodies, lipophilic probe-functionalized Fe^{3+}-doped polymeric nanomaterials, and ferricyanide chromogenic substrates on the paper substrate. Through manual folding and sliding, the device sequentially triggered EV immunocapture, probe-membrane fusion, acid-triggered release of Fe^{3+}, and Prussian blue chromogenic reaction. The colorimetric signals were recorded with a smartphone for EV protein profiling.

This platform showed a LoD of 3×10^3 particles mL^{-1} for EV detection using 20 μL of sample, with a total assay time of 30 minutes. By simultaneously analyzing five EV surface proteins, including prostate-specific membrane antigen (PSMA), prostate-specific antigen (PSA), protein tyrosine kinase 7 (PTK7), epithelial cell adhesion molecule (EpCAM), and epidermal growth factor receptor (EGFR), the platform achieved precise discrimination among healthy donors, prostatitis patients, and prostate cancer patients, attaining a diagnostic accuracy of 97.5%. Chauhan *et al*. [47] developed a barrier-free microfluidic paper analytical device (BF-μPAD) for multiplex colorimetric detection (Figure 2B). The device consisted of two stacked paper membranes with distinct wicking rates, where the top layer distributed fluid and the bottom layer immobilized detection reagents, eliminating the need for wax or physical barrier patterning. Fluid transferred vertically from the top to the bottom layer, leading to uniform colorimetric signals and no cross-contamination. The BF-μPAD rapidly distributed samples to 20 reagent spots within 30 seconds and the LoD was more than 3.5-fold lower than that of conventional μPADs. The platform enabled simultaneous colorimetric detection of salivary thiocyanate, protein, glucose, and nitrite, representing a promising tool for point-of-care testing with good signal uniformity, detection specificity, and ease of operation.

Other microfluidic platforms have also been proposed for colorimetric biochemical analysis. Liang *et al*. [48] reported a self-powered microfluidic colorimetric device for fully automated, quantitative testing of multiple liver function markers (Figure 2C). All reagents were pre-loaded in the rigid-flexible composite chambers of the device, and pressurized gas was used as driving energy. A mechanical pressure-sensing mechanism was utilized for fluid manipulation without user operation, thus completing entire detection processes, including reagent release, vortex mixing, and parallel reactions. Using 180 μL of whole blood, this microfluidic device demonstrated simultaneous colorimetrical quantification of nine liver function markers (including albumin and total protein) within 4 min. AbdElFatah *et al*. [49] reported an automated microfluidic platform integrated with nano-plasmonic enhanced colorimetric sensing for rapid multiplexed detection of pathogens. This device used 3D-printed negative pressure suction cups for sample lysis, sequential reagent release, and mixing, allowing for "sample-in-answer-out" analysis within 13 min. It achieved a LoD of 5×10^3 copies mL^{-1} for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA and 95% diagnostic accuracy in clinical specimens.

Chen *et al*. [76] developed an integrated microfluidic platform for multiplexed detection of human norovirus. The construction of a dynamically confined space based on a sucrose hydrogen-bonding network enabled one-pot cascade recombinase polymerase amplification (RPA) and loop-mediated isothermal amplification (LAMP). This assay, termed DORLA, eliminated aerosol contamination and reaction incompatibility associated with traditional two-step protocols. By integrating specific receptor-mediated viral capture, nucleic acid extraction, and DORLA detection on a single microfluidic chip, naked-eye colorimetric identification of norovirus genotypes GI and GII simultaneously was realized within 1 h, with LoD of 1×10^4 copies mL^{-1} and 1×10^3 copies mL^{-1}, respectively. Jalali *et al*. [50] designed a nanoplasmonic colorimetric microfluidic platform based on refractive index response. The system enabled parallel identification of 10 bacterial species and MIC determination of 15 antibiotics directly from urine samples within 36 minutes. Wang *et al*. [51] designed a centrifugal microfluidic chip for EV-based breast cancer diagnosis. This chip contained a decanting structure, a siphon valve, a triangular micropillar array, and an immunomagnetic bead capture chamber. Through precisely controlled rotational speeds, the chip sequentially accomplished blood cell sedimentation and

separation, siphon-driven plasma transfer, size-exclusion removal of residual cells, cluster of differentiation 63 (CD63) magnetic bead-mediated immunocapture of EVs, and a chromogenic reaction for parallel colorimetric detection.

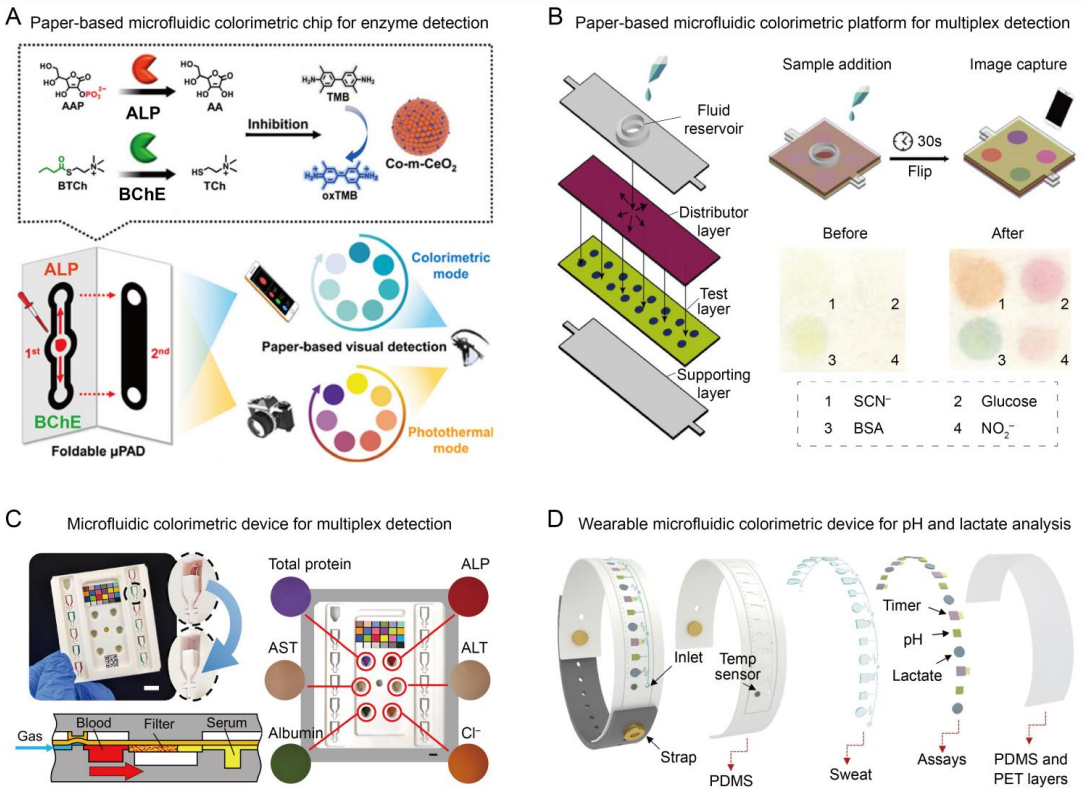

Figure 2. Microfluidic colorimetric assays for biochemical analysis. **(A)** Schematic diagram of a paper-based microfluidic colorimetric device for enzyme detection. Reproduced with permission [45]. Copyright 2024 American Chemical Society; **(B)** Schematic of a paper-based microfluidic colorimetric platform for multiple analytes. Reproduced with permission [47]. Copyright 2021 American Chemical Society; **(C)** Schematic illustration of a microfluidic colorimetric device for the detection of multiple liver function markers. Reproduced with permission [48]. Copyright 2025 The American Association for the Advancement of Science; **(D)** Schematic of a wearable microfluidic colorimetric device for pH and lactate analysis. Reproduced with permission [77]. Copyright 2024 The American Association for the Advancement of Science.

Recently, colorimetric microfluidic platforms have been extended to wearable devices for real-time biochemical analysis. Cho *et al.* [77] developed a flexible microfluidic wristband for continuous, time-resolved analysis of sweat pH and lactate levels (Figure 2D). To achieve continuous monitoring, a colorimetric timer based on the dissociation kinetics of maltodextrin-iodine complexes was designed. Two sensors were integrated: a plasmonic lactate sensor using silver nanoplates and a covalently immobilized dye-based pH sensor. Through a sequence of capillary burst valves in the microchannels, sweat sequentially filled multiple isolated reservoirs, allowing reconstruction of dynamic pH and lactate profiles over the entire exercise period from a single post-exercise chip image. Wang *et al.* [78] reported an artificial intelligence-assisted wearable microfluidic colorimetric sensing system for simultaneous, noninvasive detection of multiple small molecules and ions in tears, including vitamin C, pH, and Ca^{2+}. The flexible PDMS microfluidic patch automatically collected tears via capillary channels and directed

them into four separate reaction chambers pre-loaded with specific colorimetric strips to generate distinct color responses. To address interference from ambient color temperature and tear pH fluctuations on colorimetric readout in practical applications, a cloud-based data analysis system was implemented using a multi-channel convolutional recurrent neural network. Trained on large datasets covering diverse pH and illumination conditions, the deep learning model enabled automatic dual correction of predicted concentrations. After calibration, the coefficients of determination between predicted and actual concentrations for all four targets exceeded 0.99, markedly improving the detection accuracy and data reliability of wearable colorimetric sensors in real-world scenarios.

In summary, microfluidic colorimetric assays offer the distinct advantages of simple instrumentation, naked-eye readout, and low cost, making them well-suited for point-of-care diagnostics and resource-limited settings. However, colorimetric detection remains susceptible to external factors such as ambient light interference, camera-dependent variation, color calibration, and background color from biological samples, which can compromise the reproducibility and quantitative accuracy of the readout. In addition, the relatively limited sensitivity and narrow linear ranges constrain their application in quantifying trace-level targets. Future developments may focus on integrating smartphone-based imaging with standardized color correction algorithms, along with signal amplification strategies, to improve robustness and sensitivity while preserving the simplicity of microfluidic colorimetric detection.

4. Microfluidic fluorescence assays

Fluorescence detection transduces the concentration of analytes into the emission intensity of fluorescent probes, such as fluorophores, quantum dots, or fluorescent proteins. Compared with colorimetric readouts, fluorescence detection offers superior sensitivity and a wider dynamic range. Microfluidics enhances these assays by shortening the optical path length and reducing light scattering from the bulk solution, which effectively lowers background fluorescence and improves the signal-to-noise ratio. Moreover, multiple-channel design, low sample/reagent consumption, and compatibility with compact optical components, such as light-emitting diodes (LEDs), optical filters, complementary metal-oxide-semiconductor (CMOS) detectors, or smartphone cameras, make microfluidic fluorescence platforms well-suited for sensitive, quantitative, and high-throughput biochemical analysis.

Mo *et al*. [79] developed a microfluidic fluorescence device for noninvasive detection of β-hydroxybutyrate in sweat. This chip contained bifurcated microchannels connected to six reservoirs via capillary bursting valves (CBVs) for fluorescent probe in the reaction chambers, this platform realized real-time, noninvasive, wearable monitoring of the key metabolic small molecule β-hydroxybutyrate, showing high consistency with clinical gold standards. Kaushal *et al*. [52] reported an integrated microfluidic fluorescence immunosensor for automated quantitative detection of C-reactive protein (CRP). The microfluidic cartridge was pre-loaded with quantum dot-labeled detection probes and magnetic bead-labeled capture probes in separate microchannels. Upon sample introduction, finger-actuated fluid pouches sequentially released the sample and reagents, while V-shaped bubble traps prevented air blockage to ensure smooth fluid transport. The sample then mixed with both probes, allowing the formation of sandwich immunocomplexes on the magnetic beads. A magnet positioning slot captured the bead-immunocomplexes, and unbound reagents were removed by on-chip washing using the same finger-actuated pouches. Finally, the fluorescence signals from the quantum dots were analyzed with a portable imaging system for CRP quantification. The device achieved LoD as low as 17×10^{-3} ng mL^{-1}

in saliva and 2×10^{-2} ng mL^{-1} in serum, outperforming conventional ELISA in both operational simplicity and assay speed. Wu *et al*. [53] developed a herringbone-based microfluidic chip for fluorescent detection of EVs (Figure 3A). The herringbone structure generated chaotic convection to enhance collision frequency between EVs and the modified substrate, and thus enabled highly efficient capture of biotin-labeled EVs. After target recognition with programmed death-ligand 1 (PD-L1) antibodies and signal generation with β-galactosidase, quantitative analysis of PD-L1 protein on EVs was achieved, with a LoD of 9.95×10^{3} ng mL^{-1}. Yu *et al*. [80] further developed a microfluidic platform for multi-omic analysis of neutrophil-derived EVs (NEVs). The chip contained a herringbone micromixer and a magnetic bead immunocapture chamber, in which cluster of differentiation 66b (CD66b) antibody-conjugated magnetic beads were used to specifically enrich $CD66b^{+}$ NEVs from serum, and CD63 aptamers were used for NEV quantification. Following on-chip thermal lysis, the released aptamers together with miR-223-3p and miR-425-5p encapsulated in NEVs simultaneously triggered triplex rolling circle amplification-molecular beacon fluorescent detection. This strategy achieved parallel *in situ* analysis of surface markers and intraluminal miRNAs in a single EV subpopulation, with a LoD as low as 10^{4} particles mL^{-1}. Deng *et al*. [81] reported a thermomicrofluidic assay for the detection of tumor-derived EVs (tEVs) in a one-step manner. In this assay, the serum sample was mixed with aptamer probes and polyethylene glycol (PEG), and then introduced into microchambers for laser irradiation. Under this condition, fluorescent aptamer probes could selectively detect tEVs by recognizing the dual-protein co-expression on EVs. Meanwhile, under the laser-induced temperature gradient and PEG concentration gradient, EVs were rapidly enriched at laser position within 10 min through directional diffusiophoretic forces, which amplified the fluorescence signals of EVs. By analyzing the fluorescence signal at the laser position, this method achieved specific detection of tEVs and showed potential for non-invasive diagnosis of prostate cancer.

Centrifugal microfluidics provides an automated solution for high-throughput fluorescent biochemical analysis. Zhang *et al*. [54] developed a centrifugal microfluidic chip integrated with clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9/Cas13a for amplification-free dual detection of methicillin-resistant Staphylococcus aureus and influenza A H1N1 virus. The chip employed centrifugal force to uniformly distribute sample and CRISPR reagents into an array of microwells, where the orthogonal trans-cleavage activities of Cas9 and Cas13a toward DNA and RNA targets cleaved FAM- and Cy5-labeled fluorescent reporter probes, achieving sub-copy sensitivity within 20 minutes. In 20 simulated clinical samples, the platform increased the positive detection rate by 33.3% compared to quantitative PCR (qPCR). Xu *et al*. [82] designed a hand-powered centrifugal microfluidic platform for simultaneous human papillomavirus (HPV) genotyping. Combined with a smartphone micro-imaging system and deep learning for image recognition, the platform achieved ultrasensitive detection of HPV subtypes in multiple samples. When applied to 100 clinical samples, this platform attained a diagnostic accuracy exceeding 95%. This work promoted practical application of microfluidic nucleic acid testing in decentralized community settings. Tian *et al*. [55] developed a fully automated centrifugal microfluidic system for sample-to-answer viral nucleic acid detection (Figure 3B). This system integrated sample lysis, nucleic acid extraction, and reverse transcription loop-mediated isothermal amplification (RT-LAMP) amplification into a centrifugal microfluidic disc with 21 independent reaction units. A customized instrument was constructed to automate all procedures, including reagent injection, centrifugal fluid manipulation, temperature control and fluorescence signal readout. This

microfluidic platform enabled "sample-in-answer-out" detection of SARS-CoV-2 RNA within approximately 70 minutes, with a LoD of 2 copies per reaction. Recently, Woo *et al*. [56] designed an automated centrifugal disc device (SpinEx) for multiplexed fluorescent detection of EVs directly from liquid biopsy samples. The disc integrated on-disc chromatography, centrifugal fluid transfer and microbead-based EV capture with immunolabelling, enabling automated EV isolation and EV protein profiling from whole blood samples within 75 minutes. In a clinical study of 221 plasma samples, SpinEx-based EV protein profiling successfully discriminated cancer from non-cancer samples with 90% accuracy and 97% specificity, and classified five tumour types with 96% accuracy, demonstrating its clinical utility for non-invasive cancer diagnosis and classification.

Droplet microfluidics encapsulates single molecules or single cells in picoliter droplets, effectively concentrating fluorophores and enhancing fluorescence signals. This capability is particularly advantageous for detecting rare events, such as single molecules and single cells. Liu *et al*. [57] reported a droplet microfluidics-based single-exosome-counting immunoassay (droplet digital ExoELISA) for absolute quantification of cancer-derived exosomes. The platform employed magnetic beads to form single-exosome sandwich immunocomplexes labeled with enzymatic reporters. The immunocomplexes were then encapsulated into uniform microdroplets to generate discrete fluorescent signals for digital counting. This assay achieved a LoD down to 1×10^4 particles mL^{-1} and exhibited a dynamic range of five orders of magnitude. By analyzing the glypican 1 (GPC-1)-positive exosomes in clinical plasma samples, this assay allowed for the precise differentiation of cancer patients from healthy individuals and those with benign breast disease, with an overall accuracy of 100%. Cai *et al*. [58] combined microfluidic single-cell droplet encapsulation with dual-responsive nanoprobes for ultrasensitive detection of circulating tumor cells (CTCs). The platform co-encapsulated nanoprobes and CTCs into uniform picoliter droplets, where high intracellular miRNA-21 levels and acidic microenvironment jointly activated Zn-DNAzyme to restore fluorescence for CTC detection. The system successfully detected as few as 5 CTCs mL^{-1} in spiked human blood samples, and was further applied to count CTCs in clinical whole blood samples from patients with colorectal cancer, hepatocellular carcinoma, and cervical cancer. Cong *et al*. [59] reported a droplet microfluidic platform based on fluorescence imaging for the analysis of vascular endothelial growth factor (VEGF) secreted by single cells (Figure 3C). The SA-coated cells, capture probes, and reporter probes were encapsulated into droplets via a microfluidic chip. Within the droplet, the capture probes were conjugated to the cells through biotin, and then VEGF secreted by the cells acted as a 'bridge' to link the capture probes and reporter probes. The VEGF secretion level of single cells was directly determined by fluorescence intensity. This platform revealed that cancer cells (HeLa, MCF-7) released significantly higher VEGF than normal cells (H8), and cancer cells exhibited higher heterogeneity in VEGF levels.

DMF, which manipulates discrete droplets via electrowetting-on-dielectric (EWOD), offers a reconfigurable and sample-efficient platform for fluorescence-based biochemical assays. Ji *et al*. [60] developed an integrated droplet digital RPA platform for the rapid and absolute quantification of viruses (Figure 3D). The chip incorporated a thin-film transistor array comprising 129,600 individually addressable electrodes and employed an artificial intelligence (AI)-based path-planning algorithm for high-throughput parallel manipulation of nanoliter-scale droplets. The entire workflow, including nucleic acid extraction, digital partitioning, isothermal amplification, fluorescence imaging, and Poisson statistical analysis, was executed automatically with the DMF platform. The platform showed

sample-to-answer analysis of viral nucleic acid within 60 minutes, with a LoD of 1.69 copies per reaction. The platform enabled accurate differentiation between influenza A virus (IAV) and influenza B virus (IBV), and the quantitative results were highly consistent with those from qPCR. In another work, Bai *et al*. [83] developed a DMF platform for multiplexed screening of respiratory pathogens. This platform adopted a mature multiplex PCR strategy with dehydrated pre-stored reagents for nucleic acid amplification, which enabled fully automated and sensitive PCR amplification without manual reagent addition. Through optimization of L-junction electrodes and an embedded thin-film heater, the platform achieved "sample-in-answer-out" pathogen detection within 80 minutes, with the LoD ranging from 200 to 628 copies mL^{-1}. Li *et al*. [61] reported a dual digital immunoassay platform that incorporated digital microfluidics with CRISPR/Cas13a cascade amplification for sensitive fluorescent detection of protein biomarkers. Using EWOD manipulation, single-molecule immunocomplexes were spatially isolated into a million-scale microwell array, with each microwell serving as an isolated reaction chamber for digital counting of single immunocomplexes. After CRISPR/Cas13a signal amplification, the platform achieved attomolar (aM) sensitivity for N-terminal pro-brain natriuretic peptide (NT-proBNP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α). In 30 clinical serum samples, the method successfully quantified low-abundance NT-proBNP that was undetectable by standard ELISA, demonstrating its great potential for ultrasensitive protein biomarker detection.

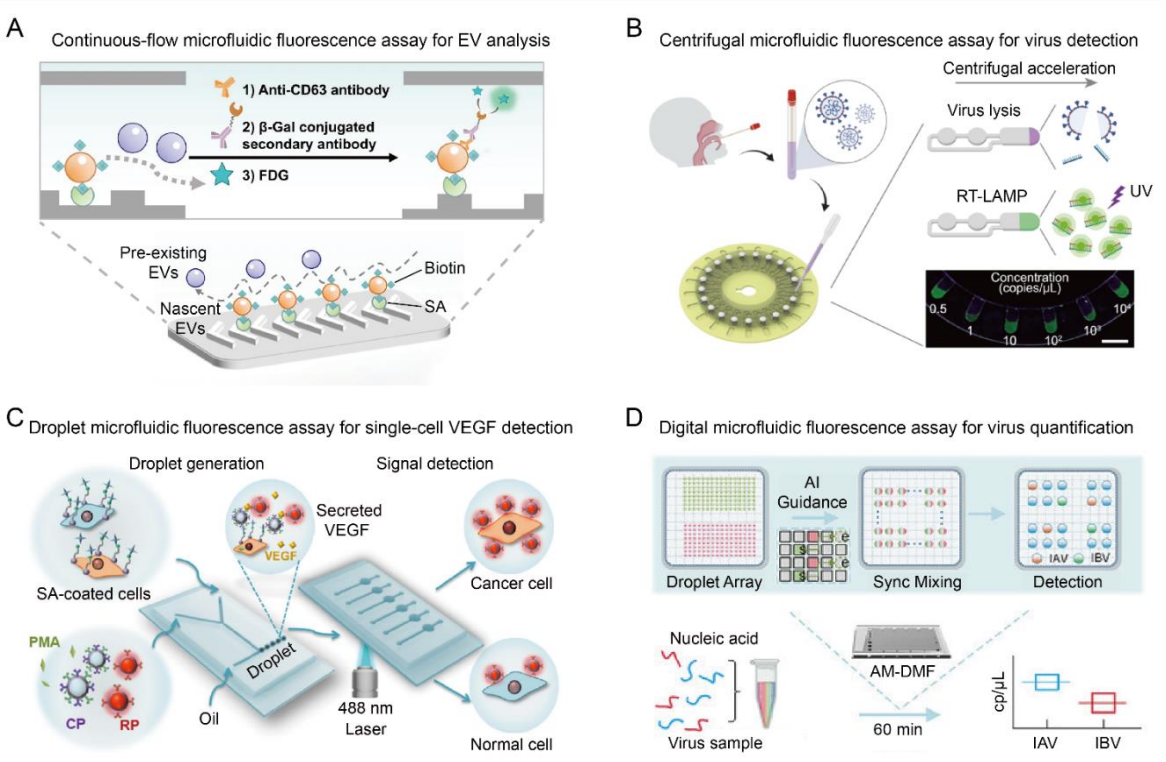

Figure 3. Microfluidic fluorescence assays for biochemical analysis. **(A)** Schematic diagram of a microfluidic fluorescence assay for EV analysis. Reproduced with permission [53]. Copyright 2023 Springer Nature; **(B)** Schematic of a centrifugal microfluidic fluorescence assay for viral nucleic acids detection. Reproduced with permission [55]. Copyright 2020 Springer Nature; **(C)** Illustration of a droplet microfluidic fluorescence assay for single-cell VEGF detection. Reprinted with permission [59]. Copyright 2022 American Chemical Society; **(D)** Workflow of a digital microfluidic fluorescence assay for virus quantification. Reproduced with permission [60]. Copyright 2025 Wiley-VCH GmbH.

Collectively, microfluidic fluorescence assays provide superior sensitivity, broad dynamic range, and excellent compatibility with various microfluidic devices. These features make fluorescence the modality of choice for high-resolution single-molecule and single-cell analysis, as well as multiplexed detection. Nevertheless, the requirement for external light sources, optical filters, and detectors adds instrumental complexity, and issues such as photobleaching and autofluorescence can interfere with signal stability. Advances in fluorophore engineering and portable optical modules are expected to further expand the utility of microfluidic fluorescence detection.

5. Microfluidic SERS assays

SERS is a spectroscopic technique that amplifies the Raman signals of analytes by several orders of magnitude through plasmonic effects on metallic nanostructures (e.g., gold or silver nanoparticles). This enhancement enables the detection of biomolecules down to single-molecule levels, while providing sharp, fingerprint-like spectral signatures that are highly specific to molecular structures. These intrinsic features make SERS an attractive tool for biochemical analysis, particularly when high sensitivity and multiplexing capability are required. Microfluidics-based SERS assays have been a versatile and ultrasensitive platform for detection of small molecules, proteins, nucleic acids, and even single cells.

Zhou *et al*. [84] designed a self-driven microfluidic chip that integrated lateral flow and vertical flow (SeDM-LV chip) for sensitive detection of IL-6. In this chip, anodic aluminum oxide membranes were used instead of traditional nitrocellulose membranes, offering improved detection sensitivity, uniformity, and anti-hook effect capability. The entire detection process was fully automated under capillary force. Using serpentine channels to enhance mixing with gold-core silver-shell alloy SERS nanotags, this chip achieved dual-mode readout of IL-6 within 5 minutes, with a naked-eye LoD of 0.1 ng mL^{-1} and a SERS LoD as low as 9.5×10^{-4} ng mL^{-1}. Zhang *et al*. [85] developed a microfluidic-based SERS platform for detecting protein biomarkers. By leveraging the nano-mixing effect generated by asymmetric electrode arrays to enhance immunoreaction efficiency, simultaneous SERS detection of the four biomarkers was realized on a single chip. Combined with machine learning classification, this assay achieved precise discrimination of lung cancer subtypes with an area under the curve (AUC) of 0.98. Wu *et al*. [62] reported a hand-powered SERS-microfluidic device for amplification-free detection of circulating tumor DNA (ctDNA) in whole blood. The device employed a finger-actuated vacuum chamber to generate negative pressure, driving the whole blood sample through an integrated filtration trench, where blood cells were removed and target ctDNAs were captured on a SERS substrate functionalized with hairpin probes. In the presence of the target mutant sequence, the hairpin probe underwent a conformational transition from a hairpin structure to a linear structure. The Raman reporter molecules labeled on the probe moved away from the substrate surface and weakened the SERS signal, thereby achieving a signal-off sensing mode. The entire workflow could be completed within 35 minutes. Using a hairpin probe-based "signal-off" sensing strategy, the chip showed a LoD of 100 fM for the EGFR E746-A750 mutation in whole blood and successfully discriminated positive from negative clinical lung cancer samples.

Chen *et al*. [63] developed a microfluidic-based SERS chip for label-free detection of EVs (Figure 4A). In this chip, EVs were conjugated with anti-CD9 antibody-functionalized probes within an S-shaped mixing channel for Raman signal enhancement. After EV capture in the downstream micropillar array trapping chamber, the intrinsic SERS fingerprint spectra of EVs derived from four different cell sources were detected. Combined with a deep learning model, this platform achieved a classification accuracy

of 97.88%. Zhou *et al*. [86] reported a microfluidic SERS platform for the multiplexed detection of surface glycosylation modifications on non-small cell lung cancer EVs, extending microfluidic-based SERS detection of EVs from protein levels to the dimension of glycan modifications. Zhan *et al*. [87] developed a hierarchically nanostructured microfluidic SERS chip for CTC capture and *in situ* phenotypic analysis in whole blood (Figure 4B). The platform employed an SU-8 nanodendritic pyramidal microcone array deposited with gold film (SNPMA/Au), and was further modified with aptamers for dual recognition through specific molecular binding and topological matching. CTCs were pre-enriched by deterministic lateral displacement, and then captured on the nanostructured substrate in a shuttle-shaped chamber. Antibody-conjugated SERS nanoprobes targeting specific biomarkers were introduced to label the captured cells, allowing for *in situ* single-cell phenotyping via SERS mapping without cell release. The platform detected CTCs from all seven clinical prostate cancer patient samples and none from healthy controls.

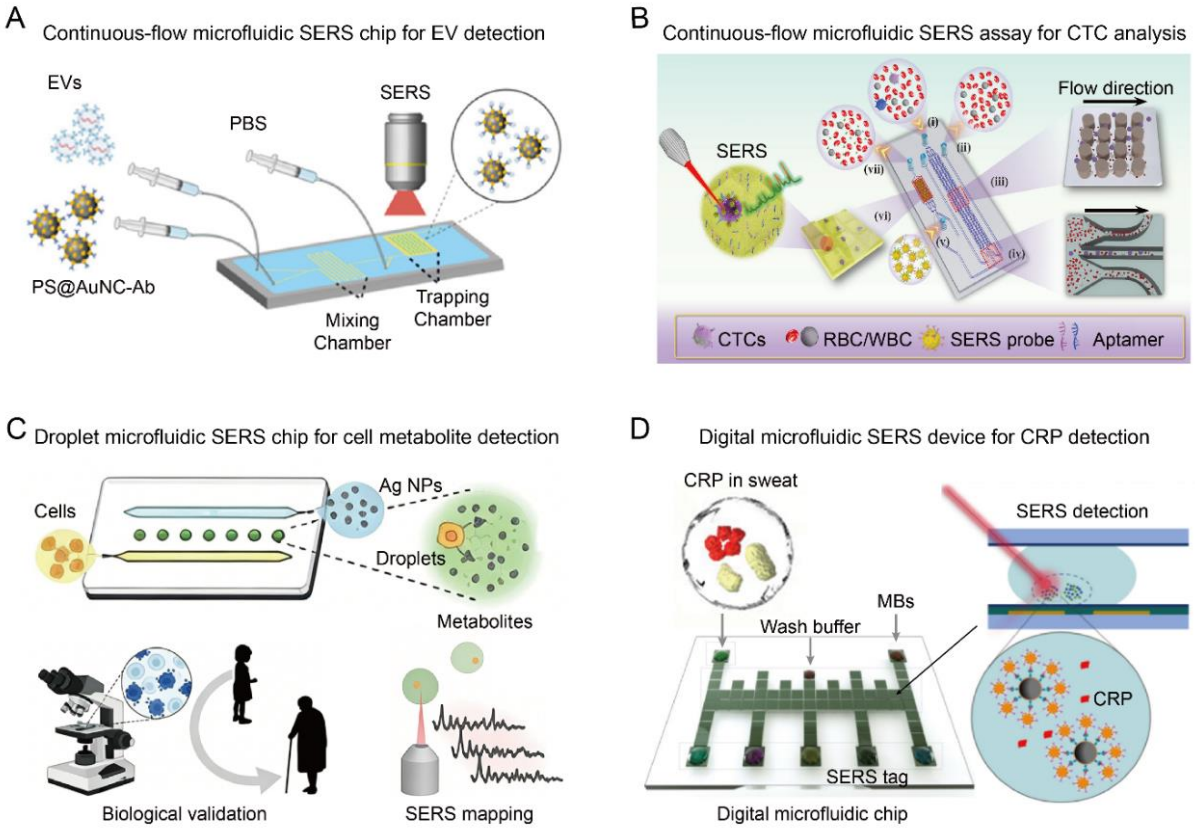

Figure 4. Microfluidic SERS assays for biochemical analysis. **(A)** Schematic illustration of a continuous-flow microfluidic SERS chip for EV detection. Reproduced with permission [63]. Copyright 2025 American Chemical Society; **(B)** Schematic of a continuous-flow microfluidic SERS assay for CTC analysis. Reproduced with permission [87]. Copyright 2025 American Chemical Society; **(C)** Illustration of a droplet microfluidic SERS platform for cell metabolite detection. Reproduced with permission [88]. Copyright 2024 Wiley-VCH GmbH; **(D)** Schematic illustration of a digital microfluidic SERS device for CRP detection. Reproduced with permission [64]. Copyright 2026 American Chemical Society.

Liu *et al*. [88] reported a droplet microfluidic single-cell metabolic analysis platform for investigation of metabolic heterogeneity in senescent fibroblasts (Figure 4C). The device performed

single-cell compartmentalization, intracellular metabolite release, and loading of silver nanoparticles through slipping operations, enabling SERS detection of metabolites from individual cells within enclosed microwells. The platform could discriminate different cancer cell lines and identify 12 differential metabolites between young and aged fibroblasts. Spermine was validated as a potent inducer of cellular senescence across multiple cell types. Xiong *et al*. [64] developed a digital microfluidics-based automated SERS platform for the non-invasive detection of CRP in sweat (Figure 4D). This platform used gold-coated Fe_3O_4 magnetic nanoparticles as capture substrates and gold nanostars as SERS tags. The entire detection process, including magnetic separation, washing, enrichment of "AuMNPs/CRP/SERS tag" complexes, and *in situ* Raman signal acquisition, was performed through EWOD manipulation. This platform completed the entire assay within 20 minutes using only 30 μL of sample, with a LoD as low as 7.7×10^{-4} ng mL^{-1}. The application of a one-dimensional convolutional neural network model significantly improved both the accuracy and the robustness of quantitative CRP analysis.

Overall, microfluidic SERS assays combine the high sensitivity and molecular fingerprint specificity of SERS with the sample handling and integration capabilities of microfluidics, enabling ultrasensitive detection and multiplexed analysis in complex biological samples. However, several challenges such as substrate batch-to-batch variability, spectral interference, and quantitative reproducibility remain. Ongoing developments in machine learning-assisted spectral analysis and advanced nanofabrication techniques are expected to mitigate these limitations and accelerate clinical translation.

6. Microfluidic electrochemical assays

Electrochemical detection is another classical biochemical analysis method that transduces the concentration of analytes into measurable current, potential, or impedance signals via redox reactions on electrode surfaces [89,90]. Unlike optical methods that require external light sources and detectors, electrochemical analysis is amenable to miniaturization, as electrodes and potentiostats can be readily integrated into compact, low-power devices [91,92]. Combining microfluidics with electrochemistry allows reproducible sample delivery to electrode surfaces, minimizes mass transport limitations, and improves signal stability. In addition, microfluidic chips can be patterned with multiple working electrodes, achieving high-throughput, multiplexed detection with low sample and reagent consumption. Moreover, such miniaturized microfluidic electrochemical systems can be easily coupled with portable potentiostats and wireless communication, facilitating on-site, real-time, and point-of-care electrochemical analysis. These features collectively position microfluidic electrochemical platforms as a sensitive, low-cost, and highly portable toolkit for biochemical analysis.

Liu *et al*. [65] developed an electrochemical microfluidic aptasensor array based on laser-induced graphene (LIG/Au) electrodes for the simultaneous detection of three pesticides: fipronil (FIP), thiamethoxam (THI), and acetamiprid (ACE) (Figure 5A). This device contained multiple electrode sensing elements on a single microfluidic chip fabricated by laser-induced graphene. During parallel detection, aptamers specifically bound to their respective targets, releasing complementary DNA strands that hybridized with hairpin-structured padlock probes. These probes were circularized by T4 DNA ligase to initiate rolling circle amplification (RCA) for generating long single-stranded DNA products containing a large number of hairpin structures. The RCA products were captured on the corresponding LIG/Au electrodes via DNA hybridization, and combined with methylene blue to produce amplified electrochemical signals. The device reached LoD ranging from 0.14 to 0.18 ng mL^{-1} for

the detection of three pesticides, and the chip exhibited good reproducibility, high stability, and satisfactory analytical performance in assessing pesticide residues. Tang *et al*. [66] proposed a high-throughput electrochemical microfluidic immunoarray for the detection of protein biomarkers (Figure 5B). A 32-sensor chip was fabricated by wet-etching the gold layer of compact discs. An 8-port manifold was integrated to construct a detection system with 256 individually addressable sensors. For protein biomarker detection, the serum sample was first captured offline by magnetic nanoparticles loaded with horseradish peroxidase (HRP), and then loaded into the chip. After automatic sample delivery and washing through microfluidic channels, the levels of four prostate cancer biomarkers in serum, including PSA, PSMA, IL-6, and platelet factor 4 (PF-4), were simultaneously quantified through differential pulse voltammetry (DPV). Najjar *et al*. [93] reported a 3D-printed microfluidic platform for the concurrent electrochemical detection of SARS-CoV-2 RNA and anti-SARS-CoV-2 antibodies in saliva. The microfluidic device contained a sample lysis chamber, a polyethersulfone (PES) membrane-based nucleic acid capture region, a LAMP amplification reservoir, and a CRISPR reaction chamber. Fluid transport was driven by a miniature peristaltic pump for automated execution of RNA extraction, isothermal amplification, and Cas12a-mediated signal transduction at the electrode surface. By measuring the oxidation peak current signals of SARS-CoV-2 RNA, a LoD of 800 copies mL^{-1} was obtained in clinical saliva samples, providing a promising strategy for point-of-care testing of SARS-CoV-2.

Zou *et al*. [67] devised an electrochemical microfluidic system featuring a homotypic cancer cell membrane-biomimetic interface for subtype-specific profiling of breast cancer-derived EVs. This device contained a four-channel parallel microfluidic chip, and the gold electrode in each channel was functionalized with breast cancer cell membranes to create a biomimetic capturing surface for selective enrichment of subtype-matched EVs via homotypic affinity interactions. After EV capture, silver nanoparticle-labeled antibodies against cluster of differentiation 47 (CD47) were added for EV labeling, and quantitative EV detection was achieved by analysis of the acid-induced release of Ag^{+} ions through DPV. This platform showed a LoD of 239 particles mL^{-1} toward MCF-7 EVs and precisely stratified breast cancer patients by molecular subtype. Lin *et al*. [94] developed an electrochemical microfluidic platform for *in situ* dynamic monitoring of reactive oxygen species (ROS) at the single-cell level. The method utilized a hydrodynamic flow confinement area generated by the microfluidic apertures for precise cell manipulation, followed by *in-situ* electrochemical sensing of hydrogen peroxide released from the targeted cell using a platinum-modified carbon microelectrode. The platform integrated fluidic control, single-cell stimulation, and amperometric detection within a single probe, allowing for localized chemical treatment and immediate response recording at the single-cell level with high sensitivity and minimal interference. It achieved a LoD of 2.71 μM for H_2O_2 and can track ROS dynamics within seconds after stimulation. This method was applied to monitor ROS release during global ferroptosis induction and local membrane damage repair in single cells, revealing the regulatory roles of glucose and calcium ions in oxidative stress. Zhu *et al*. [95] further expanded an electrochemical microfluidic platform into high-throughput label-free single-cell multiparametric phenotypic identification. The method employed a microfluidic serpentine channel to achieve inertial focusing of single cells, and used four pairs of face-to-face electrodes with a two-current differential method for detection, thereby generating unique impedance signals with four alternating peaks. By integrating particle focusing, multi-frequency impedance sensing, and machine learning-based classification, this device enabled high-throughput, label-free single-cell phenotyping with minimal positional dependence, while 29

cellular dielectric parameters could be extracted per cell at a throughput of 0.1 mL min^{-1}. Using this platform, particles of 7–20 μm and three types of tumor cells (A549, MCF-7, and SW480) were precisely characterized, and discrimination between white blood cells and tumor cells was achieved with the highest accuracy of 99.1%.

Compared with other microfluidic platforms that rely on complex channel networks, DMF is more compatible with electrochemical detection. Zhao *et al*. [96] designed a programmable magnetic digital microfluidic (PMDMF) platform with an electrochemical detection system for glucose analysis. The platform used a microcoil array to drive an N52 permanent magnet, which in turn generated a localized high-intensity magnetic field to actuate magnetic droplets on a superhydrophobic 3D-printed microfluidic chip with programmable trajectories. The PMDMF platform integrated magnetic droplet manipulation and electrochemical detection on-chip, enabling automated sample routing and real-time sensing. It achieved a LoD of 6.5 μM for glucose within a linear range of 0.01–0.25 mM. Using this platform, glucose in artificial sweat was automatically detected across five sample wells in a continuous manner. The recovery rates ranged from 88.1% to 113.5 %, demonstrating its potential for point-of-care testing. Naorungroj *et al*. [68] constructed a digital microfluidic electrochemical platform for automated amplification-free detection of HPV16 DNA. The platform integrated droplet manipulation, merging, mixing, localized heating, and electrochemical detection on a single chip. When combined with a target-induced hairpin opening and exonuclease III (Exo III)-assisted signal amplification strategy, the platform automated the entire workflow from sample loading to signal readout within 75 min. The platform showed a LoD of 1 fM for HPV16 DNA with a linear range from 1 fM to 10 pM. Using this platform, clinical cervical swab samples were tested, and all HPV16-positive and -negative specimens were correctly discriminated. Lo *et al*. [69] reported a digital microfluidic electrochemical device for isolation-free detection of PD-L1-positive EVs in cell culture supernatants and human plasma (Figure 5C). This device captured EVs using magnetic beads, followed by on-chip elution and detection of PD-L1 expression using a sandwich electrochemical immunosensor with gold nanoparticle-modified electrodes and poly-HRP signal amplification. By integrating magnetic bead functionalization, EV capture, washing, elution, and electrochemical detection all on a single chip, the microfluidic device automated the entire sample-to-result workflow with a sample consumption of only 20 μL. Using this platform, melanoma patients were distinguished from healthy controls based on plasma $PD\text{-}L1^{+}$ EV detection.

Recently, microfluidic electrochemical assays have been adopted for real-time, non-invasive biomarker monitoring in wearable formats. Ye *et al*. [97] reported a skin-adhesive microfluidic-based biosensor combined with target-induced strand-displacement aptamer switches for automated, noninvasive monitoring of estradiol in sweat. The chip utilized iontophoretic gels for sweat storage and capillary burst valves for volume and timing control of sweat into micro-reservoirs. Using AuNPs-MXene-modified working electrodes, the aptamer recognized estradiol to release methylene blue-labeled ssDNA, which was captured by the counter electrode to produce a “signal-on” response, with a LoD as low as 0.14 pM. Tu *et al*. [98] reported a wireless, patch-type microfluidic system for CRP monitoring. The device vertically integrated an iontophoretic sweat extraction module, microfluidic reagent storage and mixing channels, and laser-engraved graphene sensor arrays on a flexible substrate. The self-driven flow of sweat sequentially enabled detection antibody reconstitution, immunocomplex formation, passive washing, and electrochemical signal readout, without manual sample loading or washing steps. This wearable patch showed a LoD of 8 pM. Mi *et al*. [99] developed a microfluidic wearable electrochemical

sensor for noninvasive and multiplexed analysis of metabolites and electrolytes in human sweat (Figure 5D). The wearable platform integrated a 3D-printed PDMS microfluidic chip for rapid capillary-driven sweat collection and spontaneous sample routing to the sensing area. The chip contained a screen-printed flexible electrode array, a uric acid (UA) sensor, a polyaniline-based pH sensor, and a valinomycin-based K^+ sensor. Using the chip, real-time multiplexed on-body analysis of UA, pH, and K^+ was performed in sweat during aerobic exercise. The relationship between urine UA and sweat UA levels after consuming purine-rich foods was further investigated.

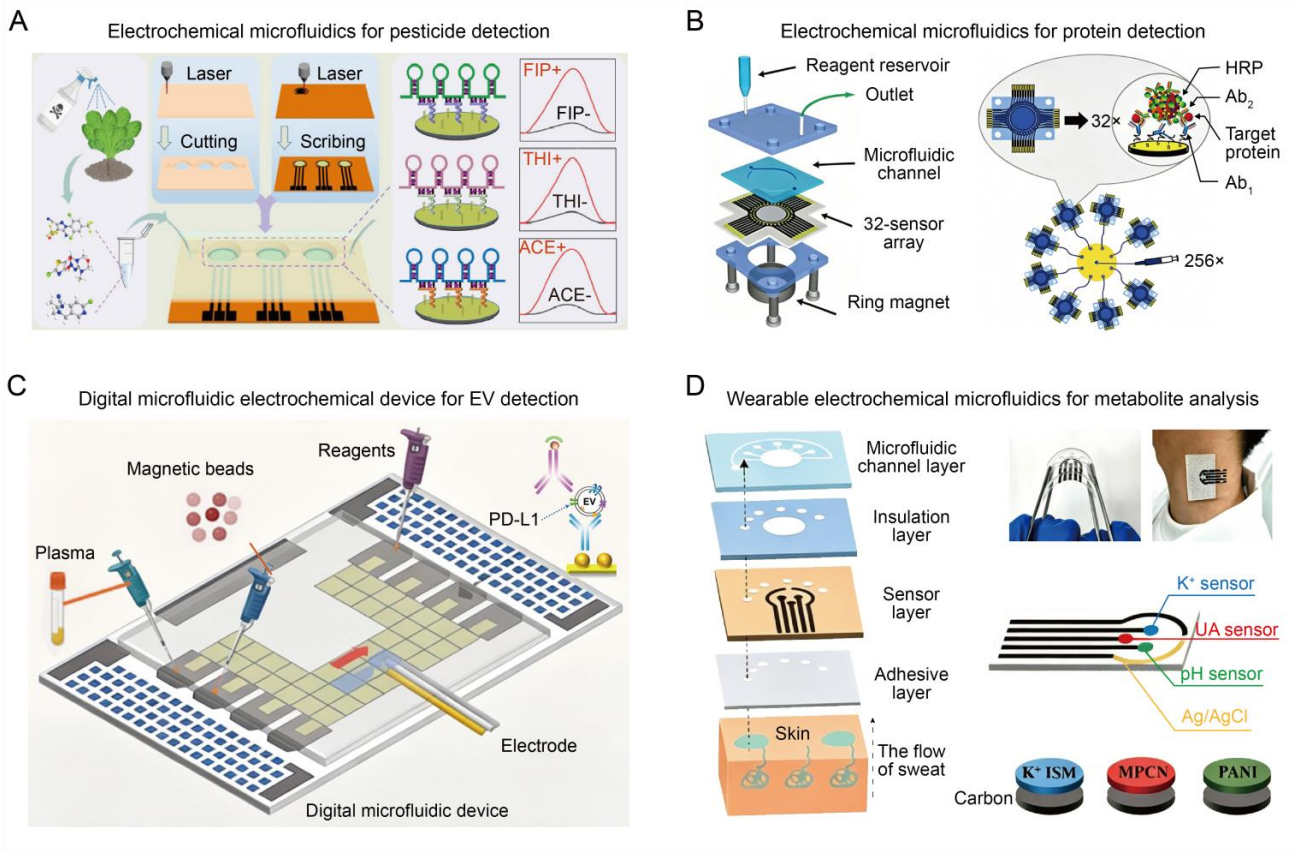

Figure 5. Microfluidic electrochemical assays for biochemical analysis. **(A)** Schematic diagram of an electrochemical microfluidic device for pesticide detection. Reproduced with permission [65]. Copyright 2026 American Chemical Society; **(B)** Schematic of an electrochemical microfluidic platform for protein detection. Reproduced with permission [66]. Copyright 2016 American Chemical Society; **(C)** Illustration of a digital microfluidic electrochemical device for EV detection. Reproduced with permission [69]. Copyright 2025 Wiley-VCH GmbH; **(D)** Schematic illustration of a wearable electrochemical microfluidic device for sweat metabolite analysis. Reproduced with permission [99]. Copyright 2024 American Chemical Society.

Taken together, microfluidic electrochemical assays offer a highly sensitive, miniaturizable platform for biochemical analysis, particularly suited for wearable devices, real-time monitoring, and point-of-care applications. However, challenges remain in terms of long-term stability, biofouling, sensor drift, and reproducibility across different electrode batches. Future efforts toward surface functionalization strategies, reference electrode stabilization, and disposable chip designs will be key to translating electrochemical microfluidic platforms from laboratory prototypes to commercial products.

7. Microfluidic mass spectrometric assays

Mass spectrometry (MS) is a versatile analytical technique that identifies and quantifies target molecules by measuring the mass-to-charge ratios of ionized species [100,101]. Unlike optical or electrochemical methods that typically require target-specific probes, MS can simultaneously profile hundreds of compounds in complex biological matrices in a label-free manner, making it especially suitable for high-throughput analysis of complex samples [102,103]. However, conventional MS analysis often relies on labor-intensive sample preparation and offline separation steps, which limits its high-throughput potential for biochemical applications. Microfluidics, with its ability to integrate sample pretreatment, purification, chemical reaction, and separation onto a single chip, can seamlessly couple with electrospray ionization (ESI) or matrix-assisted laser desorption/ionization (MALDI) interfaces via specialized emitters or target plates [104–106]. The microfluidic-MS combination significantly reduces sample consumption, shortens analysis time, and minimizes human intervention, facilitating high-throughput, automated, and sensitive detection of small molecules, lipids, peptides, and even intact proteins directly from biological samples.

Zhang *et al*. [70] developed a microfluidics-based MS platform for EV protein profiling. Employing 4D-DIA mass spectrometry, they identified over 2000 proteins from serum small EVs isolated from patients with esophageal squamous cell carcinoma, from which 14 differentially expressed candidate biomarkers were extracted and prioritized. Ma *et al*. [71] reported an inertial microfluidics-based MS platform for CTC detection in blood sample (Figure 6A). In this approach, cells were first labeled with phenylboronic acid-functionalized gold nanoparticles targeting overexpressed sialic acid on the cell surface. Inertial focusing in a multi-stream microfluidic chip was performed to drive larger tumor cells toward the central channel for direct MS detection, while smaller leukocytes were removed through side streams. Finally, the isolated tumor cells were detected by inductively coupled plasma MS (ICP-MS) analysis of gold nanoparticles. The method was applicable to various circulating tumor cells with both epithelial and mesenchymal origin, and achieved a LoD of 28 cells mL^{-1}. Xing *et al*. [107] reported an open microfluidic extraction method that integrated MS and DNA-mediated RCA for EpCAM analysis in tumor cells. Two open microfluidic chips were designed: one was an open-space chip with hydrophilic regions for on-chip cell culture, aptamer modification, RCA, and denaturant-induced probe release without closed channels or complex pumping systems, and the other was a regional open microfluidic device for localized collection of DNA probes from individual tumor cells for direct MS analysis. This method successfully distinguished HepG2 tumor cells from normal HUVECs and was further applied to evaluate the drug efficacy of 5-fluorouracil on tumor cells.

Zhang *et al*. [108] designed a microfluidics-based MS system for simultaneous analysis of single-cell marker expression and drug uptake. The method utilizes biofunctionalized nanoprobes (BioNPs), conjugating 6-FAM-Sgc8 aptamers on a single gold nanoparticle to selectively bind PTK7 on target cells, followed by laser-induced fluorescence (LIF) detection of PTK7 expression and ICP-MS measurement of ^{195}Pt to reveal oxaliplatin uptake in individual cells. The spiral microfluidic chip integrated periodic high-curvature structures and size-restriction pillars, enabling cells focusing into a stable single-file stream under extremely low flow rates through secondary flow effects. A hydrodynamic filtration structure was used to remove free probes and matrix interference online. The microfluidic chip was connected to ICP-MS via a horizontal interface, greatly improving single-cell

delivery efficiency and achieving a throughput of 500 cells per minute. Using this platform, heterogeneous PTK7 expression and drug uptake profiles were mapped across nine tumor cell lines, and distinct oxaliplatin-response signatures among single CTC from ten breast cancer patients were revealed. Xing *et al*. [109] advanced microfluidic MS into label-free dynamic single-cell metabolism studies, and revealed single-cell heterogeneity in glutamic acid and fatty acid metabolism in A549 and other cell lines. Zhang *et al*. [110] further designed an integrated system consisting of a sandwich-structured microfluidic chip coupled with ESI MS for real-time monitoring of metabolic and proteomic changes during the filamentation of extended-spectrum beta-lactamase-producing *E. coli* under antibiotic stimulation. Bacteria were continuously cultured and stimulated with antibiotics inside the chip; after *in situ* lysis, metabolites were directly infused into MS analysis. This platform allowed synchronous profiling of metabolic and protein networks under bacterial stress, revealing the upregulation of the c-di-GMP signaling pathway.

Digital microfluidics have also been widely integrated with MS. Zhao *et al*. [72] established a digital microfluidics-based MS platform for rapid enrichment and lipidomic profiling of EVs from ultra-low-volume samples (Figure 6B). Microfluidic device streamlined magnetic bead functionalization, EV capture, multi-step washing, and direct lipid extraction with organic solvents, into a 15-minute workflow, with a sample consumption less than 2.5 μL. The treated sample was further subjected to nano-flow liquid chromatography-MS analysis. Using this platform, 349 lipid species were identified from HeLa cell-derived EVs, and distinct lipid signatures of EVs secreted by M0 and M2 macrophages were also uncovered. Li *et al*. [73] reported an integrated digital microfluidics platform coupled with laser desorption ionization mass spectrometry (DMF-LDI-MS) for rapid and automated microbial metabolic analysis. This configuration eliminates the need for complex ESI interfaces, allowing direct analysis of dried droplets from the DMF chip. The system achieved a LoD of 2.86×10^{-7} M for verapamil with a dynamic range of three orders of magnitude, and performed on-chip bacterial fingerprinting and metabolic profiling using only 1 μL of sample within 8 minutes. The platform successfully differentiated clinically isolated bacterial strains and identified alanyl-alanine as a potential biomarker for distinguishing Gram-negative from Gram-positive bacteria. Yan *et al*. [111] developed a temperature-responsive agarose-based digital microfluidic platform (TRA-DMF) combined with liquid chromatography-tandem mass spectrometry (LC-MS/MS) for trace and single-cell proteomic analysis. The DMF chip integrated single-cell capture, lysis, reduction/alkylation, and enzymatic digestion into a single process. By leveraging the solid-liquid phase transition property of agarose, digestion products were encapsulated into solid microspheres upon cooling, which resolved the incompatibility between oil-phase microfluidics and MS and facilitated contamination-free product transfer to downstream LC-MS/MS. The platform identified over 4000 protein groups from 50 293T cells and up to 826 protein groups from a single 293T cell with good reproducibility.

Although the above studies realized the application of microfluidic MS in single-target protein analysis and dynamic omics of bacterial populations, both lack the ability to resolve spatial heterogeneity in tissues. The parallel-flow projection and transfer learning across omics data (PLATO) framework reported by Hu *et al*. [112] fundamentally overcomes this limitation by integrating microfluidic sampling, MS-based proteomics, and deep learning-assisted spatial reconstruction, enabling high-resolution spatial proteomic mapping of tissue sections (Figure 6C). This framework employs a parallel microchannel chip to perform regional enzymatic digestion and sampling on serial tissue sections along two orthogonal

directions. Thousands of protein expressions across the entire tissue can be obtained with only dozens of LC-MS/MS runs. Using spatial omics data from intermediate sections as references, a transfer learning algorithm reconstructs one-dimensional channel projections into two-dimensional spatial distribution maps. Microfluidics replaced the laborious point-by-point sampling of traditional laser capture microdissection (LCM) with ultra-low-throughput sampling, improving spatial resolution to 25 μm. This approach identified spatially distinct tumor subtypes and microenvironmental protein signatures in breast cancer tissues, establishing a new paradigm for advancing microfluidic-mass spectrometry technology from compositional analysis to spatial localization.

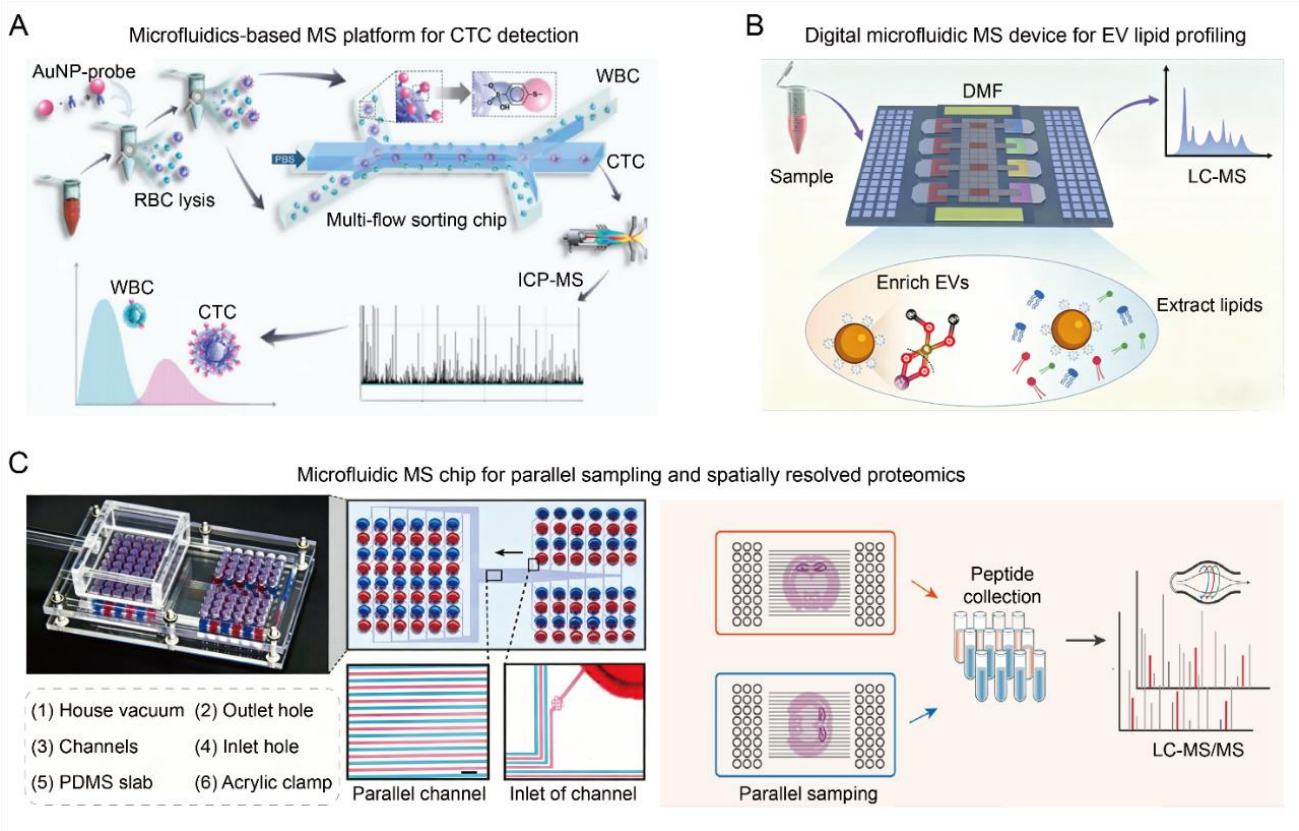

Figure 6. Microfluidic mass spectrometric assays for biochemical analysis. **(A)** Schematic of a microfluidics-based MS platform for CTC detection. Reproduced with permission [71]. Copyright 2025 American Chemical Society; **(B)** Schematic diagram of a digital microfluidic mass spectrometric device for EV lipid profiling. Reproduced with permission [72]. Copyright 2026 American Chemical Society; **(C)** Illustration of a microfluidic mass spectrometric chip for parallel sampling and spatially resolved proteomics. Reproduced with permission [112]. Copyright 2025 Elsevier.

In summary, microfluidic MS assays uniquely leverage the label-free, high-throughput, and broad-coverage capabilities of MS while addressing its conventional limitations in sample consumption, pretreatment complexity, and automation. This combination enables comprehensive profiling of small molecules, lipids, peptides, and proteins from minute sample volumes, with recent advances extending to single-cell and spatial proteomics. Nevertheless, current systems still require expensive, bulky mass spectrometers and skilled operators, which restrict their use to centralized laboratories. Continued innovation in chip-to-MS interfaces, automated data processing pipelines, and integration with

separation techniques will be essential to realize the full potential of microfluidic MS analysis in clinical and biological research.

8. Conclusion and perspective

Microfluidics provides a useful tool for biochemical analysis, addressing the limitations of conventional platforms such as bulky instrumentation, time-consuming procedures, and inadequate sensitivity for low-abundance targets [113]. By enabling precise manipulation of nanoliter-to-picoliter fluids within micron-scale channels, microfluidics significantly enhances mass and heat transfer, accelerates reaction kinetics, and integrates multiple analytical functions, from sample pretreatment to signal readout, onto a single chip. Here, we have reviewed the successful harnessing of these advantages across five major readout modalities. Colorimetric microfluidic platforms, particularly paper-based devices, offer low-cost, instrument-free point-of-care testing with visible readouts. Fluorescence-based systems provide superior sensitivity and dynamic range, enabling single-molecule and single-cell analysis through droplet and digital microfluidics. SERS microfluidic chips deliver ultrasensitive, fingerprint-like molecular spectra, achieving LoD down to the attomolar and single-particle level. Electrochemical microfluidic assays combine inherent miniaturization and portability with high sensitivity, facilitating wearable and real-time biomarker monitoring. Mass spectrometry coupled with microfluidics allows label-free, high-throughput profiling of hundreds of compounds from minute sample volumes, with recent advances even enabling high-resolution spatial proteomics. Collectively, these platforms have demonstrated robust performance across a wide spectrum of biological targets, including small molecules, proteins, nucleic acids, EVs, and cells, offering a novel technological foundation for diverse fields ranging from basic life science research and clinical diagnostics to environmental monitoring [114,115]. Despite the considerable progress in microfluidics-based biochemical analysis presented in this review, several fundamental challenges still exist in this field [116]. First, each readout modality has inherent trade-offs: colorimetry is simple but has limited sensitivity; fluorescence and SERS are sensitive but require sophisticated instrumentation; electrochemistry is rapid but suffers from electrode fouling; MS is highly capable but relies on bulky, expensive equipment. Second, most devices still depend on external pumps or complex peripherals, limiting their use beyond well-equipped laboratories. Third, the path from prototypes to products is slowed by a lack of standardized fabrication protocols and insufficient clinical validation.

In the future, several directions could be pursued to advance the practical application of microfluidic biochemical assays. The adoption of emerging low-cost manufacturing techniques, such as injection molding, roll-to-roll processing, and 3D printing, could significantly improve the reproducibility and cost-effectiveness of device production [117]. Meanwhile, the exploration of new materials beyond PDMS, including thermoplastics, paper, or hybrid substrates, could enhance the mechanical robustness, optical clarity, and surface compatibility of microfluidic chips, making them more adaptable to diverse detection scenarios [118]. Moreover, hybrid systems that combine multiple readout modalities on a single chip could provide more comprehensive results and information from a single assay, improving analytical accuracy and expanding the utility of the platform. Besides material and modality integration, microfluidic chip architecture and fluidic manipulation strategies could be further optimized to extend functional capabilities, such as multi-step reactions and temporal control of reagent delivery, which would reduce dependence on external infrastructure and make the devices more suitable for use in non-laboratory settings [119]. Finally, the establishment of standardized fabrication protocols and

quality control metrics would not only improve batch-to-batch reproducibility but also expedite the translation of academic innovations into commercially viable products, ultimately accelerating the transition of microfluidic biochemical assays from laboratory to real-world applications.

In addition, the adoption of AI is providing new opportunities for microfluidic biochemical analysis [120]. Beyond its well-recognized role in signal classification, AI can assist in image and spectral analysis for automated and objective readout, reducing subjectivity in readout interpretation and enabling rapid analysis even on portable devices. AI models are also well suited for signal correction, as they can compensate for environmental variations, background interference, and device-to-device inconsistencies that often compromise reproducibility. Furthermore, in the design phase, machine learning can guide the rapid optimization of channel geometries, droplet generation parameters, or electrode layouts through predictive simulation, significantly shortening development cycles [121]. During manufacturing, AI-driven pattern recognition can detect fabrication defects and ensure batch-to-batch consistency. For clinical applications, AI offers the ability to integrate multi-parametric sensor data with patient records and reference databases, providing actionable diagnostic recommendations [122,123]. Collectively, these capabilities make AI a valuable component of next-generation microfluidic biochemical analysis platforms.

Wearable microfluidics represents a promising frontier for future biochemical analysis, yet its practical application remains hindered by several challenges, including reliable biofluid sampling, long-term device stability, dependence on batteries, and real-time data transmission [124]. Developing flexible and breathable substrate materials, antifouling surface coatings, and stable signal readout over extended periods should be considered [125]. Further advances in fluid handling, such as capillary or vacuum-driven flow, are also needed to reduce reliance on external pumps. Meanwhile, biofuel cells and triboelectric nanogenerators that convert physiological activities into electrical power offer a promising route to eliminate external batteries [126]. As for data transmission, low-power electronics and wireless communication modules (e.g., Bluetooth) would enable continuous data streaming to portable devices, facilitating remote health monitoring and timely intervention [127,128]. With these advances, wearable microfluidic systems could eventually achieve truly continuous, maintenance-free health monitoring in daily life.

With continued progress in materials, fabrication, system integration, and data analytics, microfluidic biochemical analysis is well positioned to provide powerful, accessible tools for personalized medicine, noninvasive health monitoring, and next-generation diagnostics.

Declaration of generative AI and AI-assisted technologies

The authors did not use generative AI or AI-assisted technologies in the writing of this manuscript.

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Authors' contribution

Conceptualization, Jiashu Sun and Jinqi Deng; investigation, Jiale Lv and Shuai Zhao; writing—original draft preparation, Jiale Lv and Shuai Zhao; writing—review and editing, Jinqi Deng and Jiashu Sun; visualization, Shuai Zhao and Jiale Lv; project administration, Jiashu Sun; funding acquisition, Jiashu Sun, Jinqi Deng, and Shuai Zhao. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

Jiashu Sun holds the position of Editorial Board Member for *Life Analysis* and has not peer reviewed or made any editorial decisions for this paper.

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