Lab-on-a-chip electrical multiplexing techniques for cellular and molecular biomarker detection

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Signal multiplexing is vital to develop lab-on-a-chip devices that can detect and quantify multiple cellular and molecular biomarkers with high throughput, short analysis time, and low cost. Electrical detection of biomarkers has been widely used in lab-on-a-chip devices because it requires less external equipment and simple signal processing and provides higher scalability. Various electrical multiplexing for lab-on-a-chip devices have been developed for comprehensive, high throughput, and rapid analysis of biomarkers. In this paper, we first briefly introduce the widely used electrochemical and electrical impedance sensing methods. Next, we focus on reviewing various electrical multiplexing techniques that had achieved certain successes on rapid cellular and molecular biomarker detection, including direct methods (spatial and time multiplexing), and emerging technologies (frequency, codes, particle-based multiplexing). Lastly, the future opportunities and challenges on electrical multiplexing techniques are also discussed.

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I. INTRODUCTION

Cellular and molecular biomarkers govern the biological process and indicate the biological states of the target subjects. Rapid and accurate detection of biomarkers is vital for disease diagnosis,^{1–6} environmental monitoring,^{7–11} and biological research.^{12–16} Because of the heterogeneous nature of biological phenomena, a biological process or disease development is typically governed by multiple types of molecular biomarkers and affects multiple types of cells. Hence, the detection of a single cellular or macromolecular biomarker alone is usually insufficient for providing enough information to study the biological process or diagnose diseases.^{17,18} Evidences showed that accurate, rapid detection of multiple molecular^{18–25} and cellular^{26–29} biomarkers can dramatically improve the accuracy of disease diagnosis (e.g., diabetes and cancer). In addition, rapid measurement of multiple responses of cells under specific pharmaceuticals or simulations will greatly accelerate new drug discovery³⁰ and facilitate stem cell research.^{31–34} Furthermore, high throughput detection of biomarkers can lead to decreased sample/reagent consumption,³⁵ shortened analysis time,³⁶ amplified signals,^{37–39} and reduced cost.^{23,36}

To date, many biological samples such as blood and urine, are routinely tested via separated lab tests. Compact, disposable, and cheaper biosensors (e.g., microfluidics lab-on-chip devices) offer new approaches to replace the traditionally time-consuming laboratory tests with higher sensitivity and improved specificity.^{40–42} Among various sensing methods (e.g., optical, electrical, magnetic, etc.) for lab-on-a-chip devices, electrical sensing methods have been widely used because they have simple structures and require less external hardware, which are ideal to be integrated into the miniaturized lab-on-chip devices.

There is an urgent need to develop lab-on-a-chip devices with multiple sensing channels/elements that can detect multiple biomarkers with high throughput and short analysis time. Signal multiplexing is the key to achieve these goals. To date various electrically multiplexed lab-on-chip devices have been developed to (1) acquire comprehensive information (biomarker types, concentrations, etc.) from the heterogeneous sample and (2) achieve high throughput and

rapid analysis of biomarkers. In this paper, we will review various electrical multiplexing techniques (including the sensor array and the advanced algorithm) that had achieved certain successes on rapid cellular and molecular biomarker detection.

II. ELECTRICAL BIOMARKER DETECTION METHODS

Electrical detection of biomarkers has been widely adopted because it requires few external equipment, simple signal processing, and provides higher scalability over other existing measurement systems, including optical, magnetic, and thermoelectric biosensors. Electrical detection possess numerous advantages making it a popular biosensing method: (1) electrical biosensors typically have simpler structures and are more cost effective than other biosensing methods but with relatively fast response times,⁴³ (2) electrical biosensors can work in situations of turbid biofluids with an optically absorbing and fluorescing compound, where optical sensors would have problems,^{44,45} (3) electrical biosensors are easy to miniaturize so that only a small amount of analyte is needed, (4) in comparison to optical detection methods, electrical detection does not need time consuming fluorescence or radioactive labeling, (5) electrical biosensors are robust, and can work in relatively harsh environments, and (6) electrical detection can also be easily integrated with magnetic and optical sensors, accomplishing comprehensive tasks impossible for magnetic, optical, and thermoelectric sensors.^{46,47} Despite the numerous advantages, there are still some disadvantages of electrical biosensors: (1) the electron transfer between the measured target and the electrode surface is often inefficient, which causes difficulties in achieving very high sensitivity, (2) electrical biosensors are typically prone to nonspecific binding, electrode fouling, and electromagnetic noises, which may create false positives, and (3) the pH and ionic strength in biofluids might differ significantly, which may affect the response of certain types of biosensors such as immunosensors.⁴⁵ Nevertheless, micro and nano fabrication technologies have been used to shrink the dimensions of electro-biosensor elements for higher sensitivity of electrical biosensors;⁴³ new surface modification techniques are being developed to minimize the false positive problem.^{46–48}

To detect biomarkers (cellular or molecular), each electrical biosensing method typically has three vital steps: biomarker analyte recognition, signal (e.g., impedance) transduction, signal detection, and biomarker quantification. Based on the objectives of biomarker analysis (i.e., concentration/size/counts), various electrical sensors and their sensing principles are briefly reviewed below.

Electrochemical (EC) immunosensors have been used for detecting antibody against virus,^{47,48} cancer biomarkers,^{49,50} pesticides,⁵¹ microorganisms,⁵² and protein biomarkers.⁵³ The sensing principle of a typical electrochemical immunosensor is shown in Fig. 1(a). The electrochemical (EC) immunosensor is based on capturing the biomarkers via specific antigen/antibody binding. By converting the labels (e.g., enzyme-labelled antibody) to a electroactive product or by capturing the analyte on the electrode surface, an impedance change is induced, indicating the concentration of target biomarkers.⁵⁴ The impedance change is then quantified via two

FIG. 1. Schematic for (a) electrochemical immunosensor and (b) impedance pulse sensor.

methods: (1) voltammetry or amperometry, which measures the current (DC or pseudo-DC) as a function of applied voltage and (2) electrochemical impedance spectroscopy (EIS), which measures the impedance under a DC biased AC input, either with a wide frequency range or at a fixed frequency. Details of the fundamental and advanced EC sensors can be found from other review papers.^{54–58} Note that the bulk impedance change measured by EC sensors is a function of the biomarker concentration.

On the other hand, without any label, the presence of a target analyte can also alter the impedance of a micro/nano fluidic channels/pores because of electrical field distortion by the target analyte. This allows single particles or biomarkers (protein/cells) to be sized and counted one by one in terms of the induced impedance pulses. Within an impedance pulse sensor [Fig. 1(b)], when the target objects (i.e., proteins/cells/particles) travel through a fluidic aperture/pore/channel, the electrical field is distorted, causing changes in the impedance of the channel; an instantaneous pulse signal (voltage/current pulse) can thus be measured. Note that when a DC voltage is applied to the channel, the impedance pulse sensor is simplified to a resistive pulse sensor (or called the Coulter counter). By functionalizing the sensing channel or micro-particles, biomarker recognition can be achieved for specific biomarker detection. The impedance pulse sensing principle has two major advantages: (1) the pulse signals contain rich information (size, surface charge, and transit time) of the biomarker, allowing for localized impedance analysis with extremely high sensitivity (i.e., single molecular/cell/particle analysis) (2) it allows measurement in a continuous flow, with potentials for real-time biomarker monitoring. Detailed reviews about impedance pulse sensing can be found from other review papers.^{41,59–63}

Despite the differences in analyte recognition principles, if an electrical biomarker sensing array is used, each array needs to differentiate signals from individual sensors/sensing channels via signal processing/multiplexing strategies. In this paper, we focus on reviewing various multiplexing strategies (i.e., spatial/time/frequency/codes/particle-based multiplexing) aiming at detection of various biomarkers (i.e., nuclear acids, macromolecular biomarkers/proteins, and cells). Reviews on immunosensors for detecting bio-objects including pathogens,⁶⁴ viruses,⁶¹ cardiac biomarkers,⁵⁷ protein disease biomarkers⁶⁵ etc., can be found elsewhere.

III. SPATIAL MULTIPLEXING

Spatial multiplexing is one of most popular multiplexing methods, which integrates an array of sensors at various locations; each sensor contains a set of electrodes to quantify the target biomarker from the individual sensors.

A. Detection of multiple analytes

Simultaneous measurement of multiple biomarkers can greatly facilitate disease diagnosis and cell research. Integrated devices containing multiple sensing elements aiming at detecting different biomarkers have attracted increasing attentions. Wilson *et al.* used an integrated sensor to detect seven important tumor markers including alpha-fetoprotein (AFP), ferritin, carcinoembryonic anti-gen (CEA), hCG- β , CA 15-3, CA 125, and CA 19-9.^{66,67} As shown in Fig. 2(a), the sensor consists of an array of spatial-separated electrodes, W1-W8. Seven different antigens were immobilized on W1-W7, and W8 was used as a control electrode to detect the non-specific binding background. The detection procedures included sample incubation, washing with a blocking buffer, and adding an enzyme substrate. Current response from each working electrode (W1-W8) was recorded as a function of time, while a constant potential was applied to the electrode. Because each sensing elements were spatial separated, diffusional crosstalk between electrodes can be eliminated. The sensor's precision and accuracy were comparable to those of commercial single analyte assays. The spatial multiplexing concept was also applied to integrating sensors that detect nucleic acid (i.e., ssDNA). Levine *et al.* fabricated a sensor array to measure multiple DNA targets via a spatial multiplexed CMOS (Complementary metal-oxide-semiconductor) array [Fig. 2(b)].^{68,69} Within the 5 mm $\times$ 3 mm CMOS chip, the authors integrated a 4 $\times$ 4 array of gold working electrodes, control amplifiers, and current-input analog-to-

FIG. 2. (A) Photography of the spatial multiplexed electrochemical assay, with eight working electrodes (W1-W8), one counter electrode (CE), one reference electrode (RE), and electrical contacts (EC). Reproduced with permission from Wilson and Nie, *Anal. Chem.* **78**, 2507 (2006). Copyright 2006 American Chemical Society. (B) Schematic of the CMOS sensor array. Each array site consists of a square gold electrode and a dual-slope analog-to-digital converter (ADC). (C) Illustration of the EC sensor for multiple salivary biomarker detection, with electrodes for both mRNA and protein detection. (D) Illustration of the multiplexed EC Immunoassay consists of a Nylon sheet (a), silver ink (b), a graphite auxiliary electrode (c), an Ag/AgCl reference electrode (d), working electrodes (e, f), and an insulating dielectric (g). Reproduced with permission from Lai *et al.*, *Anal. Chem.* **81**, 9730 (2009). Copyright 2009 American Chemical Society.

digital converting circuits. Each working electrode surface was modified with a specific ssDNA probe; the DNA targets were modified with single ferrocene (Fc) redox labels. Thus, the binding of DNA targets on each electrode surface can be detected via redox reactions using cyclic voltammetry. The authors demonstrated that during the hybridization process, real time current peaks were generated from each working electrode with negligible crosstalk. Huang *et al.* also explored the possibility of integrating microfluidics sensor on a compact CMOS device.⁷⁰

Similarly, Henry *et al.* built a multiplexed chip that detected five different DNA breast cancer markers (ACTB, PLAU, EGFR, ERBB2, and ESR1) in terms of detecting their hybridizations with the specific probes on separate gold working electrodes.⁷¹ Methylene blue (MB), an electroactive guanine specific marker, was used to react with the increased bases after hybridization, which induced a current increase. Hence, the hybridization process can be quantified from the measured current changes; a detection limit of 3 nM was achieved. Similarly, Manickam investigated a fully integrated CMOS biosensor, consisting of a 10×10 sensing array for detecting DNA hybridization via electrochemical impedance spectroscopy (EIS).⁷²

For real clinical applications with a specific diagnosis purpose, detecting the combinations of biomarkers, including both RNA and specific protein biomarkers, can greatly improve diagnostic accuracy. Wei *et al.* demonstrated an array of EC sensors for detection of oral cancer via simultaneous measurement of IL-8 mRNA and IL-8 proteins from saliva [Fig. 2(c)].⁷³ The accuracies of the two EC sensors were approximately 90% for both mRNA and protein, which is very close to that measured by Polymerase chain reaction (PCR)/enzyme-linked immunosorbent assay (ELISA). The gold electrodes were functionalized by the hairpin probe for IL-8 mRNA and biotinylated human IL-8 monoclonal antibody. 56 real saliva samples were tested on the device, including 28 oral cancer samples and 28 control samples; results from the device agreed well with the parallel analysis results via conventional PCR and ELISA.

In addition to integrating multiple sensing elements within one device, efforts have been made to improve the performance (high sensitivity, easier sample preparation, etc.) and simplify the fabrication of the device. To improve the detecting sensitivity, multiple groups

demonstrated various probing methods to enhance the electron transfer process. Lai *et al.* showed a multiplexed immunoassay to detect CEA and AFP [Fig. 2(D)].⁷⁴ With the nanoparticle probe-labeled signal antibody that enhanced the electron transfer process, the signals were amplified such that wide linear ranges and low detection limits were achieved. Two different antibodies, anti-CEA and anti-AFP antibody (Ab1) were applied to the corresponding working electrodes of W1 and W2. After adding samples containing CEA and AFP, current responses at W1 and W2 were recorded for simultaneous, quantitative measurement of CEA and AFP. Similarly, Ko *et al.* fabricated three single electro-immuno-sensors on one microchip to detect three cancer biomarkers, alpha-fetoprotein (AFP), carcinoembryonic anti-gen (CEA), and prostate-specific antigen (PSA), where gold nanoparticles and silver enhancer were used to amplify the electrical signals.⁷⁵ Nanoelectrode arrays were also fabricated for multiplexed detection of DNA.⁷⁶ Similar to other electrical biosensors, the above biosensors rely on the specific interaction between the detection probes and the target biomarkers for selective detection. However, nonspecific binding may occur on the electrode surface, and could cause unwanted impedance changes and false positives. The non-specific binding issue can be suppressed by coating non-fouling materials or treating the electrodes with a site-blocking solution, at the price of adding extra processing steps.^{77,78} More details about the false positive sources can be found in Table I.

To overcome the limitation of substrate conductivity (i.e., gold electrode), semiconductor nanowire-based FETs were employed for ultrasensitive electrical detection of biomolecular interactions.^{77–79} Instead of using the traditional electrochemical sensor based on the metal electrode, Zheng *et al.* developed a highly sensitive, label-free, and multiplexed field-effect devices to detect cancer markers (f-PSA, CEA, and mucin-1).^{77,78} The sensing principle of the device is shown in Fig. 3(A). Each individual field effect transistor consisted of silicon nanowires as the gate region that was modified with a specific antibody. The specific binding reaction at the gate region caused a change in the drain-source current and the conductance, from which the corresponding target biomarker can be identified, and its concentrations can be estimated. The device was proven to have a high selectivity (0.9 pg/ml) with negligible crosstalk. Moreover, compared with the devices that measure impedance change across a pair of electrodes, nanowire devices (as field-effect transistors) measured the complementary conductance change of the nanowire upon biomarker binding, which was reported to have effectively differentiated the false-positive signals induced by nonspecific binding. A review paper by Rahong *et al.* covered recent developments in nanowire based sensors for biomarker detection.⁸⁰

To overcome the tedious sample preparation and long incubation procedures, Wu *et al.* demonstrated a reagentless electrochemical immunosensor that can perform the electric field driven incubation on the chip [Fig. 3(B)].⁵³ By applying a driving potential to the device, an electric field was produced nearby the electrode interface, which generated an external electrophoretic force to accelerate the transport of charged antigen molecules to the electrodes. Hence, the immunoreactions were accelerated and the incubation time was limited within 2 min. A rapid and multiplexed detection of four tumor markers (CA 153, CA 125, CA 199, and CEA) was demonstrated.

Recently, there is a growing need to migrate the matured microfluidics sensing technologies to platforms (i.e., paper platform) that can be easily fabricated. Wu *et al.* showed a paper based electrochemical immuno-device for the detection of four cancer biomarkers [Fig. 3(C)].⁸¹ On a piece of square paper-A, eight carbon working electrodes and the conductive pads were screen-printed. Similarly, the reference (made of Ag/AgCl) and counter (made of carbon) electrodes were printed on another piece of square paper-B. After stacking the two paper layers and functionalizing the working electrodes with specific antibodies, the device was used for multiplexed detection of four cancer biomarkers (CEA, AFP, CA125, and CA153). With the demonstration of this cost-effective approach for multiplexed biomarker sensing, advanced devices and platforms made of cost effective materials are expected to be developed for easy, cheap, and disposable applications. Comparisons between different device materials can be found in one review paper,⁸² where the device performance and complexity were discussed.

TABLE I. Comparison of various electrical multiplexing methods used for biomarker sensors.

References	Author	Method	# of channels	Target biomarkers	LOD	Multiplexing ^a preparation	Specificity	False positive sources	Ways to reduce false positive reading
66, 67	Wilson	Spatial multiplexing	7	AFP, ferritin, CEA, hCG-b, CA 15-3, CA 125, and CA 19-9.	3 ng/ml	Electrode functionalization	Specific	1. Background noise 2. Nonspecific protein binding	Coat areas between electrodes with CYTOP
68, 69	Levine	Spatial multiplexing	4 × 4	DNA	50 pM	Electrode functionalization	Specific	Nonspecific DNA binding	Create monolayer on the working electrodes
71	Henry	Spatial multiplexing	4 × 4	ACTB, PLAUR, EGFR, ERBB2 and ESR1	3 nM	Electrode functionalization	Specific	Nonspecific protein binding	Coat electrodes with ethanolamine hydrochloride
72	Manickam	Spatial multiplexing	10 × 10	protein-G	10 ng/ml	Electrode functionalization	Specific	Nonspecific DNA binding	NA
78	Zheng	Spatial multiplexing	2–3 (effective)	f-PSA, CEA, mucin-1	3 pg/ml	Nanowire functionalization	Specific, robust to electrical noise	Diffusion of viral particles None specific binding	Distinguish nonspecific binding by analyzing duration of the conductance change
50	Wu	Spatial multiplexing	4	CA153, CA125, CA199, CEA	0.06, 0.03, 0.10 U/ml, 0.04 ng/ml	Electrode functionalization	Specific	Nonspecific absorption	Incubate sensor with 0.1 M Tris-HCl (pH 7.2) containing 1% bovine serum albumin for 20 min
81	Wu	Spatial multiplexing	4 (effective)	CEA, AFP, CA125, CA153	1.5×10^{-4} ng/ml, 3.7×10^{-5} U/ml, 2.6×10^{-5} U/ml, 2.0×10^{-5} ng/ml	Electrode functionalization	Specific	Nonspecific absorption	1. Add control groups, 2. Treat working electrodes with pH 7.4 PBS containing 0.5% BSA
89	Ge	Time division multiplexing	4 × 6	CEA, AFP, CA125, CA153	0.01, 0.01, 0.05 and 0.05 ng/ml	Electrode functionalization	Specific	Nonspecific protein binding	NA
91	Yang	Time division multiplexing	4 × 4	2-propanol	5 mM	Electrode functionalization	Specific	Nonspecific binding Electrical noise	Design low noise circuit to minimize charge injection
93	Lin	Frequency division multiplexing	2	Low and high density Lipoproteins	1 mg/dl	Frequency tuning for ideal sensitivity	Specific	Nonspecific protein binding	Block reactive site with 1% ethanolamine.
94	Le Belle	Frequency division multiplexing	2	Interleukin-12, tumor necrosis factor- α	3.9 pg/ml and 60 pg/ml	Frequency tuning for ideal sensitivity	Specific	Nonspecific protein binding	1. Add control groups, 2. block reactive site with 1% ethanolamine

TABLE I. (Continued.)

References	Author	Method	# of channels	Target biomarkers	LOD	Multiplexing ^a preparation	Specificity	False positive sources	Ways to reduce false positive reading
95, 96	Jagtiani	Frequency division multiplexing	4	Polystyrene particles	Single particle counting	Electrical behaviour characterization	Nonspecific	Electrical crosstalk between channels	Share a common electrode with a fixed DC potential (signal insulation)
97	Meissner	Frequency division multiplexing	3	MCF-7 cells	NA	Electrical behaviour characterization	Nonspecific	Electrical crosstalk between sub-sensors	NA
98, 99	Balakrishnan	Frequency division multiplexing	5 (effective)	NB4, AP-1060, NALM-1 cells, Primary bone marrow	Single cell detection	Unique fluidic node patterning	Specific	Nonspecific binding	Identify specific interaction with a cutoff threshold
100	Liu	Code multiplexing	2 (effective)	HUVECs	2% target/non-target cell ratio; single cell detection	Cell labeling	Specific	Nonspecific binding	Add control groups with non-target cells
101	Wood	Code multiplexing	8	T cells	Single cell detection	Unique electrode patterning	Specific	Nonspecific binding	NA
102, 103	Liu	Code multiplexing	4	HeyA8 ovarian cancer cell	Single cell counting	Unique electrode patterning	Nonspecific	Electrical noise	NA
105	Bai	Particle multiplexing (effective)	2	Platelet-derived growth factor, thrombin	8 pM, 11 pM	Particle functionalization	Specific	Nonspecific absorption	Block the unspecified sites by incubation with 1 mg GOD and 1 mg HRP
106	Carbonaro	Particle multiplexing	2	Human GM-CSF and G-CSF antigen	15 ng/ml	Particle functionalization	Specific	Nonspecific binding	Add control groups with nonspecific antigen
112	Han	Particle multiplexing (effective)	4	Human ferritin and mouse anti-rabbit IgG	3.1 ng/ml and 5.2 ng/ml	Particle functionalization	Specific	Nonspecific binding Nonspecific attachment	Add control groups with nonspecific biomarker

^aPreparation steps to enable applying signal multiplexing.

FIG. 3. (A) Illustration of the multiplexed detection of cancer marker proteins by three silicon-nanowire devices in an array. Devices 1, 2, and 3 are fabricated from similar nanowires, and then differentiated with distinct mAb receptors (1, red; 2, green; 3, blue) specific to three different cancer markers. Reproduced with permission from Zheng *et al.*, Nat. Biotechnol. **23**, 1294 (2005). Copyright 2005 Nature Publishing Group. (B) Schematic of the EC immunosensor array, with electric field-driven incubation. Reproduced with permission from Wilson and Nie, Anal. Chem. **78**, 2507 (2006). Copyright 2008 American Chemical Society. (C) Photographs for the fabrication process of the microfluidic paper-based electrochemical immuno-device. Reproduced with permission from Wu *et al.*, Biosens. Bioelectron. **52**, 180 (2014). Copyright 2013 Elsevier B.V.

B. High throughput detection

Higher throughput detection allows fewer step preparation, less reagent consumption, and shorter measure time. Spatial multiplexing methods (parallel detection) were explored to increase detection throughput, especially for microparticle counting and cellular biomarker detection. Jagtiani *et al.* studied a resistive-pulse sensor that utilized four sensing micro apertures to detect and count micro scale particles simultaneously [Fig. 4(A)].⁸³ The device consisted of four peripheral reservoirs and a central reservoir. Each peripheral reservoir was connected to the central via a micro-scale aperture, forming a micro Coulter counter for

FIG. 4. Schematic of the (A) high throughput resistive-pulse sensor with four sensing macroapertures. Reproduced with permission from Jagtiani *et al.*, Meas. Sci. Technol. **17**, 1706 (2006). Copyright 2006 IOP Publishing Ltd. (B) Multi-channel differential resistive pulse sensor for high throughput particle detection. Reproduced with permission from Song *et al.*, Electrophoresis **36**, 495 (2015). Copyright 2015 John Wiley & Sons, Inc.

microparticle detection. Polymethacrylate (PM) particles, Rocky Mountain Juniper pollen, and Cottonwood pollen were used to test the sensors. The device can not only detect size and concentration of the particles, but can also differentiate pollens and the PM particles from the resistive pulse polarity induced by the particle surface charge. More importantly, the counting efficiency has been improved by approximately 300%, compared to the single-channel device.

Similar devices consisting of parallel Coulter counting channels were fabricated to detect pollens,⁸⁴ microparticles,⁸⁵ airborne mineral dust,⁸⁶ cancer cells,⁸⁷ as well as double-stranded DNA⁸⁸ with high throughput. Song *et al.* demonstrated a differential resistive pulse sensor for high throughput particles detection with an improved signal to noise ratio.⁸⁵ Figure 4(B) shows the sensing principle of the device, where seven detecting channels and one shared reference channel were fabricated within a single microfluidic chip. Because the size of the reference channel is identical to all other detecting channels, the baseline voltage over the reference channel was deducted from the voltage change measured from other sensing channels. With the differential detection, the signal to noise ratio can be improved by ten times; microparticles as small as $2\ \mu\text{m}$ can be detected. In addition, an average throughput of $10\ \mu\text{l}/\text{min}$ was achieved by using the seven parallel sensing channels. Although the primary goal of the above devices is to improve the detection throughput, the sensing channels or electrodes can be functionalized for specific detection of cellular biomarkers.

Because the spatial multiplexing method is essentially reproduction of one individual sensor element, the sensitivity, signal to noise ratio, and detection limit remain the same as the single sensing element. Therefore, the spatial multiplexing methods can (1) easily improve the detection throughput, or detect multiple analytes, while being immune to the crosstalk effect and (2) maintain the same performance compared with their single sensor counterparts without specially designed structures/electrodes. On the other hand, this method lacks the scalability for a higher degree of integration (i.e., over 10 sensors), because each sensor requires a separate set of signal acquisition hardware, generating a large amount of data and taking a long time for data processing. Alternatively signals from multiple sensors can be combined into a single waveform using various signal multiplexing strategies in combination with biomarker recognition methods, to increase device scalability, reduce the data size, and shorten the processing time.

IV. TIME DIVISION MULTIPLEXING

Time-division multiplexing is a well-established method in telecommunications, which combines multiple signals from a sensor array into one signal. Each signal is acquired within a fixed time duration, and can be separated from the combined signal in terms of time segments. Time division multiplexing is an effective way to reduce the size of data generated from multiple channel detections. The method can be easily implemented using commercial multiplexer where signals from separated channels are routed to a common data acquisition system. Because of the advantages including simple circuitry and negligible crosstalk, time-division multiplexing has been recently utilized in biomarker sensing.^{89,90} Ge *et al.* developed a paper-based device containing a 4×6 immuno-sensor array, and a circuit including the commercial multiplexer to read out the individual current output from the addressable single sensors.⁸⁹ As illustrated in Fig. 5(A), the device consisted of two paper layers, where eight working electrodes were screen-printed on paper-A, and a pair of counter and reference electrode (Ag/AgCl) was printed on the paper-B. By using the two multiplexers, the current signal from each working electrode was detected in sequence (row 1 to row 4, and column 1 to column 6) with a 1 s readout time. With only one set of measurement setup, the measurement circuit has been greatly simplified. Detection and quantification of four cancer biomarkers including the carcinoem-bryonic antigen (CEA), the alpha-fetoprotein (AFP), the cancer antigen 125 (CA125), and the carbohydrate antigen 153 (CA153) were demonstrated with limit of detection (LOD) at 0.01, 0.01, 0.05, and 0.05 ng/ml, respectively.

Additionally, the time multiplexing method has been combined with CMOS to detect current signals from the addressable working electrodes.^{91,92} Yang *et al.* developed a microsystem

FIG. 5. (A) Layered structure of the device and schematic diagram of the addressable electrode array detection system. Reproduced with permission from Ge *et al.*, Chem. Commun. **48**, 9397 (2012). Copyright 2012 The Royal Society of Chemistry. (B) Schematic of the surface electrode array and the onchip potentiostat comprised of a switched capacitor amperometric readout block, an electrode potential drive block, and a multiplexer. (C) A paper-based electrochemical biosensor array (1×8) and the schematic diagram of the potentiostat architecture. Reproduced with permission from Zhao *et al.*, Sci. Technol. Adv. Mater. **14**, 054402 (2013). Copyright 2015 National Institute for Materials Science.

platform for detecting multiple biomarkers including enzymes. The platform used multiple bioelectrochemical interfaces whose outputs were time multiplexed and measured with an on-chip amperometric readout system. As shown in Fig. 5(B), the microsystem was composed of a silicon chip with CMOS instrumentation circuits and an enzyme functionalized electrode array. The $0.5\text{-}\mu\text{m}$ on-chip CMOS readout electronics included a compact potentiostat supporting input currents ranging from 6 pA to $10\text{ }\mu\text{A}$, to accommodate diverse biosensor interfaces. A time-division multiplexer was used for multiplexing signals from different reservoirs/biointerfaces. The multiplexed signal was demultiplexed through a switched capacitor amperometric readout block. To demonstrate the performance of the device, secondary alcohol dehydrogenase (2° ADH) enzyme was tested as the model dehydrogenase. The sensitivity obtained using the on-chip potentiostat was $0.62\text{ }\mu\text{A mM}^{-1}\text{cm}^{-2}$. Time-division multiplexing was also utilized for the metabolic biomarker array. Zhao *et al.*⁹⁰ developed a paper-based electrochemical biosensor array for multiplexed detection of physiologically relevant metabolic biomarkers, shown in Fig. 5(C). A paper-based 1×8 array interfaced with a custom-made potentiostat including a time-division multiplexer was demonstrated for detecting eight metabolic biomarkers simultaneously.

For the time division multiplexing methods, to accurately sample the current/voltage response, continuous signals must be divided by the multiplexer into discrete time segments for sampling. Therefore, this method is essentially a data reduction method. While it allows signal acquisition from multiple sensing elements, the time multiple method would be limited for applications with highly dynamic pulse signals that requires continuous measurement with high sampling frequency.

V. FREQUENCY DIVISION MULTIPLEXING

Electrochemical impedance spectroscopy (EIS) measures the impedance response under AC excitation. Measurements from multiple EIS sensors/channels can be multiplexed into a combined signal if each sensor is measured under a specific frequency (i.e., frequency division multiplexing). The combined signal can subsequently be demultiplexed to acquire individual signals by frequency division. Recently, the LaBelle group studied the potential of multiplexed EIS biomarker detection by analyzing the imaginary impedance.^{17,93,94} Demirok *et al.* demonstrated that by conjugating gold nanoparticles to a specific antibody, the optimal frequency (at which the largest impedance change occurred) for EIS can be tuned.¹⁷ They found that the optimal frequency was shifted by 10.0, 4.8, and 1.0 Hz, while gold nanoparticle sizes were changed by 5, 10, and 20 nm, respectively. Thus, the frequency overlapping between two biomarkers' response can be minimized for multiplexed detection. To further address the signal overlapping

issue, Lin *et al.* developed a unique EIS analysis strategy that analyzed the imaginary impedance to decouple the convoluted signal generated from the device [Fig. 6(a)]. Two biomarkers (low and high density lipoproteins/LDL and HDL) were co-immobilized on the same gold disk electrode. The authors found the imaginary impedance peaks of Biomarker 1 and biomarker 2 occurred at different frequencies with minimal overlapping. By correlating the imaginary impedance with the biomarker concentration, the optimal frequency can be determined to obtain the highest imaginary impedance change. Afterward, two individual signals induced by the two biomarkers can be deconvoluted from the combined signal output; the antibody concentrations can be estimated from the imaginary impedance amplitude. While optimal frequency can be tuned differently by conjugating different gold nanoparticles, this new detection strategy is promising for multiple biomarker detection.

The frequency division multiplexing method has also been applied to the Coulter counting devices (resistive pulse sensors) to improve the throughput. Jagtiani *et al.* invented a frequency division signal multiplexing method to detect cells by measuring the resistive pulses (momentary current changes) from four microchannels simultaneously.⁹⁵ As shown in Fig. 6(b), voltages for individual sensing channels were modulated with different encoding frequencies controlled by external function generators; signals from all channels were combined into a single output signal. The combined signal can then be demodulated in terms of the encoding frequencies; current signals from individual channels were then recovered. From the recovered current signals, the cell size and concentration can be calculated. With this method, cells passing through all channels were detected in parallel. Hence, the detection throughput can be significantly increased. This multiplexing method only needs to measure one combined signal, dramatically reducing the data size and complexity of the detection electronics. Despite the promises, the major limitation is that to accurately measure the sizes of the cells, the encoding

FIG. 6. (A) Schematic representation of two biomarker's impedance signals and the predicted signal when co-immobilized on the same sensor. The black and white circles represent the optimal frequency of biomarker 1 and biomarker 2, respectively. Reproduced with permission from Lin *et al.*, Biosens. Bioelectron. **89**, 743 (2017). Copyright 2016 Elsevier B.V. (b) Design concept of a four-channel frequency division multiplexed Coulter counter, electrical equivalent circuit of the sensor, and the schematic for signal processing steps. Reproduced with permission from Jagtiani *et al.*, J. Micromech. Microeng. **21**(6), 065004 (2011). Copyright 2011 IOP Publishing Ltd. (c) Schematic of the frequency-multiplexing impedance sensor (FMIS), and a simplified impedance magnitude-frequency spectrum according to a triple FMIS sensor. Reproduced with permission from Meissner *et al.*, Lab Chip **12**, 2712 (2012). Copyright 2012 The Royal Society of Chemistry.

frequencies need to be selected within a range where the resistive behavior of the micro Coulter counter dominates over the capacitive effect.⁹⁶ Within the narrow range of the resistance-dominant frequency, only a limited number of channels (up to 10) can be used; the improvement in throughput is limited. In addition, the encoding signal was applied to each channel via a central encoding electrode in the middle of the channel; hence, this method may not be applicable to sensing channels where it is difficult to fabricate central electrodes (e.g., nanopores or nanochannels).

Instead of generating encoding signals via an external source, the electrode geometry was also engineered to supply the input signal with a unique encoding frequency. Meissner *et al.* presented a frequency-multiplexing impedance sensor (FMIS) for simultaneous detections of up to three individual microfluidic channels by using only one electrode pair [Fig. 6(c)].⁹⁷ Instead of using three pairs of electrodes, the authors designed one pair of electrode consisting of three sub-segments; each sub-segment electrode had a unique width and spacing. Because of the geometry difference, each pair of sub-segment electrodes had different double layer capacitance. Thus, each sub-segment (modeled as an RC circuit) corresponded to a specific peak resistance frequency (PRF). Therefore, signals from the three separated sub-electrode pairs can be modulated via three unique PRFs and demodulated from the combined output waveform. To validate the design concept, the authors tested the resistance of phosphate-buffered saline (PBS) buffers with different concentrations, using both a mono sensor (individual sensing with one pair of sub electrodes) and a double electrode sensor (frequency multiplexed sensing using two pairs of sub electrodes). The results showed no evident difference between the two methods, indicating the validity of the frequency multiplexing approach. Next, with two pairs of sub-electrodes working in parallel, two cellular impedance evolution processes were successfully monitored from the multiplexed measurement. These tests proved that the frequency multiplexing impedance sensor is capable of screening multiple channels at the same time.

Recently, a unique method (node pore sensing, NPS) for creating the encoding frequency was proposed by Sohn's group, where the signals with different frequencies were generated by the passage of the cells.⁹⁸ Figure 7 shows the sensing principle. The current response from the sensor was modulated with frequencies determined by nodes with unique patterns (i.e., pores with different spacing). Thus, the combined signals included multiple frequency components (f_{high} and f_{low}), corresponding to the transition of a cell through different sections of the channel. Afterward, signals from each sensing pore can be recovered by applying fast Fourier

FIG. 7. (a) An image of the node pore sensor. (b) The current pulse produced as the colloid travels through the device. The high (shaded in red) and low (shaded in green) frequency, f_{high} and f_{low} , correspond to the sequence of nodes in the device. (c) The fast Fourier transform frequency spectrum of the total signal after ten duplications of the data. Reproduced with permission from Balakrishnan *et al.*, Lab Chip 13, 1302 (2013). Copyright 2013 The Royal Society of Chemistry.

transform (FFT) to the combined signal output. Particle size can then be accurately determined, with a dramatically improved dynamic range (tens of nanometers to several microns). Also, because the encoding frequencies were governed by the channel geometry and flow rate, this device required minimal external equipment for signal generation and acquisition. Nevertheless, to accurately control the frequency, delicately designed nodes patterns need to be exactly fabricated, and the flow rate must be precisely controlled.

Compared to the spatial and time multiplexing methods, frequency division methods have three major advantages: (1) only one combined waveform (consisting of multiple frequency components) needs to be sampled. Hence the data acquisition setup can be simplified and the data size is dramatically reduced, (2) demultiplexing algorithms (i.e., FFT, band pass filtering) can be easily applied to recover the frequency encoded signals instead of using complex hardware, (3) the encoding frequencies can be tuned either externally (i.e., via function generators) or internally (i.e., via various modified antibodies/electrodes/fluidic channels). Note that different from frequency modulation in telecommunications which measures the frequency change of the signals, the above-mentioned frequency multiplexing methods simply use different AC signals as the carrier waves, where the characters (concentration/size) of the biomarker are measured from the impedance change. One significant application of frequency division multiplexing is impedance cytometry where multiple cell populations can be detected from the combined impedance changes induced by them under different frequencies. More details can be found from a review on single cell impedance cytometry.⁴¹

VI. CODES MULTIPLEXING

Recently, impedance pulse signals were patterned to barcode-like waveforms, which were used for multiplexed detection of biomarkers. Balakrishnan *et al.* introduced a device for cell surface marker profiling by identifying the patterned current pulse signals [Fig. 8(a)].⁹⁹ The microfluidic device consists of five sections separated by node pores; each section was functionalized by one specific antibody to identify a cell surface marker. The sensing mechanism is as follows: a cell with a specific antigen interacts with functionalized antibodies in the particular segment, and therefore travels more slowly through that section than through other sections. Thus, a longer current pulse is generated within the barcode-like waveform. Experiments showed the method can (1) differentiate target cells from the mixed population and (2) accurately measure the percentage of target cellular subpopulations (i.e., blast-cell population) by analyzing both cell surface markers and cell size. The device has the potential for detecting more markers by adding additional sections. However, to identify a specific marker, a tedious channel functionalization process is necessary. One challenge of many electrical biosensors is that the antibody functionalized channels or electrodes may generate a response from surface fouling or nonspecific adsorption, which could possibly cause false positives and shorten the device life cycle. The false positive sources for the devices reviewed in this article are given in Table I.

To overcome that limitation, Liu *et al.* developed a microfluidic device to detect single cells via the impedance pulse width change induced by the magnetic interactions.¹⁰⁰ The device consists of two identical micro Coulter counters, and an external magnet mounted close to the 1st stage Coulter counter [Fig. 8(B)]. The sensing mechanism is as follows: a target cell, conjugated with magnetic beads via specific antigen/antibody binding, travels slower at the first stage than at the second stage due to the larger magnetic attraction force at the first stage. Thus, by comparing the voltage pulse width (i.e., transit time) at each stage, cellular biomarkers can be detected. The results demonstrated that human umbilical vein endothelial cells (HUVECs) were successfully distinguished from rat adipose stem cells (rASCs). Note that instead of quantifying the absolute value of the transit time change, these methods simply draw a binary decision (positive/negative) on each cell in terms of the travel time difference through the second counters, which is similar to digital codes (1 or 0). Also, because of the specific affinity selection using functionalized magnetic beads, this device offers high flexibility for multiple biomarker detections by simply changing the antibodies. With the use of the functionalized magnetic

FIG. 8. (A) Schematic of the node-pore device functionalized with antibodies in the channel between the nodes. As a cell transits the device, the modulated current pulse produced (bottom) reflects the interactions between cell surface markers and the functionalized segments. (B) Schematic showing the sensing principle of the magnetic bead assay for single cell detection. Reproduced with permission from Liu *et al.*, PLoS One 12(2), e0172697 (2017). Copyright 2017 PLOS Corporation. (C) Bar code readout of the microfluidic device with patterns electrodes. Reproduced with permission from Wood *et al.*, Lab Chip 7, 469 (2007). Copyright 2007 The Royal Society of Chemistry. (D) Design concept of the microfluidic CODES device, with digitally coded microfluidic channels. Measured electrical signals, templates, and their correlation. Reproduced with permission from Liu *et al.*, Lab Chip 16, 1350 (2016). Copyright 2016 The Royal Society of Chemistry.

particles, this method eliminates the need for surface functionalization on microchannels or electrodes, and thus offers higher reliability.

In contrast to creating the codes by controlling the transit time, sensing electrodes were also patterned to generate addressable barcodes-like pulses. Wood *et al.* developed an electronic approach to detect cells flowing through different channels, based on the barcode pulses generated by the patterned electrodes.¹⁰¹ The electrode patterns and corresponding impedance changes are shown in Fig. 8(C). The mechanism is as follows: each barcode electrode includes 10 patterned metal encoding elements (bits): a metal stripe encodes a “1” and a gap between stripes encodes a “0.” Signals from each channel can be identified by (1) counting the number of peaks that correlated with the number of metal encoding strips and (2) comparing the real signal with the known templates of each channel, until the pair with the lowest error score (absolute difference) can be matched. With two parallel channels, the authors achieved a high throughput detection of 1000 counts per second. Moreover, the authors successfully

functionalized the channels with antibodies (goat anti-mouse IgG and streptavidin–biotin complex), such that parallel analysis for surface markers could be realized in the future. The authors predicted that by doubling the encoding bit from 10 bits to 20 bits, 220 (over a million) distinct patterns could be encoded. However, if a large number of patterns are encoded, signal overlapping may become a huge obstacle for scaling up the method.

To address the signal overlapping issue, Liu *et al.* demonstrated a microfluidic CODES technology based on the code division multiple access (CDMA) principle to simultaneously resolve signals from a combined waveform.^{102,103} The CDMA technology, widely used in telecommunications, achieves signal multiplexing by modulating signals from individual channels with different orthogonal digital spreading codes; individual signals can be recovered by cross-correlating the multiplexed signal with standard templates, i.e., the measured analog square pulse signals representing each digital spreading code. The sensing principle of the microfluidic CODES method is shown in Fig. 8(D). On the microfluidic device, finger-encoding electrodes with unique patterns (according to digital spreading codes) were used for generating the digital spreading codes (called Gold sequences), to modulate current signals from individual sensing channels. Figure 8(D) shows the sensing principle. In channel 1, the electrode is patterned according to the digital spreading codes (i.e., 1010110). When a cell passes through channel 1, a normalized analog signal can be measured and defined as template 1. Similarly, templates from other channels can be created. Hence, the autocorrelation coefficient between template 1 and itself (R11) exhibits a maximum amplitude at 1, while the autocorrelation coefficients between template 1 and other templates are lower than 0.5, meaning that templates are orthogonal to each other. Therefore, signals from individual channels can be identified by correlating the combined signal with each unique standard template signal interactively; from the autocorrelation peak amplitude and location, and the corresponding template, the original current signal can be estimated and recovered. A subsequent interference cancellation scheme can be used for multiple overlapped signal detection. From the recovered current signals, the sizes and count of cells from each sensing channel can be obtained simultaneously. The microfluidic CODES method can also work with non-orthogonal codes. Although the experiment was conducted on a 4-channel device, this method can be scaled to incorporate additional channels on the same chip. Similar to the frequency division multiplexing method, CODES method may have difficulties for recovering signals from a large number of channels (say 100 channels), because of the residual errors accumulation during the iterative signal recovery processes. Additionally, to generate the unique digital codes, electrodes with unique patterns must be fabricated within the sensing channel; such an encoding electrode is difficult to be fabricated inside nanoscale pores or nano channels for detection of nanoscale particles. Note that barcode type multiplexing methods have also been combined with optical detection, which is reviewed elsewhere.¹⁰⁴

VII. PARTICLE BASED MULTIPLEXING

Micro/nano scale particles can not only provide a large number of active sites, but also enhance electron transfer and improve the detection sensitivity of the EC sensor.^{38,39} In addition, micro/nano particles can be functionalized with a specific antigen/antibody, and participate in the target recognition process via specific immuno-binding reactions, offering new opportunities for biomarker detection and signal multiplexing.^{17,105} The use of micro and nano particles has three major advantages: (1) with high surface to volume ratios, these beads offer more protein binding sites than the surface of conventional microtiter plates/electrodes. This allows a high dynamic range of protein capturing/recognition. (2) Beads with unique magnetic properties can bind with target cells after specific surface modification, such that magnetic-activated cells can be separated, manipulated, and identified. (3) The size distribution of micro/nano particles can be precisely controlled as a new detectable parameter, which can be related to protein concentrations and be utilized for multiplexing.

The characters of affinity microparticles (i.e., size change, magnetic properties) can be measured and correlated with biomarker concentrations. Carbonaro and Sohn studied a resistive

pulse sensor to detect two antigens (human G-CSF and GM-CSF antigens) on a single chip, which measured the size change of latex colloids [Fig. 9(a)].¹⁰⁶ The target biomarker (antigen) led to an increased diameter of a colloid, upon specifically binding with the primary antibody on the colloid surface. The authors demonstrated that after the sandwich assay was complete (target antigen bind with antibody functionalized colloid), the diameter of the colloids was increased by 5 nm. Additionally, two individual pores were fabricated on a same microfluidic chip to detect human GM-CSF antigens and human G-CSF at the same time. This method demonstrated the concept of identifying biomarkers from particle size change. However, because the size change is caused by binding of antibodies, the size change is small (~ 5 – 10 nm). To detect such a small change, nanoscale channels were fabricated using advanced nanofabrication technology, which led to a very low throughput.

Recently microparticle based immuno-aggregation methods^{107–112} have been developed to (1) amplify the size change caused by specific binding and (2) enable multiplexed detections of multiple biomarkers. Han *et al.* have used microparticles with different sizes and magnetic property as platforms for immuno-aggregation.¹¹² The method can quantify concentrations of multiple biomarkers simultaneously based on measuring the immuno-aggregates of antibody functionalized microparticles (Ab-MPs). Further Han *et al.* developed a two-stage micro Coulter counter [as shown in Fig. 9(b)], to differentiate and count the Ab-MP aggregates triggered by two different types of biomarkers. The authors demonstrated two ways for multiplexed detection: (a) multiplexing based on particle size difference. At the 1st stage, Ab1-MP1 doublets and Ab2-MP2 doublets can be differentiated from the measured size distribution, and the concentration of two biomarkers can be measured from the volume fraction of the doublets. And (b) multiplexing based on the particle magnetic property difference. MP2 were magnetic particles while MP1 were non-magnetic particles; hence Ab2-MP2s and their aggregates were captured in the chamber where an external magnetic field was applied. Only non-magnetic particles (Ab1-MP1s) and their aggregates were detected by the 2nd stage micro Coulter counter, while the 1st stage counter detected all particles and aggregates regardless of their magnetic property. The difference in counts measured by the 1st and 2nd stage counter was the magnetic particle aggregates, which indicated the concentration of the BM2. Using both multiplexing methods, the authors demonstrated detections of mouse anti-rabbit IgG (BM1) and human ferritin (BM2) from a single test with various concentrations: 3.1 to 10 4 ng/ml and 5.2 ng/ml to 208 ng/ml, respectively. The two-stage device is capable of measuring up to 4 types of biomarkers while additional types of

FIG. 9. (a) Illustration of the sandwich assay performed on the surface of a colloid. When a colloid passes through a pore with diameter comparable to that of the colloid, a decrease in current is measured. Reproduced with permission from Carbonaro and Sohn, *Lab Chip* 5, 1155 (2005). Copyright 2005 The Royal Society of Chemistry. (b) Schematic of the multiplexed immuno-aggregation assay and design concept of the two-stage resistive pulse sensor. A mixture of antibody functionalized microparticles (Ab-MPs) containing two probes, Ab1-MP1 and Ab2-MP2, is used to detect and measure the concentrations of two biomarkers, BM1 and BM2, via immuno-aggregation; the immuno-aggregation is analyzed by a pair of resistive pulse sensors (RPSs) separated by a capture chamber. Reproduced from *Biomicrofluidics* 10, 24109 (2016). Copyright 2016 AIP Publishing LLC.

biomarkers can be detected by adding additional stages. While the method is based on specific MP aggregation due to antibody-antigen binding, a small amount of nonspecific attachment and aggregation were still observed. The authors suggested using antifouling materials on the surface to reduce the non-specific attachment/aggregation. This method is promising to serve as a useful tool for rapid detection of multiple biomarkers in biomedical research and clinical applications. The performance (sensitivity, detection limits, false positives sources, etc.) of the electrical biosensors reviewed in this article are summarized in Table I.

Other non-electrical, particle-based multiplexing methods measuring the difference between beads' properties (i.e., size/magnetic property),^{113–117} were also developed. Tekin and Gijs published a general review paper on utilizing magnetic beads in microfluidic systems for ultrasensitive protein detection.¹¹⁸ Reverte published a review paper covering usage of nanomaterials to improve electrochemical biosensors' performance.⁵⁶

VIII. DISCUSSION AND OUTLOOK

Electrical multiplexing techniques for lab-on-chip biomarker detections have been reviewed in this paper. With these methods, detections of multiple analytes and higher throughput have been achieved. As an example, spatial and time multiplexing have been widely applied to many integrated electrochemical sensor arrays and commercial instruments. However, there are still several long-standing problems limiting their applications for practical applications. While electrical impedance biosensors detect the local impedance change caused by the specific binding of the recognition probe with the target biomarker, the detection accuracy and reliability are affected by nonspecific binding/attachment, electrode fouling, and short life cycle of the probes. These issues can be minimized by using advanced probe/label materials (e.g., nanoparticle probe⁷⁴), and employing emerging sensing elements (e.g., nanowire^{77,78}), as well as utilizing anti-fouling coatings^{119,120} that can suppress/eliminate the false positives. These sensors may serve as replacements and supplements for commercially available biomarker analysis platforms with higher accuracy and throughput, without sacrificing reliability.

On the other hand, while low cost lab-on-a-chip devices have large potentials to be used for the resource poor scenarios, steps of sample preparation, data acquisition, and signal processing must be minimized. To this end, the emerging multiplexing techniques, including frequency, codes, and particle-based methods, are useful to simplify the detections and suit the need for field applications. For the frequency and codes based multiplexing methods, there is a growing trend to generate the encoding signals from the chip or even by the analyte itself, which will greatly reduce dependency on the external hardware. In the meanwhile, algorithms for multiplexing/demultiplexing the combined signals need further development and optimization for faster analysis and higher throughput, which are two keys for field applications. Also, to improve the specificity and reduce sample preparation, novel methods to functionalize the sensing channels or microelectrodes are highly desired owing to the difficulty in modifying surfaces within a constraint space in micro/nano fluidics devices. Notably, particle-based multiplexing methods have been fast growing and are shifting their roles from facilitating the detection to serving as a platform for biomarker recognition and quantifications. In the future, more characteristics of the particles can be explored as new indicators for multiplexed detections of different types of biomarkers.

With (1) accurate and reliable sensing mechanisms, (2) scalable and simple multiplexing strategies, and (3) new opportunities brought by advanced functional materials, fabrication processes, and signal processing algorithms, lab-on-chip electrical multiplexing methods will continue offering accurate, sensitive, and high throughput solutions for both fundamental and applied biomarker detections.

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