Emerging optofluidic technologies for biodiagnostic applications

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Abstract
The unprecedented global COVID-19 pandemic strongly argues the critical need for innovative diagnostic tools meeting the requirement of test speed, accuracy, and throughput. Owing to the integration of optics and microfluidics technologies, optofluidic technology enables highly precise flow manipulation and highly sensitive signal detection at the microscale, thus offering the promising potential for developing biodiagnostic applications. Research toward this direction is fast growing into an emerging area. In this paper, we give an overview of emerging optofluidic technologies for biodiagnostic applications with the focus on three common types of biomarkers: nucleic acid, protein, and cell. We conclude by discussing the challenges, opportunities, and future perspectives of this field.

KEYWORDS
biodiagnosis, biosensor, microfluidics, optofluidics
1 | INTRODUCTION

Microfluidics technology has been rapidly growing and widely applied in the fields of chemical and biological sensing over the past three decades since its onset in the early 1990s. However, many of these developed microfluidic tools still rely on bulky and complicated peripheral control and detection equipment. Since 2005, the concept of “optofluidics” has been proposed in several publications, with the basic idea of integrating optical technologies with microfluidic systems to enable more flexible, compact, and accurate analysis. Since then, this field has been evidently blossoming and advancing in many aspects, ranging from the novel design and fabrication of on-chip optical components, to the integration of optical and fluidic system, and to the various analytical applications. Several previous excellent reviews have comprehensively summarized the past developments of optofluidic technologies including the fundamentals and an extended list of applications. In this review, we placed the focus on the developments of optofluidic technologies for biodiagnostic applications, providing interested readers with a timely update of this growing field.

Early diagnosis is one of the most important approaches to permit preventive health care for many diseases. Nucleic acid and protein biomarkers are the two most common classes of biomarkers for disease diagnosis with high specificity. In addition, cell-based biomarkers have drawn increasing attention in recent years because the relevant cellular properties are revealed to be useful for disease diagnosis and assessment of other health problems. Although these diagnostic tests can be performed using conventional instruments available in centralized clinical laboratories, high cost and long turn-around time make them ill-suited for routine testing in small clinics or even self-testing at home. As such, point-of-care (POC) testing is a key enabling approach to address these issues. The integration features of optofluidic technology can (1) accelerate the reaction speed due to the short diffusion distance, high surface-to-volume ratio, and efficient heat transfer; (2) improve the sensitivity due to the efficient mass transfer in a small volume and enhanced light–matter interaction using on-chip optical modules such as optofluidic laser; (3) reduce the cost due to the lower reagent and sample consumption; (4) miniaturize the diagnostic system because of the integrated optics in chips. Those advantages make the optofluidics a promising solution for biodiagnosis. The advances of optofluidic technology will be useful for addressing global health issues such as the current COVID-19 pandemic in many aspects, from the direct detection of the COVID-19 virus using optofluidic genetic detection system to antibody-based blood diagnosis test using optofluidic protein detection tool, and to antibody screening for therapeutic purpose using optofluidic cell analysis platform. Here, we review the developments of optofluidic technologies used in biodiagnostic applications covering all three classes of biomarkers, that is, nucleic acid, protein, and cell.

2 | OPTOFLUIDIC GENETIC ANALYSIS FOR BIODIAGNOSTIC APPLICATIONS

The genetic testing is a common and indispensable approach in biodiagnosis. It can effectively identify the infectious agents and determine the genetic mutations. In this section, we will review the development of microfluidic and optofluidic techniques for genetic testing with biodiagnosis purposes. We will first introduce the development of genetic testing methods based on microfluidics and polymerase chain reaction (PCR). Although many of these microfluidic PCR techniques do not integrate the on-chip optical components; strictly speaking, they may not be suitable to be classified as optofluidic techniques, we think it will be useful to give an overview on this field considering PCR’s significant role in genetic testing-based biodiagnosis. Then we will review the amplification-free nucleic acid detection methods based on various optofluidic platforms.

2.1 | Microfluidic PCR-based genetic testing for biodiagnostic applications

PCR is a biotechnology that can rapidly amplify a specific DNA sample through three thermal cycling processes: denaturation, annealing, and extending. Since the early microfluidic PCR device reported in 1993, this field has developed a lot in recent years because the microfluidic PCR systems possess attractive advantages over the traditional tube-based PCR test; for example, on-chip sample preparation, low sample consumption, rapid reaction, and high throughput. Depending on whether the reagent solution is continuous or partitioned, microfluidic PCR systems can be classified into two categories: the continuous-flow PCR and the digital PCR.

For continuous-flow PCR, recent advances have been made toward several directions: (1) Operation simplification and system integration. For example, Tachibana et al. developed continuous-flow PCR in a microfluidic device, where self-propelled fluid transportation was realized by capillary forces. The device was fabricated on a Si wafer and the hydrophilic surface was achieved via thermal oxidation. The amplifications of β-actin, AH1pdm influenza virus, and 16S rDNA of Escherichia coli genomic DNA was validated using this chip, but the detection
was still using the off-chip gel electrophoresis method. Li et al. developed an all-in-one microfluidic PCR instrumental system, which incorporates a membrane-deflection pumping method, on-chip electrophoresis, and fluorescent detection, allowing automatic sample injection and signal detection.\cite{16} The device successfully amplified three periodontal pathogens (*Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia*). Nguyen et al. developed a similar device for PCR amplification, but instead of using electrophoresis, a surface plasmon resonance (SPR) optical fiber sensor was integrated into the device to enable label-free detection of the DNA amplicon (Figure 1A).\cite{17} The DNA of *Salmonella* spp. was amplified using this system within 30 min.\cite{2}

Simple device fabrication and easy thermal cycling control. In this regard, Salman et al. used a micromilling and thermal fusion bonding to fabricate a microfluidic PCR device.\cite{18} The thermal cycling was realized by shunting the device between three double-side temperature zones. Successful detection of *Escherichia coli* was verified in this low-cost and portable device. In another study, Trinh and Lee simply used the polytetrafluoroethylene (PTFE) tube as the capillary for PCR reaction, which was sandwiched by two glass slides using PDMS as fixation agent.\cite{19} Two well-known foodborne pathogens, *Escherichia coli* and *Salmonella* spp., were amplified within 20 min in this device. Fernandez-Carballo et al. chose a cost-effective roll-to-roll embossing method to fabricate a microfluidic reverse transcription PCR device, providing a solution for the mass production of devices.\cite{20} This chip includes several different zones for reagent mixing, reverse transcription, polymerase activation, and thermal amplification, allowing the detection of RNA-based viral pathogens such as Zika virus and chikungunya virus.

Compared with continuous-flow PCR, the digital PCR device divides the reaction solution into a large number of small partitions, allowing the absolute quantitation of the samples by counting number of partitions with positive signals. The droplet and microwell array are the two most common formats to generate small partitions for digital PCR. Various developments have emerged to improve both digital PCR formats. The droplet digital PCR (ddPCR) dispenses the reagent solution into small water-in-oil droplets. Recent efforts have been devoted to improving the ddPCR performance and developing new effective approaches to generate droplets. Bian et al. used...
the mineral oil-saturated polydimethylsiloxane (PDMS) to fabricate the ddPCR device, which can prevent the droplet evaporation that often causes the failure of PCR amplification.\textsuperscript{[21]} By utilizing two different fluorescent probes and optimizing the reagent composition, the device can simultaneously detect two types of pathogens, the *Escherichia coli* O157 and *Listeria monocytogenes* with a single molecule resolution. In addition to the using the classic T-junction method to generate droplets, other novel methods have recently been proposed to generate PCR droplets at high speed and high uniformity. Li et al. used a capillary with beveled outlet interface connecting with a pump to directly eject nanoliter droplets into oil phase.\textsuperscript{[22]} The size of droplets could be fine-tuned by adjusting the inner diameter of the capillary and the flow rate. Pan et al. applied a microfluidic impact printer technology to print well-patterned nanoliter droplets on a hydrophobic surface.\textsuperscript{[23]} After covering the droplet array with oil, the PCR amplification could be performed. The device revealed the different levels of *p53* gene between colon cancer tissues and adjacent nontumorous tissues. The microwell array-based digital PCR is another widely used digital PCR system that has been applied for biodiagnosis. Efforts have been devoted toward improving the digitization efficiency. In microwell-based digital PCR, filling the reagent solution into the microwell dead zone is a challenging task. The vacuum pressure is usually adopted to drive the solution into the microwells, but the efficiency sometimes is problematic. To address this issue, Zhou et al. proposed an alternating pull–push strategy to facilitate this process. A valve was placed at the outlet end of the chip. The valve was firstly closed to allow the vacuum pulling the solution into microwells, followed by opening the valve and allowing the air pressure to further push the solution into the microwell. By periodically repeating this process, the solution can be rapidly filled into the microwells with almost 100% sample digitization efficiency, which will be useful for analyzing the samples with small volume, such as the single-cell analysis. Some other studies explored new applications of microwell-based PCR devices. For example, Wu et al. cleaved the unmethylated DNA strands while keeping the methylated ones intact before PCR amplification, thus allowing assessment of the DNA methylation level. Using this method, they demonstrated different methylation levels of two tumor suppressor genes (*PCDHGB6 and HOXA9*) between the tumor tissues and the adjacent nontumorous tissues from patients with early-stage lung adenocarcinoma. Dueck et al. developed an integrated digital PCR instrument to automate the solution digitalization, thermal cycling, and signal-detection processes. This device was validated by quantifying non-small cell lung carcinoma rare genetic mutants (EGFR T790M). However, the samples still need to be prepared manually. Further integration of the sample processing module that enables sample to answer testing will be attractive for diagnostic applications.

Overall, the microfluidic PCR technology is becoming more integrated and mature, which will maintain its important role and broaden its use in biodiagnosis.

### 2.2 Optofluidic amplification-free genetic testing for biodiagnostic applications

Although PCR is a powerful tool for genetic testing, the nucleic acid amplification process requires dedicated sample preparation and complicated instrument. The PCR detection is usually slow and costly. The direct detection of genetic materials without amplification is an attractive approach to meet the low-cost and fast-detection requirement in POC testing. The optofluidics is an enabling technology to achieve the amplification-free detection. Indeed, various optofluidic platforms have been reported to detect the virus agents or gene mutations for biodiagnostic purpose. In this section, we will review these optofluidic tools based on different optical techniques. Table 1 lists an overview of various optofluidic technologies for amplification-free genetic testing.

Nowadays, the SPR sensor has become a gold standard for label-free measurement of the kinetics and affinities of the biomolecule. SPR happens in the interface between a thin metal layer and a dielectric layer and is sensitive to the interfacial refractive index change. Most commercial SPR systems are based on the classical Kretschmann configuration in which a prism is used to couple light into the surface plasmons. While this approach is sensitive, it requires precise optical alignment and bulky instrument. Different types of optofluidic approaches provide promising solutions to address this issue. For example, on-chip waveguide\textsuperscript{[24]} and fiber-based waveguide\textsuperscript{[25]} SPR sensor have been developed to couple the light into surface plasmons. The refractive index change induced by the analyte interactions on the surface can be easily detected by monitoring the power change of the output light. Recently, the nanoplasmonic devices that integrate subwavelength nanohole arrays into metallic film have emerged as a distinctive type of biosensor as they allow direct coupling of the perpendicularly incident light into plasmons and the extraordinary transmission signals in plasmonic nanohole arrays enables spectral measurements with high signal-to-noise ratios. Yanik et al. developed an antibody-coated optofluidic nanoplasmonic device to directly and specifically detect live viruses from biological samples (Figure 1B).\textsuperscript{[26]} Spectral shift of the transmitted light is clearly observed when the virus binds to the sensing surface. The
**TABLE 1**  Summary of optofluidic amplification-free genetic testing for biodiagnostic applications

| Detection targets | Optofluidic technology | Main results | Advantages | Ref. |
|-------------------|------------------------|--------------|------------|-----|
| Small enveloped RNA viruses (vesicular stomatitis virus and pseudotyped Ebola) and large enveloped DNA viruses (vaccinia virus) | Nanohole array-based nanoplasmonic sensor | The sensor could detect the viruses with low detection limits (<$10^7$ pfu/ml) and a dynamic range from $10^6$ to $10^9$ pfu/ml | Little to no sample preparation; direct coupling of the perpendicularly incident light into the sensor | [26] |
| Filamentous bacteriophage M13 | Whispering gallery mode (WGM) resonator in a microring sensor | The sensor could detect M13 with high specificity and sensitivity. The detection limit is $2.3 \times 10^3$ pfu/ml and the dynamic range spanned seven orders of magnitude | High sensitivity and wide detection dynamic range | [28] |
| DNA melting analysis | Optofluidic ring resonator laser | Based on the sharp phase transition between laser emission and fluorescence as the DNA melts, the sensor could distinguish between the target and the single-base mismatched DNA of up to 100 bases long | Rapid and sensitive | [29] |
| Influenza viruses H1N1, H2N2, and H3N3 | A single multimode interference (MMI) waveguide to create excitation spot patterns | The chip can detect multiplex single virus based on either single labeling or combinatorial fluorescent labeling | The detection is based on time-dependent fluorescence signal, thus no spectral emission filters are required | [30] |
| Zika nucleic acid | | | | |
| Cancer DNA biomarkers in blood | | | | |
| Single-strand DNA | Planar photonic crystal waveguide | Demonstrate the detection of DNA hybridization with an estimated detection limit as low as 19.8 nM | The detection is real-time and label-free | [31] |

Detection of three types of virus (VSV, PT-Ebola, and Vaccinia) in PBS and mimicked biological media (cell growth medium + fetal calf serum) has been demonstrated.

The whispering gallery mode (WGM) resonator is a specific type of optical sensor that has attracted intensive interest recently due to its extreme sensitivity.[27] The high sensitivity of WGM systems come from the repeated interactions of light with the matters as it circulates along the edge of the resonator. The optofluidic platforms have been developed to implement the WGM resonator in detecting the genetic materials. Zhu et al. developed an optofluidic ring resonator (OFRR) to realize label-free detection of virus (Figure 1C).[28] A quartz capillary was heated and pulled to form the OFRR with 150 μm OD. The inner surface of the capillary coated by the virus antibodies can efficiently and specifically trap the viruses that pass through the capillary. A tapered fiber that contacts the capillary perpendicularly coupled the input laser into the ring resonator to form WGMs at certain wavelength, resulting in a reduction of the laser power at the output. The shift of resonance wavelength caused by the RI change of the waveguide surface due to the virus–antibody binding was used to determine the virus concentration with high sensitivity. The sensor could detect the filamentous bacteriophage M13 with a detection limit of $2.3 \times 10^3$ pfu/ml.

DNA melting curve analysis is a useful process to characterize the dissociation dynamics of double-stranded DNA (dsDNA) by heating, which is very useful to detect gene mutations and single-nucleotide polymorphisms. The traditional method to assess the DNA melting curve is based on the fluorescent change as temperature increases. However, the thermal dynamic difference between target and mismatched DNA is usually very small and diminishes with the increase in sequence length. Lee and Fan developed an OFRR to amplify the dynamic difference.[29] The DNA and saturation dye solution were filled into the OFRR as the laser gain medium. As the dissociation of dsDNA with the temperature increases, the lasing threshold condition could not be maintained, resulting in the termination of the laser emission. This laser signal difference is much higher than the fluorescent signals, thus enabling accurate determination of the normal DNA and mismatched DNA.

Aside from high sensitivity and specificity of one single biomarker, the detection of multiple biomarkers is another key approach to increase the diagnosis accuracy. Recently, a novel wavelength division multiplexing strategy based
on a multimode interference (MMI) waveguide has been developed to enable multiplexed single biomolecule detection on an optofluidic chip (Figure 1D).[30] The spatially separated spot patterns generated by the interference of different waveguide modes in the solid MMI waveguide can be used to excite the fluorescent-labeled bioparticles that are transported into the intersection part of a fluidic microchannel and this MMI waveguide. The fluidic channel also acts as a liquid-core antiresonant reflecting optical waveguide (ARROW) to guide the emission light to the detector. When fluorescent-labeled analyte particles pass through the detection area, a time series of signal will be captured. The number of signal peaks is the same as the number of the excitation spots. As different wavelengths have different number of spots in the detection zone, the number of signal peaks could be used to distinguish the wavelengths without using emission filters. Multiple analytes labeled with different single or combinational fluorescent dyes in the sample can be resolved by analyzing the timeseries signal patterns. This platform has been well applied in the detection of different influenza A subtypes,[30] cancer DNA biomarkers in blood,[31] and Zika nucleic acid and protein biomarkers.[32]

Recently, the integration of optofluidics with photonic crystal (PC) cavities has drawn increasing attention for biosensing applications.[33] PC is a periodic nanohole structure in dielectric materials with high refractive index. This structure allows some wavelengths to pass while stopping the other wavelengths; the groups of these wavelength modes are called pass bands and stop bands. A PC cavity can be formed by modifying or removing some points in the orderly arranged hole arrays. The PC cavity has high quality factor Q because it can efficiently confine the light in the cavity. The resonant wavelength is highly sensitive to the refractive index variations in the surrounding medium. The research of using PC cavities for genetic testing has been reported but this area is still in its early stage. Hsiao and Lee conducted a computational study showing the capability of a PC nano-ring resonator for biomolecule sensing including DNA.[34] Toccafondo et al. experimentally demonstrated the detection of single-strand DNA (ssDNA) using a planar PC optofluidic sensor.[35] The specific detection was based on hybridization of the target ssDNA with the complementary DNA strand, which was previously functionalized on waveguide surface. The detection limit was estimated to be 19.8 nM.

3 OPTOFLUIDIC PROTEIN ANALYSIS FOR BIODIAGNOSTIC APPLICATIONS

Bioanalytical assays for detecting specific protein biomarkers or measuring total concentration of proteins including enzyme-linked immunosorbent assay (ELISA), bichonic acid assay, Bradford protein assay, and Lowry protein assay are reliable and straightforward pathways for diagnosis of inflammatory disease and pathogenic infection. ELISA is regarded as a gold standard for immunoassay, which is widely applied for disease evaluation and cancer detection such as monitoring cardiovascular disease,[36–38] Alzheimer,[39] and rheumatoid arthritis.[40] Traditionally, the assay of protein is mainly conducted in the commercial testing kits in which relevant reagents including secondary antibody and substrate solution are provided, and the assay is conducted in a 96-well plate that is coated with antigen/antibody to capture target proteins or other molecules. However, traditional assays have complex and tedious operating procedures. To make it easy, the assay is partially or fully done with the assistance of various instruments, including plate washer, plate reader, luminometer, and automated workstation. The cost of the expensive instruments would undoubtedly account for the expense of the assay. Additionally, to guarantee a reliable assay, the amount of samples and reagents used in the assay is large. The system of high volume in the static incubation condition takes a long time to accomplish the reaction. Even so, the detection limit of the traditional assay is high.

To overcome the general problems in the traditional protein assays, microfluidics technology provides an alternative way for rapid, simple, high-sensitivity, and low-cost assay. As the reaction occurs in a tiny, dynamic flowing system, only a small amount of samples and reagents are needed and the entire assay time could be significantly shortened.[41,42] Due to the flexible and compact design of the microfluidic chip, complex assays can be conducted in the portable and low-cost instruments, which are associated with the microfluidic chips.[43–45] Optofluidics technology as a superior type of microfluidic technology integrates add-on features of optical functions onto a single microfluidic chip, avoiding using external bulky optics. While featuring compact integration, optofluidic-based protein assay can also significantly enhance the assay sensitivity and improve the detection limit. Table 2 lists an overview of biodiagnostic applications based on various optofluidic protein analysis technologies.

3.1 Optofluidic label-requiring protein analysis for biodiagnostic applications

Optofluidic chips integrated with optical detectors are very suitable in the chemiluminescence immunoassay that is one of the most popular schemes for protein analysis. In the assay, the chemiluminescent substrate interacts with enzyme label that is associated to a secondary antibody,
| Target analyte | LOD | Assay time | Label-free assay | Detection mechanism | Optofluidic technology | Ref. |
|---------------|-----|------------|-----------------|-------------------|-----------------------|-----|
| CRP           | 1.4 nM (theoretical value) | – | No | Fluorescence detection | Silicon-based PIN photodiode packaged in the chip | [46] |
| Mouse IgG     | 0.182 pmol/cm² (calculated value) | 30 minutes | No | Chemiluminescence and colorimetry detection | An array of a-Si:H photodiodes (200 × 200 μm) integrated on the chip | [47] |
| Staphylococcal enterotoxin B | 0.5 ng/ml | 30 minutes | No | Chemiluminescence detection | Organic photodiode integrated onto a biosensor chip | [48] |
| IL-6          | 1 fg/ml | 1.5 hours | No | Fluorescence and laser emission | Optofluidic laser-based ELISA in which laser onset time reflects the enzyme–substrate reaction | [49] |
| Rabbit IgG    | 180 pg/ml | 20 minutes | No | Laser emission | Optofluidic laser-based turbidimetric inhibition immunoassay | [50] |
| Ro/SS-A, Jo-1, centromere protein B, thyroglobulin, and DNA topoisomerase-1 (ccl-70) | fM regime | – | Yes | Fluorescence detection | Plasmonic protein microarrays | [51] |
| *Escherichia coli* and *Staphylococcus aureus* | 105 CFU/ml | 20 minutes | Yes | SPR angle shifting | Microfluidic-integrated surface plasmon resonance | [52] |
| IgG2          | 500 ng/ml | Real-time | Yes | Phase response of the plasmonic resonances | Plasmonic gold nanohole array | [53] |
| R6G           | $10^{-11}$ mol/L | Real-time | Yes | SERS response | Microfluidic chip with integrated Teflon AF1600 surface liquid core optical waveguide modified with nanogold | [54] |
| Dipicolinic acid | 50 nM | Real-time | Yes | SERS response | Microfluidic channel integrated three-dimensional liquid-core (silver nanocolloids suspended in the deionized water) and liquid-cladding (2,2,2-trifluoroethanol) waveguide | [55] |
| N-acetylaspartate, glial-fibrillary acidic protein, and S100B | 1 fM | Real-time | Yes | Raman intensity | Micrometer pillars covered with a plasmon-active nanometric gold layer integrated in the optofluidic chip | [56] |
| CA125, HER2, and CA15-3 | 620 fg/ml for HER2 antigen, 0.55 U/ml for CA125, and 1.84 U/ml for CA15-3 | Real-time | Yes | Total internal reflection angle and excitation angle of photonic crystal surface wave | One-dimensional photonic crystal (planar multiplayer stack) chip | [57] |

(Continues)
| Target analyte | LOD   | Assay time | Label-free assay | Detection mechanism | Optofluidic technology | Ref. |
|---------------|-------|------------|-----------------|---------------------|------------------------|-----|
| β-actin       | 255 ng/ml | Real-time | Yes             | Peak wavelength shifting induced by change in the local refractive index | Guided-mode resonance biosensor | [66] |
| CRP           | 3.2 ng/ml | Real-time | Yes             | Peak wavelength shifting induced by change in the local refractive index | Guided-mode resonance biosensor | [64] |
| Dinitrophenyl | 75 ng/ml | Real-time | Yes             | Detection of light intensity in both transmission and reflection modes | Guided-mode resonance biosensor | [65] |
| Troponin (TNNT1), procalcitonin (PCT), and CRP | 10 pg/ml for TNNT1 and PCT, and 1 pg/ml for CRP | Real-time | Yes | Position shifting in optical resonance | Chirped guided-mode resonance biosensor | [67] |

and the reaction gives off fluorescence light without any excitation. The emission of the fluorescence can be directly acquired by on-chip optical detectors, for example, silicon photodiodes [46,47] and organic photodiodes [48]. Because the photodiode is closely attached to the reaction area, signal loss and background noise in the detection system can be significantly eliminated. Sensitive detection of low-concentration analytes can be achieved by the integrated optical detector. It is worth noting that inexpensive, easy-to-fabricate and high-sensitive optical detector is preferred to meet the requirement of low-cost assay and mass production of the disposable optofluidic chips. In comparison, organic photodiodes present excellent features, because they can be realized on diverse materials such as glass and plastic by using low-cost fabrication methods. The integrated optical detector is applicable for hybrid chemiluminescence and colorimetric detection of fluorescein isothiocyanate or horse radish peroxidase-labeled antibody with concentrations of nanomolar (nM) to micromolar (μM) range. [47] The detection limit can be further improved by suppressing the dark current of the on-chip photodetector.

The optofluidic laser provides an extraordinary platform for protein detection with ultrahigh sensitivity. [11,49] The assay is conducted in the microfluidic channel, whose top and bottom are coated with reflective layers, forming a Fabry–Pérot cavity whose operating principle is illustrated in Figure 2A. Meanwhile, the cavity is under the illumination of the external excitation. The optofluidic laser starts to emit light when the concentration of the reaction product reaches a threshold. The schematic diagram of the optofluidic laser-based ELSIA is shown in Figure 2B. When the concentration of the analyte is low, it takes a long time for the reaction between enzyme and substrate. Thus, instead of emission intensity as a measurement, the laser onset time (i.e., the time duration when the laser emits the light) that is inversely proportional to the enzyme concentration, is used as a sensing signal. The optofluidic laser-based ELISA is very sensitive to a low-analyte concentration and has a large dynamic range. In the ELISA of interleukin-6, the detection limit of 1 fg/ml and the dynamic range of six orders of magnitude were realized. [11] Besides ELISA, the optofluidic laser can be used in the turbidimetric inhibition immunoassay, which could be used for quantification of protein concentration. [50] The antigen–antibody complexes mixed with the gain material were injected into the cavity of the laser. In the demonstration, IgG concentration was measured. A detection limit of 180 pg/ml along with the dynamic range of five orders of magnitude was achieved.

### 3.2 Optofluidic label-free protein analysis for biodiagnostic applications

In addition to integrating optical components in the optofluidic chip for direct observation of the fluorescent biomarkers, harnessing optical resonance by embedding nanostructure in the chip based on micro/nano-fabrication techniques is another extraordinary approach for high-sensitive protein analysis. Most importantly, protein analysis based on optical resonance phenomenon, which relies on the variation of the light resulting from the occurrence of protein reaction on the nanostructure, can realize real-time and label-free diagnostics.

Optofluidic technology utilizing SPR effect is an outstanding candidate for label-free proteomic analysis. [51–53]
and the related technology has been commercialized (e.g., SPRi platform from Horiba Scientific and Biacore SPR systems from Cytiva). The operating principle of SPR sensor and SPR-based biosensors is illustrated in Figure 2C,D. In the SPR optofluidic chip, a thin metal layer is coated on the substrate where protein can be immobilized for detection. When the substrate is excited by polarized light, an evanescent wave is generated and interacts with the protein. The change in dielectric refractive index near the metal surface has a significant response over the SPR phenomenon. By monitoring spectral shifts and intensity changes of the reflected light, high-sensitivity and broad dynamic-range protein detection can be realized. Furthermore, the dynamic change in molecular mass can be measured in real time so that binding stoichiometry and kinetics of the interaction between ligand and analyte can be evaluated. In addition to spectral shifts and intensity changes of the plasmonic resonances, the phase response is another important physical property that could be exploited for detecting atomically thin topographical changes. A nanostructured gold nanohole array, which could enhance phase signal from extremely small volumes near the surface
(<100 nm), over a large area was developed as a plasmonic biosensor on which thousands of microarray elements could be simultaneously monitored.[54] In the demonstration, the compact plasmonic biosensor could detect IgG2 of concentration as low as 500 ng/ml.

Surface-enhanced Raman spectroscopy (SERS) is an alternative surface-sensitive technique, which exploits interaction of molecules and nanostructured metallic surface to enhance Raman intensity. Metal nanoparticles or nanocolloids as SERS enhancement media could be immobilized on the surface of microchannels or suspended in reagents to interact with the analytes. SERS spectrum could be collected under the excitation illumination via liquid waveguide.[55,56] An optofluidic chip integrated with a SERS-active substrate, on which submicrometer pillars were electrohydrodynamically fabricated and covered with a plasmon-active nanometric gold layer, was employed to detect representative biomarkers, that is, N-acetylaspartate, glial-fibrillary acidic protein, and S100B, for traumatic brain injury. A linear relationship was achieved with good correlation between Raman intensity and biomarker concentration within the range of 1 fM to 100 nM.[57] The SERS-integrated optofluidic chip had the potential to help the diagnosis of traumatic brain injury.

PC has a nanostructure where refractive index is periodically modulated. Due to the existence of photonic band gap, PCs present excellent wavelength-selection capability. One-dimensional (1D) PC structure in periodic multilayer stacks could be simply constructed by film coating on the surface of the substrate.[58] A multilayer 1D PC-based optofluidic chip was demonstrated for multiplex detection of one ovarian cancer biomarker cancer antigen 125 (CA125) and two breast cancer biomarkers human epidermal growth factor receptor 2 (HER2) and cancer antigen 15-3 (CA15-3). High-sensitive detection (detection limit of 620 fg/ml for HER2 antigen, 0.55 U/ml for CA125, and 1.84 U/ml for CA15-3) is achieved.[59] Besides multilayer 1D PC, guided mode resonance (GMR) filter, known as resonant waveguide grating, is another type of PC consisting of 1D or two-dimensional periodic grating nanostructure that can couple far-field light to a leaky-waveguide mode in the structure and yield a resonant signal at a specific wavelength.[60,61] The operating principle of a GMR filter is illustrated in Figure 2E. Resonance condition changes with the local refractive index of the surrounding media. Compact GMR filters can operate in both transmission and reflection modes and easily be integrated into the optofluidic chip, forming a GMR optofluidic biosensor. Figure 2F demonstrates the GMR optofluidic biosensor-based platform for protein assay. In the label-free GMR optofluidic biosensor, resonance wavelength shifts with the concentration of protein attached to the nanostructure. The GMR optofluidic biosensor exhibits low detection limit and broad linear dynamic range.[62–66] Thanks to the advanced microfabrication techniques, multiple GMR sensors could be fabricated in a single optofluidic chip[67] in which multiple biomarkers, troponin, procalcitonin, and C-reactive protein, could be tested in parallel with low detection limits of 10 pg/ml.

It is indeed a great leap from the traditional protein assay to the optofluidics-based protein analysis techniques, which presents lots of advanced features including low limit and broad dynamic range of detection, rapid processing time, and low sample and reagent volumes. Despite that, more efforts are necessary to further improve optofluidic biosensors for proteomic bioanalytics. The throughput of the assay could be enhanced. It would be efficient to realize multiplexed protein assay for multiple samples simultaneously. In addition, most optofluidic biosensors have to work with peripheral instruments for illumination, light collection, spectrum analysis, and signal processing. For example, in the case of GMR optofluidic biosensing, polarized incident light matching the waveguide mode is required and optical spectrum analysis is needed for determination of peak wavelength. It is also an enormous challenge to build high-precision, portable, and low-cost instruments to fulfill the requirements of optofluidic protein analysis.

4 | OPTOFLUIDIC CELL ANALYSIS FOR BIODIAGNOSTIC APPLICATIONS

Cells are the basic and functional units of organisms. The characterization of cell properties is an essential part of life science research. The phenotypic differences between cells from healthy people and diseased patients have been extensively studied, and many biochemical or biophysical cell properties have been recognized as useful biomarkers for disease diagnosis. Because of the high heterogeneity of cells, two important trends for better cell analysis are single-cell analysis and high throughput. Optofluidics technology has shown great potential in this area, which possesses unique advantages such as noninvasive optical cell manipulation and multiple cell property detection. Huang et al. have nicely summarized the optofluidic-based cell analysis techniques into three categories: cell manipulation, treatment, and property detection.[68] In this section, we will review the development of optofluidic platforms for diagnosis applications using cell-based biomarkers. We will first briefly introduce the common optofluidic techniques that have been applied for cell analysis. Then we will review the optofluidic developments for detecting three different types of widely investigated cell-based biomarkers: cell deformability, cell refractive index, and circulating tumor cell (CTC). Table 3 lists an overview of
TABLE 3 Summary of optofluidic cell analysis for biodiagnostic applications

| Cell property          | Optofluidic technology                                      | Target cells                                                                 | Main results                                                                                                                                                                                                 | Ref.     |
|------------------------|-------------------------------------------------------------|------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------|
| Cell deformability     | Dual laser beam stretcher using two fibers                  | RBCs and PMNs; normal breast epithelial cells (MCF-10), nonmetastatic (MCF-7) and metastatic (modMCF-7) cancer cells | RBCs showed a much higher deformability than PMNs; MCF-7 about five times more deformable than MCF-10, while modMCF-7 could be stretched about twice as much as MCF-7                                                                 | [87]     |
|                        |                                                              | Primary human keratinocytes (PHK) and oral squamous cell carcinomas (OSCC); normal oral cell lines and oral cancer cell lines | OSCC > 3.5 times more deformable than PHK; the cancer cell lines were >2.5 times more deformable than the normal cell lines                                                                                     | [88]     |
|                        | Dual laser beam stretcher using integrated on-chip waveguides | The metastatic (A375P) and highly metastatic (A375MC2) human melanoma cells  | A375MC2 cells were more deformable than A375P. A375MC2 could be purified from the cell mixture, and its concentration increases from 50% to 85% by setting an appropriate threshold of the deformability                                      | [91]     |
|                        | Using both optical gradient force and fluidic drag force to realize cell stretching | Rabbit healthy RBCs and RBCs treated with glutaraldehyde                      | Mechanical characterization of RBCs at ~1.5 cells per second in a continuous-flow mode; the stretcher could distinguish between healthy RBCs and RBCs treated with glutaraldehyde                                           | [92]     |
| Cell refractive index  | On-chip cavity laser                                         | Five types of cancer cells: HeLa, PC12, MDA-MB-231, MCF-7, Jurkat            | The refractive index of five cancerous cells has been determined to range from 1.392 to 1.401, which is larger than the typical value of the normal cells of 1.35–1.37                                                                 | [94]     |
|                        | Graphene-based total internal reflection                     | Jurkat cells and lymphocytes                                                 | This sensor could accurately detect small quantity of Jurkat cells among normal lymphocytes at single-cell level                                                                                           | [95]     |
|                        | CTC                                                          | Cell lines AU-565 and RAMOS Real blood sample from patients with breast cancer | The chip could quantify the cell lines and CTCs from real blood sample, and the results correlate well with the benchtop flow cytometry                                                                          | [98]     |
|                        | Optical fiber-based fluorescent detection                    | Breast cancer cell line MCF-7 and blood sample                               | The infrared laser can separate CTC-RBC clusters due to the size and refractive index increase, with a recovery rate exceeding 90% and a 92% purity                                                                 | [99]     |
|                        | Off-chip laser and PMT                                        | The CTCs in the mouse blood circulation system directly connected to the chip via an implanted arteriovenous shunt | The fluorescently labeled CTCs from the genetically engineered mouse model could be longitudinally separated over 4 days                                                                                        | [100]    |

various optofluidic technologies for cell-based biodiagnostic applications.

4.1 Common optofluidic techniques for cell analysis

The first important application of optofluidic techniques is cell fluorescent detection. Compared with the traditional fluorescent microscope, the optofluidic devices provide a more flexible and compact solution by integrating the optical components into the microfluidic device. Schelb et al. developed an all-polymer chip with integrated optical waveguides and microfluidic channels.[69] This paper described a relatively simple method to fabricate the device. The microfluidic channels were formed using deep UV photochemical degrade, followed by chemical etching on PMMA material. The optical waveguides were also generated by irradiation of lower energy deep UV on the PMMA, causing the consequent changing of the refractive
The fluorescence detection of phospholipids and cells were demonstrated in different configurations. In one configuration, the samples were deposited inside a microfluidic channel where the optical waveguides were placed on two sides of the channel and the light transmitted through the channel was the excitation source. In another configuration, the samples were directly coated on top of the waveguide, and the evanescent field of the optical mode acted as the excitation source. In another study, Yokokawa et al. integrated all the necessary optics including prism, cylindrical microlens, and optical fiber into a microfluidic device to set up total internal reflection fluorescence microscopy for real-time cell observation with high S/N ratio.

Another large area where the optofluidic device plays an important role is the use of optical forces for cell manipulation. Since first reported in 1970 by Dr. Ashkin, the optical tweezer has been widely applied in manipulating the cells in terms of trapping, moving, and stretching the cells. In contrast to use high N.A. objective to focus the laser beam in a traditional optical tweezer setup, the optofluidic devices use either embedded optical fiber or integrated waveguide to guide the laser beam into the desired position and manipulate the cells. Two common types of optical cell traps are the single-beam and dual-beam laser traps. The single-beam setup uses the gradient force generated by a single focused laser beam to trap cells, while the dual-beam setup uses two counter-propagating and divergent laser beams to trap the cells. The single-beam setup can easily move the trapped cells and does not require the precise laser alignment. The dual-beam setup can stretch the cells by increasing the laser power, making it a promising tool for cell mechanical property measurement. In addition to the laser beam, the evanescent field has been harnessed to manipulate the cells using integrated waveguides in the optofluidic chip. Recently, Shi et al. developed several novel optical techniques for nanoparticle manipulation; for example, sorting of Mie chiral particles using optical lateral forces, linear sorting of sub-50-nm nanoparticles with nanometer-precision using the synchronized optical force and flow drag force, and massive trapping and sorting of nanoparticles using a near-field optical potential well array. Particularly, some of their studies were used for cell manipulation. In one study, they demonstrated the nanoparticle hopping in an optofluidic lattice, which was generated by direct coupling of the laser into the microfluidic channel via a microquadrangular lens. The nanoparticle hopping was then harnessed to directly and selectively quantify the binding efficiency of antibodies and bacteria, offering a new strategy for probing the cell–biomolecule interactions at single-cell level. Later, the same group developed an optofluidic nanophotonic sawtooth array (ONSA) device to sort different bacteria based on the shape. Light was coupled into the silicon nanowaveguide array under the microfluidic channel, generating an array of coupled hotspots. They demonstrated that the spherical Staphylococcus aureus could be stably trapped in the hotspots, while the rod-shaped Escherichia coli would escape from the ONSA due to their alignment in one hotspot and twist to the other spot. Aside from the direct light forces, other forces that are induced by light have been used to manipulate the cells. One example is that a bubble generated by a pulsed laser can be used to perturb the cell path inside a microfluidic channel, and this ultrafast switching mechanism has been used for high-throughput cell sorting. Another worth-mentioning technique is the optoelectronic tweezers (OETs). OET is usually composed of a light-sensitive substrate and another normal electrode. Only when light is introduced to a spot in the substrate, that spot becomes conductive and a dielectrophoretic (DEP) force that can manipulate the cells. Unlike optical tweezers, OET does not require a high intensity and coherent light source. Furthermore, the OETs can take advantage of the existing electronic technologies such as complementary metal oxide semiconductor (CMOS) and digital micromirror device (DMD) to enable optical addressability, which has made it a promising and practical solution for massive cell manipulation. Indeed, OET technology has been commercialized; for example, the specialized OET platforms made by the Berkeley lights have found many cell-based applications such as antibody discovery, cell line, and cell therapy development.

Recently, by combing the microfluidic channel for minute chemical delivery and the micro-LED for light irradiation, a novel type of optofluidic platform has emerged that allows simultaneous optogenetic and pharmacological neuromodulation in a well-controlled manner. Some researches incorporated micropumping, wireless charging, and wireless communication technologies, making the whole device light and portable enough to be implanted in small mice. This optofluidic platform will provide new opportunities for both the basic research and therapy of the nervous system.

### 4.2 Optofluidic detection of cell deformability as a disease biomarker

There is increasing evidence that cell deformability is a useful and label-free biomarker for determining abnormal cell characteristics such as metastatic potential, cell differentiation, and cell activation. The traditional contact-based techniques for cell deformability measurement include micropipette aspiration, microsphiltration, and atomic force microscopy, etc. Those methods usually suffer from measurement artifacts and low throughput.
More recently, hydrodynamic stretching has become a promising approach for measuring cell deformability in a high throughput manner.\cite{Wu2018} In addition to these techniques, optical stretchers use the gradient/scattering force to trap and stretch cells with a moderate throughput (higher than the contact-based methods but lower than the hydrodynamic method). Compared with the hydrodynamic method, the optical method does not require precise flow control. Furthermore, the optical tweezer can trap the cells, easily adjust the stretching intensity and duration, and even rotate the cells by rotating the laser modes. These unique features provide more accurate control and valuable information for the characterization of cell deformability. In this section, we will review the optofluidic techniques used to study the cell deformability as a diagnostic biomarker. The most common optical stretcher uses a dual laser beam setup, where two divergent counter-propagating laser beams can trap the cells at low laser intensities, and then stretch the cells at higher intensities due to forces induced by momentum transfer. Lincoln et al. developed a deformability-based flow cytometry based on this type of optical stretching.\cite{Lincoln2006}

Optical fibers were integrated into the chip to deliver laser beams on both sides of the microfluidic channel. A splitter was used to split the output of the laser source into two fibers, each fiber having half of the initial beam. The cells were observed using a phase-contrast microscope. This device showed RBCs have much higher deformability than polymorphonuclear cells, which could be expected as the RBCs lack internal structures. Then, the authors demonstrated the device could be used to monitor the malignant transformation of human breast epithelial cells, using normal human breast epithelial cells (MCF-10) and their cancerous counterparts: the nonmetastatic MCF-7 and the metastatic modMCF-7 induced by treating MCF-7 using phorbol esters. It was found that MCF-7 can be stretched about five times more than MCF-10, while modMCF-7 can be stretched about twice of MCF-7. The same group later used this technology to study the mechanical properties of oral cancer cells and acute promyelocytic leukemia cells.\cite{Lincoln2006a,Lincoln2006b} These results suggest that the optical cell deformability measurement could become a noninvasive biomarker for cancer diagnosis and monitoring. The potential difficulty of a fiber-based dual-beam stretcher is that it requires precise alignment of two fibers. Several methods have been developed to address or avoid this issue. Lai et al. developed a PDMS device with air chambers located on three sides of the fiber buried channel.\cite{Lai2009} By applying pressure to the air chambers, the wall between the air chamber and fiber channel could be moved to adjust the position of the fibers, thereby allowing precise alignment of the double fibers. Furthermore, a DEP force was applied to lift the cells in the fluidic channel to the beam center to improve cell trapping efficiency. Instead of using optical fibers, integrated waveguide could be fabricated directly on the chip to eliminate the alignment problem. Yang et al. used femtosecond laser to directly write the waveguides and fluid channels on the chip, and develop an optofluidic cell sorting device based on the cell deformability.\cite{Yang2016} Dual laser beams were coupled into the waveguide on the two sides of the fluidic channel. Cell deformability was evaluated by real-time imaging and analysis, followed by guiding the cells into different outlets by adjusting the power of laser beams. This effectiveness of this device was demonstrated by extracting a sample population of highly metastatic cells from a heterogeneous cell mixture. In traditional settings, flow needs to be stopped when performing the cell stretching test, which limits the throughput. More recently, Yao et al. utilized the combined effect of optical gradient force and fluidic drag force to realize cell stretching test in a continuous flow mode (Figure 3A).\cite{Yao2018} Only one laser beam is required, which also eliminates the waveguide alignment requirement of the dual-beam cell stretching system.

### 4.3 Optofluidic detection of cell refractive index as a disease biomarker

Refractive index is an important biophysical property of a single cell, usually related to other cell biophysical parameters such as mass, protein concentration, elasticity, and certain metabolic activities. Many studies have investigated the refractive index difference between normal cells and abnormal cells. The change of the refractive index has been recognized as a useful biomarker for diseases such as cancer, malaria, and anemia.\cite{Wu2019} Compared with normal cells, cancer cells usually have a greater refractive index because more protein stays in its nucleus to adapt to rapid cell division. Similarly, the concentration of hemoglobin inside an RBC affects its refractive index, which can be used to diagnose some related diseases such as anemia and malaria. Several optofluidic devices have been developed to characterize the refractive index of different cells and assess its capability in disease diagnosis. Liang et al. developed an integrated microchip, which includes a laser diode, a microlens, a mirror, and a microfluidic channel to form a cell refractive index detection system.\cite{Liang2018} The microfluidic channel acted as a part of the external laser cavity. The refractive index of the cell could be determined by detecting the change of emission frequency and power of laser when the cell passes through the beam. The PDMS planar lens was fabricated with the microfluidic channel in the same soft lithography process. Electrodes were patterned on the glass substrate to power the laser diode and drive the microflow. The refractive index of several cancer cell lines...
FIGURE 3  Examples of optofluidic platforms for cell analysis applications. (A) Schematics of the optofluidic “tweeze-and-drag” cell stretcher, which combines the optical gradient force and fluid drag force to study cell deformability (reproduced from [11] with permission from the Royal Society of Chemistry). (B) Schematics of an optofluidic sensor for measuring cell refractive index based on the polarization-dependent absorption of graphene under total internal reflection (reproduced from [95] with permission from the American Chemical Society). (C) An in vivo flow cytometry for CTC detection and sorting (reproduced from [100] with permission from United States National Academy of Sciences).

(HeLa, PC12, MDA-MB-231, MCF-7, and Jurkat) has been determined to range from 1.392 to 1.401, which is larger than the typical value of the normal cells of 1.35–1.37. In a more recent study, Xing et al. developed a graphene-based optical sensor for ultrasensitive detection of the refractive index of a single cell (Figure 3B). A graphene layer was sandwiched between a PDMS microfluidic layer and a quartz substrate. Under a total internal reflection configuration, the strong broadband absorption of graphene exhibits different reflectance for transverse electric and transverse magnetic modes, which is sensitive to refractive index of the surrounding media. The detection range of the refractive index can also be adjusted by changing the incidence angle. The system was successfully validated by distinguishing Jurkat cells from normal lymphocytes when the cell mixture passed through the detection region.
4.4 Optofluidic detection of CTC as a disease biomarker

A CTC is a cell that escapes from a primary tumor and enters the blood circulation. CTCs can spread to distant organs and form new tumors. The phenotypic analysis of CTCs can help early diagnosis of cancer and allow personalized treatments. Many microfluidic technologies have been developed for CTC enrichment and detection based on different mechanisms including filtration, centrifugation, magnetism, and immunoaffinity.\[96,97\] In this section, we focus on reviewing the optofluidic platforms that integrated the optical detection and microfluidic cytometry setup for CTC characterization in which the optical signal has been applied in either CTC detection or CTC separation steps. In CTC detection, Pedrol et al. designed a microfluidic device with a 3D-flow focusing function and integrated optics for the fluorescent quantification of CTCs.\[95\] Optical fibers were inserted to the chip as the waveguides for the excitation and emission light. The signal collecting fiber was oriented at 45\(^\circ\) to the excitation fiber to decrease the background. Two pairs of such fibers were placed along the channel to detect both red and green fluorescence. Two cell lines (AU-565 and RAMOS) that express different levels of two common CTC markers HER2 and EpCAM were selected to test the performance of the device. The detected ratio of these two cell types using different mixed samples agreed well with the standard cytometry. The test results using real samples from healthy donors and breast cancer patients with different metastatic degrees also validated this device’s ability in defining the health status of the patient. It is often preferable to separate CTCs from the blood for further molecular characterization using the advanced profiling tools such as single-cell RNA sequencing (scRNA-Seq). The optical force is an ideal actuation force for cell separation because it is noncontact and noninvasive. However, because CTCs and normal blood cells are similar in size and refractive index, the difference in optical forces exerted on them is usually small, thereby the CTCs and normal cells cannot be separated precisely. To address this issue, Hu et al. developed a method to generate large CC-RBCs (RBC-conjugated CTC) with the help of folic acid.\[99\] Due to the increased size and refractive index of the CC-RBCs, CTCs can be easily separated from the normal bloodstream using optical force. The RBCs in the collected CC-RBCs can be later removed by RBC lysis buffer. Compared with the other immunomagnetic conjugation methods, the RBC conjugation uses the donors’ own cells, which might cause less influence on the cells. The device could separate the MCF-7 cells from spiked blood samples, with a recovery rate exceeding 90\% and a 92\% purity. While many optofluidic tools for CTC research use blood samples in vitro, some studies have demonstrated the idea of direct detection and isolation of CTCs from the bloodstream, which is called in vivo flow cytometry. In a recent study, Hamza et al. developed an optofluidic real-time cell sorter that can continuously collect fluorescently labeled CTCs from a genetically engineered mouse model (Figure 3C).\[100\] An arteriovenous shunt was implanted on the back of a mouse, allowing for continuous blood withdrawal from the left carotid artery and return through the right jugular vein. The bloodstream was connected to a microfluidic chip with one single inlet and two outlets. Two closely spaced laser beamlines illuminated the main flow channel for detecting the fluorescently expressed CTC as well as calculating the velocity of the cells. Two valves in the downstream of the chip controlled the bloodstream either going back to the mouse body or going to the CTC collection tube. This device allowed the CTCs to be separated in low blood volumes. A secondary single CTC sorting chip was then used to further enrich CTCs from the collected blood and perform downstream characterization using scRNA-Seq.

Overall, the development of optofluidic techniques enables the characterization of different properties of individual cell in an accurate and high-throughput manner, thus laying the foundation for the use of these cell properties for disease diagnosis. Further research effort and clinical validation will be needed to enable automated sample processing and to assess the accuracy and specialty of these cell-based biomarkers.

5 CONCLUSIONS

In this review, we summarized the development of optofluidic technologies for the identification and quantitative detection of nucleic acids, proteins, and cell properties as diagnostic biomarkers. Owing to integration and miniaturization, these optofluidic systems showed promise to improve test speed, accuracy, and throughput. Looking forward, these systems still face many challenges before they could be adopted in clinical practice, while the efforts to address these challenges present exciting opportunities. For example, the precise integration and alignment of micro-optical components into the chip remains difficult. On-chip monolithic fabrication of optical components has shown promises to address this issue; for example, the planar lens fabricated using a lithographic process,\[94\] and waveguide formation using femtosecond laser direct writing technique.\[101\] However, optical performances of the optofluidic components, such as numerical aperture of optofluidic lenses, sensitivity, and bandwidth of on-chip photodetectors, and light coupling efficiency for feeding
light into waveguide, still need to be improved, which will require advances in material science, optical engineering, and micro/nanomanufacturing.

In addition, many current optofluidic systems still require various external instruments to aid flow control and signal detection. It is a great challenge to develop all-in-one systems for conducting the entire assay. In the near future, the integration of all the optics, electronics, and flow control components into a portable system, together with the automated signal readout and analysis, would be a promising option to enable POC diagnosis. Furthermore, with the development of optofluidic technology, integrating cost-effective optical components, for example, organic photodiodes[48] and reproducible lasers,[49] and combining pump-free microfluidic technology, for example, capillary-driven microfluidics,[102,103] onto a disposable biochip for rapid, high-sensitivity and low-cost assay would bring a bright future for biodiagnostic applications, which requires joint efforts from academia and industry.

Identification and characterization of single bio-objects can provide ultimate resolution for biodiagnosis, which usually requires the ability to manipulate small single objects. While the trapping of single cells using laser beam is very efficient, the manipulation and detection of single biomolecule are not easy because the diffraction limit is beyond the nanoscale size of biomolecules. By integrating nanostructures such as GMR filters, nanowaveguides, and nanoholes into the device, the nanoplasmonic and photonic crystal resonator-based nanophotonic biosensors demonstrate the capability to manipulate and detect the nano-objects including the biomolecules.[26,35] However, the potential of advanced nanophotonic devices as analytical platforms for biodiagnosis applications, for example, metasurface-based molecular biosensing, has not yet been fully explored. Most of the efforts have been devoted to developing and characterizing the optical properties of the nanostructures and only basic proof-of-concept biosensing applications have been demonstrated.

As seen in Section 4, various cell-based biomarkers have provided invaluable information for disease diagnosis. Many of them are based on the “passive” biophysical properties of cells such as cell number, cell deformability, and cell refractive index. Recent studies have shown that some “active” cell functions (e.g., cell migration)[104,105] present promising potential for disease diagnosis and assessment at a higher biological level. However, the complicated cell operation and bulky microscopic imaging system is still a major challenge to establish the cell functional assays for disease diagnosis in clinical practice. We envision the optofluidic systems will provide a promising approach for transforming the cell functional assays into disease diagnosis.

Based on these analyses, we envision further development in the following aspects will critically facilitate the development of optofluidic-based biodiagnostic applications: (1) novel conceptual design and advanced manufacturing technique of on-chip optical components, for example, lens, light sources, filters, gratings, and photodetectors; (2) exploration of nanophotonics including the underlying principle and extraordinary nanofabrication techniques for ultrasensitive biosensing; (3) development of integrated systems that allows rapid and cost-efficient sample-to-result POCT; (4) smart design of optofluidic chips for high-throughput multiplex assays. Efforts toward these directions will help realize the full potential of optofluidic technologies for biodiagnostic applications and aid its wide adoption for clinical practice in the future.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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